EXPERIMENTAL PROOF OF BALANCED GENETIC LOADS IN DROSOPHILA[1]

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GENETIC loads, consisting mainly of quasi-recessive deleterious genes or gene complexes, scattered throughout the karyotype but concentrated chiefly in the autosomes, have been found in all species of Drosophila studied in this regard. The influence of these genetic loads on the fitness of their heterozygous carriers is still insufficiently understood. The recessivity may or may not be complete. The heterozygous carriers of some of the components of the genetic load are inferior in fitness to the noncarriers, though much superior to the homozygotes. These, as well as the completely dominant and completely recessive components, are parts of the mutational genetic load, maintained in the population chiefly by recurrent mutation. On the other hand, some parts of the genetic load may be overdominant, and may produce heterosis, increased fitness or adaptive value, in their heterozygous carriers. These are components of the balanced genetic load, controlled chiefly by natural selection. To discriminate between the mutational and the balanced fractions of the genetic load has been a difficult task indeed.

MULLER (1950 and later work) believes the mutational load to be by far the most important, and cites the hypomorphic nature of most mutant genes as an argument against common occurrence of superior fitness in heterozygotes. However in sickle cell anemia, the best substantiated case of balanced polymorphism in man, the heterozygotes are intermediate between the homozygotes in the composition of their hemoglobin, and probably in resistance to malaria; they excel only in a single trait—fitness in certain environments. The experiments of DOBZHANSKY and WRIGHT (1941), STERN, CARSON, KINST, NOVITSKI and UPHOFF (1952), CORDEIRO (1952), PROUT (1952), WALLACE and KING (1952), CORDEIRO and DOBZHANSKY (1954), WALLACE (1960) and others showed that some of the genes or gene complexes which are lethal in double dose are deleterious but others are heterotic in heterozygotes. GREENBERG and CROW (1960) suggested that the degree of dominance of deleterious genes in heterozygotes may be an inverse function of the loss of fitness in homozygotes and, hence, that all components of the genetic load may be about uniformly deleterious when heterozygous. In the experiments of DOBZHANSKY, KRIMBAS and KRIMBAS (1960, Table 3), lethals showed no evidence of reducing the viability of heterozygotes; FALK

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(1961) took this to be evidence in favor of the greater dominance of subvital mutations.

The possibility of using the same data to support diametrically opposed conclusions makes a reconsideration of the situation imperative. The present study describes sources of error which mimic the effects of partial dominance. These sources of error have not been taken into account in previous studies, although once recognized they are easily removed from the data. The analysis outlined in the pages that follow shows that in the maintenance of fitness of Drosophila populations balanced polymorphism, heterozygote superiority, plays an important role.

Experimental Techniques

The data here analyzed are those obtained from experimental populations of *Drosophila melanogaster* and from natural populations of *Drosophila pseudoobscura*. Earlier publications describing these populations are WALLACE 1956, SPASSKY, *et al.* 1960, and DOBZHANSKY, *et al.* 1960. The experimental technique involved crossing flies from the populations to be analyzed to laboratory flies which have the chromosomes of a certain pair marked by convenient dominant mutant genes and provided with crossover suppressors. The constitution of the laboratory flies may be represented as D_1/D_2, wherein D_1 and D_2 are mutants easily visible in heterozygotes but lethal in homozygotes. Single sons from the progeny, $D_1/+$, are backcrossed to D_1/D_2 females from the stock, $D_1/+$ and $D_2/+$ females and males are then selected and inbred, so that in the following generation the zygotes are formed in the ratio:

$$1D_1/D_2 : 1D_1/+ : 1D_2/+ : 1+/+$$

The $+/+$ class may either carry in duplicate the same chromosome derived from the wild ancestor (wild-type homozygote), or two chromosomes derived from different ancestors (wild-type heterozygote); the $D_1/+$ and $D_2/+$ classes carry a wild chromosome and a laboratory chromosome with a mutant marker (mutant heterozygote); the D_1/D_2 class carries no wild chromosomes of a given pair at all, and may thus serve as a standard of comparison. The mutants D_1 and D_2 were, of course, different in different experiments, depending upon the species and the chromosome used (*CyL* and *Pm* in the second chromosome of *D. melanogaster*, *Ba* and Δ, Delta, in the second, and *BlSc* and *L* in the third chromosome of *D. pseudoobscura*). For more details, see the original articles referred to above.

The standard experimental procedure has been to classify the chromosomes derived from natural or experimental populations according to the viability of the $+/+$ homozygotes; with sufficient numbers of flies raised this can be done with a fair degree of precision. It is most convenient to measure this viability as the ratio of the numbers of the $+/+$ and D_1/D_2 flies obtained in the cultures (see HALDANE 1956). This ratio varies from zero, for chromosomes which are lethal when homozygous, to values higher than one. The different values reflect, in the main, the genetically conditioned differences in viability effects of tested chromosomes, although sampling errors and variations in experimental conditions make their contributions to the observed array of values. It would seem a simple matter

to test for the possible effects of wild chromosomes in mutant heterozygotes by computing $D_1/+:D_1/D_2$ and $D_2/+:D_1/D_2$ ratios in different cultures. In actual fact the situation is complicated for a number of reasons. For example, the viabilities of wild homozygotes and mutant heterozygotes within individual cultures are correlated since each is based on the number of D_1/D_2 flies in that culture (see LEVENE in DOBZHANSKY, KRIMBAS and KRIMBAS 1960). Still other biases are discussed below together with adjustments needed for correction.

Effects of environmental variations: In our experiments the viabilities of various classes of flies are measured through observations on ratios of flies with and without certain mutant markers. Now, if data for an experiment have been gathered at two or more separate times environmental heterogeneities may produce spurious correlations between the viabilities of certain classes. An example of this effect can be cited in experiments carried out at the Biological Laboratory, Cold Spring Harbor. Although results obtained over a period of several years were highly repeatable, it could nevertheless be shown that the frequencies of wild-type flies in cultures underwent seasonal as well as occasional sporadic fluctuations. These fluctuations may have been caused by changes in the moisture content of the food. For example, flies with curly wings (*Cy*) may be trapped relatively oftener in moist than in dry food. The percentage of wild-type flies observed in counts would necessarily decrease or increase slightly as the frequency of trapped curly winged flies goes up or down.

The essential feature of such experimental errors is that they affect wild-type flies—homozygotes and heterozygotes—similarly not because of genotype, but because of phenotype. If this type of error is not recognized (and Curly need not be the only mutation that gives rise to the problem), it introduces a bias into one's results. Thus, tests which yield higher than average frequencies of wild-type flies will yield higher than average frequencies of homozygotes as well as higher than average frequencies of heterozygotes. Conversely, tests performed under conditions which yield fewer than average wild-type flies will yield both fewer homozygotes and fewer heterozygotes. If in analyzing the data this situation is not recognized, the resultant correlation between viability effects of chromosomes in homozygous and heterozygous condition will be erroneously interpreted as manifestation of partial dominance.

To illustrate that this is a real problem, some data obtained from six laboratory populations of *D. melanogaster* studied at Cold Spring Harbor by three workers are presented in Table 1. The data of this table concern chromosomes obtained from population in the period from sample 138 to 183. Consecutively numbered samples were taken from these populations at two week intervals; consequently, this period represents about a year and a half. Populations 5, 6, and 7 were nearly five years old at the start of this interval; populations 17, 18, and 19 were slightly less than one year in age.

For each population and for each worker an overall average viability of quasi-normal homozygotes and of heterozygotes has been computed (Table 1). From the averages of quasi-normal homozygotes and of heterozygotes calculated for individual samples, each sample has been assigned to a "quadrant" (Table 2).

TABLE 1

Average frequency ratios in three experimental populations of Drosophila melanogaster *obtained by three workers I, III, and IV*

Population no.	Homozygotes I	Homozygotes III	Homozygotes IV	Heterozygotes I	Heterozygotes III	Heterozygotes IV
5	0.89	0.98	0.92	1.04	1.11	1.03
6	0.86	0.84	0.84	1.15	1.13	1.10
7	0.91	0.95	0.88	1.05	1.10	1.02
17	0.93	0.94	0.91	1.07	1.10	1.03
18	0.94	0.98	0.93	1.07	1.10	1.06
19	0.86	0.93	0.89	1.08	1.15	1.09

TABLE 2

Distribution of individual samples relative to the mean viabilities shown in Table 1 (Further explanation in text)

Population no.	Worker	Quadrants 1	Quadrants 2	Quadrants 3	Quadrants 4	Total samples
5	I	1	1	2	1	5
5	III	2	2	4	3	11
5	IV	2	4	2	3	11
6	I	2	2	1	0	5
6	III	2	2	3	1	8
6	IV	2	4	1	4	11
7	I	3	1	2	0	6
7	III	3	2	6	1	12
7	IV	2	5	2	3	12
17	I	2	1	3	0	6
17	III	4	0	7	1	12
17	IV	1	5	3	3	12
18	I	3	0	3	1	7
18	III	3	2	6	2	13
18	IV	4	2	3	4	13
19	I	2	2	0	2	6
19	III	5	1	5	1	12
19	IV	4	3	2	3	12
Totals		47	39	55	33	174
Workers I and III only		32	16	42	13	103

Quadrant 1 includes samples in which both the homozygote and the heterozygote averages of the sample exceed their corresponding overall averages; quadrant 2, average of homozygotes of the sample exceeds the overall homozygote average but the average of heterozygotes is smaller than its grand average; quadrant 3, both the homozygote and heterozygote averages of the sample are smaller than the corresponding grand averages; quadrant 4, average of the homozygotes of the sample is smaller than the overall average for homozygotes, but the average of heterozygotes is larger. The bottom line of Table 2 indicates clearly that most samples fall in quadrants 1 and 3; in the case of two workers the pattern is

especially clear. Thus, the viabilities of homozygotes and heterozygotes do rise and fall together from sample to sample.

One might suppose that within populations the gradual replacement of old alleles by new ones with exceptionally beneficial effects on the viability would lead to the results of the kind described above. The experimental conditions of these populations exclude this possibility. Populations 5, 6, and 7 were old, chronically irradiated populations, long since at equilibrium frequencies for lethals and other deleterious gene mutations; populations 17, 18, and 19 were populations derived from populations 5 and 6 some nine to ten months before the year and a half under discussion. Even for the latter populations the general trend of average viabilities of all wild-type flies was downward rather than the reverse. It is extremely unlikely that any phenomenon other than secular changes in environmental conditions—some cyclic, others sporadic—need be invoked to explain the data shown in Tables 1 and 2.

RESULTS

Preliminary analysis of the effect of wild chromosomes on the viability of mutant heterozygotes: The samples taken from the experimental laboratory populations of *D. melanogaster* and analyzed with the aid of the *Cy L-Pm* method contain in the aggregate 4,261 second chromosomes tested for viability in homozygous and in heterozygous combinations. Since each wild-type chromosome was tested in two heterozygous combinations (*Cy L*/+ and *Pm*/+), the total data available for analysis consists of 8,522 heterozygous combinations.

These tests were made over a period of several years, hence it is necessary to remove the possibility that temporal environmental fluctuations of the sort revealed in Tables 1 and 2 may have affected the results. Fortunately, this possibility can be removed. The basic "unit" of work in these experiments consisted of the tests made by one worker in one sample period on chromosomes from a single population. The number of chromosomes in each such unit has varied somewhat but is generally very nearly 25; these are tested as 25 homozygotes and 25 wild-type heterozygotes where the latter are constructed as $+^1/+^2$, $+^2/+^3 \ldots +^{24}/+^{25}$, and $+^{25}/+^1$. It happens that each unit contained one or more lethal chromosomes; thus, we have at our disposal a means for removing the bias resulting from uncontrolled environmental fluctuations.

Within what we have called a "unit" of work, the chromosomes are classified into ten categories according to the viabilities of their homozygous carriers (0–0.20, 0.21–0.40, 0.41–0.60, . . . 1.61–1.80, 1.81+). Next, the average viabilities of *CyL*/+ and *Pm*/+ heterozygotes carrying wild-type chromosomes of these various categories, but developing in cultures yielding wild-type heterozygotes, are computed. Following this (and still within the unit), the average viability of the *CyL* heterozygotes carrying wild-type chromosomes of the 0–0.20 category is taken to be 1.000, and the average viabilities of *CyL* heterozygotes carrying the wild-type chromosomes of all other categories are adjusted accordingly. Similarly in the case of the *Pm* heterozygotes; those heterozygous for lethal and near lethal wild-type chromosomes (0–0.20) are assigned a viability of 1.000

and the average viabilities of the others are adjusted proportionately. These adjustments are made within units; the final viabilities computed are the averages of all of these adjusted values. By this maneuver we eliminate seasonal fluctuations, sporadic influences, as well as systematic differences between different workers. Furthermore, we reduce the viabilities of *CyL*/+ and *Pm*/+ flies, which otherwise differ considerably, to a common viability scale.

A summary of the data is given in Table 3. Three aspects of the table are worth noting. First, the numbers of tests representing most categories of wild-type chromosomes are substantial ones. Second, starting at the bottom of the viability scale (0–0.20), it can be seen that the chromosomes of the first six categories give almost identical average viabilities in heterozygous combinations. Third, the viability (1.042) of the category 1.21–1.40 is significantly higher than 1.000, and the average of that category together with all subsequent ones is also significantly greater than 1.000.

Thus, we see no evidence for partially dominant effects of chromosomes with pronounced deleterious effects on the viability of homozygous individuals. We do, however, as did DOBZHANSKY, KRIMBAS and KRIMBAS (1960), detect an apparent tendency for chromosomes giving homozygotes of the highest viabilities to give also heterozygotes with superior viabilities (our Table 3 is analogous to Table 3, page 749, in DOBZHANSKY, KRIMBAS and KRIMBAS 1960). It is worth emphasizing that the heterozygous combinations we are discussing here are those of wild-type chromosomes from populations and genetically marked chromosomes of laboratory origin. Wild-type heterozygotes formed by random combinations of chromosomes from the populations present a separate problem; WALLACE (1960, 1962) has given reasons for believing that in the latter combinations lethals and semilethals may slightly increase the viability of heterozygotes.

Genetic load and balanced heterosis: The analysis described above represents

TABLE 3

Effects of various wild-type second chromosomes of D. melanogaster *on the viability of mutant* (CyL *and* Pm) *heterozygotes*

Homozygotes	Populations 5	6	7	17	18	19	Average	Tests
0 –0.20	1	1	1	1	1	1	1	4762
0.21–0.40	1.037	0.986	0.985	1.057	0.947	1.003	1.000	281
0.41–0.60	0.990	1.075	0.981	0.990	1.016	0.965	1.000	319
0.61–0.80	0.968	1.050	0.971	1.011	0.998	0.978	0.993	827
0.81–1.00	1.040	0.982	0.990	1.005	1.017	0.987	1.004	1315
1.01–1.20	1.018	1.017	1.010	1.054	1.008	0.975	1.013	686
1.21–1.40	1.144	1.144	1.017	1.021	1.042	1.023	1.042	235
1.41–1.60	0.980	....	0.920	0.999	1.016	0.867	0.982	60
1.61–1.80	1.194	0.924	0.955	....	1.003	1.396	1.032	25
1.81+	0.928	....	....	....	1.150	....	1.106	12

The leftmost column shows the viability effects of the chromosomes in homozygotes; the following six columns report the viabilities of *Pm*/+ and *CyL*/+ heterozygotes in six experimental populations, recomputed as explained in the text so that the heterozygotes carrying lethal or near-lethal wild chromosomes (the class 0–0.20) are given the viability of 1.000; the second column from the right gives the grand averages for all the populations; the last column indicates the numbers of tests on which these averages are based.

an improvement over most earlier studies on the effects of deleterious genetic variant in heterozygous condition. Nevertheless, the data here obtained for *D. melanogaster*, and those of DOBZHANSKY, KRIMBAS and KRIMBAS (1960) for *D. pseudoobscura*, disclose a peculiar situation. Although the chromosomes which are lethal, semilethal, and subvital in homozygous condition give uniformly viable heterozygotes, the rare chromosomes which are supervital when homozygous give the highest viabilities in heterozygotes. But if so, why are such supervitals rare in populations? Why has natural selection not made them prevalent? DOBZHANSKY, KRIMBAS and KRIMBAS surmised that the ostensible high viability of the heterozygotes for "supervitals" is a result of an interaction of these wild chromosomes with laboratory chromosomes carrying mutant markers. This suggestion is not satisfactory, since such special interactions are unlikely to occur in two different species and involve a variety of laboratory chromosomes.

The difficulty lies in that, despite appearances to the contrary, the scale of viabilities used for chromosomal homozygotes is not strictly comparable with that applied to heterozygotes. In a test of over 4,000 cultures some estimates of homozygous viabilities are bound to be exceptionally high as the result of chance deviations, or because of rarely-encountered favorable culture conditions. If a number of cultures yielding exceptionally high viabilities of homozygotes are picked out, as for example those having yielded ratios between 1.61 and 1.80 in Table 3, and if we then compute the viability of heterozygotes carrying these same chromosomes, the latter will almost certainly be lower than the former. The average computed for the heterozygotes will regress toward some mean; that of the classified homozygotes, on the contrary, will not regress. It has been chosen because it represents a particular—and, in the example given, an exceptionally high—viability. The two viabilities are not comparable. However, if replicate cultures of homozygotes were available, an average viability for homozygotes comparable to that of the heterozygotes could be computed from these additional cultures. For those chromosomes that fell between 1.61–1.80 in our Table 3, the average viability calculated for independently replicated cultures would be directly comparable to the average calculated for heterozygotes; the former would regress toward the mean of homozygotes of the sort which tend to fall within the selected interval just as the latter would regress toward the mean of heterozygotes carrying these particular chromosomes.

Unfortunately, among the tests involving *D. melanogaster* replicate cultures are available for only one sample of each of three populations (population 17–sample 158; 18–159; 7–160). The results of an analysis of these three samples are listed in Table 4. The number of cultures available in these samples was not sufficient to permit distinguishing many classes of the viabilities; chromosomes have been classified into only nine categories (0, 0.01–0, 10, 0.11–0.50, 0.51–0.70, 0.71–0.90, 0.91–1.10, 1.11–1.30, 1.31–1.50, 1.51+) according to their effect on the viability when homozygous. We call these the "ordered" homozygotes, to indicate that we have ordered them according to their observed viabilities. The second column of Table 4 gives the average viabilities observed in duplicates of those cultures which were classified into the nine categories. An examination of

TABLE 4

Effects of various wild-type second chromosomes of D. melanogaster *on the viability of homozygous and heterozygous individuals*

Ordered homozygotes	Replicate homozy-gotes	n	Mutant heterozy-gotes	n	Adjusted homozy-gotes	Adjusted heterozy-gotes
0	0.01	131	0.97	517	.01	1.01
0.01–0.10	0.01	32	0.94	129	.01	.98
0.11–0.50	0.26	25	0.99	98	.27	1.03
0.51–0.70	0.59	37	0.99	149	.61	1.03
0.71–0.90	0.73	87	0.95	352	.76	.99
0.91–1.10	0.85	59	1.00	236	.89	1.04
1.11–1.30	0.95	33	1.02	132	.99	1.06
1.31–1.50	0.94	6	1.03	26	.98	1.07
1.51+	0.96	2	1.23	10	1.00	1.28

The viabilities of the homozygotes in certain "ordered" categories are compared with the average viabilities of the homozygotes observed in replicate cultures, and with the average viabilities of the mutant (*CyL* and *Pm*) heterozygotes carrying the same chromosomes. n = the number of entries upon which each average is based.

this column reveals why a new type of analysis is necessary! Within categories involving chromosomes of extremely low viability, ordered and duplicate cultures yield nearly identical average viabilities; in categories involving higher average viabilities those calculated for the duplicate cultures increase more slowly than the ordered homozygotes. Finally the averages for duplicate cultures become virtually constant, despite the continued increase in the classification of the ordered homozygotes. The relation between ordered and duplicate cultures is shown in Figure 2. (The standard of viability, 1.000, used in columns 1 and 2 of Table 4 and in Figure 2 is that of *CyL/Pm* class; there were too few cultures in these tests to permit the use of our improved calculation.)

The average viability of heterozygotes carrying chromosomes classified as having various effects on the viability are listed in the fourth column of Table 4. It can be seen that the viabilities of the heterozygotes, though smaller than the highest categories of ordered homozygotes, are *not* smaller than the viabilities calculated for duplicate cultures. This fact has been emphasized in the last two columns of Table 4 by setting the maximum viability of replicate homozygotes equal to 1.00 and by adjusting all other homozygote and mutant heterozygote viabilities correspondingly. However, there is evidence that heterozygotes carrying chromosomes of the highest ordered categories have the highest average viabilities; this agrees with the results shown in Table 3 and with those of DOBZHANSKY, KRIMBAS and KRIMBAS. The relation between the viabilities of heterozygotes and homozygotes of duplicate cultures—a relation based on truly equivalent viability scales—is shown in Figure 3.

The striking feature of Figure 3 is the abrupt break in the curve which occurs just as the viabilities of the homozygotes and heterozygotes are approaching equality. This break has been emphasized in the figure by including data from Table 3 for comparison with the curve presented in Figure 1. Unfortunately, the viability of duplicate homozygotes must be taken from Table 4 since these are the

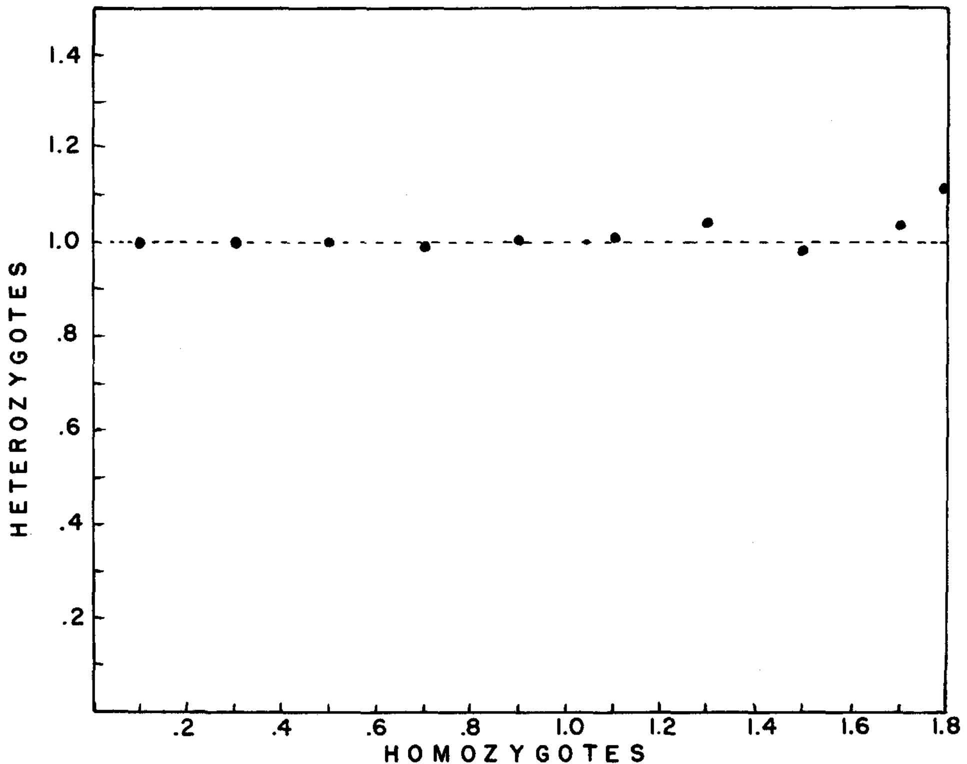

FIGURE 1.—Graph showing the relation between viability effects of various chromosomes in homozygous and heterozygous condition (based on columns 1 and 8 of Table 3).

only duplicate cultures available. In this curve we see that the first six points of Table 3, the six which had virtually identical heterozygote viabilities, are the points which form the horizontal portion of the curve in Figure 3; the other points lie on the ascending portion of the curve. This abrupt change contrasts sharply with the situation depicted in Figure 1. (To include the data from Table 3 in Figure 3 it was necessary to reconvert the viabilities of heterozygotes to a scale based on *CyL/Pm* as a standard of 1.000 and then to make the additional adjustment given in Table 4.)

The indication that the viabilities of heterozygotes not only surpass those of the homozygotes in every category, but, more remarkably, appear to diverge from the latter at the upper range of the viability of homozygotes is extremely suggestive. To verify the results obtained with the relatively limited material on *D. melanogaster*, we have reanalyzed the more extensive data of DOBZHANSKY, KRIMBAS and KRIMBAS (1960) on *D. pseudoobscura*. These workers observed the proportions of four classes of flies, D_1/D_2, $D_1/+$, $D_2/+$, and $+/+$, in nearly 1000 tests of second and third chromosomes, each test having been made with three replications. Any one of the three replicate cultures may be regarded as containing the "ordered" homozygote, and the two replicates furnish the "replicate"

homozygotes; these tests thus yield almost 5300 observations. A summary of the data is shown in Table 5 and Figure 2.

TABLE 5

Effects of various wild-type second and third chromosomes of D. pseudoobscura *on the viability of homozygotes*

"Ordered" homozygotes	Second chromosomes Replicate homozygotes	n	Third chromosomes Replicate homozygotes	n	Combined Replicate homozygotes	n
0	0.012	313	0.024	307	0.018	620
0.01–0.10	0.059	122	0.124	107	0.089	229
0.11–0.20	0.222	33	0.209	33	0.216	66
0.21–0.50	0.541	183	0.568	240	0.556	423
0.51–0.80	0.897	432	0.887	524	0.891	956
0.81–1.00	0.998	526	0.994	632	0.995	1158
1.01–1.20	0.997	359	1.069	468	1.038	827
1.21–1.40	1.079	231	1.095	299	1.088	530
1.41–1.60	1.130	94	1.150	138	1.142	232
1.61–1.80	1.101	34	1.210	88	1.180	122
1.81+	1.151	53	1.199	79	1.180	132

The viabilities of the homozygotes in certain "ordered" categories are compared with the viabilities of the homozygotes observed in replicate cultures. n = number of observations.

The situation in *D. pseudoobscura* revealed in Table 5 is analogous to that shown in Table 4 for *D. melanogaster*. Chromosomes which act as homozygous lethals in one culture do the same in replicate cultures. Semilethal and subvital chromosomes behave similarly, or regress slightly towards the mean in replicate cultures; such regression becomes very striking for the ostensibly supervital chromosomes. The behavior of the second and the third chromosomes is alike.

The following steps in the analysis of the data for *D. pseudoobscura* are quite analogous to those for *D. melanogaster*. The viabilities of the "replicate" homozygotes shown in Table 5 are expressed in fractions of that of the most viable class (1.151 for the second chromosome, 1.210 for the third chromosome, and 1.180 for the combined data). The resulting figures are shown in Table 6 in the columns "Adjusted homozygotes." The columns "Mutant heterozygotes" in Table 6 give the average viabilities of the heterozygotes carrying the wild chromosomes of different kinds and the mutant markers, *Ba* or Delta for the second, *BlSc* or *L* for the third chromosomes. These figures, and their combined average, are obtained by averaging the viabilities of the mutant heterozygotes observed in the cultures which are the replicates of those of the homozygotes in the "ordered" categories. They are based on numbers of observations twice those shown in Table 5. This is so because each replicate culture contains only one homozygous class (+/+), but two heterozygous classes (D_1/+ and D_2+).

We now adjust the observed viabilities of the mutant heterozygotes to make them comparable to the adjusted homozygotes. The crude figures for the mutant heterozygotes in Table 6 are divided by 1.151 for the second, by 1.210 for the third chromosome, and by 1.180 for the combined data (cf. Table 5). The rela-

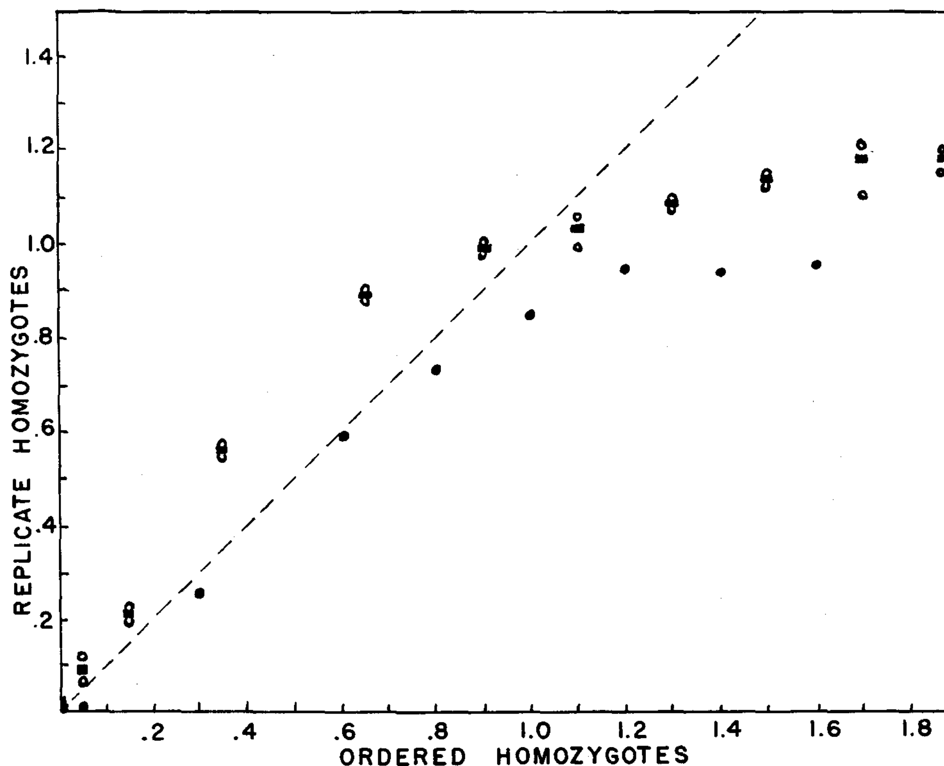

FIGURE 2.—Graph showing the relation between viability effects of chromosomes in homozygotes classified according to observed viability (ordered homozygotes) and in homozygotes of replicate cultures. Solid circles based on columns 1 and 2 of Table 4; solid squares based on columns 1 and 6 of Table 5; open circles represent second and third chromosomes of *D. pseudoobscura* treated separately.

tive viabilities of the heterozygotes so obtained are given in Table 6 in the columns "Adjusted heterozygotes," and are shown in Figure 4. The curves depicting the relations between the effects of different chromosomes on the viabilities of homozygotes and heterozygotes are essentially horizontal, until the viabilities of the two genotypes approach equality; before reaching the point of equality, however, the curve for heterozygotes turns upward abruptly. The viabilities of mutant heterozygotes for *D. pseudoobscura* nearly equal the maximum observed for homozygotes; those heterozygotes which carry ordered chromosomes of highest viability have viabilities greater than the maximum for homozygotes. We should point out once more that the pattern seen in these studies is that observed in "artificial" heterozygotes, those carrying a laboratory chromosome with a mutant marker, and a wild-type chromosome of different origin. We might add that it would have been possible in the studies on both *D. melanogaster* and *D. pseudoobscura* to remove the viability effects of the dominant marker genes themselves. This would have required one more transformation of viabili-

TABLE 6

Relation between the effects of various wild-type second and third chromosomes of D. pseudoobscura *on the viabilities of the homozygotes and of mutant-carrying heterozygotes*
Further explanation in text

"Ordered" homozygotes	Second chromosomes Adjusted homozygotes	Second chromosomes Mutant heterozygotes	Second chromosomes Adjusted heterozygotes	Third chromosomes Adjusted homozygotes	Third chromosomes Mutant heterozygotes	Third chromosomes Adjusted heterozygotes	Combined Adjusted homozygotes	Combined Mutant heterozygotes	Combined Adjusted heterozygotes
0	0.010	1.127	0.979	0.019	1.125	0.930	0.015	1.126	0.954
0.01–0.10	0.051	1.046	0.909	0.102	1.119	0.925	0.075	1.080	0.915
0.11–0.20	0.193	1.051	0.913	0.173	1.129	0.933	0.183	1.090	0.924
0.21–0.50	0.470	1.132	0.983	0.469	1.099	0.908	0.471	1.113	0.943
0.51–0.80	0.779	1.120	0.973	0.733	1.089	0.900	0.755	1.103	0.935
0.81–1.00	0.867	1.135	0.986	0.821	1.113	0.920	0.843	1.123	0.952
1.01–1.20	0.866	1.093	0.950	0.833	1.142	0.944	0.880	1.121	0.950
1.21–1.40	0.937	1.150	0.999	0.905	1.166	0.964	0.922	1.159	0.982
1.41–1.60	0.982	1.211	1.052	0.950	1.227	1.014	0.968	1.220	1.034
1.61–1.80	0.956	1.294	1.124	1.000	1.352	1.117	1.000	1.336	1.132
1.81+	1.000	1.219	1.059	0.991	1.306	1.079	1.000	1.271	1.077

ties; since it would have increased the viability of heterozygotes by only 3–5 percent, such an additional transformation did not seem necessary.

DISCUSSION

Genetic loads in populations of sexual outbreeding species have several components, among which the mutational and the balanced are the principal ones. Mutational loads consist of deleterious dominant, semidominant, and recessive genes, the presence of which in populations is the result of recurrent mutation. Some genes and gene complexes exhibit however a heterotic "overdominance," making the heterozygotes superior in fitness to both corresponding homozygotes. Such heterotic genes and gene complexes are maintained in populations by natural selection in a state of balanced polymorphism. The continuous production of the relatively unfit homozygotes in these populations gives rise to balanced genetic loads.

The relative magnitudes of the mutational and balanced loads are in doubt and in need of further study. Many plant and animal breeders find little evidence of heterotic overdominance in their materials (MATHER 1955); additive effects of favorable dominants and unfavorable recessives seem to account for most of the inheritance of yield in corn (ROBINSON and COMSTOCK 1955). MORTON, CROW and MULLER (1956) have interpreted some data on inbreeding effects in man to mean that the balanced load is unimportant, a conclusion questioned by NEEL (1958), SCHULL (1958) and NEEL and SCHULL (1962). Similarly, GREENBERG and CROW (1960) claim that the genetic loads in Drosophila are preponderantly mutational, a conclusion at variance with those of DOBZHANSKY, PAVLOVSKY, SPASSKY and SPASSKY (1955), DOBZHANSKY, KRIMBAS and KRIMBAS (1960), and others.

The results of the analysis reported in the present article show, we believe unambiguously, that the balanced components of the genetic loads are not unimportant in the natural and experimental populations of Drosophila which we have studied. If the genetic loads were exclusively mutational, then the viabilities of the homozygotes and heterozygotes would, in their upper ranges, converge on a single value. This is so because, in the absence of heterotic overdominance, the most highly viable homozygotes should be those with fewest deleterious mutations, approaching the classical textbook model of a genotype with all favorable dominants and no unfavorable recessives. The "best" chromosomes found in a population would then form homozygotes equal in viability to the most viable heterozygotes. Furthermore, heterozygosity in itself would not offer heterozygotes any basis for exceeding the best homozygotes. But this is not what we have found.

At the upper range of viabilities, the homozygotes and heterozygotes *diverge*, the latter being distinctly superior to the former (see Figures 3 and 4). This is evidence for heterotic overdominance. (Many authors restrict the word "overdominance" to interactions of single pairs of alleles; since we are working with chromosomes, and thus with gene complexes, "balanced heterosis" may be a

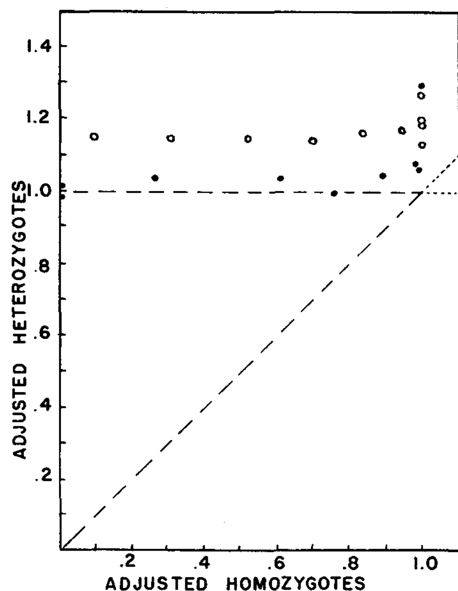

FIGURE 3.—Relation between viability effects of various second chromosomes of *D. melanogaster* in homozygous and heterozygous condition. The viability of homozygotes is based on that found in replicate cultures. (Solid points based on last two columns of Table 4; open circles show column 8 of Table 2 (modified as explained in text) plotted against adjusted homozygotes of Table 4.)

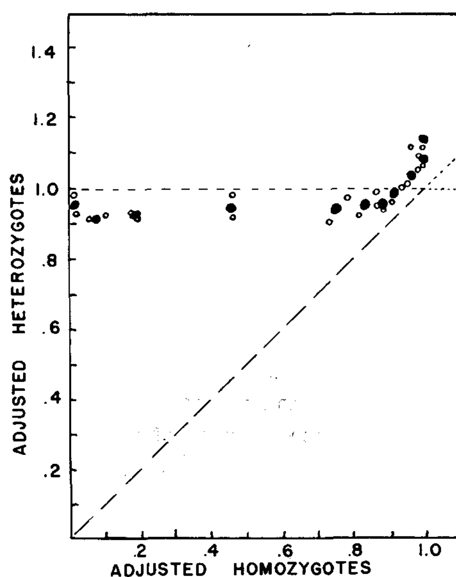

FIGURE 4.—Relation between the viability effects of various second and third chromosomes of *D. pseudoobscura* in homozygous and heterozygous condition. Solid circles based on combined data given in Table 6 (adjusted figures); open circles represent second and third chromosomes treated separately.

more appropriate term. We may note, however, that the divergence of the via-bilities of homozygotes and heterozygotes in the upper viability range cannot be explained unless heterozygosity is contributing to high viability.)

How important is the balanced heterosis we have observed? How many gene loci are involved? These are two different questions and their answers must be sought in different ways. In the absence of balanced heterosis, the viabilities of homozygotes and heterozygotes should converge on a single value; this value should lie on the diagonals drawn in Figures 3 and 4. To explain the divergence actually observed, one must assume that the experimental curves in these figures have been displaced upward, that is, that the viabilities of heterozygotes exceed those expected by some 15–20 percent. This displacement, we suggest, must be ascribed to some form of overdominance, perhaps the cumulative effects of many heterozygous loci or, alternatively, the major effects of fewer loci. There may or may not be epistatic interactions between these heterozygous loci as well.

To estimate the number of loci involved in bringing about the upward displacement of heterozygous viabilities requires specially designed experiments. One such design is that described by WALLACE (1958a,b). On the basis of his findings WALLACE inferred that the number of loci involved in balanced heterosis is probably large; MULLER and FALK (1961) and FALK (1961) failed to confirm these findings. The problem remains an open one.

SUMMARY

Experiments on the effects in heterozygous condition of genes and gene complexes which are lethal, semilethal, or subvital when homozygous are beset with difficulties. The most insidious one is a bias due to correlations between the frequencies of the homozygotes and of the heterozygotes in different cultures, caused by similar reactions of certain phenotypes to environmental fluctuations. Such correlations may be erroneously interpreted as evidence of partial dominance of some of the components of the genetic loads in populations. The data of WALLACE on experimental laboratory populations of *Drosophila melanogaster*, and of DOBZHANSKY and colleagues on natural populations of *D. pseudoobscura* are analyzed with the view to elimination of the disturbing biases.

No evidence is found of any losses of viability in heterozygotes carrying chromosomes which are lethal, semilethal, or subvital when homozygous. It should, of course, be noted that the chromosomes analyzed came from populations subjected for many generations to natural selection; newly arisen mutants may well be different in their behavior.

Chromosomes which are supervital when homozygous (i.e., produce exceptionally vigorous homozygotes) seem to impart high viability to heterozygotes as well. A more careful analysis shows however that the heterozygotes generally exceed in viability even the most highly viable homozygotes. In the upper part of the viability range the viabilities of the heterozygotes and homozygotes actually diverge.

The classical hypothesis of population structure postulates that heterozygotes for pairs of alleles are intermediate between the homozygotes in phenotypic

traits, including fitness. This is contradicted by the observed divergence of the viabilities of the heterozygotes and homozygotes at the upper extreme of the viability range. It is inferred that this divergence reflects an upward displacement of the viability of heterozygotes resulting from heterotic, overdominant genes or gene combinations.

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