GENETICS OF NATURAL POPULATIONS. XII. EXPERIMENTAL REPRODUCTION OF SOME OF THE CHANGES CAUSED BY NATURAL SELECTION IN CERTAIN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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THE PROBLEM

RAPID changes in the genetic composition occur in populations of *Drosophila pseudoobscura* which inhabit certain localities on Mount San Jacinto, California (DOBZHANSKY 1943). These changes are cyclic and connected with the succession of the year's seasons. The genetic variable involved is the gene arrangement in the third chromosome. Three gene arrangements are common among the third chromosomes of the San Jacinto populations. One of them, called Standard (abbreviated ST), is most frequent in the populations in winter and in early spring, reaches its lowest frequency in early summer, and increases in frequency during middle and late summer. The second, Chiricahua (abbreviated CH), shows a cycle opposite to that of ST. The third, Arrowhead (AR), tends to follow a path resembling that of CH but with less regularity. Inversion homozygotes and heterozygotes occur in the populations with frequencies which are close to those which are expected if the carriers of the different gene arrangements mate at random.

Analysis of the data has led to the working hypothesis according to which the changes in the relative frequencies of the gene arrangements are induced by natural selection in response to the seasonal alterations in the environment. The gene arrangements may be in themselves adaptively neutral (that is, free from position effects), but they contain different gene complexes which make their carriers adapted to different seasonal environments (DOBZHANSKY 1943). Since the changes in the composition of the populations are considerable and rapid, the chromosomal types concerned must be subject to intense selection pressures. The selective advantages and disadvantages that must be postulated are, indeed, high enough to justify an attempt to detect them in laboratory experiments. The present article reports the results of experiments designed to test the validity of the above hypothesis.

APPARATUS

The problem of creating experimental populations of Drosophila even remotely comparable to the free-living ones is not easy. Attempts to solve it by increasing the size of the container in which the flies are bred are unsatisfactory. No matter how large a container, and how much food it may hold, time comes when the population must be transferred to fresh medium, and this presents as yet insuperable difficulties of the sampling technique. The nearest approach to a successful solution of the problem is that of L'HÉRITIER

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and TEISSIER (1933) and L'HÉRITIER (1937), who devised population cages in which fresh food is introduced and from which the worked-out food is removed, frequently enough for the size and the age distribution of the population in the cage to remain approximately stationary. We had the privilege also of inspecting a very different model of population cage built by Mrs. G. W. DIEDERICH.

Population cages used in the present experiments (fig. 1) are a modification of the L'HÉRITIER and TEISSIER model. The cage is a wooden box with out-

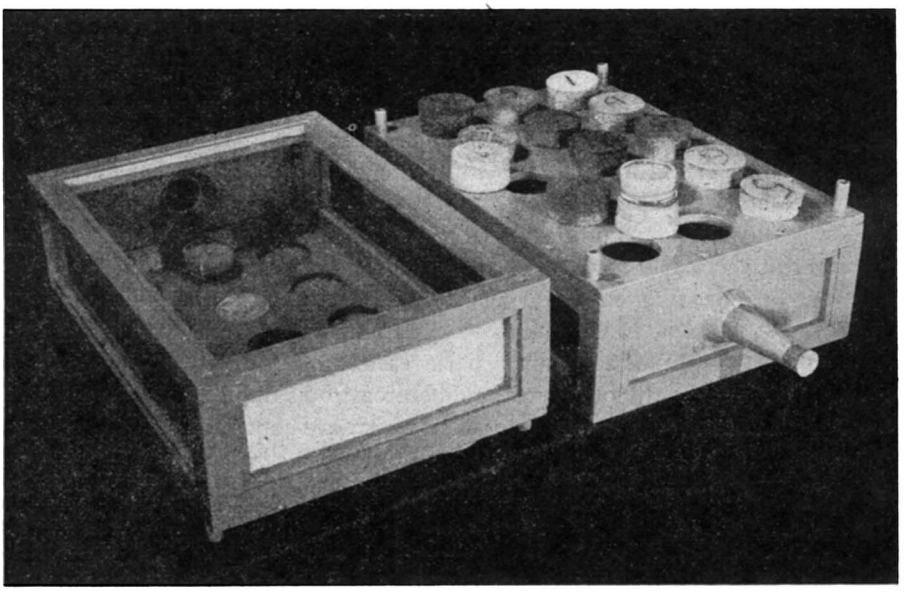

FIGURE 1.—The population cage. Left—view from above; right—view from below.

side dimensions $17 \times 12 \times 5\frac{1}{2}$ inches. The bottom has 15 circular openings $2\frac{1}{4}$ inches in diameter closed by tightly fitting corks. The corks are provided with wire loops that hold in place 2×1 inch Stender jars filled with cornmeal-molasses-agar culture medium. The top of the box and the long sides have glass windows. One of the shorter sides has a window for ventilation covered with wire and gauze nettings to prevent entry and escape of flies. The opposite side has a metallic funnel closed by a cork; this funnel serves as an opening through which flies can be introduced and withdrawn from the cage, and also for cleaning the glass and moistening the food while flies are breeding in the cage.

A known mixture of desired genetic types of flies, together with two or three jars with food, is introduced into the cage at the beginning of the experiment. Jars with fresh food are added, and those with worked-out food removed, at desired intervals. The withdrawal of a cork, removal and replacement of jars with food, and insertion of the cork back into its place can be done with few or no flies escaping. If the number of flies in the cage at the

beginning of an experiment is small, the population increases very rapidly to numbers compatible with the quantity of food in the medium, and thereafter fluctuates within relatively narrow limits. The size of the definitive population at room temperature, and with one jar of fresh food inserted into the cage on alternate days, is of the order of 3000–5000 flies per cage. This population is reached in general within one generation from the introduction of the parent flies into the cage.

The greatest difficulty of experiments with population cages is that any mite infection that may develop in the cage is uncontrollable. As soon as mites appear, the experiment must be terminated and the cage with its contents sterilized by heat. A relatively minor difficulty is that the glass windows of the cage eventually become opaque because of the fly excreta. The glass is cleaned with the aid of a wad of cotton on a wire introduced through the funnel in the side of the cage. The food in the jars inside the cage may become too dry, in which case it is moistened with weak yeast suspension injected through a glass tube.

MATERIAL

All the flies that served as material for the experiments were descendants of individuals collected at Piñon Flats, Mount San Jacinto, California, in the summer of 1942. Chromosomes with Standard, Arrowhead, Chiricahua, Tree-Line, and Santa Cruz gene arrangements are encountered in the populations of that locality, the first three arrangements being much more frequent than the others, particularly than the fifth. Observations extending over four breeding seasons (1939–1942) showed that the relative frequencies of these gene arrangements change from month to month as indicated in table 1 and figure 2 (for further details see DOBZHANSKY 1943).

TABLE 1

Seasonal changes in the percentage frequencies of the different gene arrangements in the third chromosomes of the population of Piñon Flats, California. The symbol "n" in this and all following tables indicates the numbers of the chromosomes examined.

MONTH	ST	AR	CH	OTHERS	*n*
March	47.2	18.1	28.6	6.0	496
April	46.8	24.9	23.6	4.6	449
May	33.6	29.0	31.3	6.0	642
June	29.2	30.6	35.9	4.3	630
July–August	42.3	26.3	27.3	4.1	388
September	47.3	22.2	26.6	3.9	338
Oct.–Dec.	47.3	26.7	21.2	4.8	330

The frequency of ST is lowest in June, increases during the summer, remains uniformly high during autumn and winter, and decreases in spring. That of CH is highest in June, wanes in summer, is low in winter, and waxes during spring. AR behaves like CH, but the trends are less regular. No significant changes are established for the relatively rare Tree Line and Santa Cruz chromosomes.

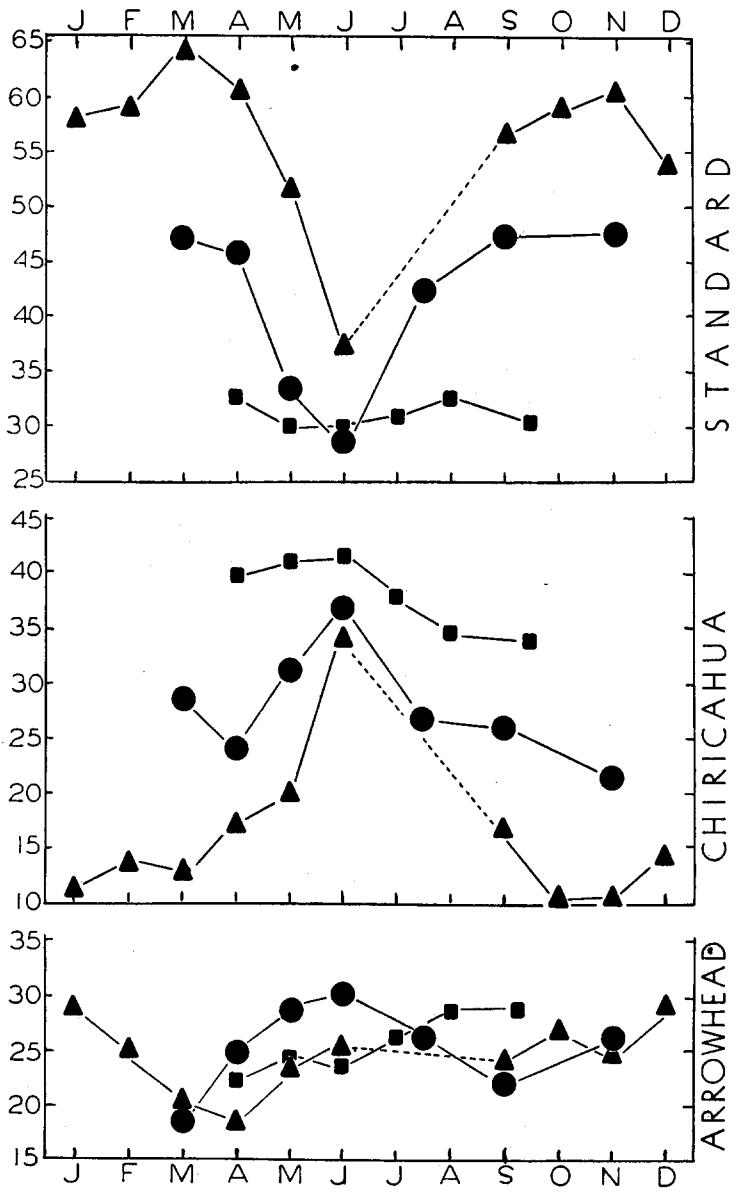

FIGURE 2.—Changes observed in the populations of *Drosophila pseudoobscura* on Mount San Jacinto, California. The figures on the left indicate the percentage frequencies of the three gene arrangements (Standard, Chiricahua, and Arrowhead); the letters at the top and at the bottom stand for the months of the year; triangles—the Andreas Canyon population; circles—the Pinon Flats population; squares—the Keen Camp population. Combined data for four years of observation.

A collection of strains descended from progenitors captured at Piñon Flats has been maintained in the laboratory since 1942; each strain is derived from a single female impregnated by one or more males. Since each female and each male progenitor carried two third chromosomes, which might have had different gene arrangements and gene contents, a strain may harbor four or more kinds of third chromosomes. However, after many generations in the laboratory, some of the ancestral chromosomes may be broken up by crossing over or lost by inbreeding, even though the strains are perpetuated in mass cultures. Theoretically, every strain kept in the laboratory should eventually become homozygous for a single kind of third chromosome. That such an uniformity has not been reached in at least some of the strains is attested by the fact that they continue to carry two or even three chromosomes with different gene arrangements. Retention in laboratory stocks of variability brought in from natural populations is observed even in strains which have have been in captivity for a decade and longer without precautions against inbreeding; some of these stocks still carry third chromosomes with different gene arrangements. A possible reason for this maintenance of variability is that many wild chromosomes carry recessive mutant genes which are deleterious when homozygous; there is a strong selection against homozygosis.

THE INITIAL POPULATION OF AN EXPERIMENTAL CAGE

In the experiments described in this report a fly population with known frequencies of different types of third chromosomes was introduced into a cage, and the incidence of these chromosome types in the progeny of the initial population was observed from time to time. The gene arrangements were determined through examination of the chromosomes in the salivary gland cells of larvae, which were, of course, killed in the process. Nevertheless, given a collection of strains with the desired gene arrangements, populations can be prepared with any initial frequencies of these chromosome types.

For this purpose, two or three dozen pair matings are made from the flies of the strains available. Eight fully grown larvae are taken from the offspring of each pair, their salivary glands stained in acetic orcein, and the gene arrangements in their third chromosomes determined by microscopic examination. With three gene arrangements, ST, AR, and CH, the following twenty-one types of matings are possible (the gene arrangements in the two chromosomes of a zygote are separated by the sign /).

PARENTS	OFFSPRING	PARENTS	OFFSPRING
ST/ST, ST/ST	ST/ST	CH/CH, CH/CH	CH/CH
ST/ST, AR/AR	ST/AR	CH/CH, ST/AR	ST/CH, AR/CH
ST/ST, CH/CH	ST/CH	CH/CH, ST/CH	ST/CH, CH/CH
ST/ST, ST/AR	ST/ST, ST/AR	CH/CH, AR/CH	AR/CH, CH/CH
ST/ST, ST/CH	ST/ST, ST/CH	ST/AR, ST/AR	ST/ST, ST/AR, AR/AR
ST/ST, AR/CH	ST/AR, ST/CH	ST/AR, ST/CH	ST/ST, ST/CH, ST/AR, AR/CH
AR/AR, AR/AR	AR/AR	ST/AR, AR/CH	AR/AR, ST/AR, ST/CH, AR/CH
AR/AR, CH/CH	AR/CH	ST/CH, ST/CH	ST/ST, ST/CH, CH/CH
AR/AR, ST/AR	AR/AR, ST/AR	ST/CH, AR/CH	CH/CH, ST/AR, ST/CH, AR/CH
AR/AR, ST/CH	AR/ST, AR/CH	AR/CH, AR/CH	AR/AR, AR/CH, CH/CH
AR/AR, AR/CH	AR/AR, AR/CH		

The chromosome constitution of the parental pair in each mating thus becomes known through examination of the chromosomes of eight or more of their offspring. The proportions of individuals with different combinations of chromosomes in the whole offspring of the pair is easily deduced. Thus, if one parent is ST/ST and the other ST/AR, half of the offspring are ST/ST and the other half ST/AR; if the parents are ST/AR and ST/AR, the offspring are one-quarter ST/ST, one-half ST/AR, and one-quarter AR/AR. When the adult offspring hatch, the desired numbers of flies are taken from each bottle, the sexes are separated, and the flies are aged for three to four days, then placed into the cage. A simple addition shows the most probable numbers of "chromosomes" with each gene arrangement introduced into the population (each fly, of course, has two "chromosomes"). These numbers in the initial populations in the different experiments are indicated in table 2.

TABLE 2

Conditions in the different experiments.

EXPERIMENT NO.	STARTED	TEMP.	LIGHT	INITIAL NUMBER OF FLIES	NUMBER OF CHROMOSOMES			KINDS OF CHROMOSOMES		
					ST	AR	CH	ST	AR	CH
1	Dec. 8, 1942	Room	Light	480	280	320	360	7	9	8
2	Dec. 8, 1942	Room	Light	480	280	320	360	7	9	8
3	Feb. 1, 1943	16½°	Dark	830	605	495	560	10	6	9
4	Feb. 3, 1943	21°	Dark	620	437	400	403	9	8	9
5	Feb. 3, 1943	25½°	Dark	585	399	372	399	9	6	9
6	Jan. 19, 1944	25½°	Dark	456	253	312	347	6	6	7
7	Jan. 19, 1944	16½°	Dark	503	303	331	372	6	6	7
8	Jan. 22, 1944	25½°	Dark	258	242	—	274	4	—	5
9	Jan. 22, 1944	16½°	Dark	226	207	—	245	4	—	5
10	June 20, 1944	Room	Light	±5000	?	?	?	6	6	7
11	June 20, 1944	Room	Light	±5000	?	?	?	4	—	5
12	June 28, 1944	Room	Light	1027	393	783	878	8	7	9
13	July 18, 1944	Room	Light	1390	813	—	1967	4	—	7
14	July 18, 1944	Room	Light	1002	619	1385	—	5	6	—
15	July 26, 1944	Room	Light	1464	—	930	1998	—	4	7
16	Sept. 30, 1944	16½°	Dark	897	1214	374	206	9	6	5
17	Sept. 30, 1944	16½°	Dark	562	934	—	190	5	—	4
18	Oct. 23, 1944	Room	Light	1356	539	1178	985	9	7	6
19	Nov. 15, 1944	Room	Light	639	489	—	789	5	—	6

Many chromosomes in natural populations of *D. pseudoobscura* carry recessive genes or gene complexes which, when homozygous, modify the viability, fertility, or development rate of the flies (DOBZHANSKY, HOLZ, and SPASSKY 1942). Changes that may be observed in the composition of the population of an experimental cage need not necessarily be ascribed to the properties of the chromosomes with different gene arrangements; such changes may be provoked also by differences in the gene contents of the chromosomes,

which may be independent of the gene arrangement. We are interested, however, in the viability of carriers not of individual third chromosomes but of the ST, AR, and CH chromosomes as groups. Therefore it became necessary to eliminate or minimize the disturbing effects of the gene contents that vary from chromosome to chromosome with the same gene arrangement. To this end, several strains of flies with the same gene arrangement were used; in other words, the ST, AR, and CH chromosomes in the initial population of every experimental cage were derived from several different flies collected on Mount San Jacinto, frequently at different times, weeks or even months apart. The population which develops in a cage contains many individuals homozygous for the ST, AR, and CH gene arrangements. But a majority of these structural homozygotes carried two *different* chromosomes with somewhat different gene contents—that is, they were genic heterozygotes. Table 2 shows the numbers of "kinds of chromosomes" with each gene arrangement in the several experiments. In computing these numbers, it was assumed that every strain contains one and only one kind of third chromosome with each gene arrangement found in that strain. Since more than a single kind were doubtless present in some of the strains, the numbers shown in table 2 are minimum values.

SAMPLING

In order to determine the frequencies of chromosomes with each gene arrangement at different times in the experimental populations, samples were taken at approximately monthly intervals in each cage. The surface of the food in the Stender jars inserted into a cage becomes covered with fly eggs in about 24 hours. A bit of this food was cut out and placed in a regular culture bottle. Extra yeast was added when middle-sized larvae appeared. When the larvae were fully grown, their salivary glands were stained in acetic orcein; examination of the configuration of the third chromosome in one such larva permits determination of the gene arrangements in two third chromosomes, the maternal and the paternal ones. The examination of sufficient larvae should provide a fair estimate of the composition of the population in the cage.

A source of error, however, must be considered. The eggs deposited in a jar come from some but not from all the flies in the cage. To make the samples as representative as possible, the monthly samples of 300 chromosomes were sub-divided into six (seven in the early experiments) subsamples. That is, a chip of food with eggs was taken on each of six successive days, and the chromosomes of 25 larvae from each subsample were examined. This gave six groups of 50 chromosomes each, a total of 300 chromosomes. Comparison of the subsamples constituting a sample should indicate the extent to which the samples are representative of the population of a cage; χ^2's were computed to measure the homogeneity of the subsamples in 38 different samples. Among the 38 χ^2's, 15 had the probability 0.5 or higher of occurring by chance; 12 had probabilities ranging from 0.5 and 0.1; four probabilities from 0.1 to 0.05; three probabilities between 0.05 and 0.02; two between 0.02 and 0.01; and two probabilities just below the 0.01 level. The variability of the sub-

samples constituting a sample, therefore, was in excess of what might be expected owing to chance alone, but only slightly so. A sample may be considered a fair measure of the status of the population of a cage at the time when it is taken.

It follows from the above that the process of taking the monthly sample in an experimental cage occupies nearly a week. In tables 3 to 10 the dates of the samples are given in terms of ten-day periods; E, M, and L before the name of the month indicate the first, second, and third ten-day period, respectively. The entries in tables 3 to 10 labelled "Initial" indicate the composition of the initial population of the experimental cage, as deduced from the chromosomal constitution of the parent flies (see above). The lack of the "Initial" entries in Experiments 10 and 11 (tables 6, 10) is due to the fact that these experiments represent continuation at room temperature of Experiments 7 and 9 (tables 5, 8) carried at a lower temperature; the initial populations in Experiments 10 and 11, therefore, are the same as the final populations in the cages in Experiments 7 and 9, respectively, and their compositions are indicated by the "Control" entries.

In most experiments, a "Control" sample was taken about a week after the introduction of the initial fly population into the cage. The eggs collected at that time are bound to come from the initial population, since the F_1 generation begins to hatch in no less than two weeks from the start of the experimental cage. The incidences of the several gene arrangements in the Control sample therefore should be the same as in the initial population. Yet, more or less significant differences were observed in the second, fifth, and ninth experiments. It is possible that these differences were due merely to sampling errors. On the other hand, it should be kept in mind that the composition of the initial population is inferred from that of its parents, while the Control sample describes the genetic constitution of the eggs being deposited in the cage about a week after the beginning of the experiment. In other words, a whole generation elapses between the points to which these two sets of data are relevant. If the carriers of some gene arrangements are more and of others less favored in certain environments, differences between the Initial and Control populations may be real. It may be noted in this connection that the cultures from which the flies of the initial populations came in all experiments developed at room temperature. Since at that temperature ST chromosomes are more viable than CH chromosomes (see below), control samples are liable to show higher frequencies of ST and lower ones of CH than the corresponding initial populations. This is what is actually found wherever the two are appreciably different.

In natural populations the carriers of the different gene arrangements interbreed at random. The frequencies of inversion homozygotes and heterozygotes are in accord with expectations based on the Hardy-Weinberg theorem (DOBZHANSKY and EPLING 1944, and other work). The same holds for our samples of the populations of the experimental cages. Only the gametic frequencies of the gene arrangements are reported in tables 3 to 10, the bulkier zygotic data being kept on file.

LENGTH OF THE LIFE CYCLE

Almost from the time the initial population is introduced into an experimental cage, the number of larvae in the jars is much greater than can mature on the food available. Under these rigorous conditions the duration of the development from egg to adult is greater in experimental cages than it is in environments more nearly approaching optimum. The mass hatching of adults from pupae takes place 24 to 26 days after the insertion of the jar with food into the experimental cage at 25½°C, in about 28 to 31 days at 21°C, and in about 35 to 37 days at 16½°C. Some flies hatch both earlier and much later than the times indicated, but it is the date of mass hatching that is most important in this investigation, and the above figures represent the best estimates based on visual observation of jars in different experimental cages. The longevity of the flies in the cages is probably smaller than it would be under optimal conditions, and a minority of members of the initial population live long enough to meet the F_1 individuals appearing in the cage.

TABLE 3

Percentage frequencies of the different gene arrangements. First experiment—abundant food, second experiment—food scarce.

TIME	EXPERIMENT 1				EXPERIMENT 2			
	ST	AR	CH	n	ST	AR	CH	n
Initial	29.2	33.3	37.5		29.2	33.3	37.5	
Control	27.1	35.0	38.0	266	34.5	39.9	25.6	258
E January 1943	47.3	30.8	21.9	370	44.5	37.2	18.3	328
E February 1943	42.4	38.6	19.0	342	46.0	35.3	18.6	354
M April 1943	54.3	27.1	18.6	376	50.5	29.4	20.1	364
M May 1943	54.2	19.2	26.5	426	55.8	28.9	15.3	398
M June 1943	52.9	27.1	20.1	384	53.7	33.0	13.3	300

RESULTS

Among the 19 experiments performed, no two were sufficiently alike to be properly considered replications. Some of the variables are given in table 2.

The first and second experiments (table 3) were designed to test the possible influence of the food regime. In the first experiment, two jars with fresh food were given every three days. Since an experimental cage has 15 food jars (see above), each jar in the first experiment remained in the cage for 22 to 23 days and when removed contained many unhatched pupae. This puts a prize on rapid development, because all slowly developing individuals are eliminated. In the second experiment, one jar with fresh food was introduced every five days. Therefore, each jar remained in the cage for 75 days, and when finally removed, it contained no live larvae or pupae and the food was entirely consumed. The two cages stood side by side in a room which was kept very warm —25–27°C during the day, from a fraction to three degrees lower during the night. The initial populations of the cages, started in early December of 1942,

contained approximately equal proportions of ST, AR, and CH chromosomes. The frequencies of ST rose and those of CH dropped with time in both cages. In about two months from the start, in early February of 1943, the frequency of ST reached 42 to 46 percent and CH declined to about 19 percent; AR remained at about the initial level (table 3). In other words, the frequency of CH was reduced in two to three generations to about half of its former value. From early February to the middle of June, when both experiments were discontinued because of mite infection, no sharp changes occurred in the populations; the samples show a further increase of the frequency of ST and a slight decline of AR in Experiment 1, and an increase of ST and a decline of CH in Experiment 2. No consistent differences in the trends which could be ascribed to the different food regimes became apparent in the two experiments. As shown below, the waxing of the frequencies of ST and the waning of CH occurred in all experiments conducted at intermediate and high temperatures.

TABLE 4

Percentage frequencies of the different gene arrangements. Third experiment—16½°C, fourth experiment—21°C, fifth experiment—25½°C.

TIME	EXPERIMENT 3				EXPERIMENT 4				EXPERIMENT 5			
	ST	AR	CH	n	ST	AR	CH	n	ST	AR	CH	n
Initial	36.5	29.8	33.7		35.2	32.3	32.5		34.1	31.8	34.1	
Control	34.7	31.2	34.1	320	41.4	31.4	27.2	360	44.2	37.1	18.7	310
M April 1943	50.0	22.3	27.7	300	45.0	32.0	23.0	300	54.0	31.7	14.3	300
M May 1943	—	—	—		46.0	29.3	24.7	300	55.7	33.7	10.7	300
M June 1943	36.0	32.0	32.0	300	45.0	29.7	25.3	300	56.1	30.1	13.8	362
M July 1943	—	—	—		47.6	39.6	12.8	250	57.3	30.7	12.0	300

Experiments 3, 4, and 5 were started early in February of 1943 in order to test the influence of temperature (table 4). The cages were placed in incubators at 21° and 25½°C and in a cold room at 16½°C. One jar of fresh food was given every four days in Experiment 3, every three days in Experiment 4, and on alternate days in Experiment 5; consequently, the jars remained in the cages 60, 45, and 30 days respectively, which at the low, intermediate and the high temperatures suffices for a majority of the surviving larvae and pupae to become transformed into imagoes. The initial populations contained about equal proportions of ST, AR, and CH chromosomes. Rapid increases in the frequencies of ST and decreases of CH, with AR remaining at about the same level, were observed in the fourth and fifth experiments (table 4). The changes seemed to be more rapid in the experiment (the fifth) conducted at the higher than in that (the fourth) conducted at the intermediate temperature, but the difference between the control sample and the initial population in Experiment 5 makes this uncertain. The result of Experiment 3 is somewhat ambiguous. Here the control sample coincided very well with the presumed composition of the initial population; a sample taken two months after the start, in the

middle of April of 1943, showed an ostensible rise of ST and a drop of CH; no sample was taken in May, but the mid-June sample (when the experiment was discontinued on account of mite infection) agreed almost exactly with the control and with the initial population (table 4).

TABLE 5

Percentage frequencies of the different gene arrangements. Sixth and eighth experiments—25½°C seventh and ninth experiments—16½°C.

TIME	ST	AR	CH	n	ST	CH	n
		EXPERIMENT 6				EXPERIMENT 8	
Initial	27.7	34.2	38.1		46.9	53.1	
Control	29.0	28.3	42.7	300	52.0	48.0	300
L February 1944	34.0	31.0	35.0	300	60.3	39.7	300
M March 1944	45.7	26.0	28.3	300	63.3	36.7	300
M April 1944	44.3	21.0	34.7	300	69.3	30.7	250
		EXPERIMENT 7				EXPERIMENT 9	
Initial	30.1	32.9	37.0		45.8	54.2	
Control	34.7	23.0	42.3	300	59.0	41.0	300
L March 1944	35.7	26.3	38.0	300	48.3	51.7	300
L April 1944	33.8	25.2	41.0	290	51.0	49.0	300
E June 1944	35.7	24.7	39.7	300	46.3	53.7	300

To establish beyond doubt the influence of the temperature factor indicated by the preceding experiments, four cages were started in January of 1944 (Experiments 6, 7, 8, and 9, table 5). Cages Nos. 6 and 8 were placed in an incubator at 25½°C, and Nos. 7 and 9 in a cold room at 16½°C. Since the foregoing experiments indicated that the frequencies of ST and CH are more subject to change than those of AR, cages Nos. 8 and 9 were populated by flies with ST and CH chromosomes only. All three gene arrangements were introduced into cages Nos. 6 and 7. Slightly more CH than ST chromosomes were put into the initial mixtures in all four experiments under consideration. The frequencies of ST rose rather sharply in the two experiments at the higher temperature, even though these experiments lasted only three months, till mid-April of 1944. The frequency of CH dropped in the eighth experiment, while in the sixth both AR and CH appear to have shared in the decline (table 5). In the eighth experiment the frequency of CH chromosomes was equal to or greater than that of ST chromosomes in January, but in April ST chromosomes were twice as frequent as CH. Contrasting with the results of the experiments at the high temperature, no appreciable and consistent changes in the frequencies of the gene arrangements occurred in the seventh and ninth experiments which lasted for five months (January–June) but which were carried on in the cold room (table 5). It may be noted that the initial populations of cages Nos. 6 to 9 consisted of sibs. The difference in the results can be ascribed only to temperature.

In June of 1944, experimental cages Nos. 7 and 9 were taken out of the cold

TABLE 6

Percentage frequencies of the different gene arrangements. Tenth to 12th experiments.

TIME	EXPERIMENT 10				EXPERIMENT 11			EXPERIMENT 12			
	ST	AR	CH	n	ST	CH	n	ST	AR	CH	n
Initial	—	—	—		—	—		19.1	38.1	42.7	
Control	35.7	24.7	39.7	300	46.3	53.7	300	22.0	38.3	39.7	300
L July 1944	38.7	25.7	35.7	300	61.7	38.3	300	28.0	40.6	31.4	300
M August 1944	52.3	19.7	28.0	300	66.3	33.7	300	39.7	35.3	25.0	300
M September 1944	48.3	22.3	29.3	300	63.7	36.3	300	50.7	30.3	19.0	300

room and placed first in a room with an open window, where they experienced the vagaries of the summer temperatures, and in late August they were transferred to an incubator at 25°C. The numbers of these cages were changed to 10 and 11, respectively. When removed from the cold room, the cages contained numerically fully developed populations. The last (June) samples in cages Nos. 7 and 9 (see table 5) may therefore be regarded as control samples in cages Nos. 10 and 11 (table 6). The temperature change was soon reflected in the incidence of the gene arrangements. Already the July samples indicated an upward trend for ST and a downward one for CH, and by mid-August the changes became quite significant. The experiments had to be discontinued in September on account of an infection; the last samples did not differ from the August ones.

TABLE 7

Percentage frequencies of the different gene arrangements. Thirteenth to 15th experiments.

TIME	EXPERIMENT 13			EXPERIMENT 14			EXPERIMENT 15		
	ST	CH	n	ST	AR	n	AR	CH	n
Initial	29.2	70.8		30.9	69.1		31.8	68.2	
L August 1944	54.7	45.3	300	48.3	51.7	300	43.0	57.0	300
M September 1944	58.3	41.7	300	55.3	44.7	300	42.0	58.0	300

Late in June of 1944, a mixture of flies in which CH and AR chromosomes were each about twice as frequent as ST ones was introduced into cage No. 12 (table 6). The environmental conditions in the 12th experiment were the same as in the 10th and the 11th (see above). In a month, in late July, the frequency of ST rose from 19 percent (or from 22 percent if the control sample is taken as the starting point) to 28 percent, and the frequency of CH fell from 43 percent (or 40 percent) to 31 percent. By mid-September ST rose to 51 percent and CH fell to only 19 percent; the frequency of AR dwindled relatively slightly. Thus in only three months—that is, in three to four generations—the frequency of ST was more than doubled, and that of CH reduced to less than half of the initial value.

It is clear that under the conditions of these experiments ST chromosomes displace CH chromosomes at high but not at low temperatures. The behavior of AR chromosomes is erratic: they either hold their own or else lose in competition with ST. To test this point further, in July of 1944 experiments 13, 14, and 15 were designed in which only two gene arrangements were introduced into each cage: ST and CH in the 13th, ST and AR in the 14th, and AR and CH in the 15th (table 7). The cages were kept first in a laboratory room with closed windows, where the populations were injured by the summer heat, then in a well ventilated laboratory room, and finally in an incubator at 25°C. By mid-September all cages developed a mite infection and had to be destroyed; thus, the experiments lasted less than two months. The results, nevertheless, are fairly clear. In the 13th experiment the frequency of ST doubled while that of CH fell from 71 percent to 42 percent. This is consistent with the results of the eighth and the 11th experiments (tables 5 and 6), in which the ST and CH gene arrangements were competing at a high temperature; at the low temperature (experiment 9, table 5, and experiment 19, table 9) the relative frequencies of ST and CH remained constant. In the 14th experiment (table 7) ST increased in frequency from 31 percent to 55 percent, while AR correspondingly dwindled from 69 percent to 45 percent. It follows that at high temperatures chromosomes with ST gene arrangement displace those with AR, provided at least, that CH chromosomes, which are still weaker competitors than AR, are absent. A comparison of the results of the 13th and the 14th experiments suggests, if the data are taken at their face value, that the displacement of CH by ST is more rapid than the displacement of AR by ST; the difference, however, is not statistically significant. The result of the 15th experiment (table 7) is not satisfactory: the August sample indicates that AR chromosomes displace CH in the absence of ST, which is consistent with the expectation, but the September sample failed to show a further drop in the frequency of CH.

The results of the experiments so far described are of two kinds. Namely, in the experiments conducted at the higher temperatures the frequencies of ST chromosomes increase and those of CH decrease, while at 16½°C the proportions of ST, AR, and CH gene arrangements show no perceptible alterations. It is known, however, that in the population of the Piñon Flats locality from which the ancestors of the experimental flies came, a third kind of behavior of the gene arrangements is observed every spring season: the frequency of ST drops and that of CH increases. An unsuccessful attempt to reproduce experimentally this last type of change has been made. It is very probable that neither homozygotes for ST (ST/ST) nor for CH (CH/CH), but the heterozygotes ST/CH are the most favored genetic constitution. If so, the changes in the populations will proceed toward equilibrium values at which the relative proportions of the three genotypes will give the maximal degree of fitness of the population. The equilibrium values may, of course, be different at different temperatures. Now, in experiments 1 to 17 the initial populations contained either equal proportions of ST and CH, or else ST was less frequent than CH. It is possible therefore that at the low temperature these initial

populations happened to contain just about the equilibrium proportions of the gene arrangements. If so, no striking changes in the composition of the populations could be expected in these experiments. Accordingly, experiments 16 and 17 were started at 16½°C with initial populations containing considerably more ST than CH chromosomes (table 8). The populations were kept in the cold room from September 30, 1944, till February 12, 1945, without consistent changes in the frequencies of the gene arrangements being observed. This suggests that the adaptive values of the homo- and heterozygotes for the three gene arrangements are very nearly alike in experimental cages kept at 16½°C.

TABLE 8

Percentage frequencies of the different gene arrangements. Sixteenth and 17th experiments. Temperature 16½°C.

TIME	EXPERIMENT 16				EXPERIMENT 17		
	ST	AR	CH	n	ST	CH	n
Initial	67.7	20.9	11.5		83.1	16.9	
L October 1944	76.3	9.3	14.3	300	86.7	13.3	300
M November 1944	63.0	19.7	17.3	300	87.7	12.3	300
L December 1944	66.0	16.3	17.6	300	89.7	10.3	300
E February 1945	61.0	24.3	14.7	300	86.3	13.7	300

STATISTICAL EVIDENCE OF SELECTION

A statistical study of the changes in chromosome frequency has been based on a grouping of the experiments according to the chromosomes present and the temperature. The largest group involved all three chromosomes at 25° (experiments 1, 2, 5, 6, 10, 12, and 18). These provide data on the changes in 30 intervals, disregarding the estimates of the initial compositions of the boxes. Experiments 3, 7, and 16 (eight intervals) involve all three chromosomes at 16.5°. Experiments 8, 11, 13, and 19 (13 intervals) involve Standard and Chiricahua at 25°, and experiments 9 and 17 (6 intervals) involve the same chromosomes at 16.5°. There remain three isolated experiments, No. 14 involving Standard and Arrowhead at 25°, No. 15 involving Arrowhead and Chiricahua at 25°, and No. 4 involving all three chromosomes at 21°. These three experiments will not be considered here beyond calling attention to the fact that the changes are in the directions expected from the other experiments.

In making a statistical analysis, it is necessary to reduce the observed changes in chromosome frequency to rates per generation. These were estimated on the assumption of an interval of 3.5 weeks between generations in the experiments at 25° and an interval of 5.2 weeks in those at 16.5°.

The first question is the statistical significance of any changes in composition that may indicate selection. Both the average rate of change per generation and the regression of rate of change on chromosome frequency must be considered. A significant average rate of change obviously indicates a selective

process, but even if the average does not differ significantly from zero, a significant regression indicates a selective process which changes in amount or even in direction as chromosome frequency changes.

The principal results in the four groups of experiments referred to above are shown in table 10. Here n is the number of intervals, $\bar{q}$ is the mean chromosome

TABLE 9

Percentage frequencies of the different gene arrangements. Eighteenth and 19th experiments. Room temperature.

TIME	EXPERIMENT 18				EXPERIMENT 19		
	ST	AR	CH	n	ST	CH	n
Initial	19.9	43.6	36.5		38.3	61.7	
M November 1944	33.3	27.3	39.3	300	—	—	
M December 1944	37.7	28.7	33.7	300	53.0	47.0	300
M January 1945	39.3	30.0	30.7	300	63.3	36.7	300
L February 1945	44.3	30.0	25.7	300	60.3	39.7	300
L March 1945	42.0	39.0	19.0	300	65.3	34.7	300
L April 1945	46.7	30.3	23.0	300	65.3	34.7	300
E June 1945	56.4	27.3	16.3	282	70.4	29.6	250
L July 1945	50.3	31.7	18.0	300	72.0	28.0	300

frequency; $\bar{\Delta q}$ is the mean rate of change of chromosome frequency per generation; $b_{\Delta q \cdot q}$ is the regression of rate of change on chromosome frequency, and $\sigma^2_{\Delta q \cdot q}$ is the variance of Δq estimated for constant q. The significance of $\bar{\Delta q}$ and $b_{\Delta q \cdot q}$ are determined from their ratios (t) to their standard errors ($SE_{\bar{\Delta q}}$ and SE_b, respectively), using Students' probabilities for small numbers.

TABLE 10

Statistical analysis of the experimental data (further explanation in text)

	TWO TYPES AT 25° STANDARD (*vs.* CHIRICAHUA)	THREE TYPES AT 25°			TWO TYPES AT 16.5° STANDARD (*vs.* CHIRICAHUA)	THREE TYPES AT 16.5°		
		STANDARD	ARROWHEAD	CHIRICAHUA		STANDARD	ARROWHEAD	CHIRICAHUA
n	13	30	30	30	6	8	8	8
$\bar{q}$	.5492	.4201	.3182	.2617	.7040	.4927	.2169	.2904
$\bar{\Delta q}$	+.0472	+.0391	−.0122	−.0268	−.0125	−.0156	+.0167	−.0011
$SE_{\bar{\Delta q}}$	.0061	.0076	.0068	.0072	.0162	.0190	.0110	.0102
t	7.77	5.17	1.80	3.74	.77	.82	1.53	.11
Prob.	<.001	<.001	.05–.10	<.001	.40–.50	.40–.50	.10–.20	>.90
$b_{\Delta q \cdot q}$	−.346	−.387	−.394	−.261	+.036	−.237	−.614	−.055
SE_b	.057	.074	.116	.079	.092	.119	.176	.095
t	6.13	5.21	3.40	3.31	.39	1.99	3.48	.58
Prob.	<.001	<.001	<.01	<.01	.70–.80	.05–.10	.01–.02	.50–.60
$\sigma^2_{\Delta q \cdot q}$	.00048	.00172	.00139	.00154	.00163	.00290	.00096	.00083
$\bar{q}(1-\bar{q})/300$	.00079	.00078	.00071	.00062	.00059	.00075	.00055	.00065
F	.61	2.21	1.95	2.51	2.77	3.88	1.74	1.28
Prob.	.05–.20	<.001	<.01	<.001	.01–.05	<.001	.05–.20	>.20

The variance of Δq for given q is compared with that expected from accidents of sampling in a sample of 300 by Fisher's test. The following formulae were used:

$$\bar{q} = \sum q/n$$
$$\overline{\Delta q} = \sum \Delta q/n$$
$$b_{\Delta q \cdot q} = (\sum q\Delta q - \overline{\Delta q}\sum q)/(\sum q^2 - \bar{q}\sum q)$$
$$\sigma^2_{\Delta q \cdot q} = [(\sum \Delta^2 q - \overline{\Delta q}\sum \Delta q) - b^2_{\Delta q \cdot q}(\sum q^2 - \bar{q}\sum q)]/(n-2)$$
$$SE^2_{\overline{\Delta q}} = \sigma^2_{\Delta q \cdot q}/n$$
$$SE^2_b = \sigma^2_{\Delta q \cdot q}/(\sum q^2 - \bar{q}\sum q).$$

From inspection of table 10 it may be seen that there were highly significant changes in chromosome frequency in the experiments at 25° in which all three chromosomes were involved and also in those involving only Standard and Chiricahua, in spite of the small number of cases in the latter. In both sets, Standard tended to increase and Chiricahua to decrease within the range of values of chromosome frequencies in the experiments. There were, on the other hand, no significant changes in chromosome frequency in the experiments at 16.5°. This does not imply that there were no changes but merely that any changes were too small to be detected with confidence in the small number of intervals in these sets of experiments.

The regression of rate of change on chromosome frequency was highly significant for all chromosomes in the experiments at 25°. In all cases, the slope is negative and such as to indicate a reversal in the direction of change at some point. In other words, there is a tendency for any chromosome to increase in frequency when rare and decrease when sufficiently common. In the experiments at 16.5° there is one apparently significant result. The rate of change of Arrowhead falls off very rapidly as its frequency increases (probability between .01 and .02). There is a suggestion of a similar trend in the case of Standard (probability between .05 and .10) but not in the case of Chiricahua.

The estimated variance of Δq for given q is actually less, though not significantly, than expected by chance in a sample of 300 in the experiments involving only Standard and Chiricahua at 25°. On the other hand, it is consistently and highly significantly greater for the three chromosomes in the experiments involving all three at 25°. The excess could be accounted for by limitation of the number of parents but could also be due to unknown factors that affected the conditions of selection differently in the various experiments and intervals. If due solely to limitations in the effective number of parents a rough estimate of this effective number (N) may be made by equating the excess $[\sigma^2_{\Delta q \cdot q} - \overline{q(1-q)}/300]$ to $\overline{q(1-q)}/2N$, the variance expected from this cause. The estimate came out 124 from the data on Standard, 157 from that on Arrowhead, and 113 from that on Chiricahua, with an average of 131. It is probable, however, that other factors (density, condition of food, etc.) are the ones that are important.

There is a highly significant excess variance of Δq for given q at 16.5° in

the case of Standard, not borne out by corresponding excesses in the cases of Arrowhead and Chiricahua where all three are involved. There is also excess variance at this temperature where only Standard and Chiricahua are involved. There can be little doubt that in most cases the rate of change of chromosome frequency, at a given frequency, varies more than accounted for by mere sampling errors.

ESTIMATES OF SELECTIVE VALUES

It is of interest next to attempt to determine the nature of the selective process and the values of the selection coefficients. Unfortunately the data at hand do not yield a unique interpretation.

The simplest assumptions are that the relative selective values of the genotypes are the same at all chromosome frequencies and that they are the same for males and females. We shall consider first the sets of experiments involving only Standard and Chiricahua at 25°. Selection must favor the heterozygotes over both homozygotes, under the above hypotheses, if there is a point of equilibrium (*cf.* FISHER 1922). Let s be the selective disadvantage of homozygous Standard and t that of homozygous Chiricahua, relative to the heterozygotes.

Genotype	*Frequency* (f)	*Selective Value* (W)	
ST/ST	q^2	$1 - s$	
ST/CH	$2q(1 - q)$	1	(1)
CH/CH	$(1 - q)^2$	$1 - t$	

It can easily be found that Δq is related to q by the following formula (*cf.* WRIGHT 1931)

$$\Delta q = q(1 - sq - \overline{W})/\overline{W}, \qquad \overline{W} = 1 - sq^2 - t(1 - q)^2 \tag{2}$$

$$\Delta q = -q(1 - q)[sq - t(1 - q)]/\overline{W} \tag{3}$$

$$s = \frac{1}{q}\left[1 - \left(1 + \frac{\Delta q}{q}\right)\overline{W}\right] \quad \text{from (2)} \tag{4}$$

$$t = \frac{1}{(1 - q)}\left[1 - \left(1 - \frac{\Delta q}{(1 - q)}\right)\overline{W}\right]. \tag{5}$$

The equilibrium point, $\hat{q}$, is that at which $\Delta q = 0$, with both chromosomes present.

$$\hat{q} = t/(s + t) \quad \text{from (3)}. \tag{6}$$

In this set of experiments, observations were made at values of q ranging between .292 and .663. The observed values of Δq do not diverge conspicuously from the linear regression line, $\Delta q = .2375 - .3465q$. This indicates equilibrium at about $\hat{q} = .685 (= .2375/.3465)$, slightly above the highest value of q in the data. No great confidence can be placed in this, however, because of the theoretical curvilinear relation of Δq to q. The best estimate that can be made from the linear regression is probably that obtained by equating the

regression coefficient to the slope of the tangent of the theoretical curve at the mean value of q.

$$\frac{d\Delta q}{dq} = \frac{\Delta q}{q} - q\left[\frac{s}{\overline{W}} + \frac{(1 - sq)}{\overline{W}^2}\frac{d\overline{W}}{dq}\right] \quad \text{from (2)}$$

$$= 2\left(1 + \frac{\Delta q}{q}\right)\left(1 - \frac{\Delta q}{1 - q}\right) - 1 - \frac{1}{\overline{W}} \quad \text{using (4) and (5)} \qquad (7)$$

Assuming that $\frac{d\Delta q}{dq}$ at $\bar{q}$ is approximated by $b_{\Delta q \cdot q}$ and substituting $\bar{\Delta q}$ and $\bar{q}$ for Δq and q respectively, $\overline{W}$ at $\bar{q}$ may be estimated from the reciprocal of the following expression.

$$\frac{1}{\overline{W}} = 2\left(1 + \frac{\Delta q}{q}\right)\left(1 - \frac{\Delta q}{1 - q}\right) - 1 - b_{\Delta q \cdot q}. \qquad (8)$$

Estimates of s and t can now be made by the same substitutions with the help of the estimate for $\overline{W}$ at $\bar{q}$. For Standard and Chiricahua at 25°

$$\begin{aligned} \overline{W} \text{ at } \bar{q} &= .7747 \\ s &= .289 \\ t &= .680 \\ \hat{\bar{q}} &= .702. \end{aligned} \qquad (9)$$

For the best estimates, however, it is necessary to use the method of least squares. As the expression for Δq in terms of q is not linear with respect to the parameters s and t (equation 3), the solution must be determined by iteration. Using trial values of s and t to estimate $\overline{W}$ for each observation (*cf.* (2)), values of s and t in the numerator of (3) can be obtained that minimize the squared deviations of observed and estimated Δq. The estimates of $\overline{W}$ may then be readjusted and the process repeated until the values of s and t calculated for the numerator agree sufficiently with those tried in the denominator. Strictly, the observations should be weighted by the reciprocals of their variances (that is, in proportion to $q(1-q)$), but as these weights would range only from 4.0 to 4.8 in this data, weighting may be ignored without serious error, in order to avoid the considerable complication which it would introduce into the calculations.

Let $y = \Delta q$, $x_1 = q^2(1-q)/\overline{W}$ and $x_2 = q(1-q)^2/\overline{W}$.

The deviations (δ) of the 13 observations from the theoretical values can be written as follows by substitutions in (3).

$$\delta = y + x_1 s - x_2 t. \qquad (10)$$

Minimizing $\sum \delta^2$ by putting $\frac{\partial \sum \delta^2}{\partial s}$ and $\frac{\partial \sum \delta^2}{\partial t}$ equal to zero gives the normal equations:

$$\begin{aligned} (\sum x_1^2)s - (\sum x_1 x_2)t &= -\sum x_1 y \\ -(\sum x_1 x_2)s + (\sum x_2^2)t &= \sum x_2 y. \end{aligned} \qquad (11)$$

The results of successive trials, starting from the estimates of s and t, arrived at from the regression coefficient, were as follows in terms of the values tried in the denominator and calculated (calc.) for the numerator of (3).

	1ST TRIAL		2ND TRIAL		3RD TRIAL		FINAL
	TRIED	CALC.	TRIED	CALC.	TRIED	CALC.	ESTIMATES
s	.289	.3083	.2987	.3047	.3031	.3038	.304
t	.680	.7037	.6919	.6967	.6949	.6948	.695

The equilibrium point from the final estimates is $\hat{q}=.696$.

The values, $s=.30$, $t=.70$, $\hat{q}=.70$, are as accurate as the data warrant. The solid line in figure 3 shows the theoretical curve for Δq based on these values in comparison with the 13 observations.

This theory obviously fits the data as well as can be expected, but it involves the assumption that selection acts in the same way on males and females. This is not necessarily the case. Selection may occur in such factors as mating activity or fecundity rather than in mortality. It is not likely that there would be the same differentials between genotypes in the males and females in mating and fecundity. It is thus necessary to consider the effect of differences in the selective values of the sexes. Fortunately the results differ little from those obtained by assigning the average for each genotype to both sexes, as noted by WRIGHT (1942).

Let $q_f(=q+\delta q)$ be the frequency of ST in eggs and $q_m(=q-\delta q)$ be that in sperms, and let $W_{11}+\delta W_{11}$ be the selective value of genotype ST/ST in females and $W_{11}-\delta W_{11}$ be that in males, and let similar symbols be used for other selective values. Thus q is the average frequency of ST and the W's are average selective values referred to above.

		Selective values		
	Frequency (f)	*Females* (W_f)	*Males* (W_m)	*Average*
ST/ST	$q^2-\delta^2q$	$W_{11}+\delta W_{11}$	$W_{11}-\delta W_{11}$	W_{11}
ST/CH	$2q(1-q)+2\delta^2q$	$W_{12}+\delta W_{12}$	$W_{12}-\delta W_{12}$	W_{12} (13)
CH/CH	$(1-q)^2-\delta^2q$	$W_{22}+\delta W_{22}$ z	$W_{22}-\delta W_{22}$	W_{22}

The frequencies of Standard in eggs ($_1q_f$) and sperms ($_1q_m$) that function in producing the next generation are as follows, using $\overline{W}_f$ for $\sum W_f f$ and $\overline{W}_m$ for $\sum W_m f$.

$$\begin{aligned} {}_1q_f &= \{[q^2-\delta^2q][W_{11}+\delta W_{11}]+[q(1-q)+\delta^2q][W_{12}+\delta W_{12}]\}/\overline{W}_f \\ {}_1q_m &= \{[q^2-\delta^2q][W_{11}-\delta W_{11}]+[q(1-q)+\delta^2q][W_{12}-\delta W_{12}]\}/\overline{W}_m. \end{aligned} \quad (14)$$

Let $_1q=(1/2)(_1q_f+{}_1q_m)$ be the average frequency of Standard after a generation and $_1\delta q=(1/2)(_1q_f-{}_1q_m)$ be the deviation in females from this average, and let $\overline{W}_f=\overline{W}+\delta\overline{W}$ and $\overline{W}_m=\overline{W}-\delta\overline{W}$.

$$\begin{aligned} {}_1q = \{&[\overline{W}W_{11}-\delta\overline{W}\delta W_{11}][q^2-\delta^2q] \\ &+[\overline{W}W_{12}-\delta\overline{W}\delta W_{12}][q(1-q)+\delta^2q]\}/[\overline{W}^2-(\delta\overline{W})^2] \end{aligned} \quad (15)$$

$$\begin{aligned} {}_1\delta q = \{&[\overline{W}\delta W_{11}-W_{11}\delta\overline{W}][q^2-\delta^2q] \\ &+[\overline{W}\delta W_{12}-W_{12}\delta W_{12}][q(1-q)+\delta^2q]\}/[\overline{W}^2-(\delta\overline{W})^2]. \end{aligned} \quad (16)$$

It may be noted that the above value of $_1q$ differs from the expression $[W_{11}q^2+W_{12}q(1-q)]/\overline{W}$, expected if selection acts in the same way on males and females, only by terms that are of the second degree with respect to sex differences.

On starting from any initial values of q and δq, the values in subsequent generations can be found by repeated application of (15) and (16).

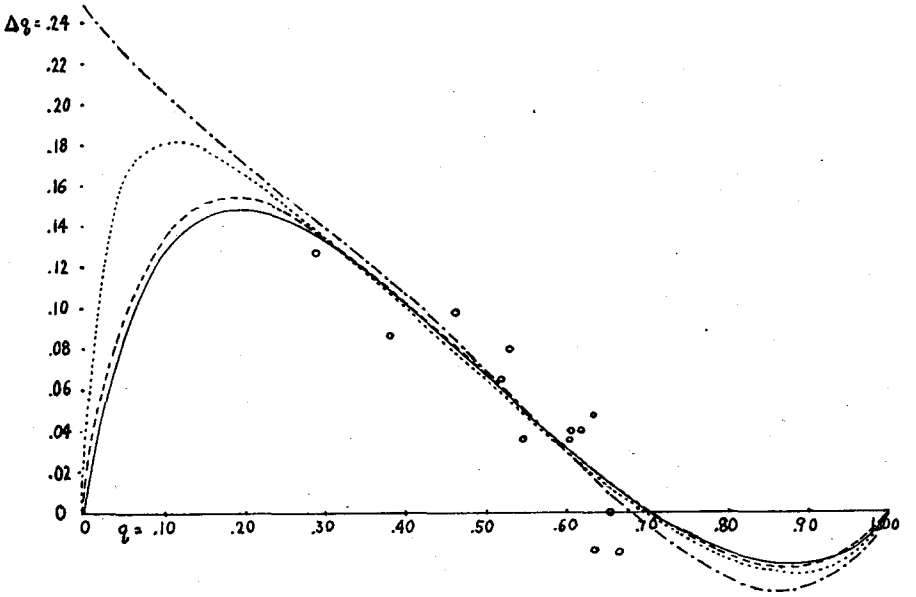

FIGURE 3.—The circles show the amounts of change per generation (Δq) in the frequency (q) of Standard chromosomes in the experiments which involved only Standard and Chiricahua at 25°. The solid line represents the theoretical relation of Δq to q that fits best under the hypothesis that the heterozygotes have a constant selective advantage over both homozygotes and that the sexes are alike in this respect. The broken line represents the same hypothesis except that the homozygotes of one sex are supposed to have considerably more selective disadvantage in relation to the homozygotes than do those of the other sex. The line in dot and dash is the case in which the relative selective disadvantage of the homozygotes in one sex is as extreme as possible. The dotted line represents the hypothesis that the heterozygotes are intermediate between the homozygotes but that each homozygote is favored when rare and opposed when sufficiently common. All hypotheses fit about equally well in the region covered by the experiments.

The effects of considerable differences between the sexes with respect to selection were tested by taking the same average values of W_{11}, W_{12}, and W_{22} arrived at above but assuming δW_{11} and δW_{22} both to be .10.

Genotype	*Selective Vales*			
	W_f	W_m	W	
ST/ST	.80	.60	.70	(17)
ST/CH	1.00	1.00	1.00	
CH/CH	.40	.20	.30	

The calculations were started from q=.01, δq=0 and q=.99, δq=0.

q	δq	Δq	q	δq	Δq	q	δq	Δq	
.01	0	+.0252	.99	0	−.0043	.8264	+.0142	−.0249	
.0352	−.0110	+.0725	.9857	+.0020	−.0061	.8015	+.0144	−.0221	
.1077	−.0285	+.1375	.9796	+.0028	−.0084	.7794	+.0143	−.0187	
.2452	−.0425	+.1475	.9712	+.0038	−.0113	.7607	+.0138	−.0152	(18)
.3927	−.0321	+.1062	.9599	+.0052	−.0147	.7455	+.0133	−.0121	
.4989	−.0153	+.0673	.9452	+.0068	−.0185	.7334		−.010	
.5662	−.0046	+.0425	.9267	+.0086	−.0222				
.6087	+.0014	+.0275	.9045	+.0104	−.0250				
.6362	+.0048	+.0183	.8795	+.0121	−.0266				
.6545		+.012	.8529	+.0134	−.0265				

The relation of Δq to q, based on these values, is shown as a broken line in figure 3. This curve differs only slightly from the solid line which shows the relation of Δq to q with the same average selective values of genotypes, but no sex difference. Thus the data are fitted substantially as well by both hypotheses.

It seemed next of interest to make the sex difference in selection value as great as possible in the homozygotes without ever exceeding the heterozygotes and subject to acceptance of the same average values, $W_{11}=.70$, $W_{12}=1.00$ and $W_{22}=.30$. This involves the assumption that CH/CH does not reproduce at all in one sex.

	Selection Value			
Genotype	W_f	W_m	W	
ST/ST	1.00	.40	.70	
ST/CH	1.00	1.00	1.00	
CH/CH	.60	0	.30	(19)

In this case Δq approaches .25 as q approaches 0. The values of q and δq were calculated starting from q=0 and q=.99.

q	δq	Δq	q	δq	Δq	
0	0	.25	.99	+.005	−.0070	(20)
.25	−.25	.1563	.9830	+.0070	−.0114	
.4063	−.0938	.1070	.9715	+.0115	−.0177	
.5133	−.0423	.0630	.9538	+ 0180	−.0256	
.5763	−.0098	.0376	.9282	+.0263	−.0335	
.6139	+.0070	.0228	.8947	+.0352	−.0391	
.6367	+.0161	.0142	.8556	+.0426	−.0400	
.6509	+.0213	.0089	.8157	+.0466	−.0363	
.6598	+.0243	.0057	.7794	+.0468	−.0298	
.6655	+.0262	.0037	.7496	+.0446	−.0227	
.6692	+.0273	.0024	.7269	+.0413	−.0164	
.6715	+.0280	.0015	.7106	+.0382	−.0114	
.6731	+.0285	.0010	.6992	+.0356	−.008	
.6741	+.0288	.0007				

A curve from these values is plotted in dot and dash in figure 3. It differs greatly from the solid line at both low and high values of q, but in the region represented by the observations (q=.292 to .663) it does not differ much.

There is therefore no reason from the observations at hand to rule out the possibility of very great selective differences between the sexes. It would require such experiments as the competitive mating of two types of males with each type of female and competitive mating of two types of females with each type of male to distinguish these hypotheses.

So far we have assumed that the relative selective values of the genotypes remain the same at all gene frequencies. This is not necessarily, or even probably, the case where selective mating is in question, and it becomes desirable to consider the consequences where the W's are functions of q. For simplicity we shall assume that sex differences are sufficiently small that they may be ignored. The general formula for Δq is as follows (WRIGHT 1942).

$$\Delta q = q(1-q)\sum W\frac{df}{dq}\Big/2\overline{W}, \qquad \overline{W} = \sum Wf. \tag{21}$$

If the W's, as reproductive rates, involve a function of q that is the same for all, this cancels in (21) leaving Δq the same as if the function were not involved. Thus populations with the same rates of change of gene frequencies may differ widely in the values of q at which the population reproduces most rapidly ($\overline{W}$ maximum), and these points are not in general at the point of equilibrium.

There is no such cancelling if the W's involve different functions of q. It must suffice here to illustrate by one hypothesis how widely the selective values may differ from those arrived at on the assumption that they are independent of q, and still fit the data.

Assume that the heterozygotes, instead of always being superior to both homozygotes, are always exactly intermediate in selective value, but also that the selective values of the homozygotes fall off linearly as the corresponding chromosome frequencies rise.

Genotype	*Frequency* (f)	*Selective Value* (W)	
ST/ST	q^2	$1 + a - bq$	
ST/CH	$2q(1-q)$	1	(22)
CH/CH	$(1-q)^2$	$1 - a + bq$	

$$\overline{W} = 1 - (a - bq)(1 - 2q) \tag{23}$$

$$\Delta q = q(1-q)(a - bq)/\overline{W} \tag{24}$$

$$\hat{q} = a/b \tag{25}$$

$$a - bq = \Delta q/[q(1 - q + (1 - 2q)\Delta q] \text{ from } (23), (24) \tag{26}$$

A rough determination of values of a and b to fit the observations under this hypothesis can be made by taking $\hat{q}$ as .70 (hence $a = .70b$ from (25)) and substituting $\bar{q}$ and $\overline{\Delta q}$ in place of q and Δq in (26).

$$\begin{aligned} a &= .902 \\ b &= 1.288. \end{aligned} \tag{27}$$

Substitution of these values in (24) gives the dotted line in figure 3. This curve differs considerably from the solid line at low values of q, but differs

only slightly in the region covered by the observations and thus is a mathematically possible interpretation. How widely this hypothesis differs from that which fits the data on the hypothesis that selective values are independent of gene frequency may be seen from the following table.

Selective values (W)

	W's CONSTANT	W's VARY AS IN (22)						
		q=0	.10	.30	.50	.70	.90	1.00
ST/ST	.70	1.90	1.77	1.51	1.26	1.00	.74	.61
ST/CH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
CH/CH	.30	.10	.23	.49	.74	1.00	1.26	1.39

An hypothesis of this nature is one that might well be approximated in nature in cases in which a species occupies a heterogenous environment. Each genotype may be favored by selection when rare and unable to occupy fully the ecological niches to which it is best adapted, but selected against when so abundant that it must in part occupy ecological niches to which it is less well adapted than other genotypes. This particular hypothesis is perhaps improbable under the conditions of the experiments, but the possibility that the W's depend on different functions of the gene frequencies is not improbable. This could be tested only by special experiments.

In the most extensive experiments, three types of chromosomes and consequently six genotypes were present. The analysis is much more complicated than where only two types of chromosome are present, and we shall restrict consideration to the hypothesis that selective values are independent of gene frequencies and that sex differences may be ignored.

As a first approach, the two chromosomes, Arrowhead and Chiricahua, which tended to decrease in frequency in the data, may be grouped together in opposition to Standard, which tended to increase in frequency. Let q refer to the frequency of Standard, s to its selective disadvantage when homozygous, and t to the selective disadvantage of Arrowhead and Chiricahua combined in comparison with the heterozygotes that involve Standard. Substitution in (8) yields

$$\begin{aligned} \overline{W} &= .7016 \text{ at } \bar{q} \\ s &= .555 \\ t &= .596 \\ \hat{q} &= .518. \end{aligned} \tag{29}$$

In this case, t is the weighted average of the selective disadvantages of AR/AR, AR/CH, and CH/CH relative to ST/AR and ST/CH combined. Using subscripts 1, 2, and 3 for Standard, Arrowhead, and Chiricahua, respectively, and indicating selective disadvantages of genotypes by double symbols, we may write

$$t = .596 = (s_{22}q_2^2 + 2s_{23}q_2q_3 + s_{33}q_3^2)/(1 - q_1)^2. \tag{30}$$

If now it be assumed that the heterozygote AR/CH is at no selective disadvantage relative to the other heterozygotes (that is, $s_{23}=0$), we can estimate roughly the average selective disadvantages of the AR/AR and CH/CH by putting these the same in the above equation and substituting $\bar{q}_1=.4201$, $\bar{q}_2=.3182$ and $\bar{q}_3=.2617$ (from table 10) for q_1, q_2, and q_3, respectively. But this yields the impossible value, $s_{22}=s_{33}=1.17$ which implies that AR/AR and CH/CH suffer fates 17 percent worse than death on the average.

This lumping together of different genotypes however, is not a very satisfactory procedure, especially since it is obvious from the data that Arrowhead and Chiricahua do not react alike. Consider next the hypothesis that the three heterozygotes are equivalent in selective value ($W_{12}=W_{13}=W_{23}=1$) but that the homozygotes suffer diverse selective handicaps (s_1, s_2 and s_3).

Genotype	*Frequency*	*Selective Value*	
ST/ST	q_1^2	$1-s_1$	
ST/AR	$2q_1q_2$	1	
ST/CH	$2q_1q_3$	1	(31)
AR/AR	q_2^2	$1-s_2$	
AR/CH	$2q_2q_3$	1	
CH/CH	q_3^2	$1-s_3$	

$$\overline{W} = 1 - s_1q_1^2 - s_2q_2^2 - s_3q_3^2 \tag{32}$$

$$\Delta q_1 = q_1\left[\frac{1-s_1q_1}{\overline{W}} - 1\right] \tag{33}$$

$$\Delta q_2 = q_2\left[\frac{1-s_2q_2}{\overline{W}} - 1\right]$$

$$\Delta q_3 = q_3\left[\frac{1-s_3q_3}{\overline{W}} - 1\right]$$

$$\frac{\partial \overline{W}}{\partial q_1} = -2\left[s_1q_1 + s_2q_2\frac{\partial q_2}{\partial q_1} + s_3q_3\frac{\partial q_3}{\partial q_1}\right]. \tag{34}$$

$$\frac{\partial \Delta q_1}{\partial q_1} = \frac{\Delta q_1}{q_1} - q_1\left\{\overline{W}s_1 + (1-s_1q_1)\frac{\partial \overline{W}}{\partial q_1}\right\}\Big/\overline{W}^2. \tag{35}$$

After some reduction and noting that since $q_1+q_2+q_3=1$, $1+\frac{\partial q_2}{\partial q_1}+\frac{\partial q_3}{\partial q_1}=0$

$$\frac{\partial \Delta q_1}{\partial q_1} = 1 - \frac{1}{\overline{W}} + 2\frac{\Delta q_1}{q_1} - 2q_1\left(1+\frac{\Delta q_1}{q_1}\right)\left(\frac{\Delta q_1}{q_1}+\frac{\Delta q_2}{q_2}\frac{\partial q_2}{\partial q_1}+\frac{\Delta q_3}{q_3}\frac{\partial q_3}{\partial q_1}\right). \tag{36}$$

Assuming that all observed values of q are in the region in which relations are approximately linear, an approximate value of $\overline{W}$ may be obtained by replacing the q's and Δq's by their mean values and replacing $\frac{\partial q_2}{\partial q_1}$ by

the regression coefficient $b_{q_2q_1}(=-.2657)$, $\frac{\partial q_3}{\partial q_1}$ by $b_{q_3q_1}(=-.7343)$ and $\frac{\partial \Delta q_1}{\partial q_1}$ by $b_{\Delta q_1 q_1}(=-.3865)$. With these assumptions, $\overline{W}$ comes out .7091 for the set of mean gene frequencies. The values of s_1, s_2, and s_3 can now be estimated form (33).

$$\begin{aligned} s_1 &= .536 & W_{11} &= .464 \\ s_2 &= 1.000 & W_{22} &= .000 \\ s_3 &= 1.389 & W_{33} &= -.389. \end{aligned} \tag{37}$$

The estimated value of s_1 (.536) does not differ much from that obtained by lumping Arrowhead and Chiricahua (.518). The selection against AR/AR is complete and that against CH/CH is impossible, 38.9 percent greater than complete elimination. The weighted average selective disdvantage of AR/AR, AR/CH and CH/CH comes out .584, in approximate agreement with .596 where these were lumped. It appears to be impossible to account for the results on the hypothesis that the heterozygotes are all equivalent. The selective values must be allowed more latitude.

Genotypes	*Frequencies*	*Selective Values*
ST/ST	q_1^2	W_{11}
ST/AR	$2q_1q_2$	W_{12}
ST/CH	$2q_1q_3$	W_{13}
AR/AR	q_2^2	W_{22}
AR/CH	$2q_2q_3$	W_{23}
CH/CH	q_3^2	W_{33}

(38)

$$\overline{W} = W_{11}q_1^2 + 2W_{12}q_1q_2 + 2W_{13}q_1q_3 + W_{22}q_2^2 + 2W_{23}q_2q_3 + W_{33}q_3^2 \tag{39}$$

$$\begin{aligned} \Delta q_1 &= q_1\left[\frac{W_{11}q_1 + W_{12}q_2 + W_{13}q_3}{\overline{W}} - 1\right] \\ \Delta q_2 &= q_2\left[\frac{W_{12}q_1 + W_{22}q_2 + W_{23}q_3}{\overline{W}} - 1\right] \\ \Delta q_3 &= q_3\left[\frac{W_{13}q_1 + W_{23}q_2 + W_{33}q_3}{\overline{W}} - 1\right]. \end{aligned} \tag{40}$$

At equilibrium $\Delta q_1 = \Delta q_2 = \Delta q_3 = 0$

$$\begin{aligned} W_{11}\hat{q}_1 + W_{12}\hat{q}_2 + W_{13}\hat{q}_3 - \overline{W} &= 0 \\ W_{12}\hat{q}_1 + W_{22}\hat{q}_2 + W_{23}\hat{q}_3 - \overline{W} &= 0 \\ W_{13}\hat{q}_1 + W_{23}\hat{q}_2 + W_{33}\hat{q}_3 - \overline{W} &= 0. \end{aligned}$$

$\overline{W}$ with its quadratic terms can be eliminated, leaving a series of linear equations to solve for two variables $\hat{q}_1$ and $\hat{q}_2$ (noting that $\hat{q}_3 = 1 - \hat{q}_1 - \hat{q}_2$). An analogous system of $(n-1)$ simultaneous linear equations would appear with n instead of three alternatives. In the present case let

$$K_1 = W_{22}W_{33} - W_{13}W_{22} - W_{12}W_{33} + W_{12}W_{23} + W_{13}W_{23} - W_{23}^2$$
$$K_2 = W_{33}W_{11} - W_{12}W_{33} - W_{13}W_{11} + W_{23}W_{13} + W_{12}W_{13} - W_{13}^2 \quad (42)$$
$$K_3 = W_{11}W_{22} - W_{23}W_{11} - W_{13}W_{22} + W_{13}W_{12} + W_{23}W_{12} - W_{12}^2.$$

Then

$$\hat{q}_1 = K_1/(K_1 + K_2 + K_3)$$
$$\hat{q}_2 = K_2/(K_1 + K_2 + K_3) \quad (43)$$
$$\hat{q}_3 = K_3/(K_1 + K_2 + K_3).$$

It may be noted that in the special case considered earlier in which the selective values of all heterozygotes are 1, the condition that $\Delta q_1 = \Delta q_2 = \Delta q_3 = 0$ gives at once $s_1q_1 = s_2q_2 = s_3q_3 = 1 - \overline{W}$ which gives

$$\hat{q}_1 = \frac{1}{s_1} \Big/ \sum \frac{1}{s} \quad (44)$$

with analogous formulae for $\hat{q}_2$, $\hat{q}_3$ (and for other q's if there are more than three alternatives).

Returning to the case in which no restriction (other than constancy) are placed on the W's, the observation equations are as follows (from 40)

$$q_1 + \Delta q_1 = (q_1^2W_{11} + q_1q_2W_{12} + q_1q_3W_{13})/\overline{W}$$
$$q_2 + \Delta q_2 = (q_1q_2W_{12} + q_2^2W_{22} + q_2q_3W_{23})/\overline{W} \quad (45)$$
$$q_3 + \Delta q_3 = (q_1q_3W_{13} + q_2q_3W_{23} + q_3^2W_{33})/\overline{W}.$$

It is convenient to let $y_1 = q_1 + \Delta q_1$, $y_2 = q_2 + \Delta q_2$, $y_3 = q_3 + \Delta q_3$, $x_{11} = q_1^2/\overline{W}$, $x_{12} = q_1q_2/\overline{W}$ etc. The quantity to be minimized is then

$$\sum \delta^2 = \sum [(y_1 - x_{11}W_{11} - x_{12}W_{12} - x_{13}W_{13})^2]$$
$$+ \sum [(y_2 - x_{12}W_{12} - x_{22}W_{22} - x_{23}W_{23})^2] \quad (46)$$
$$+ \sum [(y_3 - x_{13}W_{13} - x_{23}W_{23} - x_{33}W_{33})^2].$$

The six normal equations with the unknown selective values written for convenience above are as follows:

W_{11}	W_{12}	W_{13}	W_{22}	W_{23}	W_{33}	
$\sum x_{11}^2$	$+ \sum x_{11}x_{12}$	$+ \sum x_{11}x_{13}$	$+ 0$	$+ 0$	$+ 0$	$= \sum x_{11}y_1$
$\sum x_{11}x_{12}$	$+ 2\sum w_{12}^2$	$+ \sum x_{12}x_{13}$	$+ \sum x_{12}x_{22}$	$+ \sum x_{12}x_{23}$	$+ 0$	$= \sum x_{12}y_1 + \sum x_{12}y_2$
$\sum x_{11}x_{13}$	$+ \sum x_{12}x_{13}$	$+ 2\sum x_{13}^2$	$+ 0$	$+ \sum x_{13}x_{23}$	$+ \sum x_{13}x_{33}$	$= \sum x_{13}y_1 + \sum x_{13}y_3$
0	$+ \sum x_{12}x_{22}$	$+ 0$	$+ \sum x_{22}^2$	$+ \sum x_{22}x_{23}$	$+ 0$	$= \sum x_{22}y_2$
0	$+ \sum x_{12}x_{23}$	$+ \sum x_{13}x_{23}$	$+ \sum x_{22}x_{23}$	$+ 2\sum x_{23}^2$	$+ \sum x_{23}x_{33}$	$= \sum x_{23}y_2 + \sum x_{23}y_3$
0	$+ 0$	$+ \sum x_{13}x_{33}$	$+ 0$	$+ \sum x_{23}x_{33}$	$+ \sum x_{33}^2$	$= \sum x_{33}y_3.$

These could be reduced to five, taking one of the W's as standard, since only the relative values are in question. The iteration process necessitated by the use of trial values of the W's in estimating $\overline{W}$ for each observation however, is facilitated by allowing all of the W's to vary. As before, no attempt was made at differential weighting of the observations.

The initial trials were based on the estimates of $W_{11}=(.696)$, $W_{33}(=.305)$, with $W_{13}(=1.000$ as standard), deduced from the experiments with Standard and Chiricahua only, and on preliminary deductions for W_{12}, W_{23} and W_{22}. These trial values turned out to be unsatisfactory, but approximate stability was reached at the fourth iteration. The trial values (used in $\overline{W}$) and the results from solution of the normal equations were as follows:

	1ST TRIAL		2ND TRIAL		3RD TRIAL		4TH TRIAL		FINAL ESTIMATE
	TRIED	CALC.	TRIED	CALC.	TRIED	CALC.	TRIED	CALC.	$W_{13}=1$
W_{11}	.696	.636	.656	.622	.60	.594	.59	.597	.43
W_{12}	2.027	1.911	1.950	1.868	1.76	1.785	1.79	1.797	1.30
W_{13}	1.000	1.281	1.189	1.347	1.44	1.411	1.41	1.394	1.00
W_{22}	.305	.034	.125	.053	.06	.081	.08	.073	.05
W_{23}	.965	.969	.968	.980	.96	.984	.98	.982	.71
W_{33}	.305	.388	.361	.340	.35	.275	.28	.284	.21

While complete stability is not reached, the values given in the last column (reduced to a scale on which $W_{13}=1.00$) should be sufficiently accurate for the hypothesis under consideration. The reduction in the selective value of ST/ST from .696 to .43 and for CH/CH from .305 to .21 suggests increased selection against the homozygotes in the presence of three chromosomes instead of two, or, in other words, that the selective values are to some extent at least functions of the chromosome frequencies instead of constants as here assumed. The method, however, does not yield any impossible estimates of W, although the selection against homozygous Arrowhead is indicated to be almost complete.

The frequencies of the three chromosomes at equilibrium can be calculated from the above estimates of the W's by substitutions in (42) and (43).

Standard	$\hat{q}_1=.531$
Arrowhead	$\hat{q}_2=.339$
Chiricahua	$\hat{q}_3=.130$

DISCUSSION

There can be no doubt from these experiments that the three chromosome types found in the population of Piñon Flats, Mount San Jacinto, are not alike in selective value, even though the data do not yield a unique interpretation of the selective processes that take place. Under the conditions obtaining in the experimental populations, the selective values are such that a certain set of frequencies of chromosome types is in equilibrium. Furthermore, the selective values are quite different under different conditions, temperature being at least one of the modifying agents. In a general way, this makes the observation that there are marked seasonal changes in the frequencies of the chromosome types in natural populations understandable. But a detailed application of the experimental results to the interpretation of the changes observed in nature encounters difficulties.

Parallel cyclic changes occur in the populations of Piñon Flats (4000 feet above sea level) and Andreas Canyon (800 feet). In both localities the

frequencies of Standard are lowest and of Chiricahua highest in June. The waning July population at Piñon shows some increase of Standard and a drop in Chiricahua; this process continues till September when Standard about reaches its annual maximum frequency and Chiricahua its minimum. Flies are rare in July and August at Andreas, but in September the population shows a materially increased frequency of Standard and a decreased one of Chiricahua. By analogy with the populations of experimental cages kept at 25°C, it is tempting to ascribe the waxing of Standard and the waning of Chricahua at Piñon and Andreas to the high temperatures prevailing during the summer months in these localities. The same analogy seems to account for the fact that Standard chromosomes are less frequent and Chiricahua more frequent at Piñon than in the warmest locality, Andreas. Furthermore, at Keen Camp (about 4500 feet elevation, ponderosa pine zone) Standard is still less and Chiricahua still more frequent than at Piñon (DOBZHANSKY 1943).

Again, no appreciable changes in the frequencies of the chromosomal types take place in any locality during the cool season, from September to March or April. This agrees with the finding that chromosome frequencies change little if at all in experimental cages kept at 16½°C. Although Keen is the coolest of the three localities, a similar explanation is hardly satisfactory to account for the absence at Keen of the cyclic changes of the type observed at Piñon and Andreas. The selective values of the chromosomes at Keen (and Andreas) need not be the same as those at Piñon.

The frequencies of Standard drop and of Chiricahua increase from April to June at Piñon and at Andreas. Since the temperatures are increasing during spring, the observed changes in the chromosome frequencies go in the direction opposite to that found at high temperatures in the experimental populations. The causation of these changes is at present a matter of speculation.

The sensitivity of the selective differentials to environmental changes is probably the most interesting phenomenon observed in our experiments, apart from the establishment of the fact that chromosome types found in natural populations may differ in adaptive value. As shown above, the chromosome types are equivalent or nearly so at 16½°C, but strikingly unlike at higher temperatures. An analogous extreme sensitivity to temperature has been found also in homozygotes for some individual chromosomes isolated from natural populations (DOBZHANSKY and SPASSKY 1944). It must be kept in mind that, among the mass of possible environmental variables, only temperature and the amount of food have so far been studied in population cages. It seems very improbable that the relative adaptive values of the chromosome types so greatly modified by temperature will prove to be insensitive to anything else. The changes observed at Piñon and Andreas during spring may, then, represent a selective response to factors other than temperature (for example, qualitative changes in the diet). By the same token, the causative factor of the changes taking place in the same populations in summer need not necessarily be temperature, even though temperature does induce analogous changes in population cages.

An alternative explanation has been suggested by HOVANITZ (1944)—namely, that the populations of Piñon and Andreas are swamped in summer by migration from the flourishing populations higher on the mountain, which resemble Keen in chromosome frequencies. The return of Piñon and Andreas in late summer and in fall to the frequencies of the chromosome types characteristic for the winter populations of these localities obviously cannot be accounted for by migration. It must be due to selective pressures of the sort indicated by the experiments with population cages, or even to complete extinction of the migrant population which must somehow escape crossing with the natives. Furthermore, the amount of dispersion as determined in *Drosophila pseudoobscura* by experiments in the field is not adequate (DOBZHANSKY and WRIGHT 1943). The mean square radial distance in one day from the site of release of dense populations of marked flies was 17,600m^2 with variance of distances increasing on subsequent days by about 8000m^2 per day at 72°F. The dispersion variance (close to zero below 60°F) increased by about 760m^2 for each increment of one degree Fahrenheit. These figures must be halved to give the variance of spread in one direction. Thus even assuming a temperature of 93°F, the increment of variance in a given direction of flies that emerge at one place on a certain day and spread by random flights is only about 12,000m^2 per day. In a month, the standard deviation of the group about its point of origin would be only about 600 meters. In the experiments moreover, there was evidence that about half of the flies released died in a week. Since Andreas is about 16 kilometers from Keen down the mountain, it is obvious that random dispersal from Keen at the observed rate is wholly inadequate to account for the rapid shift at Andreas to a Keen-like composition of the population. The only possibility of accounting for the May–June shift at Piñon and Andreas by migration from higher on the mountain seems to be by a directed mass movement at a rate of at least half a kilometer per day.

Cyclic changes in the composition of populations have been discovered by TIMOFEEFF-RESSOVSKY (1940) in the beetle *Adalia bipunctata*, by GERSHENSON (1945) in the hamster *Cricetus cricetus*, and by DUBININ and TINIAKOV (1945) in *Drosophila funebris*. The changes appear to be caused in all cases by natural selection in response to seasonal alterations in the environments. The situation found in *Drosophila pseudoobscura* is, thus, not unique. It furnishes, however, the only instance in which changes analogous to those taking place in natural populations can be in part reproduced in laboratory experiments.

SUMMARY

Artificial populations of *Drosophila pseudoobscura* were kept in cages constructed as a modification of the model devised by L'HÉRITIER and TEISSIER.

All the flies used in the experiments reported in this paper are descendants of parents collected at Piñon Flats, Mount San Jacinto, California. Three types of third chromosomes, Standard, Chiricahua, and Arrowhead, are common in the population of that locality. Their relative frequencies undergo

cyclic seasonal changes—namely, Standard increases and Chiricahua decreases in frequency during the summer; the opposite change takes place during spring; the relative frequencies remain constant during fall and winter.

Most of the experiments began with approximately equal numbers either of two or of three chromosome types. At 25°, with initial frequencies equal or with Chiricahua more frequent than Standard, there was a highly significant increase in frequency of Standard (where present) at the expense of either of the other types but especially of Chiricahua. On the other hand, there were no significant changes in mean frequency at 16.5°C. The rate of change at 25° showed a strong negative regression on frequency in all cases, and a probably significant regression of this sort was observed in one case (Arrowhead) at 16.5°. The data further indicate that all chromosome types are favored when sufficiently rare and opposed when sufficiently common.

The simplest hypothesis is that the heterozygotes are favored over both corresponding homozygotes and that there are no sex differences. The results in the experiments at 25° in which only Standard and Chiricahua were present can be adequately explained by postulating the relative selective values .70:1.00:.30 for ST/ST, ST/CH and CH/CH, respectively. Equilibrium is indicated at 70 percent Standard, 30 percent Chiricahua.

However, there is no assurance that selection acts alike on males and females. The mathematical theory for unequal selective values in the sexes is presented. It is found that the results differ little except in extreme cases from those found with sex equality in selection. Thus the data can be fitted substantially as well by assuming selective values 1.00:1.00:.60 for females, .40:1.00:0 for males (for ST/ST, ST/CH, and CH/CH, respectively) as under the previous hypothesis in which both sexes are selected according to the averages of those figures.

It is possible that the selective values may vary with the changes in composition of the population. The mathematical theory of variable selection coefficients is discussed briefly. An extreme hypothesis of this sort (heterozygotes always intermediate while each homozygote decreases in selective value with increase in frequency) was found to fit the data reasonably well. The selective values arrived at were (1.90−1.29q) for ST/ST, 1.00 for ST/CH and (.10+1.29q) for CH/CH, where q is the frequency of Standard (.70 at equilibrium as before).

The mathematical theory of selection among multiple alleles is considered in connection with the cases in which three chromosome types were present. The selective values at 25°, indicated by the method of least squares, are .43 for ST/ST, .05 for AR/AR, .21 for CH/CH, 1.30 for ST/AR, 1.00 for ST/CH, and .71 for AR/CH. Equilibrium is indicated at 53 percent Standard, 34 percent Arrowhead and 13 percent Chiricahua. The hypothesis that all three heterozygotes are equal in selective value gives impossible results.

The experimental results demonstrate clearly that there may be selective differences between chromosome types derived from the same locality and that

these may be of such a nature as to result in the indefinite persistence of several types in such a locality. The marked rise in frequency of Standard and decrease of Chiricahua in summer, observed to occur in nature in the locality from which the flies were collected, is analogous to the experimental reaction to high temperature, and it is tempting to compare the lack of change in artificial population at 16.5° with the constancy observed in the natural ones during fall and winter. The changes which occur in nature during spring (the reverse of those in summer) however, have not been reproduced in the population cages. Mass migration at this time from the population high in the mountains would explain the results qualitatively, but it would be difficult to reconcile this with the results of earlier experiments on the rate of dispersion of the flies in nature. It appears that there are important factors yet to be discovered.

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