GENETICS OF NATURAL POPULATIONS. XIX. ORIGIN OF HETEROSIS THROUGH NATURAL SELECTION IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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Received November 8, 1949

IN MOST parts of the geographic distribution of *Drosophila pseudoobscura*, chromosomal types which differ in gene arrangement in the third, the X, and, less frequently, in other chromosomes occur in the populations. The diversity of the gene orders is due to inversion of chromosome sections. Since the chromosomal types interbreed at random, inversion heterozygotes and homozygotes are found in nature. The observation that seasonal changes in relative frequencies of third-chromosome gene arrangements take place in populations of Piñon Flats, on Mount San Jacinto, in California, has suggested that these gene arrangements affect the adaptive properties of their carriers (DOBZHANSKY 1943). Indeed, experimental populations kept in so-called "population cages" have shown that the inversion heterozygotes which carry two third chromosomes with different gene arrangements derived from the same population are superior in fitness to the corresponding homozygotes (WRIGHT and DOBZHANSKY 1946; DOBZHANSKY 1947 a, b). The chromosomal polymorphism is, accordingly, adaptive and it is balanced. Natural selection preserves all chromosomal types in a population at equilibrium frequency levels determined by their relative adaptive values. Intrapopulational heterosis is thus maintained.

Inversion heterozygotes which carry two third chromosomes with different gene arrangements derived from the population of Keen Camp on Mount San Jacinto (some 13 miles from Piñon Flats) or from the population of Mather (300 miles away from Piñon Flats) also possess, with one exception, higher fitness than do the homozygotes. Populations of these three localities consist mostly of the same chromosomal types. Experiments with population cages showed, however, that the adaptive values of the heterozygotes and homozygotes for the same inversions are not the same in the Piñon, Keen, and Mather populations (DOBZHANSKY 1948a, b). The structural differences between the chromosomes evidently do not account for the heterosis observed in the inversion heterozygotes. It is more likely that the heterosis is an outcome of interaction of polygene complexes carried in the chromosomes with different gene arrangements. These polygene complexes are fitted together, or "coadapted," by natural selection in the course of the evolutionary process. Heterosis is a result of natural selection (DOBZHANSKY 1949).

The process of mutual adjustment of gene contents may, however, have taken place only between chromosomes which occur in the same population. Chromosomes of different populations need not be coadapted, unless these

populations often hybridize. Natural selection may have produced different polygene complexes in different populations, coadapted within but not between the populations. If this hypothesis is correct, inversion heterozygotes which carry two chromosomes derived from different populations may not show as much heterosis as is observed within these populations themselves. The present article describes experiments devised to test this hypothesis. The hypothesis has withstood the tests.

MATERIAL AND TECHNIQUE

The strains used for the purposes of the present study were mostly the same as those used previously (DOBZHANSKY 1947a, 1948b). They were derived from wild flies collected in the Piñon Flats and Mather localities in California. In addition, 12 strains collected by PROFESSOR H. SPEITH at Santa Barbara, Chihuahua, Mexico, were employed. Since wild flies are usually heterozygous for gene arrangements, pair matings were made and their progenies examined cytologically to select strains homozygous for the Standard (ST), Arrowhead (AR), and Chiracahua (CH) gene arrangements in the third chromosome (see DOBZHANSKY 1948 for further comment on this procedure). In all, 15 ST, 12 AR, and 16 CH strains of Piñon Flats origin, 8 ST, 10 AR, and 16 CH of Mather origin, and 12 CH strains of Mexican origin were employed.

Population cages of the type described by WRIGHT and DOBZHANSKY (1946), but somewhat improved in construction, were used. All experimental cages were kept at 25° C, in incubators or in a constant temperature room, with no light except that given off by the heating bulbs in the incubators.

VIABILITY OF HYBRIDS BETWEEN STRAINS OF DIFFERENT GEOGRAPHIC ORIGIN

Flies from 15 strains of Piñon Flats origin, homozygous for CH, were crossed, in regular culture bottles, to flies from 10 strains of Mather origin, homozygous for AR chromosomes. In some bottles CH flies were used as females and AR as males, and in others the reciprocal cross was made. In either case, the progeny are AR/CH heterozygotes.

On February 3, 1947, 1,217 of such heterozygotes, about equal numbers from different crosses, were placed in population cage No. 45. Their offspring (the first generation developing in the cage) will start the development consisting of 25 percent of AR/AR homozygotes, 50 percent AR/CH heterozygotes, and 25 per-cent CH/CH homozygotes. The crowding and vigorous competition in the population cage causes, however, so great a mortality between the egg and the adult stages that only a fraction of the eggs deposited give rise to adult flies. If the viability of the homo- and heterozygotes is unequal, the mortality may be differential, and the ratios among the adult flies may be different from 1:2:1. The hypothesis of differential mortality was tested by examination of the constitution of the adult flies emerging in the cage in the first generation.

Early in March, several cups crowded with pupae and mature larvae were withdrawn from cage No. 45, and the young adult flies emerging in these cups were isolated, so that the females remained virgin. These flies were outcrossed

TABLE 1

Observed numbers and viability quotients of homozygotes and heterozygotes for chromosomes with ST, AR, and CH gene arrangements of Mather (M) and Piñon Flats (P) origin. The viability of heterozygotes is taken to be unity.

	CAGE NO. 45			CAGE NO. 46		
	AR^M/AR^M	AR^M/CH^P	CH^P/CH^P	ST^P/ST^P	ST^P/AR^M	AR^M/AR^M
Observed ♀♀	34	46	10	13	41	33
Viability	1.40	1	0.39	0.58	1	1.56
χ^2	3.70	—	8.57	3.57	—	5.73
Observed ♂♂	41	65	19	21	58	45
Viability	1.22	1	0.54	0.67	1	1.47
χ^2	1.19	—	7.03	3.16	—	6.47
Observed Total	75	111	29	34	99	78
Viability	1.28	1	0.47	0.63	1	1.51
χ^2	4.29	—	15.32	6.63	—	12.15
	CAGE NO. 47			CAGE NO. 48		
	ST^M/ST^M	ST^M/AR^P	AR^P/AR^P	ST^M/ST^M	ST^M/CH^P	CH^P/CH^P
Observed ♀♀	31	49	20	37	53	10
Viability	1.19	1	0.76	1.32	1	0.33
χ^2	0.93	—	1.42	2.74	—	11.75
Observed ♂♂	38	51	11	29	53	18
Viability	1.41	1	0.39	1.03	1	0.63
χ^2	4.34	—	9.60	0.01	—	3.14
Observed Total	69	100	31	66	106	28
Viability	1.31	1	0.57	1.18	1	0.48
χ^2	4.67	—	9.28	1.63	—	14.32
	CAGE NO. 49					
	ST^P/ST^P	ST^P/CH^M	CH^M/CH^M			
Observed ♀♀	14	19	2	—	—	—
Viability	1.40	1	0.17	—	—	—
χ^2	1.49	—	6.51	—	—	—
Observed ♂♂	59	81	22	—	—	—
Viability	1.38	1	0.49	—	—	—
χ^2	5.85	—	10.34	—	—	—
Observed Total	73	100	24	—	—	—
Viability	1.38	1	0.43	—	—	—
χ^2	7.38	—	16.05	—	—	—

in individual cultures to known AR/AR homozygotes; chromosomes were examined in salivary glands of six larvae from each culture. Now, if the parent of the culture was an AR/AR homozygote, all larvae will also be AR/AR. If the tested individual was CH/CH, all larvae will be CH/AR. Finally, if the tested parent was AR/CH, half of the progeny should be AR/AR and half AR/CH; with six larvae examined, the probability is 31:32 that the two chromosomal types will actually be seen, but one thirty-secondth of the heterozygotes will be misclassified as homozygotes. The results of the tests of 90 males and 125 females from cage No. 45 are shown in table 1.

If no differential mortality occurred in the cage, the AR/AR and CH/CH classes should have been equally frequent, and half as frequent as the AR/CH class. In reality, the heterozygotes AR/CH are less than twice as frequent as AR/AR, but more than twice as frequent as CH/CH (table 1). In the environment of the population cage, the viabilities of these three classes are AR/AR $>$AR/CH$>$CH/CH. The viability of AR/CH may be taken to be unity, and the viabilities of the other classes expressed in relation to that of the heterozygotes. Let the numbers of AR/AR, AR/CH, and CH/CH be denoted a, b, and c respectively. The expected numbers of AR/AR and CH/CH are, then b/2, and the viabilities (viability quotients) of these classes 2a/b and 2c/b respectively. Because of the misclassification of 1/32 of the homozygotes (see above), the computations are slightly more complex, namely:

$$\text{Viability of AR/AR} = \frac{(a - b/62)}{(32b/62)} = \frac{62a - b}{32b},$$

and

$$\text{Viability of CH/CH} = \frac{(c - b/62)}{(32b/62)} = \frac{62c - b}{32b}.$$

It is important to test the statistical significance of the observed deviations of the viability quotients from unity, i.e., from the viability of the heterozygotes. This can be done by computing chi-squares as follows:

$$\text{For AR/AR, } \chi^2 = \frac{(a - 33b/62)^2}{(33b/62)} = \frac{(62a - 33b)^2}{2{,}046b},$$

and

$$\text{For CH/CH, } \chi^2 = \frac{(c - 33b/62)^2}{(33b/62)} = \frac{(62c - 33b)^2}{2{,}046b}.$$

Each of these chi-squares has one degree of freedom; the two chi-squares are independent of each other, because either homozygote may be more or less viable in relation to the heterozygotes. The viability quotients and their chi-squares are entered in table 1. The CH^P/CH^P homozygotes are quite significantly inferior to the AR^M/CH^P heterozygotes but the AR^M/AR^M homozygotes are superior to the heterozygotes in viability.

Four further experiments were made in which the viability of heterozygotes which carried third chromosomes of Mather and of Piñon Flats origin with different gene arrangements was compared with the viabilities of the corresponding homozygotes. In all cases, flies from several strains homozygous for desired chromosomes were intercrossed in culture bottles; between one and two thousand F_1 hybrid flies were introduced into each population cage. Among the zygotes formed in the population cages in the next generation, one-half were necessarily heterozygous for the gene arrangements, and one-quarter belonged to either of two homozygous classes. The ideal 1:2:1 ratio was, however, disturbed among the adult flies developing in the cages because of the unequal viabilitles of the three types of offspring, and, consequently, because of unequal elimination. The empirical ratios were determined by testing, for chromosomal constitution, samples of adult flies which hatched from pupae formed in the population cages. This entailed the laborious but unavoidable crossing of the flies to be tested in individual cultures to chromosomally known flies, and examining the salivary gland chromosomes in six larvae in each progeny.

Population cage No. 46 contained ST chromosomes of Piñon Flats origin and AR chromosomes of Mather origin. The reciprocal combination, ST from Mather and AR from Piñon Flats, was made in cage No. 47. Cage No. 48 had ST chromosomes from Mather and CH from Piñon Flats, and cage No. 49 ST chromosomes from Piñon Flats and CH from Mather. The results are summarized in table 1, in which the observed numbers of females and males of different chromosomal constitutions are given. Taking the viability of the heterozygotes to be 1 in all cases, the viability quotients of the homozygotes are determined with the aid of the formulae given above. Chi-squares, testing the significance of the deviations between the observed viability quotients and unity, are also given.

In every experiment, one of the homozygotes is sharply inferior in viability to the heterozygote (quotients 0.17 to 0.76). In cages Nos. 45, 48, and 49, the poorly viable homozygote is CH/CH, in No. 48, it is ST/ST, and in No. 47, it is AR/AR. The inferiority is statistically quite significant for every total (chi-squares 6.63 to 16.05), but not always for both sexes. In four experiments out of five, the viability is lowered in females relatively more than it is in males. Every experiment shows also that one of the homozygotes is more viable than the heterozygote (quotients 1.03–1.56), although the superiorities are not always significant statistically and one gains the impression that the heterozygotes are closer in viability to the more viable than to the less viable homozygotes.

As stated in the introduction, Mather and Piñon Flats are about 300 miles distant from each other, and both are located in California. It seemed desirable to study the behavior in heterozygotes of chromosomes coming from geographically more remote places. Strains homozygous for CH gene arrangement from Mexico were, accordingly, crossed to ST and AR strains from Piñon Flats. F_1 hybrid flies were placed in cages Nos. 55 and 56, and the chromosomal constitution of the adult flies of the first generation developing in the cages

was examined as described for cages Nos. 45–49. The results are summarized in table 2.

Taken at face value, cage No. 55 behaved like the cages containing chromosomes from different localities in California, *i.e.*, the viability of the heterozygotes proved to be intermediate between the homozygotes (ST^P/ST^P

TABLE 2

Observed numbers and viability quotients of homozygotes and heterozygotes for chromosomes with AR, ST, and CH gene arrangements of Piñon Flats (P) and Mexican (M) origin. The viability of heterozygotes is taken to be unity.

	CAGE NO. 55			CAGE NO. 56		
	ST^P/ST^P	ST^P/CH^M	CH^M/CH^M	AR^P/AR^P	AR^P/CH^M	CH^M/CH^M
Observed ♀♀	47	48	25	29	29	17
Viability	1.87	1	0.98	1.84	1	1.10
χ^2	18.01	—	0.01	11.92	—	0.16
Observed ♂♂	34	74	32	55	75	47
Viability	0.86	1	0.81	1.39	1	1.18
χ^2	0.74	—	1.39	5.70	—	1.26
Observed Total	81	122	57	84	104	64
Viability	1.26	1	0.87	1.53	1	1.16
χ^2	3.97	—	0.97	14.83	—	1.35

$> ST^P/CH^M > CH^M/CH^M$). The differences are, however, mostly below the conventional level of statistical significance. But in cage No. 56, a very interesting result is obtained, *i.e.*, one of the homozygotes (AR^P/AR^P) is quite significantly more viable, while the other homozygote (CH^M/CH^M) has an equal or a slightly greater viability than the heterozygote.

STRUCTURAL AND GENE HYBRIDITY

In the experiments so far described, inversion heterozygotes with two third chromosomes of different geographic origin were compared to inversion homozygotes with two third chromosomes of the same origin. In all these experiments, the inversion heterozygotes failed to show the superior viability ("heterosis") which heterozygotes usually have within a geographic strain. Now, it is possible that the gene contents of the chromosomes of different geographic origin are different regardless of the gene arrangement. For example, ST and AR chromosomes of the Mather population may have certain genes in common which distinguish them from the chromosomes of the Piñon Flats population. If so, in the experiments so far described, we compared inversion heterozygotes, which were also heterozygous for geographical gene complexes, with inversion homozygotes which carried third chromosomes of similar geographic origin. Following a suggestion made in a conversation with PROFESSOR H. J. MULLER, two other experiments were arranged, in which the inversion

homozygotes carried third chromosomes of different geographic origin, while inversion heterozygotes carried chromosomes of similar geographic origin.

Strains with ST chromosomes of Piñon Flats origin were outcrossed, in culture bottles, to AR strains of Mather origin. The F_1 flies from this cross are, of course, ST^P/AR^M heterozygotes. In other bottles, AR strains of Piñon Flats origin were crossed to ST strains of Mather origin. In these bottles, the F_1 flies are ST^M/AR^P heterozygotes. Females ST^P/AR^M were then crossed to ST^M/AR^P males and the mixture placed in population cage No. 50. Population cage No. 51 received ST^M/AR^P females and ST^P/AR^M males. The first generation developing in either population cage will then contain, at the start, four genotypes in equal numbers, namely, 1 ST^P/ST^M:1 ST^P/AR^P:1 ST^M/AR^M:1 AR^M/AR^P. The inversion homozygotes carry genes of Piñon Flats and of Mather origin, while the heterozygotes have, in their third chromosomes, either Piñon Flats or Mather genes (since the inversions strongly suppress crossing over, the recombinations in these chromosomes may be disregarded, cf. DOBZHANSKY and EPLING 1948). The chromosomal constitution of the adult flies hatching in cages Nos. 50 and 51 was determined as usual. Table 3 summarizes the data.

TABLE 3

Observed numbers and viability quotients of homozygotes and heterozygotes for chromosomes with AR and ST gene arrangements of Piñon Flats (P) and Mather (M) *origin. The viability of the heterozygotes is taken to be unity.*

		ST^M/ST^P	ST^P/AR^P AND ST^M/AR^M	AR^M/AR^P
Cage No. 50	Observed ♀♀	32	64	17
	Viability	0.94	1	0.48
	χ^2	0.13	—	8.55
Cage No. 50	Observed ♂♂	30	57	18
	Viability	0.99	1	0.64
	χ^2	0.01	—	5.03
Cage No. 51	Observed ♀♀	29	58	8
	Viability	0.94	1	0.24
	χ^2	0.11	—	16.94
Cage No. 51	Observed ♂♂	23	67	20
	Viability	0.63	1	0.54
	χ^2	4.50	—	21.46
Total	Observed	114	246	63
	Viability	0.87	1	0.46
	χ^2	2.19	—	35.25

The results in cages Nos. 50 and 51 are clearly different from all previous experiments in which chromosomes of different geographic origin were involved. They are more nearly similar to those where the chromosomes came

from the same locality. The heterozygotes as a class are superior in viability to one of the homozygous classes (AR/AR), and, taking the data at face value, also superior to the other homozygotes (ST/ST), although the viability quotients do not significantly differ from unity in the latter case. Heterozygosis for ST and AR third chromosomes from the same population results in an increase in viability, even in the presence of a geographically mixed genetic background of chromosomes other than the third.

NATURAL SELECTION IN HYBRID POPULATIONS

Analysis of the first generation of flies developed in population cages has shown that heterozygotes which carry two third chromosomes of different geographic origin are inferior in viability to at least one of the homozygotes. Now, in a population in which the heterozygotes for a gene or a gene arrangement are superior to both homozygotes, natural selection leads to an equilibrium, at which all the gene alleles or variant gene arrangements continue to be present with certain predictable frequencies. This was observed in earlier experiments on competition of third chromosomes of *Drosophila pseudoobscura* in population cages (WRIGHT and DOBZHANSKY 1946; DOBZHANSKY 1948). If, however, the heterozygotes are equal or inferior in fitness to at least one of the homozygotes, no equilibrium should be reached, and the alleles or gene arrangements which form superior homozygotes should eventually crowd out and eliminate their competitors. The population cages No. 45–49 were, therefore, continued for 13–14 months. At intervals of one to two months, samples of eggs deposited in the cages were taken, larvae coming from these eggs allowed to develop at optimal conditions in culture bottles, and 150 of the larvae from each sample used for examination of the salivary gland chromosomes. A "sample" consisted of six "subsamples" of 25 larvae each, grown from eggs taken on six successive days (for details of this technique see WRIGHT and DOBZHANSKY 1946, pp. 131–132). The resulting data are presented in tables 4–6.

TABLE 4

Percentage frequencies of AR chromosomes of Mather origin (AR^M) and CH chromosomes of Piñon Flats origin (CH^P) in population cage No. 45. E=early; M=middle; L=late.

TIME	AR^M	CH^P	TIME	AR^M	CH^P
February 2, 1947	50.0	50.0	E. August, 1947	82.3	17.7
E. March, 1947	60.7	39.3	E. September, 1947	83.7	16.3
E. April, 1947	61.3	38.7	M. November, 1947	88.7	11.3
E. June, 1947	73.3	26.7	L. January, 1948	89.7	10.3
E. July, 1947	76.3	23.7	L. March, 1948	91.0	9.0

Cage No. 45 was started in February 1947 with equal numbers of AR chromosomes of Mather origin and CH chromosomes of Piñon Flats origin. Six months later, in early July, AR chromosomes had reached the frequency of 76 percent and CH declined to 24 percent. By March 1948, AR rose to 91 percent and CH declined to 9 percent respectively. These results are clearly different

TABLE 5

Percentage frequencies of ST and AR chromosomes of Mather (M) or of Piñon Flats (P) origin in population cages Nos. 46 and 47. E=early; M=middle; L=late.

TIME	CAGE NO. 46		CAGE NO. 47	
	ST^P	AR^M	ST^M	AR^P
February 2, 1947	50.0	50.0	—	—
E. March, 1947	39.6	60.4	50.0	50.0
E. April, 1947	62.0	38.0	59.5	40.5
M. June, 1947	66.0	34.0	73.7	26.3
M. July, 1947	68.3	31.7	78.3	21.7
M. August, 1947	74.3	25.7	79.3	20.7
M. November, 1947	72.0	28.0	76.8	23.3
L. January, 1948	75.3	24.7	75.0	25.0
L. March, 1948	79.0	21.0	81.0	19.0

from what is observed in competition of AR and CH chromosomes from the same population. In Piñon Flats populations, an equilibrium is established at about 79 percent of AR, and in Mather populations at 53 percent of AR (DOBZHANSKY 1948[1]). From the data in table 4 the following estimates of the adaptive values (W) and selection coefficients (s and t) of the chromosomal types in cage No. 45 may be derived (cf. WRIGHT and DOBZHANSKY 1946 for method):

Genotype	*W*	
AR_MAR^M	1.02	$s=-0.02$
AR_MCH^P	1.00	$t=0.43$
CH_PCH^P	0.57	

The adaptive value of AR^M/AR^M homozygotes is equal or slightly superior to that of the heterozygotes, and clearly superior to the CH^P/CH^P homozygotes. The fact that CH^P chromosomes have not been eliminated in cage No.

TABLE 6

Percentage frequencies of ST and CH chromosomes of Mather (M) and Piñon Flats (P) origin in population cages Nos. 48 and 49. E=early; M=middle; L=late.

TIME	CAGE NO. 48		CAGE NO. 49	
	ST^M	CH^P	ST^P	CH^M
March 13, 1947	50.0	50.0	50.0	50.0
M. April, 1947	59.5	40.5	62.4	37.6
L. June, 1947	76.3	23.7	79.3	20.7
L. July, 1947	80.7	19.3	82.3	17.7
L. August, 1947	82.0	18.0	85.7	14.3
L. November, 1947	88.0	12.0	90.7	9.3
E. February, 1948	90.3	9.7	92.0	8.0
L. March, 1948	92.0	8.0	93.0	7.0

[1] In this paper, the adaptive values, W, of the AR/AR and CH/CH types are incorrectly given as 0.81 and 0.60 respectively (p. 598); the correct figures are 0.48 for AR/AR and 0.40 for CH/CH.

45 is not surprising. With the adaptive values indicated the experiments must be continued for at least three years longer to have a reasonable chance of CH^P chromosomes becoming extinct.

Cages Nos. 48 and 49 were started on March 13th, 1947, with equal numbers of ST and CH chromosomes of Mather and Piñon Flats origin (table 5). As early as July of the same year, the frequency of ST rose to about 80 percent, and by March, 1948, to more than 90 percent. In pure Piñon Flats populations, the equilibrium of ST and CH chromosomes is established at about 74 percent ST, and in pure Mather populations at about 77 percent ST (DOBZHANSKY 1948). From the data in table 5 the following estimates can be computed:

	Genotype	W	
Cage No. 48	ST^M/ST^M	1.025	$s=-0.025$
	ST^M/CH^P	1.00	$t=0.36$
	CH^P/CH^P	0.64	
Cage No. 49	ST^P/ST^P	1.025	$s=-0.025$
	ST^P/CH^M	1.00	$t=0.61$
	CH^M/CH^M	0.39	

The ST/ST homozygotes are equal or slightly superior in adaptive value to the heterozygotes, while the CH/CH homozygotes are much inferior. At the end of the experiments, the CH chromosomes were rare in cages Nos. 48 and 49, but much longer observation would be needed to see them eliminated completely.

The adaptive values of the chromosomal types estimated from experiments Nos. 45, 48, and 49 may be compared with the estimates of the relative viability of the same chromosomal types. The relative viability has been computed on the basis of the deviations from the binomial square rule observed among the first generation adults in the same population cages (p. 290). The comparison shows that the adaptive values and the viability quotients are not identical. Although either set of estimates is subject to large experimental errors, it appears to be more than a coincidence that the heterozygotes are about equal in adaptive value to the fittest homozygotes, while the viability of the heterozygotes is definitely intermediate between the homozygotes. That such discrepancies are real and not caused by experimental errors is clearly attested by the outcome of experiments Nos. 46 and 47 (table 6). It may be recalled that the viability quotients of the chromosomal types in cage No. 46 were found to be $AR^M/AR^M > AR^M/ST^P > ST^P/ST^P$. On this basis, one might expect that the frequency of ST chromosomes in this cage would decline with time, and eventually AR chromosomes would be the only ones present. The experiment has given a quite different result. The frequency of ST gradually rose, until an equilibrium was approached in the vicinity of 80 percent ST[2]). In cage No. 47, a trend toward an equilibrium at

[2] The value 39.6 percent ST in cage No. 46 recorded for March, 1947, in table 6 is based on the analysis of the chromosomal constitution of the adult flies of the first generation hatching in this cage (see data in table 1). It is, accordingly, not quite comparable with the other values in table 6, which are based on examination of larvae hatching from the eggs deposited by the flies in this cage. The same is true for the first counts in cages Nos. 45–49 recorded in tables 4–6.

a level close to 80 percent ST also appears to be established. The adaptive values and selection coefficients deduced from data in table 5 are as follows:

	Genotype	*W*	
Cage No. 46	ST^P/ST^P	0.93	s=0.07
	ST^P/AR^M	1.00	t=0.40
	AR^M/AR^M	0.60	
Cage No. 47	ST^M/ST^M	0.88	s=0.12
	ST^M/AR^P	1.00	t=0.63
	AR^P/AR^P	0.37	

In both cages the adaptive values are ST/AR>ST/ST>AR/AR, in other words, the reverse of the viability relations in cage No. 46 (see above). What is the basis of this inconsistency, which is merely an extreme case of the relatively minor disagreements between the estimates of the adaptive values and the viability coefficients for cages Nos. 45, 48, and 49?

The answer is that the relative viability under competition is only one of the variables which determine the adaptive value of a genotype. Our estimates of the viability describe the survival rates of the carriers of different chromosomal types between the time when zygotes are formed and the hatching of the adult from pupae. These rates need not necessarily be, and evidently are not, either quantitatively or qualitatively consonant with the duration of life of the adult, fecundity, sexual activity, and other variables which influence the adaptive value. And it is the adaptive value which determines the selective fate of a genotype.

The discrepancy here observed is not unique. WALLACE (1948) found that larvae of *Drosophila pseudoobscura* heterozygous for the "sex-ratio" condition in the X-chromosome are superior in viability to homozygous normal larvae, and these are superior to homozygous "sex-ratio" larvae. Yet, the adult longevity is the same in heterozygotes and in normal homozygotes, while "sex-ratio" homozygotes are much inferior. The normal and "sex-ratio" homozygotes are equal to each other in fecundity but inferior to heterozygotes. Similar lack of concordance in different traits was found by HEUTS (1948) in homozygotes and heterozygotes for different gene arrangements in the third chromosome of the Piñon Flats populations of *D. pseudoobscura*. In *D. polymorpha* homozygotes and heterozygotes for a certain gene can be distinguished by the color of the abdomen. DA CUNHA (1949) found in natural populations of this species deviations from the ratios demanded by the binomial square rule in favor of homozygotes, while in artificial populations of the same species the deviations are in favor of the heterozygotes. Adaptive differences between variants of a species are often so complex that superiority in one trait often goes with inferiority in other traits. The adaptive value of a variant is the result of interaction of numerous variables.

DISCUSSION

Although hybrid vigor, or heterosis, has been studied for a long time, only in recent years has it become evident that these terms (which are usually

treated as synonymous) are common names for a group of scarcely related phenomena (DOBZHANSKY 1947b, 1949; CROW 1948). The situation in *Drosophila pseudoobscura* is instructive because in this species two different kinds of heterosis are reasonably well known and understood. The first kind arises from the presence in populations of deleterious recessive mutant genes sheltered by their normal dominant alleles. Accumulation of these deleterious genes is a by-product of the mutation process. The second kind of heterosis is due to complexes of linked polygenes which give specific "heterotic" interaction effects in heterozygotes ("overdominance," cf. HULL 1946). This kind of heterosis is engendered by natural selection as a form of adaptation of the species to its environment.

Mutations that arise in any species are usually deleterious to their carriers. Now, deleterious dominant and semi-dominant mutants are eliminated relatively rapidly by natural selection; recessive and near recessive mutants accumulate in populations of outbreeding species. A majority of individuals in natural populations of *D. pseudoobscura* carry one or more deleterious recessive mutants in heterozygous condition (DOBZHANSKY, HOLZ, and SPASSKY 1942). Inbreeding makes the deleterious recessives homozygous, which results in loss of fitness in the inbred line. Intercrossing of inbred lines results in restoration of the normal level of vigor. This is not because dominant gene alleles are intrinsically more favorable than recessive ones, but because dominant alleles present in a population are rigidly controlled by natural selection.

Since physical and biotic environments are constant neither in space nor in time, a living species meets a variety of environments. It responds to the challenge of variable environments by evolving genetically controlled adaptive polymorphism. One of the forms of adaptive polymorphism is balanced polymorphism. Suppose that a population contains alternative genetic variants A^1, A^2, and A^3, and that the heterozygotes A^1A^2, A^1A^3, and A^2A^3 are heterotic, i.e., have higher net fitness (adaptive value) than the homozygotes A^1A^1, A^2A^2, and A^3A^3. The variants A^1, A^2, and A^3 may be gene alleles, linked gene complexes, or chromosomal variants, such as gene arrangements modified by inversions or translocations. Natural selection will preserve all heterotic genetic variants, even if the adaptive values of the homozygotes are very low. The greater the number of balanced alternative variants, the greater the proportion in an outbred population of highly fit heterozygotes, and the lower the frequency of the less well adapted homozygotes. It is, of course, possible that the heterozygotes may be adapted for occupation of ecological niches which are widespread in natural habitats, and the homozygotes may occupy more restricted habitats. Natural selection favors attainment of a high level of adaptedness by the population as a whole, even at the expense of producing some inferior or narrowly specialized types. Balanced polymorphism enables an outbreeding species to attain high mean fitness, and at the same time to preserve great evolutionary plasticity. To be sure, balanced polymorphism is not incompatible with some inbreeding. By a kind of genetical *tour de force*, some species of Oenothera have evolved mechanisms which combine heterosis with virtually obligatory self-pollination. Nevertheless, inbreeding in a nor-

mally crossbred species usually leads to loss of fitness, and crossing of inbred strains, to restoration of normal reproductive biology and of the normal heterotic state.

In Drosophila, the variations in the gene arrangement in chromosomes of natural populations are often, and perhaps always, connected with the maintenance of heterosis. The adaptive superiority of inversion heterozygotes to homozygotes in *Drosophila pseudoobscura* has been established by DOBZHANSKY (1943, 1947 a, b, 1949), WRIGHT and DOBZHANSKY (1946), DOBZHANSKY and LEVENE (1948), and WALLACE (1948). DUBININ and TINIAKOV (1945, 1946 a, b) demonstrated the adaptive nature of the inversions in *D. funebris*, SPIESS (1950) in *D. persimilis*, CARSON and STALKER (1947) and LEVITAN (unpublished) in *D. robusta*. The only case of balanced polymorphism in Drosophila not known to be connected with chromosomal inversions is that studied by *da Cunha* (1949) in *D. polymorpha*, a species unfavorable for cytological study.

In *D. pseudoobscura*, inversion heterozygotes which carry two chromosomes derived from the same population usually show heterosis, while heterozygotes which carry chromosomes from geographically remote populations usually do not. Heterosis is here the outcome of interaction of polygene complexes borne in the chromosomes with different gene arrangements and protected by the inversions from disintegration by crossing over in the heterozygotes. The mutual adjustment, or coadaptation, of the polygene complexes which occur in the population of a geographical region is clearly an outcome of evolutionary development controlled by natural selection. Natural selection is responsible for the origin of heterosis as well as for its maintenance. CROW (1948) has presented arguments to show that heterosis in *Zea mays* is caused largely by interaction of genes, or gene complexes, which are deleterious when homozygous, *i.e.*, by the second of the two mechanisms described above for *D. pseudoobscura*.

Other types of "heterosis" have also been recorded in the literature. The term "heterosis" is sometimes applied to situations in which hybrids show "the increased size, the excessive kinetic energy, the increased productiveness," but no evidence of increased adaptive value compared to the parental forms (SHULL 1948). It has been shown above that the adaptive values of the chromosomal types of *D. pseudoobscura* are not necessarily parallel to the relative viabilities of these types between the egg and the adult stage. It is naive to assume, as is so often implied in discussions of heterosis, that a hybrid which is larger or more rapidly growing than its parents is also more "vigorous." Surely, there must be some optimal size of the body, of its parts, optimal fecundity, and optimal rates of growth and of metabolic processes for every species. Increases above the optima result, then, in losses rather than in gains of adaptive value.

Cases were already known in the pre-Mendelian period when artificially obtained hybrids between normally outbred species exhibited "luxuriance" in size or in growth rate. It is sometimes asserted that the mule is "heterotic" compared to its parents, the horse and the ass. Artificial hybrids between normally self-pollinating and sympatric strains of certain plant species have

also been called "heterotic" because of their larger size. It is doubtful, or at least uncertain, whether these enlarged hybrids would show adaptive values superior to their parents and prove more vigorous in competition with the latter in natural habitats. The advisability of applying the term "heterosis" to cases in which heterozygotes are larger in body size, or show "increases" in any "traits," but no evidence of higher adaptive value compared to the corresponding homozygotes, is open to question. Perhaps the word "luxuriance" would be a better designation for such cases, the word "heterosis" or "euheterosis" to be used for adaptive superiority of heterozygotes to homozygotes. Whichever way the terminological problem is decided, it is clear that the mechanisms underlying euheterosis and luxuriance are quite different. Euheterosis is a product of mutation and selection pressures on populations of outbreeding species. Luxuriance is a result of gene interactions which are not causally related to the reproductive biology of the species.

Euheterosis and luxuriance occur, of course, both in wild and in domesticated forms. In the latter, euheterosis is brought about by combined action of artificial and natural selection. Just as natural selection engenders and maintains gene complexes which confer adaptive superiority on heterozygotes in outbreeding wild species, in outbred cultivated species, artificial selection establishes gene complexes which lead the heterozygotes to possess qualities desired by man. Similarly, artificial selection eliminates the unfavorable dominants and semidominants more rapidly than the unfavorable recessives. Euheterosis is always a form of evolutionary adaptation—to the environment in wild species and to the human needs or desires in domesticated ones. Luxuriance is in either case an evolutionary accident.

SUMMARY

Inversion heterozygotes in *Drosophila pseudoobscura* which carry two chromosomes derived from the same population are, as a rule, superior in adaptive value to the homozygotes. In contrast to this, inversion heterozygotes which carry two chromosomes of different geographic origin usually show no heterosis. In one of the experiments, in which the chromosomes came from remote localities (California and Mexico), the heterozygotes were even inferior to both homozygotes. The heterosis is produced, in these cases, by interaction of polygene complexes, which have become mutually adjusted, or coadapted, by natural selection in the course of the evolutionary process. Polygene complexes which occur in geographically distant localities have not undergone the process of coadaptation.

The adaptive value (net fitness) of a type is not necessarily proportional to its survival value at all developmental stages. In our experiments, some types which showed relatively higher mortalities than other types between the egg and the adult stage proved nevertheless to be adaptively superior to the latter. The adaptive values are determined, in our experiments, by observing the changes in the relative frequencies of the two types with time in populations breeding in population cages.

The causation of different forms of heterosis is discussed. Heterosis proper, or *euheterosis*, is distinguished from *luxuriance* of heterozygotes. The former is

a product of mutation and selection pressures; the latter is an evolutionary accident.

ACKNOWLEDGMENT

The writer wishes to acknowledge his obligation to DOCTOR HOWARD LEVENE for his advice regarding the statistical treatment of the data and, in particular, for the formulae used in calculation of the survival values. The selection coefficients and the adaptive values were calculated under the direction of PROFESSOR SEWALL WRIGHT. MISS CHARLOTTE MADISON, MRS. E. SCHEREMETJEVA-BRUNST, and MESSRS. M. FOSTER, B. SPASSKY, and S. ZIMMERING assisted in conducting the experiments, making the cytological preparations, and carrying out most of the calculations. All the classifications of the gene arrangements were, however, made by the writer. The ROCKEFELLER FOUNDATION and the CARNEGIE INSTITUTION OF WASHINGTON provided financial support for the work.

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