GENOTYPIC BACKGROUND AND HETEROSIS IN DROSOPHILA PSEUDOOBSCURA[1]

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NATURAL populations of many species of Drosophila vary with respect to the gene arrangement in their chromosomes. In *Drosophila pseudoobscura*, at least sixteen gene arrangements are known in the third chromosome; these arrangements differ from each other by inversions of blocks of genes. The chromosome polymorphism in this species is balanced, i.e., the inversion heterozygotes have net selective advantages over the homozygotes. This selective advantage, or heterosis, has been shown to exist both in natural populations and in experimental populations bred in laboratories (DOBZHANSKY 1943, 1947b; WRIGHT and DOBZHANSKY 1946; DOBZHANSKY and LEVENE 1948).

The genetic mechanisms which bring about the heterosis in the inversion heterozygotes need elucidation. That this heterosis is delicately balanced has been shown in a variety of ways. In the first place it depends upon the environment in which the flies live. The carriers of a certain chromosomal constitution may be either adaptively superior or inferior to the carriers of other chromosomal constitutions depending upon temperature, nutrition, and on the presence or absence in the same population of other genotypic variants (DA CUNHA 1951; DOBZHANSKY and SPASSKY 1954; LEVENE, PAVLOVSKY, and DOBZHANSKY 1954). More important still, the adaptive value of a karyotype in a given environment depends upon the geographic origin of the constituent chromosomes. When experimental populations contain chromosomes derived from the same natural population, the inversion heterozygotes are often heterotic. However, in experimental populations of mixed geographic origin heterosis may be absent (DOBZHANSKY 1949). DOBZHANSKY and LEVENE (1951) reported that on hybridizing a chromosome type known as Standard (ST) from California with one known as Chiricahua (CH) from Mexico, they first observed a tendency toward elimination of the CH chromosomes, but then a change in the process and the final establishment of equilibrium of the two types. This was the first occurrence of the development of heterosis during the course of an experiment. They also reported that in a different population cage, using Arrowhead (AR) from California against CH from Mexico, they found equilibrium with no indication in this case that the heterosis had developed during the course of the experiment.

The origin of heterosis in experimental populations of mixed geographic origin has been studied further by DOBZHANSKY and PAVLOVSKY (1953), who found that the results of replicate experiments of this kind are not wholly determinate. In some populations containing ST chromosomes from California and CH from Mexico the structural heterozygotes have become heterotic, while in other populations of the

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same kind, and kept in similar environments, CH chromosomes were eliminated. This raised the problem whether the superiority of the inversion heterozygotes over the homozygotes is due to the genes in only the third chromosomes, or to a structuring of the whole genotype. The experiments described in the present report were designed to obtain information bearing on the above problem.

MATERIAL AND METHODS

All experiments were made with 12 strains of *D. pseudoobscura* derived from wild progenitors collected at Pinon Flats, Mount San Jacinto, California, and 12 strains collected at Santa Barbara, Chihuahua, Mexico. The collections in California were made by DR. D. F. MITCHELL in 1949, and in Mexico by PROF. H. T. SPIETH in 1947. These are the same strains which were used in the work of DOBZHANSKY and PAVLOVSKY (1953).

The experimental populations were maintained in population cages of a construction somewhat different from that used in the previous investigations. The cages used in the present experiments are lucite boxes, rectangular in shape, 4½″ high, 5¼″ wide and 18″ long. In the floor of the cage are 16 holes for the insertion of food vials. Since a fresh vial is inserted three times a week, each vial remains in the cage for a period of 37 days. The generation time is estimated at 25 days. All experimental populations were kept in constant temperature rooms at 25°C. The populations were sampled once a month for the first three months and every two months thereafter. Each sample consisted of 300 chromosomes, obtained from 150 larvae, taken in 6 subsamples on six successive days, 25 larvae (50 chromosomes) per subsample. The chromosome frequencies in tables 1–4 are, thus, based on 300 observations each.

CHROMOSOME TRANSFER BETWEEN GEOGRAPHIC POPULATIONS

In order to study the effect of background genotype on heterosis it was first necessary to obtain strains in which the third chromosomes of Californian origin were transferred onto the genetic background of Mexican origin, and vice versa. This had to be done in two stages.

First, third chromosomes marked with visible dominant mutant genes, and carrying gene arrangements other than those to be used in the experiments, were crossed repeatedly to the Californian and Mexican stocks. The marked chromosome in one case had the mutants, *orange*, *Blade*, *Scute*, and *purple* (*or BL Sc pr*) and had the Standard (ST) gene arrangement. In the other series, the chromosome was marked by the mutants, *Lobed* and *orange* (*L or*) and had the Santa Cruz (SC) gene arrangement. Strains bearing each of these marked chromosomes were crossed seven times both to the Californian (C) stocks which contained the gene arrangement Arrowhead (AR) and to the Mexican (M) stocks which contained the gene arrangement Chiricahua (CH). This procedure is shown in figure 1. As this was done with both markers for the Mexican as well as the Californian stocks, there were obtained four types of stocks containing mixed chromosomes with gene markers, as shown in figure 2.

In making the above crosses, the strains with the chromosomes containing the mutant markers were outcrossed each time to a different wild strain of either the

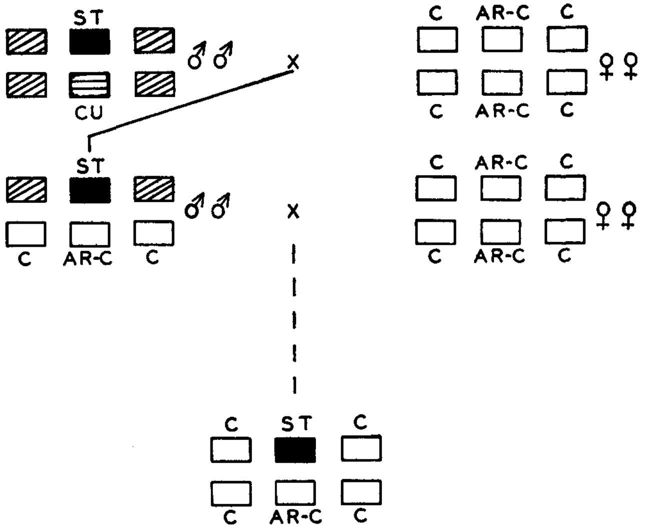

FIGURE 1.—Stage one of chromosome transfer between geographic populations. ST—Standard gene arrangement containing *or Bl Sc pr* mutants, CU—Cuernavaca gene arrangement containing recessive lethal mutant, AR-Arrowhead gene arrangement, C—California chromosomes. Cross-hatched chromosomes represent background chromosomes derived from diverse laboratory stocks. Broken lines represent five backcrosses of marker-chromosomes, with ST gene arrangement, to Arrowhead California stocks to provide an all California genotype except for marker-chromosomes.

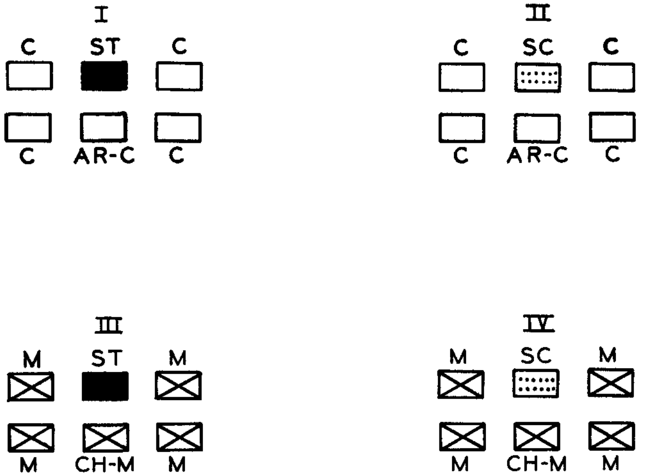

FIGURE 2.—Results of stage one of chromosome transfer between geographic populations. ST—Standard gene arrangement containing *or Bl Sc pr* mutants, AR—Arrowhead gene arrangement, C—California chromosomes, SC—Santa Cruz gene arrangement containing L or mutants, M—Mexican chromosomes, CH—Chiricahua gene arrangement. Represented are four marker-stocks containing visible mutants on either all California or all Mexican genotypes.

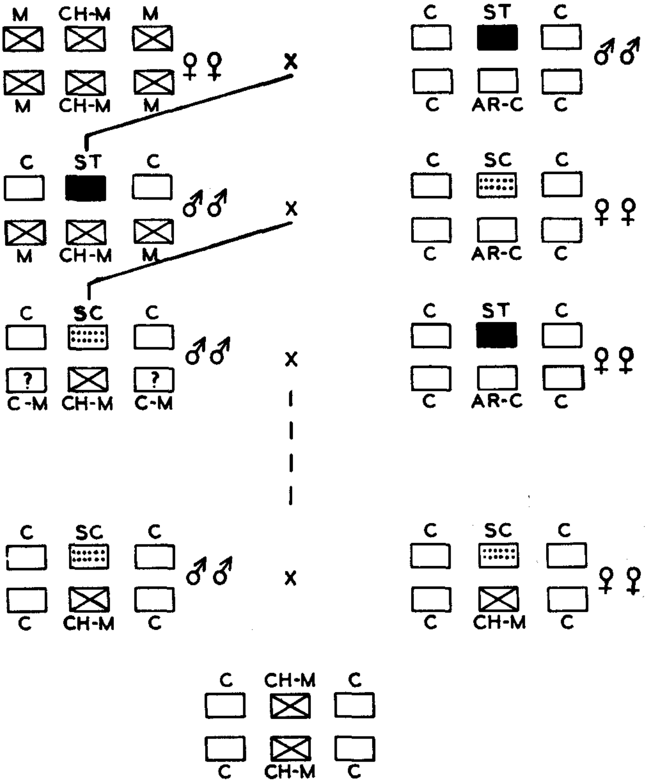

FIGURE 3.—Stage two of chromosome transfer between geographic populations. Symbols as on previous figure. Broken lines represent four more backcrosses alternately to the two marker-stocks which resulted in the experimental stocks which contained third chromosomes of one geographic origin and the remaining genotype of a different geographic origin.

Californian or Mexican origin. In this manner, strains were obtained which had the mutant markers but which also carried a variety of genetic modifiers which were present in the geographical populations used.

The second series of crosses is represented schematically in figure 3. The purpose of these crosses was to obtain strains which had the third chromosomes of one geographic origin and chromosomes other than the third of a different geographic origin. In *Drosophila pseudoobscura* the variations in gene arrangement are found mainly in the third chromosome (DOBZHANSKY and EPLING 1944), the chromosomes other than the third have usually the same gene arrangement regardless of geographic origin. For this reason, the transfers of Californian third chromosomes onto Mexico genetic background, and vice versa, had to be done by repeated backcrossing to the strains of the proper geographic origin. Seven such backcrosses were made. This means that less than 5% undesirable background chromosomes remained in the strains finally obtained.

Male flies were used whenever mixed background chromosomes were involved in

TABLE 1

Control experiments. Experimental populations containing CH and AR chromosomes of Californian origin

Month	Percent CH	
	Population D-1	Population D-2
0	50.0	50.0
1	51.1	45.0
2	41.3	35.7
3	35.7	38.3
5	39.0	34.3
7	33.7	33.7
9	28.7	29.7
11	30.3	39.3
13	27.3	28.3

order to prevent crossing over from contaminating the stocks. Third chromosome crossing over in the marker-stocks was eliminated by the constant maintenance of the marker-stocks as inversion heterozygotes. This has been shown to be a very effective crossover suppressor by DOBZHANSKY and EPLING (1948).

After obtaining the two types of third chromosomes on foreign backgrounds it was possible to place the two inversion types in competition with one another, in population cages, under any background chromosome situation. This was done by hybridizing the obtained strains with the original pure AR(C) or CH(M) strains maintained in our laboratory.

CONTROL EXPERIMENTS

Inversion heterozygotes which carry chromosomes with different gene arrangements, but derived from the same locality, usually exhibit heterosis. Thus DOBZHANSKY (1948) has shown that populations which contain AR and CH chromosomes of Pinon Flats origin reach, at 25°C, an equilibrium at about 75% AR and 25% CH. However, as stated above, it is known that the adaptive values of the heterozygous and homozygous karyotypes are exceedingly sensitive to modification by environmental agencies such as temperature, nutrition, and probably others. The experiments reported in the present paper were carried out under conditions appreciably different from those of DOBZHANSKY (1948). This was the result of the use of the plastic population cage, instead of the standard wooden model. The food vials have very different surface-volume ratios in the two types of cages with a result that the plastic cage supports an adult population which is appreciably smaller than the standard cage. Two control experiments were accordingly arranged in which the initial populations consisted of F_1 hybrids between strains homozygous for AR and for CH of Pinon Flats origin.

The results are summarized in table 1 and figure 4. Both control populations reached equilibria at about 70% AR and 30% CH. The changes in the relative frequencies of the chromosomal types observed in the two experiments were parallel, the composition of both populations remaining alike at all stages of the experiment

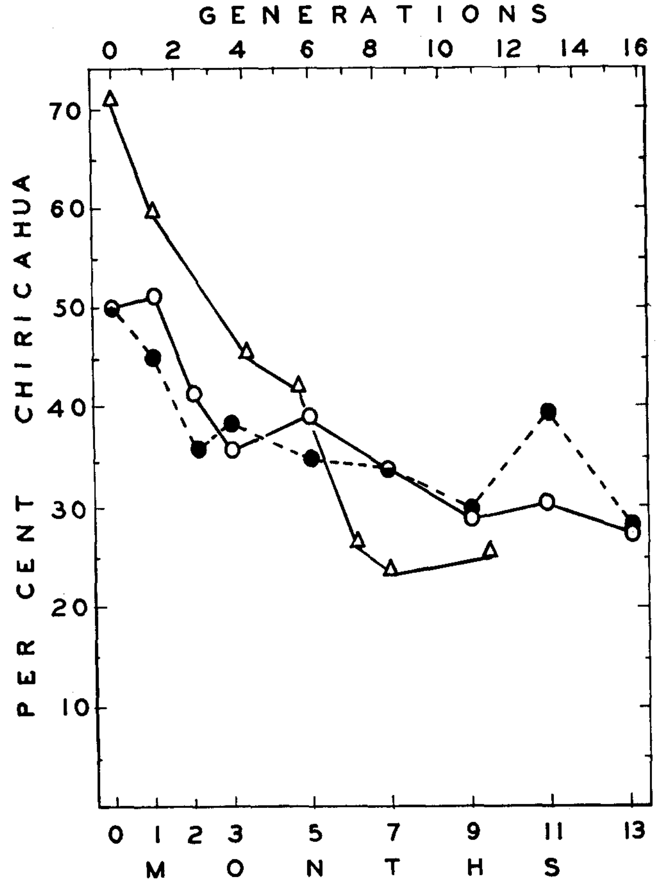

FIGURE 4.—Frequencies of chromosomes with the CH(C) gene arrangement in populations of geographically uniform origin. Population D-1—white circles, D-2—black circles, DOBZHANSKY'S 1948 data—white triangles.

within limits of sampling errors. The aggregate chi-square for the 8 pairs of samples is 11.56, which corresponds to a probability of 0.2–0.1. The greatest divergence was observed between the samples taken in the replicate experiments during the eleventh month (table 1); such a divergence or a greater one has, however, a probability of chance occurrence between 0.05 and 0.02. Figure 4 gives a graphic comparison of the outcomes of the two control experiments with that of DOBZHANSKY (1948). No appreciable differences between the experiments are apparent, although the equilibrium level in the populations described by DOBZHANSKY may be slightly lower than in the present ones. If this difference is real, it may be accounted for by the dissimilarities of the environments of the two types of cages used in these experiments.

In attempting to estimate the adaptive values of the karyotypes it was found that both the method worked out by SEWALL WRIGHT, which assumes constant selective coefficients throughout the experiment, and that of HOWARD LEVENE, which permits changing selective coefficients but in a regular fashion, give anomalous results (WRIGHT and DOBZHANSKY 1946; DOBZHANSKY and LEVENE 1951). An inspection of the data will show that there were changes in direction of selection that were both sudden and reversible. They were probably due to peculiarities of the type of cage used. This aspect of the experiment will be discussed later. As it is felt that these sudden and reversible changes in selection do not mirror the selection process it was decided to omit the obviously aberrant points and then calculate the adaptive values of the karyotypes using Wright's method. The data omitted were month #1 of population D-1 and months #2 and #11 of population D-2. The adaptive values of the karyotypes of these populations and those found by DOBZHANSKY (1948) are given below:

Karyotype	*Present experiments*	*Dobzhansky's experiments*
AR/AR	0.81	0.86
AR/CH	1.00	1.00
CH/CH	0.53	0.48

POPULATIONS WITH CALIFORNIAN GENETIC BACKGROUND

Strains with CH third chromosomes of Mexican origin and with other chromosomes mostly of Californian origin, were obtained as described above. These strains were crossed to others of pure Californian descent, but homozygous for the AR gene arrangement in the third chromosomes. Two replicate experimental populations were made using the F_1 hybrid flies. In these populations the genotype is, then, mostly derived from the Californian race, except that the third chromosomes are CH from Mexico and AR from California. The changes observed in these populations are summarized in table 2 and figure 5.

Both populations eventually reached equilibria at about the levels 70–75% AR and 25–30% CH, which is close enough to the equilibrium observed in the control

TABLE 2

Populations containing AR chromosomes of Californian origin, CH chromosomes of Mexican origin, and Californian genetic background

Month	Percent CH	
	Population A-1	Population A-2
0	50.0	50.0
1	43.3	43.7
2	41.7	32.3
3	47.7	38.3
5	32.3	30.7
7	34.7	29.0
9	39.0	31.7
11	21.7	23.0
13	21.3	19.7
15	23.3	27.0
17	25.0	31.7
19	22.0	27.3

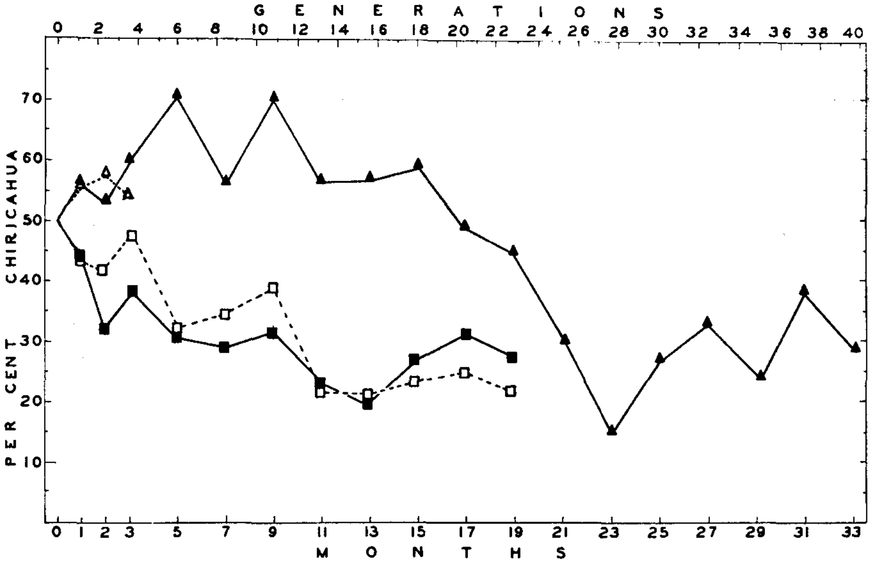

FIGURE 5.—Frequencies of chromosomes with the CH(M) gene arrangement in populations containing California genetic background: population A-1—white square, A-2—black squares; and populations containing Mexican genetic background: population B-1—white triangle, B-2—black triangle.

experiments (fig. 4). However, the frequency of CH chromosomes in the population A-1 decreased at first more slowly than in A-2, although the final equilibrium in A-1 seems even lower for CH than in A-2 (table 2 and fig. 5). The differences between the two populations are statistically significant in the samples taken during the second and third months (chi-squares 5.61 and 5.33 respectively, corresponding to probabilities around 0.02). Although in later samples the differences are no longer significant, the aggregate chi-square is 21.59, which, for 11 degrees of freedom, corresponds to a probability between 0.02 and 0.01.

Inspection of figure 5 suggests that the course of selection in the two populations under consideration is, on the whole, less regular and uniform than in the control populations (fig. 4). A sharp drop in the frequency of CH chromosomes between the ninth and eleventh months seems especially striking. The reason for this drop is not clear. It may have been caused by a sudden change in the genetic composition of the populations themselves, if a highly fit AR homozygote arose at that time. However, since a sharp drop in the percentage of CH(M) occurred in many of the other cages at this time, it is felt that the causative agent may have been environment. The drop coincided with a spell of very hot weather. Although the population cages were kept in a constant temperature room at 25°C the hot spell resulted in an increase of humidity in the constant temperature room and in a lush growth of molds in the cages. However, in spite of the irregularities noted in the selection process, it is apparent that in all populations with Californian background, AR from California and CH from Mexico form a heterotic combination.

POPULATIONS WITH GEOGRAPHICALLY MIXED GENETIC BACKGROUNDS

The parents of the experimental populations reported in this section were F_1 hybrids between strains in which the third chromosomes with CH gene arrangements of Mexican origin were transferred onto Californian genetic background, and strains with AR third chromosomes of Californian origin transferred to the Mexican genetic background. The resulting populations had, then, Mexican CH and Californian AR third chromosomes, and a great variety of genetic backgrounds resulting from assortment and recombination of Californian and Mexican chromosomes. The events observed in these populations are reported in table 3 and figure 6.

In contrast to the experiments previously described, the frequencies of the AR and CH chromosomes fluctuated in both populations around the starting frequency for more than 15 generations, after which the populations were terminated. The two replicate populations remained rather similar throughout (the aggregate chi-square is 11.37, corresponding to a probability between 0.1 and 0.2). What is more important is that the final frequencies of the AR and CH chromosomes were not appreciably different from the initial ones. This result was unexpected for two reasons. First it differs from the outcomes of the control experiments and of the experiments with the Californian genetic background. Secondly, it differs from the results observed by DOBZHANSKY and LEVENE (1951) in a population which had, like the present ones, Californian AR and Mexican CH third chromosomes and a geographically mixed genetic background. In the populations studied by DOBZHANSKY and LEVENE, the initial frequency of AR and CH chromosomes were, like in this study, 50%, but the final equilibrium was in the vicinity of 75% AR and 25% CH.

Two new populations were, accordingly, started, both with a mixed Californian-Mexican genetic background, and Californian AR and Mexican CH third chromosomes. However, in one of these new populations the initial frequencies were 20% CH and 80% AR (population C-3), and in the other 80% CH and 20% AR (popula-

TABLE 3

Populations containing AR chromosomes of Californian origin, CH chromosomes of Mexican origin, and geographically mixed genetic backgrounds

Month	Percent CH			
	Population C-1	Population C-2	Population C-3	Population C-4
0	50.0	50.0	20.0	80.0
1	46.3	46.0	20.0	64.3
2	49.3	44.3	23.3	68.7
3	54.3	49.3	21.7	65.7
5	48.3	52.0	27.7	49.0
7	48.3	44.3	24.0	43.0
9	51.7	55.0	29.0	43.3
11	50.7	43.7	25.7	35.7
13	42.3	49.3	26.0	25.0
15	—	—	—	31.3
17	—	—	—	37.0
19	—	—	—	26.0

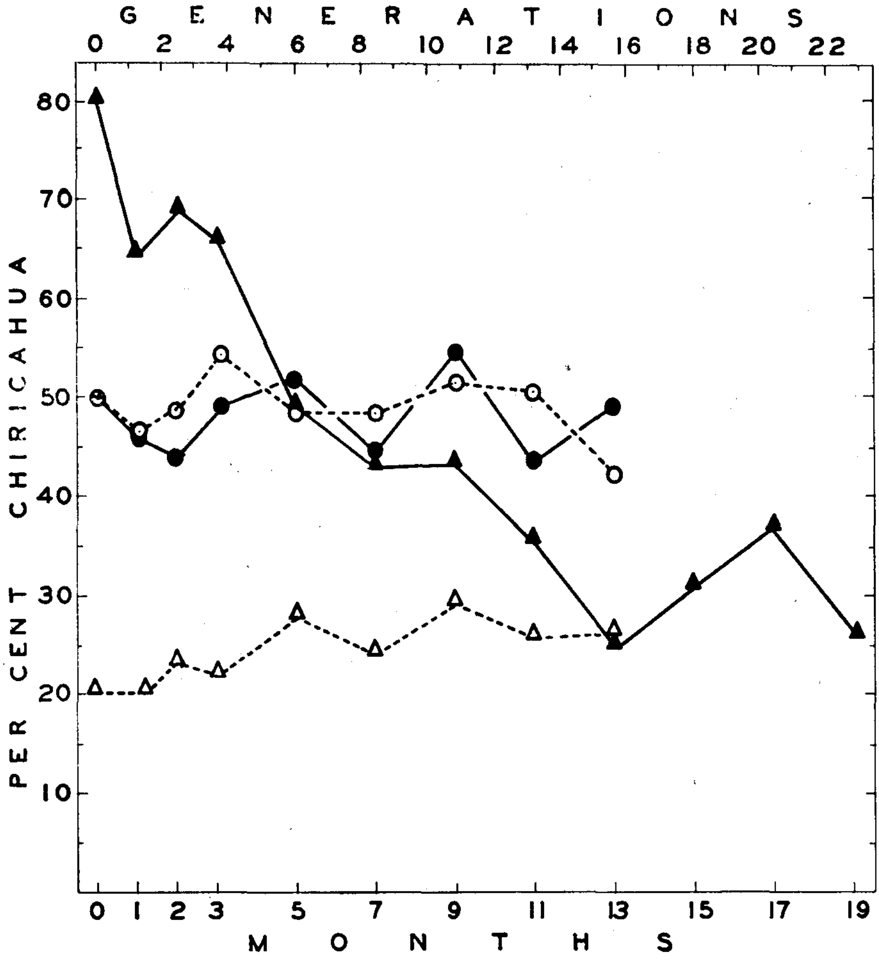

FIGURE 6.—Frequencies of chromosomes with the CH(M) gene arrangement in populations of geographically mixed genetic backgrounds. Population C-1—white circles, C-2—black circles, C-3—white triangles, C-4—black triangles.

tion C-4). In populations in which all chromosomes are of uniform geographic origin, the initial frequencies of the competing gene arrangements do not affect the final outcome. Identical or very similar equilibrium frequencies are established regardless of the initial frequencies of the chromosomes (DOBZHANSKY 1947a, 1948). The results observed in the populations C-3 and C-4 are also reported in table 3 and figure 6.

The behavior of the populations C-3 and C-4 is in agreement with that of the population described by DOBZHANSKY and LEVENE (1951), and not with that of populations C-1 and C-2. In C-4 the frequency of CH chromosomes dropped sharply, and approached an equilibrium at a level between 25 and 30 percent. In C-3 there was a slight but apparently significant increase in the frequency of CH chromosomes, up to the same equilibrium level as in C-4. Possible interpretations of these results will be discussed below.

Populations with Mexican genetic background

Populations of this kind gave the most complex and interesting results. As many as eight of them were studied, two populations having been kept for almost three years (forty generations), with periodic samples being taken. In every instance, the initial populations consisted of hybrids obtained by crossing strains of pure Mexican origin, homozygous for CH, with strains having AR chromosomes of Californian origin which had previously been transferred onto the Mexican genetic background. The observations are summarized in table 4 and figures 5 and 7.

Two populations, B-1 and B-2, (fig. 5) had the initial frequencies of the CH and AR chromosomes 50% each. B-1 was started earlier than B-2, and was kept for three months, during which it showed no appreciable changes in the karyotype composition. This seemed to parallel the behavior of the populations C-1 and C-2, which had geographically mixed backgrounds (see above). However, the later replicate, B-2, showed a significant, though very erratic, increase in the frequency of CH chromosomes, which reached the 70% level after 5 and after 9 months. However, instead of establishing and maintaining an equilibrium, the CH chromosomes began to dwindle in frequency after 15 months. The frequency of CH reached a value as low as 15% after 23 months, only to rise again to 30% two months later and fluctuated around that point for the duration of the cage.

TABLE 4

Populations containing AR chromosomes of Californian origin, CH chromosomes of Mexican origin, and Mexican genetic background

Month	Percent CH							
	Population B-1	Population B-2	Population B-3	Population B-5	Population B-7	Population B-4	Population B-6	Population B-8
0	50.0	50.0	20.0	20.0	20.0	80.0	80.0	80.0
1	55.3	56.3	24.3	15.0	16.7	74.0	60.3	60.0
2	57.3	53.0	31.7	17.7	10.3	68.3	59.3	64.7
3	53.7	60.0	28.7	22.7	16.0	62.7	40.3	50.7
5	—	70.3	31.7	36.7	21.3	59.0	42.7	52.0
7	—	56.0	28.7	36.7	13.7	49.7	41.3	44.0
9	—	70.0	29.0	33.0	18.7	49.0	31.7	34.0
11	—	56.3	29.3	24.0	—	64.0	32.7	42.0
13	—	56.7	—	20.0	—	53.0	36.3	—
15		59.0	—	47.3	—	54.7	47.0	—
17		49.0	—	24.0	—	29.3	32.3	—
19		44.7	—	39.0	—	27.3	34.0	—
21		31.0	—	—	—	34.7	—	—
23		14.7	—	—	—	39.3	—	—
25		27.0				40.3		
27		33.0				33.0		
29		24.0				31.7		
31		38.3				42.0		
33		28.7						

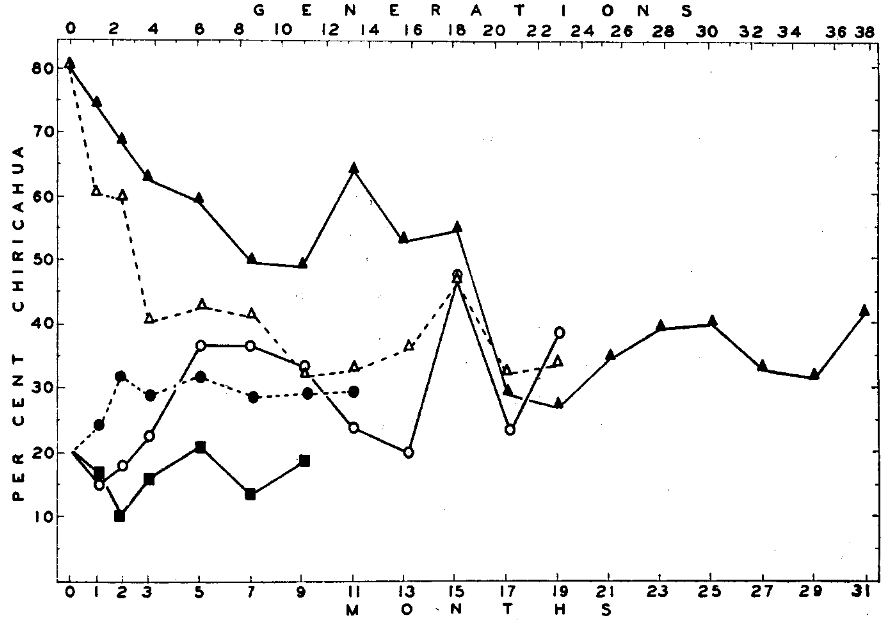

FIGURE 7.—Frequencies of chromosomes with the CH(M) gene arrangement in populations containing Mexican genetic background. Population B-3—black circle, B-4—black triangle, B-5—white circle, B-6—white triangle, B-7—black square.

Populations of similarly mixed geographic origin, but with different initial frequencies of AR and CH chromosomes, behaved quite differently. Thus, the populations B-3 and B-5 (fig. 7) were started at different times, to be sure, but both with initial frequencies of 20% CH and 80% AR chromosomes. Population B-3 reached a level of about 30% CH two months after the start. This level was preserved without significant fluctuations, as expected when a true equilibrium has become established. This was not so in population B-5. Here there was at first no change in the karyotype composition, or even a drop in the frequency of CH to the 15% level. But this trend (if it was real) became reversed, and CH rose to the 35% level and remained there from the fifth to the ninth months. Thereafter the cage behaved very erratically. There was a rather sharp drop in CH frequency to 20% by the thirteenth month, and a sudden jump to about 50% at the fifteenth month. This was followed by a drop to 24% CH and a rise to 39% CH at the next two readings.

The replicate, though not simultaneous, populations B-4 and B-6 (fig. 7) had initially 80% CH and 20% AR. In both populations the frequency of CH chromosomes dropped. However, while in B-6 the drop was reasonably (though not entirely) regular, population B-4 showed great oscillations. The frequency of CH, in B-4, at first dropped gradually, and seemed to establish an equilibrium at about 50% (with a significant jump 11 months from the start). Then after 15 months there occurred a sudden and dramatic drop to 30% and a recovery to about 40% when the population was 2 years old. Subsequently there was a return to the 30% level when the population was 29 months old and another rise to 42% CH two months later. The sudden and dramatic drop in CH frequency in B-4, noted above, coincided in time with sharp decreases in CH frequency in other populations (for example, the drop at the twenty-first month in B-2 and at the eleventh month in A-1 and A-2). The possibility that these coincident changes were caused by environmental factors has been discussed earlier in this paper.

Two phenomena observed in the experiments on populations with mixed and with Mexican genetic backgrounds would be very unusual in populations of uniform geographic origin, at least under reasonably carefully controlled environmental conditions. These phenomena are the establishment of somewhat different equilibrium levels in populations with initially similar genetic components, and the erratic progress of the selection process, especially with reversals of trends. Such phenomena have, however, been described in populations of geographically mixed origin by DOBZHANSKY and LEVENE (1951) and DOBZHANSKY and PAVLOVSKY (1953 and unpublished data). They have also been recorded by EPLING, MITCHELL and MATTONI (1953), although not ascribed by these authors to the geographic mixtures.

However, before the observations here described can be put in the same category as those of the authors just cited, other possibilities must be considered. One possibility is the effect of the use of the plastic population cages, with their populations which are numerically smaller than those obtained in the larger standard cages. It seemed barely conceivable (although not probable) that the fluctuations in the chromosome frequencies might be caused by genetic drift. Populations B-7 and B-8 were, accordingly, started and put in standard wooden cages, with Mexican CH and Californian AR chromosomes on Mexican genetic background. Population B-7 had initially 20% CH chromosomes, and thus was a replicate of B-3 and B-5; population B-8 had 80% CH chromosomes, and constituted a replication of B-4 and B-6 (table 4 and fig. 7; B-8 is not shown in graph form).

Population B-7 fluctuated between 10% and 20% CH for 9 months and was then terminated. Its mean value was about 15% CH. This population B-7, showed fluctuations about the equilibrium level but none so violent as those observed in most of the plastic cages. Cage B-8 has shown a fall in CH frequency from the initial value of 80% to about 40%. It is too early to tell whether or at what value it will reach equilibrium. It seems possible from the results in B-7 that the standard wooden cages give fewer irregular fluctuations than the plastic cages. This probem will be discussed below.

Another possible explanation of these inconsistent results concerns itself with the method of transfer of the third chromosomes to the various genetic backgrounds. Since it is impossible to be certain that the background genotype is entirely of Californian or of Mexican origin, one must rely on repeated backcrosses to eliminate the undesired background chromosomes. It is, then, possible that some chromosome sections of Californian origin were present in the populations and that there was selection for these Californian elements. This possibility seems to have some substantiation in the fact that both the early populations, which were maintained for long periods of time, and the later populations gave results indistinguishable from the populations with Californian genetic background. One also finds that the later B type populations do not show the initial plateau of 50–55% CH that the earlier populations showed.

TABLE 5

Initial dates of experiments

Population	Initial Date
D-1	10/1/52
D-2	10/5/52
A-1	10/10/52
A-2	10/15/52
C-1	1/10/52
C-2	12/15/51
C-3	3/10/53
C-4	3/10/53
B-1	10/10/51
B-2	12/5/51
B-3	4/5/52
B-5	3/20/53
B-7	11/15/53
B-4	4/10/52
B-6	3/20/53
B-8	11/15/53

DISCUSSION

The only common denominator in all the experimental populations described in the present article is that in every one of them the two kinds of chromosomes, AR and CH, tend to persist indefinitely. In no population has either chromosomal type come close to being eliminated. This result is what is expected if the Californian AR and Mexican CH chromosomes produce heterosis, i.e., make the heterozygous karyotype, AR/CH, superior in adaptive value to both homozygotes, AR/AR and CH/CH. Such heterosis could not have been predicted. To be sure, AR/CH heterozygotes were known to be heterotic in at least some environments, provided that both chromosomes are derived from the same geographic population, as in the control experiment reported in table 1 and figure 4. However, populations having ST chromosomes from California and CH from Mexico may have the latter chromosomes eliminated (DOBZHANSKY and LEVENE 1951; DOBZHANSKY and PAVLOVSKY 1953).

Beyond that, the behavior of a population is influenced by the geographic origin not only of the third chromosomes, which are being followed cytologically because they are visibly different under the microscope, but also the remainder of the karyotype, which is geographically not differentiated as to the gene arrangement. Indeed, the populations reported upon in figures 5, 6, and 7 all had Californian AR and Mexican CH chromosomes; the differences between these populations concerned the geographic origin of the rest of the karyotype, which was either Californian, or Mexican, or of mixed origin. The events in a population are determined by the degree to which the heterozygotes are superior to the homozygotes, as well as by whether the two homozygotes are equal in selective value or whether one of them is superior to the other. The relative magnitudes of the selective values have been known to depend on environmental agencies such as temperature, nutrition, and presence of other genotypes in the same medium (DA CUNHA 1951; DOBZHANSKY and

SPASSKY 1954; LEVENE, PAVLOVSKY, and DOBZHANSKY 1954). The present study shows them to be determined not only by the genes in the chromosome pair directly concerned but by the genotype as a whole.

These two relationships, the fact of heterosis and that of the influence of the genetic background, proved to be unexpectedly obscured by another phenomenon. This is a striking indeterminacy in the behavior of populations that are replicates, at least in the sense of having the same initial components. Wide divergences in the outcomes of what should have been replicate experiments can easily be seen in figures 5–7. A closer examination shows that the divergences are of at least three kinds; (1) irregular fluctuations in the frequencies of a given class of chromosomes which exceed the expected sampling errors, (2) different equilibria apparently reached by populations of qualitatively or even quantitatively similar initial composition, and (3) erratic selection trends, which make the same genetic component of a population alternately increase and decrease in frequency.

The first kind of fluctuations may be due to several possible causes. That these fluctuations seem to occur mostly in the small lucite population cages used in these experiments, and rarely if at all in the larger cages appears to be significant. The low and fluctuating numbers of adult flies in the small cages may permit some fluctuations caused by genetic drift. A more likely possibility, suggested by the work of BIRCH (unpublished) is that the adaptive values of the karyotypes may be a function of the population density, and particularly of the presence or absence of competition for food among the larvae. Indeed, the experiments of BIRCH show some irregular fluctuations which are quite similar to those in the data reported in the present article. Expansions and contractions of the populations in the small cages have repeatedly been observed, especially in connection with such things as extreme weather conditions, development of molds, etc. Sharp but reversible changes in the selection pressures could easily be induced by such expansions and contractions.

The second and third kinds of fluctuations (changes in equilibrium values and in selection trends) have been observed in populations of mixed geographic origin, and rarely if ever in populations of geographically uniform origin (DOBZHANSKY and PAVLOVSKY 1953; DOBZHANSKY and LEVENE 1951). The explanation of these interesting but complex phenomena suggested by the authors referred to is, briefly, as follows. The gene complexes in the chromosomes with different gene arrangement from the same natural population have been coadapted by a long process of natural selection to produce superior fitness in heterozygotes. The results of WALLACE (1955) and VETUKHIV (1953, 1954) as well as those described in the present article suggest that the coadaptation involves not a single pair of chromosomes, but indeed, the whole genotype. Now, in artificial populations of uniform geographic origin the coadapted genotypic system is not altered qualitatively. All that occurs are changes in the relative frequencies of certain pre-existing gene complexes carried in the chromosomes with different gene arrangements. This is not so in populations of geographically mixed origin. Here the recombination of genes derived from different populations (races) gives a great variety of genotypes, many or most of which may have never existed in nature. A process of natural selection is, then, enacted in the artificial populations. New adaptively integrated genotypes are formed, and they may be different in different populations. In a given population the selective process takes time, and different genotypes may gain prevalence at different stages of this process. The indeterminacy of the results is, then, a function of the field of potentially possible gene combinations being too great in relation to a limited size of the experimental populations.

In the experiments here described, the populations had one of the third chromosomes differ in origin from its partner as well as from the other chromosomes. Furthermore, in all instances care was taken to have several strains from each geographic region enter in the composition of each experimental population. Enough genetic variance was, thus, available (even disregarding the possibility of chromosomes or chromosome sections of other geographic origin slipping through the crosses shown in figures 1 and 3) to start a selective process of coadaptation between the gene contents of the different third chromosomes and of other chromosomes. Nevertheless, the AR/CH heterozygotes proved superior to the AR/AR and CH/CH homozygotes in all populations, as though these gene contents were coadapted to each other as they usually are when the chromosomes are of uniform geographic origin. Two possibilities may be mentioned as tentative explanations of this fact. The first is that, regardless of their geographic origins, AR and CH chromosomes interact to produce heterosis. This could happen if one of their gene arrangements first arose in a region where the other was also present, and the gene contents of these chromosomes were coadapted by natural selection. The coadaptedness of the Californian AR and Mexican CH chromosomes would, then, be a genetic relic of their former sympatric occurrence.

Another, perhaps more likely possibility, is suggested by the recent work of BRNCIC (1954), WALLACE (1955), and particularly LERNER (1954). BRNCIC and WALLACE found that juxtaposition of whole chromosomes of different geographic origins produces heterosis, which disappears when these chromosomes are broken by crossing over. LERNER has put forward the view that, at least in sexual and cross-fertilizing organisms, heterozygosis per se may be a viability stimulus. Now in the experimental populations described in the present article the third chromosomes of Californian and Mexican origins were prevented from exchanging segments because the inversion heterozygote, AR/CH, acts as a crossover suppressor (DOBZHANSKY and EPLING 1948). The inversion heterozygotes were thus necessarily heterozygous for alleles at many loci. The Californian and Mexican chromosomes with AR and CH gene arrangements carry gene complexes which have proven successful in their respective localities. As shown by BRNCIC and WALLACE, such heterozygosis results in heterosis. To be sure, the stimulating effects of allele heterozygosis may be offset by a lack of coadaptation between the gene contents of the third chromosomes and the rest of the genotype, as observed by DOBZHANSKY and PAVLOVSKY (1953) for ST chromosomes from California and CH chromosomes from Mexico. The fact that species hybrids are often inviable or sterile is conclusive evidence that heterozygosis per se does not guarantee superior fitness. Heterozygosis and coadaptation (or lack of coadaptation) may be regarded as two variables the interaction of which is responsible for the relative indeterminacy of the results of selection in the experimental populations described.

SUMMARY

Strains of *Drosophila pseudoobscura* were used which were derived from flies captured in California and which contained the Arrowhead (AR) gene arrangement, and other strains which came from Mexico and contained the Chiricahua (CH) gene arrangement in the third chromosomes. By a series of crosses, the AR chromosomes of Californian origin were transferred to the Mexican genetic background, and conversely, Mexican CH chromosomes were transferred to the Californian genetic background. Experimental populations were then formed, all of which had Californian AR and Mexican CH chromosomes; some of these populations contained, however, a Californian genetic background, others contained a Mexican genetic background, while still others contained a mixed genetic background of Californian and Mexican chromosomes.

All experimental populations remained chromosomally polymorphic, with AR and CH chromosomes eventually stabilized at certain equilibrium frequencies. The AR/CH heterozygotes were, thus, heterotic, i.e., superior in fitness to the AR/AR and CH/CH homozygotes. The position of the equilibrium level varied however, depending upon the geographic orgin of the genetic background, each gene arrangement being most frequent on its native genetic background. This second conclusion was partly obscured by the changing nature of the genetic background. Furthermore, the course of the selection process was often erratic and some of the replicate experiments produced significantly different outcomes.

The most probable interpretation of these observations is that although heterosis was formed under all the genetic backgrounds tested in these experiments, the level at which this equilibrium was achieved is a function of the genetic background of the population. The relative indeterminacy of the outcome in the experimental populations was due to the complex nature of the selective process which led to the establishment of novel adaptive genotypes from the genetic elements contributed by the hybridization of populations of different geographic origins.

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