GENETICS OF NATURAL POPULATIONS. XIV. A RESPONSE OF CERTAIN GENE ARRANGEMENTS IN THE THIRD CHROMOSOME OF *DROSOPHILA PSEUDOOBSCURA* TO NATURAL SELECTION

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INTRODUCTION

CYCLIC changes in the frequencies of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* take place in populations of some localities on Mount San Jacinto, in California (DOBZHANSKY 1943). Chromosomes with Standard gene arrangement (ST) are least frequent in June, increase in frequency during the summer, remain at a high level during autumn and winter, and decline during spring. Chromosomes with Arrowhead (AR) and particularly with Chiricahua (CH) gene arrangements undergo cycles opposite in sign to that of ST chromosomes. The supposition that these cyclic changes are due to natural selection is in accord with all the facts available, and constitutes a fruitful working hypothesis for further research.

Artificial models of natural populations can be created with the aid of population cages of the type first proposed by L'HÉRITIER and TEISSIER (see WRIGHT and DOBZHANSKY 1946 for a description of the model used). If a mixture of flies containing more than 30 percent Chiricahua and less than 70 percent of Standard chromosomes is introduced in a population cage, the frequency of ST rises and that of CH decreases generation after generation. Yet, the CH chromosomes are never eliminated completely; the population reaches an equilibrium at about 70 percent ST and 30 percent CH. If the initial population contains many CH and few AR chromosomes, the frequency of AR increases at the expense of CH until an equilibrium is established. These changes in the frequencies of the chromosomal types take place in population cages kept at temperatures above 20°C; at 16½°C no changes are observed.

The simplest hypothesis that fits the observations on the behavior of the chromosomal types in population cages is that, at temperatures above 20°C, the heterozygotes ST/CH have a higher adaptive value than ST/ST homozygotes, and these latter higher than CH/CH homozygotes. Similarly, the adaptive values of the carriers of AR and CH gene arrangements form the series AR/CH > AR/AR > CH/CH. At 16½°C the adaptive values of the homo- and heterozygotes are similar, at any rate more so than they are at higher temperatures (WRIGHT and DOBZHANSKY 1946).

However, much remains to be learned about the operation of natural selection which brings about the changes both in free-living populations and in the experimental cages. Nothing is known at present about the stage of the life

cycle of the fly at which the differential survival of the different chromosomal types take place. It is not certain that selection acts equally on females and males, or that the selection intensity remains constant regardless of the frequencies of the chromosomal types in the population (WRIGHT and DOBZHANSKY 1946). The present article reports the results of experiments designed to test further the validity of the hypothesis of natural selection in the case of the chromosomal variation in *D. pseudoobscura*, as well as to approach the more special problems just stated.

MATERIAL AND METHODS

Except when otherwise specified, the progenitors of all the flies used in this study were collected at Piñon Flats, San Jacinto, California. The collections were made partly in 1942 by the writer, but mostly in late April of 1945 by MR. ALEXANDER SOKOLOFF.

The technique of preparing the initial mixtures of flies with desired proportion of chromosomes of different kinds has been described by WRIGHT and DOBZHANSKY (1946). The same paper contains descriptions of the methods of sampling the composition of a population in an experimental cage and of cytological examination of the chromosomes.

CHANGES OBSERVED IN THE POPULATION CAGES NUMBERS 22 AND 23

On September 19, 1945, an initial population of 1366 flies was introduced in cage number 22, which was then placed in a room with constant temperature regulated at 25°C and with humidity of about 70 percent. This population contained 20.31 percent of ST and 79.69 percent of CH chromosomes. The ST chromosomes came from 20 different strains and CH chromosomes also from 20 different strains (see WRIGHT and DOBZHANSKY 1946, for comment on the importance of using several strains as source material for population cage experiments). On the same day, 1763 flies containing a mixture of 20.72 percent AR and 79.28 percent CH chromosomes were placed in cage number 23. The AR chromosomes came from 11, and CH chromosomes from 15 different strains. Cage number 23 was placed in the same constant temperature and humidity room in which cage number 22 stood.

Samples of eggs deposited in the population cages were taken in late October, late November, and late December by the usual method (WRIGHT and DOBZHANSKY 1946), and the gene arrangement was determined in 300 chromosomes in each sample. The results are summarized in table 1. In early January of 1946 both cages developed mite infections and were destroyed.

Table 1 shows that in cage number 22 the frequency of ST chromosomes rose from the initial 20.3 percent in September to 54.7 percent in December, and the frequency of CH dropped from 79.7 in September to 45.3 in December. Similarly, in cage number 23 there was an increase of the frequency of AR from 20.7 to 56.7 percent, and a decline of CH from 79.3 to 43.3 percent. There is no question that these changes are statistically significant; in fact the increases and decreases observed on successive months are also significant.

The changes observed in cages numbers 22 and 23 are in accord with the findings of WRIGHT and DOBZHANSKY (1946) in analogous experiments. The simplest hypothesis that fits both series of experiments is that, if the adaptive value of the heterozygotes ST/CH is taken to be 1.0, then the adaptive values of ST/ST and CH/CH homozygotes are, under the condition of the population cages, about 0.7 and 0.3 respectively. Accepting these values (slightly different

TABLE 1

Percentage frequencies of the different gene arrangements in the populations of the cages Nos. 22 and 23 at different times.

TIME	EXPERIMENT NO. 22			EXPERIMENT NO. 23		
	ST	CH	n	AR	CH	n
Initial (September)	20.3	79.7		20.7	79.3	
October	32.3	67.7	300	33.7	66.3	300
November	42.7	57.3	300	46.3	53.7	300
December	54.7	45.3	300	56.7	43.3	300

estimates, based on all the available data, will be found on p. 152), and taking the duration of a generation to be one month (which is a slight overestimate), one can easily compute the expected frequencies of ST in cage number 22 on different months. A comparison of the observed and expected frequencies is as follows (in percentages):

	OBSERVED	EXPECTED
September	20.3	20.3
October	32.3	35.1
November	42.7	47.0
December	54.7	54.8

The agreement is excellent. No estimates of the adaptive values of the genotypes AR/CH, AR/AR, and CH/CH in cages containing only these two gene arrangements have been made by WRIGHT and DOBZHANSKY. However, the parallelism between the changes in cages numbers 22 and 23 is so great (table 1) that the relative adaptive values of these three genotypes are probably not far from those of ST/CH, ST/ST, and CH/CH (see above). The population in cage number 22 should eventually reach the equilibrium values of about 70 percent ST and 30 percent CH chromosomes, after which the changes should no longer occur. The mite infection made it impossible to check the validity of this prediction in cage number 22; however, it has been realized in analogous experments of WRIGHT and DOBZHANSKY (1946), as well as in experiments to be reported on elsewhere.

THE HARDY-WEINBERG PROPORTIONS IN EGG SAMPLES

Provided that the flies carrying different chromosomal types interbreed at random, the zygotes produced by a panmictic natural population, or by one

confined in a population cage, must contain heterozygotes and homozygotes in proportions indicated by the formula given about forty years ago by HARDY and WEINBERG. Taking the frequency of gametes carrying ST chromosomes to be q, and of gametes with CH chromosomes to be $(1-q)$, the frequencies of the homo- and heterozygotes will be as follows:

$$q^2\ ST/ST : 2q(1-q)\ ST/CH : (1-q)^2\ CH/CH$$

However, if the different classes of zygotes survive at different rates, their proportions may deviate from the ones demanded by HARDY-WEINBERG'S formula. Thus, assuming the survival rates of the ST/ST, ST/CH, and CH/CH genotypes to be W_1, W_2, and W_3, the proportions of the different genotypes at the stage of the life cycle following that at which the selective elimination occurs will be:

ST/ST	q^2W_1
ST/CH	$2q(1-q)W_2$
CH/CH	$(1-q)^2W_3$

Disturbances of the HARDY-WEINBERG proportions may then be used to detect the occurrence of selective elimination of zygotes.

The composition of the populations in cages numbers 22 and 23, as well as in the experiments described by WRIGHT and DOBZHANSKY (1946), was examined at approximately monthly intervals. Every month pieces of the culture medium with eggs deposited on it were withdrawn from each population cage on six successive days. These eggs were placed in regular culture bottles and allowed to develop at room temperature; when small larvae appeared in the bottles they were given extra yeast to attain optimal nutrition. The salivary gland cells of 25 larvae from each culture were examined under a microscope. A monthly sample, consisting of six subsamples, includes therefore 150 larvae. The gene arrangements were determined in two chromosomes in each larva, heterozygous as well as homozygous individuals being easily distinguishable by presence of inversion loops or by the patterns of stainable discs in the chromosomes. Numbers of hetero- and homozygotes observed in all experiments involving ST and CH chromosomes are summarized in table 2, and in experiments involving AR and CH chromosomes in table 3. Gametic frequencies of the gene arrangements, that is the frequencies of each gene arrangement in the sample of gametes which gave rise to a given group of 150 larvae, can easily be determined. Each homozygote is formed, of course, by two gametes with identical chromosomes, while a heterozygote is a product of the union of two gametes with different chromosomes. A sample of 150 larvae came from 300 gametes with 300 third chromosomes. The gametic frequencies, expressed in percentages, are shown in the two rightmost columns in tables 2 and 3. Knowing the gametic frequencies one can compute, with the aid of the HARDY-WEINBERG formula, the numbers of hetero- and homozygotes expected to occur in each sample of 150 larvae. These computations involve the assumptions that the populations of the experimental cages are panmictic, and that the different classes of zygotes suffer no differential mortality between the egg stage and the

TABLE 2

Zygotic and gametic frequencies of the Standard (ST) and Chiricahua (CH) gene arrangements among the eggs deposited in the population cages.

CAGE NO.	DATE OF SAMPLE		ZYGOTIC FREQUENCIES			χ^2	GAMETIC FREQUENCIES IN PERCENT	
			ST/ST	ST/CH	CH/CH		ST	CH
		Obs	37	82	31		52.0	48.0
8	January 1944	Exp	40.5	74.9	34.5	1.33		
		Diff	−3.5	+ 7.1	−3.5			
		Obs	46	89	15		60.3	39.7
8	February 1944	Exp	54.6	71.8	23.6	8.61		
		Diff	−8.6	+17.2	−8.6			
		Obs	58	74	18		63.3	36.7
8	March 1944	Exp	60.1	69.7	20.2	0.58		
		Diff	−2.1	+ 4.3	−2.2			
		Obs	70	68	12		69.3	30.7
8	April 1944	Exp	71.4	64.2	14.4	0.65		
		Diff	−1.4	+ 3.8	−2.4			
		Obs	52	81	17		61.7	38.3
11	July 1944	Exp	57.1	70.9	22.0	2.99		
		Diff	−5.1	+10.1	−5.0			
		Obs	63	73	14		66.3	33.7
11	August 1944	Exp	65.9	67.0	17.0	1.20		
		Diff	−2.9	+ 6.0	−3.0			
		Obs	66	59	25		63.7	36.3
11	September 1944	Exp	60.9	69.4	19.8	3.36		
		Diff	+5.1	−10.3	+5.2			
		Obs	39	86	25		54.7	45.3
13	August 1944	Exp	44.9	74.3	30.8	3.65		
		Diff	−5.9	+11.7	−5.8			
		Obs	51	73	26		58.3	41.7
13	September 1944	Exp	51.0	72.9	26.1	0.00		
		Diff	0.0	+ 0.1	−0.1			
		Obs	41	77	32		53.0	47.0
19	December 1944	Exp	42.1	74.7	33.1	0.10		
		Diff	−1.1	+ 2.3	−1.1			
		Obs	56	78	16		63.3	36.7
19	January 1945	Exp	60.1	69.8	20.2	2.11		
		Diff	−4.1	+ 8.2	−4.2			
		Obs	53	75	22		60.3	39.7
19	February 1945	Exp	54.6	71.8	23.7	0.31		
		Diff	−1.6	+3.2	−1.7			
		Obs	65	66	19		65.3	34.7
19	March 1945	Exp	63.9	68.0	18.0	0.14		
		Diff	+1.1	− 2.0	+1.0			
		Obs	61	74	15		65.3	34.7
19	April 1945	Exp	64.0	68.0	18.0	1.17		
		Diff	−3.0	+ 6.0	−3.0			
		Obs	58	60	7		70.4	29.6
19	June 1945	Exp	62.0	52.1	11.0	2.94		
		Diff	−4.0	+ 7.9	−4.0			
		Obs	20	88	42		42.7	57.3
22	November 1945	Exp	27.3	73.4	49.3	5.93		
		Diff	−7.3	+14.6	−7.3			
		Obs	41	82	27		54.7	45.3
22	December 1945	Exp	44.8	74.4	30.8	1.57		
		Diff	−3.8	+ 7.6	−3.8			

TABLE 3

Zygotic and gametic frequencies of the Arrowhead (AR) and Chiricahua (CH) gene arrangements among the eggs deposited in the population cages.

CAGE NO.	DATE OF SAMPLE		ZYGOTIC FREQUENCIES AR/AR	AR/CH	CH/CH	χ^2	GAMETIC FREQUENCIES IN PERCENT ST	CH
		Obs	20	86	44		42.0	58.0
15	September 1944	Exp	26.5	73.1	50.5	4.71		
		Diff	−6.5	+12.9	−6.5			
		Obs	26	77	47		43.0	57.0
15	August 1944	Exp	27.7	73.5	48.7	0.34		
		Diff	−1.7	+3.5	−1.7			
		Obs	29	81	40		46.3	53.7
23	November 1945	Exp	32.2	74.6	43.2	1.04		
		Diff	−3.2	+6.4	−3.2			
		Obs	48	74	28		56.7	43.3
23	December 1945	Exp	48.2	73.6	28.1	0.02		
		Diff	−0.2	+ 0.4	−0.1			

stage of grownup larva at which the cytological examination is made. The expected numbers of homo- and heterozygotes, and the differences between observed and expected numbers, are shown in tables 2 and 3. Chi-squares that measure the statistical significance of these differences are also included in these tables. Each chi-square has one degree of freedom; this is because the gametic frequencies are calculated from the same observed zygotic data which are subsequently used for comparison with the zygotic frequencies expected if the HARDY-WEINBERG proportions are realized.

Among the twenty-one samples tabulated in tables 2 and 3, only one, that for February 1944 in cage number 8, shows a very significant difference between the observed and the expected frequencies of the homo- and heterozygotes. The chi-square is in this case equal to 8.61, which has a probability of chance occurrence of about 0.004. Two more samples, namely for November 1945 in cage number 22 and for September 1944 in cage number 15, show chi-squares of 5.93 and 4.71 respectively, which are ordinarily taken as indicating significant deviations between the observed and the expected figures. In all other samples the observed and expected numbers agree well enough.

However, it may be noted that among the twenty-one samples listed in tables 2 and 3 only two samples (September 1944 in cage 11 and March 1945 in cage 19) show numbers of homozygotes in excess, and numbers of heterozygotes, below the expectation. The remaining nineteen samples show greater or smaller excesses of heterozygotes and deficiencies of homozygotes. The total chi-square

for all data in table 2 is 36.6, which, for 17 degrees of freedom, has the probability of chance occurrence less than 0.004. There is consequently little doubt that the inversion homozygotes are discriminated against, and heterozygotes are favored, even among larvae raised on abundant food in culture bottles. Nevertheless, the conclusion to be drawn from the data in tables 2 and 3 is that the HARDY-WEINBERG ratios are approached rather closely. In any case, there is nothing in the data to indicate that the adaptive value of ST/ST zygotes is 70 percent, and of CH/CH zygotes only 30 percent, of the value of ST/CH zygotes, as postulated by WRIGHT and DOBZHANSKY (1946). Taking the data in table 2 as being representative of the conditions which obtain when the larvae from the eggs deposited in population cages are grown in regular culture bottles, PROFESSOR SEWALL WRIGHT has very kindly communicated to the writer the following estimates of the relative viabilities of the ST/ST, ST/CH, and CH/CH classes:

	W
ST/ST	0.907
ST/CH	1.000
CH/CH	0.782

From the data in table 3, the corresponding values for the AR/AR, AR/CH, and CH/CH zygotes are as follows:

	W
AR/AR	0.916
AR/CH	1.000
CH/CH	0.803

This is very far from the values 0.7, 1.0, and 0.3, for ST/ST, ST/CH, and CH/CH zygotes respectively, arrived at by WRIGHT and DOBZHANSKY on the basis of analysis of changes in the composition of the cage populations. The discrepancy may be explained in several ways. First of all, the hypothesis of WRIGHT and DOBZHANSKY does not postulate that the differences in the adaptive values manifest themselves through selective mortality between the egg stage when the samples are taken in population cages and the late larval stage when the examination of the chromosomes in the salivary glands is made. It is conceivable that the selective mortality occurs in the pupa stage, or that ST/ST and CH/CH flies have a lower longevity, or lower fecundity, or lower sexual activity than the adult ST/CH individuals. If so, the HARDY-WEINBERG proportions may be realized in the samples of eggs taken in the population cages, and yet the selective process may occur at later stages. Furthermore, selective mortality of larvae of the different genotypes may take place under the conditions of crowding and acute competition for food which prevail in the population cages, and not under the relatively more favorable conditions deliberately maintained in the culture bottles used for raising larvae intended for examination of the salivary gland chromosomes. Indeed, thousands of eggs are deposited in the population cages on the food in every jar inserted into a cage, but at best only hundreds of adult flies hatch in each jar. In a few days after the insertion of a fresh jar, the culture medium in population cages is

completely filled with young larvae most of which doubtless die before reaching full growth. The larvae that do grow up contain salivary chromosomes which are very unfavorable for cytological study. The technique of raising the larvae under optimal conditions is intended to make the cytological study easier. But by placing the larvae in near optimal conditions the differential mortality may well be diminished or even avoided. The fact that the composition of the population in the population cages changes very rapidly at 25°C, but not at 16½°C (WRIGHT and DOBZHANSKY 1946), shows that the differences in adaptive value between the chromosomal types are quite sensitive to environmental influences. Accordingly, a study of the HARDY-WEINBERG ratios was undertaken in flies that had developed in the population cages.

THE HARDY-WEINBERG RATIOS AMONG ADULT FLIES

On December 14, 1945, the adult flies present at that time in the population cages numbers 22 and 23 were transferred to bottles and etherized. Slightly more than one hundred males were chosen at random from amongst the flies in each cage. The males from cage number 22 were outcrossed singly to females known to be homozygous for ST chromosomes; the males from cage number 23 were likewise outcrossed to known AR homozygotes. The outcrosses were made in regular culture bottles, which were treated so as to insure an optimal development of larvae desired for cytological examination. When the larvae reached maturity, the salivary glands of six larvae from each bottle were stained in acetic orcein and examined for the condition of the third chromosomes. When two types of chromosomal structure (for example, ST/ST and ST/CH) are found among the larvae, it necessarily follows that the father of these larvae was a heterozygote carrying a ST and a CH chromosome. When all six larvae showed the same configuration in the third chromosome, the father was probably a structural homozygote. However, with six larvae studied there is still one chance in thirty-two that the father was a heterozygote but that by accident all the larvae examined were of the same chromosomal type. The observed numbers of the different chromosomal types among the males developed in the population cages are reported in tables 4 and 5 in the horizontal row labelled "old ♂♂."

On the same day, three jars containing mature ready-to-hatch pupae were withdrawn from the population cages numbers 22 and 23. The flies that hatched in these jars were collected for several days, males and females being separated so that the latter remained virgin. The flies from cage number 22 were then outcrossed singly to known ST/ST homozygotes, and those from cage number 23 to known AR/AR homozygotes. The larvae coming from the outcrosses were kept well fed, and when they matured six larvae from each cross were dissected and their salivary glands were stained in acetic orcein and examined under a microscope. Thus the zygotic chromosomal constitutions of the females and males that had developed in the population cages up to the stage of late pupa became known. The data are shown in tables 4 and 5 in the rows labelled "Young ♀♀" and "Young ♂♂."

The gametic frequencies of the gene arrangements in the samples of adult flies having developed in the population cages can easily be calculated from the zygotic data. The expected zygotic frequencies are then computed with the aid of the HARDY-WEINBERG formula. The expected numbers, and their deviations from the observed ones, are given in tables 4 and 5. Chi-squares are calculated to evaluate the statistical significance of the deviations observed. These chi-squares have one degree of freedom.

TABLE 4

Genetic structure of adult flies in population cage No. 22.

SEX AND AGE		ZYGOTIC FREQUENCIES			χ^2	GAMETIC FREQUENCIES IN PERCENT	
		ST/ST	ST/CH	CH/CH		ST	CH
Young ♀♀	Observed	31	83	16		55.8	44.2
	Expected	40.4	64.1	25.4			
	Difference	− 9.4	+18.9	− 9.4	11.24		
Old ♂♂	Observed	26	86	13		55.2	44.8
	Expected	38.1	61.6	25.1			
	Difference	−12.1	+24.2	−12.1	19.14		
Total	Observed	57	169	29		55.5	44.5
	Expected	78.5	126.0	50.5			
	Difference	−21.5	+43.0	−21.5	29.70		

The heterozygotes ST/CH are decidedly more frequent, and the homozygotes ST/ST and CH/CH less frequent, than expected. This is true for the male as well as for the female flies and for the total. The smallest chi-square, 11.24 for the young females, has a probability of chance occurrence of about 0.001. The probabilities of the deviations observed for the old males and in the total being due to chance are negligible. Table 5 shows that the observed and the expected frequencies for the young males agree well enough, but the heterozygotes are significantly more frequent than expected among young females as well as among old males. The total shows a highly significant excess of heterozygotes and a deficiency of homozygotes.

It must be noted that the excesses of heterozygotes and the deficiencies of homozygotes are very likely even greater than the data in tables 4 and 5 indicate. As stated above, the technique of distinguishing the homo- and heterozygotes was such that approximately one in every thirty-two heterozygotes must have been misclassified as a homozygote. The expected numbers of the different classes must, therefore, be corrected according to the following formulae:

ST/ST or AR/AR	$q^2+1/64[2q(1-q)]$
ST/CH or AR/CH	$62/64[2q(1-q)]$
CH/CH	$(1-q)^2+1/64[2q(1-q)]$

If these corrections are introduced, the observed and the expected values for the total in cage number 22 become as follows (compare with table 4):

	ST/ST	ST/CH	CH/CH
Observed	57	169	29
Expected	80.5	122.0	52.5
Difference	−23.5	+47.0	−23.5

For the total in cage number 23 the observed and the expected figures become as follows (compare with table 5):

	AR/AR	AR/CH	CH/CH
Observed	80	196	58
Expected	97.5	161.0	75.5
Difference	−17.5	+35.0	−17.5

It is, then, safe to conclude that, among the adult flies developed in the population cages numbers 22 and 23, heterozygotes are quite appreciably more frequent, and homozygotes less frequent, than demanded by the HARDY-WEINBERG formula. It should, however, be recalled that the HARDY-WEINBERG proportions were found to be relatively only slightly disturbed among the larvae coming from the eggs deposited in the population cages but grown under the near-optimal conditions in culture bottles (tables 2 and 3). It follows that a selective mortality takes place among the individuals homozygous for the gene arrangements ST, CH, and AR in the population cages. At just what stage of the life cycle the selective mortality occurs does not appear from the data, except that it must lie between the egg stage and the emergence of the adult insect from the pupa.

TABLE 5

Genetic structure of adult flies in population cage No. 23.

SEX AND AGE		ZYGOTIC FREQUENCIES			χ^2	GAMETIC FREQUENCIES IN PERCENT	
		AR/AR	AR/CH	CH/CH		AR	CH
Young ♀♀	Observed	24	63	13		55.5	44.5
	Expected	30.8	49.4	19.8			
	Difference	− 6.8	+13.6	− 6.8	7.57		
Old ♂♂	Observed	22	63	15		53.5	46.5
	Expected	28.6	49.8	21.6			
	Difference	− 6.6	+13.2	− 6.6	7.04		
Young ♂♂	Observed	34	70	30		51.5	48.5
	Expected	35.5	66.9	31.5			
	Difference	− 1.5	+ 3.1	− 1.5	0.27		
Total	Observed	80	196	58		53.3	46.7
	Expected	94.8	166.3	72.9			
	Difference	−14.8	+29.7	−14.9	10.66		

The finding that selective elimination of homozygotes takes place in the population cages is important because it proves that the hypothesis of natural selection is correctly applied to our data. An attempt may now be made to ascertain whether this selective mortality occurs on a scale sufficient to account for the speed of the changes in frequencies of the gene arrangements observed in cages numbers 22 and 23 (table 1), as well as in the experiments described earlier by WRIGHT and DOBZHANSKY (1946).

WRIGHT and DOBZHANSKY (1946) have published the results of four experiments (numbers 8, 11, 13, and 19) in which population cages with mixtures of ST and CH chromosomes were kept at temperatures close to 25°C. Two more experiments of this kind are described in the present article (tables 1 and 9). The results of all experiments are summarized in table 6. The column marked q_0 gives the frequencies of ST chromosomes found in the successive samples taken in each cage. The column Δq_0 shows the differences between the frequencies q_0 in the successive samples. The time intervals elapsed between the samples are indicated in weeks. Since a fly generation takes about 3.5 weeks in population cages at 25°C, the net differences, Δq_0, must be converted into differences per 3.5 week periods; the resulting figures are given in the column marked "Δq_0 per generation." The figures in the left part of the table are "Uncorrected" for the systematic error arising because a slight but perceptible differential mortality of ST/ST and CH/CH homozygotes takes place even when the larvae are raised on abundant food (see above). If this differential mortality is corrected for, the observed frequencies of ST chromosomes, q_0, give the real frequencies, q, and the "Crude Δq" and the "Δq per generation" can easily be computed (table 6).

The selective disadvantages of the homozygotes ST/ST and CH/CH, denoted s and t respectively, the adaptive values W, and the frequency of ST chromosomes, $\hat{q}$, at which the population of an experimental cage reaches equilibrium, have been computed from the data in table 6 by PROFESSOR SEWALL WRIGHT with the aid of the equations given in WRIGHT and DOBZHANSKY, 1946 (see particularly equation 11, on page 142). From the uncorrected data in table 6 the following results are obtained:

GENOTYPE	FREQUENCY	W		
ST/ST	q^2	0.766	s=0.234	
ST/CH	$2q(1-q)$	1.000		$\hat{q}$=0.724
CH/CH	$(1-q)^2$	0.387	t=0.613	

The corresponding values derived from the corrected data are:

GENOTYPE	FREQUENCY	W		
ST/ST	q^2	0.762	s=0.238	
ST/CH	$2q(1-q)$	1.000		$\hat{q}$=0.723
CH/CH	$(1-q)^2$	0.379	t=0.621	

The corrected and the uncorrected data yield substantially the same estimates. The adaptive value of ST homozygotes is, under the conditions of the experiments, approximately 76 percent of that of the ST/CH heterozygotes; the adaptive value of CH homozygotes is only 38 percent of that of the

TABLE 6

Summary of data on rate of change in the frequency of ST gene arrangement in population cages kept at temperatures close to 25°C. Further explanation in text.

EXPERIMENT NO.	INTERVAL (WEEKS)	UNCORRECTED			CORRECTED		
		q_0	CRUDE Δq_0	Δq_0 PER GENERATION	q	CRUDE Δq	Δq PER GENERATION
8	4.5	0.520	+0.083	+0.065	0.503	+0.091	+0.071
	3.0	0.603	+0.030	+0.035	0.594	+0.033	+0.038
	4.5	0.633	+0.060	+0.047	0.627	+0.065	+0.051
	—	0.693	—	—	0.692	—	—
11	5.5	0.463	+0.154	+0.098	0.440	+0.169	+0.107
	4.0	0.617	+0.046	+0.040	0.609	+0.050	+0.044
	4.5	0.633	−0.026	−0.020	0.659	−0.028	−0.022
	—	0.637	—	—	0.631	—	—
13	7.0	0.292	+0.255	+0.127	0.262	+0.270	+0.135
	3.5	0.547	+0.036	+0.036	0.532	+0.040	+0.040
	—	0.583	—	—	0.572	—	—
19	6.0	0.383	+0.147	+0.086	0.355	+0.159	+0.093
	4.5	0.530	+0.103	+0.080	0.514	+0.113	+0.088
	5.5	0.633	−0.030	−0.019	0.627	−0.033	−0.021
	4.5	0.603	+0.050	+0.039	0.594	+0.054	+0.043
	4.5	0.653	0.000	0.000	0.648	0.000	0.000
	6.0	0.653	+0.051	+0.030	0.648	+0.056	+0.032
	7.5	0.704	+0.016	+0.007	0.704	+0.017	+0.008
	—	0.720	—	—	0.721	—	—
22	4.5	0.203	+0.120	+0.093	0.175	+0.118	+0.092
	4.5	0.323	+0.104	+0.081	0.293	+0.109	+0.085
	4.5	0.427	+0.120	+0.093	0.402	+0.130	+0.102
	—	0.547	—	—	0.532	—	—
24	4.5	0.896	−0.029	−0.023	0.902	−0.029	−0.022
	4.5	0.867	−0.017	−0.013	0.873	−0.017	−0.013
	4.5	0.850	−0.050	−0.039	0.856	−0.051	−0.040
	—	0.800	—	—	0.805	—	—

ST/CH heterozygotes. These values are, however, somewhat higher than those arrived at by WRIGHT and DOBZHANSKY, 1946, on the basis of the older data (the old values are 0.7 for ST/ST and 0.3 fo CH/CH homozygotes). The equilibrium frequency of ST chromosomes is about 72 percent, instead of the old figure, 0.7, given by WRIGHT and DOBZHANSKY.

The selective disadvantages of the homozygotes (s and t) and the selective values (W) can, however, be estimated also quite independently from the above, using the deviations from the HARDY-WEINBERG proportions which are

observed among the adult flies in the population cages. Indeed, the HARDY-WEINBERG proportions are approximately, though incompletely, realized among the larvae coming from the eggs deposited in the population cages but grown in culture bottles with abundant food (see above). Among the adult flies much greater deviations from these proportions are apparent. Consequently, a differential elimination of homozygotes takes place between the egg and the adult stages.

The fundamental equations with the aid of which the adaptive values and the selective disadvantages can be calculated from the deviations from the HARDY-WEINBERG proportions among the adult flies have been generously supplied by PROFESSOR SEWALL WRIGHT. They are as follows:

$$ST/ST = nq^2 (1-s)$$
$$ST/CH = 2nq (1-q)$$
$$CH/CH = n(1-q)^2 (1-t)$$
$$\hat{q} = t/(s+t) = 0.7, \text{ from WRIGHT and DOBZHANSKY (1946)}$$

In these equations, q is the unknown frequency of ST chromosomes among the gametes from which the examined sample of the adult flies came (q_0 being the frequency after the selection has taken place, cf. table 6), s and t the selective disadvantages of ST/ST and CH/CH zygotes, n the unknown number of zygotes before selection, and $\hat{q}$ the equilibrium frequency of ST chromosomes in population cages containing mixtures of ST and CH. The data in table 4 can now be used to estimate the adaptive values, W. The observed numbers of the ST/ST, ST/CH, and CH/CH zygotes are corrected for the misclassified heterozygotes (see above). The adaptive values, W, for ST/ST and CH/CH zygotes equal (1−s) and (1−t) respectively. The results of the calculations made by PROFESSOR WRIGHT are shown in table 7.

TABLE 7

Estimates of the adaptive values of ST/ST, ST/CH, and CH/CH zygotes derived from the data in table 4.

ZYGOTE	YOUNG ♀♀				OLD ♂♂				AVERAGE W
	OBSERVED	CORRECTED	CALCULATED	W	OBSERVED	CORRECTED	CALCULATED	W	
ST/ST	31	29.7	41.6	0.714	26	24.6	37.0	0.665	0.690
ST/CH	83	85.6	85.6	1.000	86	88.8	88.8	1.000	1.000
CH/CH	16	14.7	44.0	0.334	13	11.6	53.3	0.218	0.277
n=171.2		q=0.493		q_0=0.558	n=179.1		q=0.455		q_0=0.552

The estimates of W derived from the data on females and on males are, as could be expected, somewhat different. Their averages give the most reliable values that can be secured. These values, 0.69 for ST/ST and 0.28 for CH/CH, agree remarkably well with the estimates, 0.7 and 0.3 respectively, arrived at by WRIGHT and DOBZHANSKY, 1946, from the data on changes in the frequen-

cies of ST and CH with time in the population cages. The agreement is somewhat less close with the improved estimates, 0.76 and 0.38 respectively, arrived at from the more complete data (see page 152). In any case, it is justified to conclude that the differential mortality (inferred from the data on the deviations from the HARDY-WEINBERG proportions among the adult flies in the population cages) is more than sufficient to account for the rate of change in the frequencies of ST and CH chromosomes observed with time in these population cages.

The adaptive values of the AR/AR, AR/CH, and CH/CH zygotes have been calculated by PROFESSOR WRIGHT from the data in table 5; the results are shown in table 8.

TABLE 8

Estimates of the adaptive values of AR/AR, AR/CH, and CH/CH zygotes derived from the data in table 5

ZYGOTE	YOUNG ♀♀				OLD ♂♂				YOUNG ♂♂				AVERAGE
	OBSERVED	CORRECTED	CALCULATED	W	OBSERVED	CORRECTED	CALCULATED	W	OBSERVED	CORRECTED	CALCULATED	W	W
AR/AR	24	23.0	31.7	0.726	22	21.0	28.6	0.734	34	32.9	36.0	0.914	0.804
AR/CH	63	65.0	65.0	1.000	63	65.0	65.0	1.000	70	72.2	72.2	1.000	1.000
CH/CH	13	12.0	33.3	0.360	15	14.0	36.9	0.379	30	28.9	36.2	0.799	0.542
	$n=130.0$	$q=0.494$	$q_0=0.555$		$n=130.5$	$q=0.468$	$q_0=0.535$		$n=144.4$	$q=0.499$	$q_0=0.533$		

The best estimates from the available information are adaptive values of about 0.80 for homozygous AR and about 0.55 for homozygous CH. The selection against the homozygotes appears, then, to be weaker in this case than it is for the ST and CH homozygotes in the population cages containing the latter two gene arrangements. Examination of table 1 suggests, however, that the rates of changes in the frequencies of ST and AR gene arrangements competing against CH are much the same in population cages numbers 22 and 23. If so, the adaptive values of AR/AR homozygotes and AR/CH heterozygotes should be about the same as those of ST/ST and ST/CH respectively. If this will be confirmed in future experiments, it would follow that the disturbances in the HARDY-WEINBERG equilibrium proportions shown in tables 5 and 8 are insufficient to account for the speed of the changes in the frequencies of AR gene arrangement observed with time in the population cages. If so, the explanation will have to be looked for in differences of adaptive values among the adults of different chromosomal constitution.

THE EQUILIBRIUM IN POPULATION CAGES

In all the experiments conducted at high temperatures reported by WRIGHT and DOBZHANSKY (1946) as well as in the present article, the frequencies of ST or AR chromosomes were observed to increase at the expense of CH chromosomes. In these experiments the initial populations of the cages contained either equal numbers of CH and ST or AR, or else CH chromosomes had

higher initial frequencies than their stronger competitors. However, CH chromosomes were never supplanted entirely by ST or AR; instead, whenever the population cages were kept for a sufficiently long time, an equilibrium was reached. For ST and CH, this equilibrium lies close to 70 percent ST and 30 percent CH. The existence of this equilibrium is due to the heterozygotes ST/CH exceeding in adaptive value both homozygotes. The equilibrium is reached when the average adaptive level in the population as a whole is at a maximum. An equilibrium is, however, a state which can be attained from two opposite directions. Provided that the interpretation of the above data is correct, it follows that if the initial population of a cage contains more than 70 percent ST and less than 30 percent CH, the frequency of CH must increase with time at the expense of ST. To test this, a mixture of 2235 flies containing 89.6 percent ST and 10.4 percent CH chromosomes was introduced in population cage number 24 on October 20, 1945. At least 19 "kinds" of ST and 12 "kinds" of CH chromosomes went into the mixture (cf. WRIGHT and DOBZHANSKY 1946). Samples of eggs were taken in cage number 24 in late November and late December 1945, and in late January 1946. The results obtained are reported in table 9.

TABLE 9

Percentage frequencies of ST and CH chromosomes in the population cage No. 24

TIME	ST	CH	n
Initial (October)	89.6	10.4	
November	86.7	13.3	300
December	85.0	15.0	300
January	80.0	20.0	300

In three months the frequency of CH has seemingly doubled—it increased from about 10 percent to about 20 percent. The change is just significant statistically (it is below the conventional level of significance if only the samples from November to January are taken into account). However, the observed rate of change is as fast as expected if the adaptive values of the chromosomal types ST/ST, ST/CH, and CH/CH are close to respectively 0.7, 1.0, and 0.3, as estimated by WRIGHT and DOBZHANSKY (1946). Indeed, starting with a population of 90 percent ST and 10 percent CH, one should theoretically get a population with 82.5 percent ST and 17.5 percent CH in about three generations. Unfortunately, cage number 24 contracted a mite infection in January of 1946 and could not be followed further.

The experiment just described is the first among the experiments thus far conducted with population cages in which the frequency of CH chromosomes has apparently increased at the expense of ST chromosomes. The equilibrium frequencies of the gene arrangements can be approached from both directions. An even more convincing demonstration of this has recently been obtained in experiments with population cages which contained flies descended from wild ancestors collected at Mather, California. These experiments will be reported

in detail later, and only a preliminary statement can be given here. Population cage number 29 was started on December 22, 1945, with about 70 percent ST and 30 percent AR chromosomes. In less than six months it reached an equilibrium at the level of about 55 percent ST and 45 percent AR. Population cage number 32, started on the same day as number 29, had initially about 20 percent ST and 80 percent AR. In September 1946 this cage contained 52 percent ST and 48 percent AR. It appears, then, that ST and AR chromosomes from the Mather locality tend, under the conditions of the popultaion cages, to reach an equilibrium at about 50 percent of each.

DISCUSSION

The deviations from the HARDY-WEINBERG ratios of homo- and heterozygotes observed among the adult flies in the population cages constitute a proof of differential survival among the chromosomal types involved. This proof of differential survival, and hence of natural selection, is independent of the demonstration of the action of selection by means of observations on the changes in frequencies of the gene arrangements with time in the population cages. That is, even if one did not know that the frequencies of these gene arrangements actually change under certain conditions (temperature, etc.) in the population cages, one could predict that such changes must occur.

What physiological properties of the carriers of the gene arrangements studied are responsible for the differential survival is unknown. In any case, the differential survival takes place in some environments and not in others. Relatively little differential elimination occurs when larvae are kept without crowding on abundant food (tables 2 and 3). WRIGHT and DOBZHANSKY (1946) have found no changes in the population cages kept at $16\frac{1}{2}$°C, which means that at that temperature the adaptive values of the gene arrangements differ relatively little. Observations in nature show further that while in summer ST chromosomes displace CH, in spring CH chromosomes displace ST (DOBZHANSKY 1943). This suggests that under certain conditions, not yet duplicated in the experimental population cages, carriers of CH may even be superior to those of ST. The experiment conducted in the population cage number 24, in which CH increased in frequency at the expense of ST (see above), does not reproduce the spring conditions in which the natural populations on Mount San Jacinto live. Indeed, what happened in cage number 24 was that the equilibrium of ST and CH chromosomes, which lies at about 70 percent of the former and 30 percent of the latter, was approached by a population which had originally 90 percent ST and only 10 percent CH. All other experiments so far conducted had starting frequencies of ST below and of CH above the equilibrium values. The cyclic changes which occur in nature apparently entail changes in the equilibrium values themselves. In summer the equilibrium is in favor of ST, in autumn and winter ST and CH are equivalent, while in spring the equilibrium changes in favor of CH.

Drosophila pseudoobscura is not unique in having cyclic seasonal changes in the frequencies of genetic variants in natural populations. The excellent work

of DUBININ and TINIAKOV (1945, 1946a, b) has disclosed that a certain inversion, called II-1, increases in frequency in the populations of *D. funebris* in the city of Moscow from spring till early autumn. However, the frequencies of the same inversion dwindle during winters. DUBININ and TINIAKOV were able to show that carriers of this inversion survive artificially induced hibernation less frequently than do carriers of the alternative gene arrangement. This lessened survival ability affects inversion homozygotes as well as heterozygotes. The adaptive value of the inversion II-1 is, consequently, higher than that of its alternative gene arrangement during the warmer season, but lower during hibernation. Furthermore, this inversion is quite common in urban populations but relatively rare in rural districts, indicating that its adaptive value is higher in urban than in rural environments.

The fact that in *D. funebris* both homozygotes and heterozygotes for the inversion II-1 are discriminated against during hibernation makes the genetic mechanism discovered in this species by DUBININ and TINIAKOV less perfect than the one existing in *D. pseudoobscura*. An unusually severe or prolonged winter might throw the inversion out of the population altogether, notwithstanding the fact that the same inversion has a high adaptive value under summer conditions. In *D. pseudoobscura* the inversion heterozygotes are superior in adaptive value to the corresponding homozygotes. The superiority of heterozygotes permits natural populations to respond to spatial as well as to temporal variations in the environment without depletion of their store of genetic variability; such a depletion would lead to loss of evolutionary plasticity. Indeed, the chance of accidental loss of any one gene arrangement is reduced to a minimum because the inversion frequencies reach an equilibrium at which all gene arrangements are retained. Retention of plasticity is possible, however only at a sacrifice of immediate fitness, because the plasticity entails continuous production of relatively ill-adapted inversion homozygotes. The constitution CH/CH acts, as shown above, as a semilethal at temperatures above 20°C. This opposition of plasticity and fitness has been pointed out by HALDANE (1937) and the writer (DOBZHANSKY 1937, 1938), and emphasized especially by MATHER (1943). The situation found in *D. pseudoobscura* illustrates this opposition most vividly.

Looked at from another angle, the superiority of inversion heterozygotes over the homozygotes furnishes a mechanism for the maintenance of heterosis. In a species in which a wide outbreeding is the rule, a mutant gene, or a gene complex which is deleterious to homozygotes may nevertheless be favored by natural selection if it is adaptive in heterozygotes with other genes or gene complexes. If favorable effects are produced by a complex of linked genes which taken separately are less advantageous or even deleterious, an inversion which binds together this complex acquires a selective advantage. Selection consequently favors increasing the variety of inversions with different gene complexes, because the greater the variety of inversions present in a population the less frequently will the relatively ill-adapted homozygotes be produced. Natural populations of some species of Drosophila are composed mainly of inversion heterozygotes. This is in general true of *D. pseudoobscura*; however

such species as *D. virilis*, *D. repleta*, and *D. hydei* seem to be free of inversions, (PATTERSON, STONE and GRIFFEN 1940, WHARTON 1942, WARTERS 1944) and even in *D. pseudoobscura* populations of some territories consist of inversion homozygotes. CARSON and STALKER (1947) failed to detect seasonal changes of inversion frequencies in *D. robusta* near St. Louis, and the writer has reason to believe that no such seasonal changes occur in populations of *D. persimilis* in the Sierra Nevada of California. No seasonal changes have been found in the population of *D. pseudoobscura* at Keen Camp, on San Jacinto. Comparative studies on the population structure in different species living in different environments are a promising field for investigation.

SUMMARY

A mixture of several strains of *Drosophila pseudoobscura* with roughly 20 percent of Standard (ST) and 80 percent of Chiricahua (CH) chromosomes was introduced in a population cage. In about three months this cage contained approximately 55 percent ST and 45 percent CH. The initial population of another cage consisted of roughly 21 percent Arrowhead (AR) and 79 percent CH; three months later AR rose to 57 percent and CH fell to about 43 percent.

Samples of eggs deposited by the flies in the population cages were taken, and the larvae coming from these eggs were grown under optimal conditions. Chromosomal heterozygotes (ST/CH or AR/CH) and homozygotes (ST/ST, AR/AR, CH/CH) are found among such larvae in proportions close to those demanded by the HARDY-WEINBERG formula, although there is a slight but significant excess of heterozygotes. Consequently, the flies in the population cages are panmictic with respect to the chromosomal constitution, and there is relatively little differential mortality between the egg and the late larval stages under optimal conditions.

The HARDY-WEINBERG proportions are not realized among adult flies developed in the population cages. There is a very appreciable excess of heterozygotes and a deficiency of homozygotes. A selective elimination of a part of homozygotes through differential mortality must take place in the population cages; this differential mortality occurs probably in the larval stage.

The differential mortality of homozygotes in the cage which contained ST and CH was more than sufficient to account for the speed of the changes in the frequencies of these chromosomes observed in this cage. On the other hand, in the cage with AR and CH chromosomes the differential mortality indicated by the data may or may not be sufficient. Natural selection in the population cages need not, however, be based on differential mortality alone; differences in longevity, fecundity, etc. of adult flies may also exist.

The selective process taking place in population cages leads to establishment of an equilibrium at which all the gene arrangements present in the initial population of a cage are retained. For ST and CH chromosomes this equilibrium lies at about 70 percent ST and 30 percent CH. It is demonstrated that this equilibrium can be approached both when the initial populations have less and when they have more than 70 percent ST.

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