GENETICS OF NATURAL POPULATIONS. XXVIII. THE MAGNITUDE OF THE GENETIC LOAD IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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POPULATIONS of species that reproduce sexually and by cross-fertilization usually carry some genetic variants which, either when homozygous or both in heterozygous and in homozygous condition, decrease the fitness of their carriers in at least some environments. The sum total of genetic variants of this kind, present in the gene pool of a population, constitutes its genetic load. Exploration and analysis of genetic loads in populations of the same and of different species living in the same or in different environments is one of the most urgent tasks of modern population genetics. Of late, this topic has acquired added interest, especially in connection with the problem of genetic damage that may be caused by the exposure of human and other populations to high-energy radiations. The genetic damage consists in making the genetic load of a population heavier than it was before the damage was inflicted.

Quantitative studies on genetic loads generally require special genetic tools, chromosomes with suitable "marker" genes, inversions, crossover suppressors, etc. Genetic tools of this sort are at present available only in a few species of Drosophila. It is not beyond the realm of what is possible to contrive such tools also in corn and perhaps in some other organisms, but this has not, in fact, been accomplished to date. Other and much less direct techniques will have to be used in studies on the genetic loads of human populations, and it is perhaps not unduly optimistic to hope that studies on Drosophila may be useful in pointing the way.

The pioneering studies on the genetic loads in populations of *D. melanogaster* were initiated by CHETVERIKOV (1927), DUBININ and his collaborators (1934 and later works), IVES (1945 and later), and others. In *D. pseudoobscura*, the species with which the present work is concerned, the genetic loads were investigated by STURTEVANT (1937); DOBZHANSKY and QUEAL (1938); DOBZHANSKY (1939); DOBZHANSKY, HOLZ and SPASSKY (1942); DOBZHANSKY and SPASSKY (1944, 1953, 1954); and DOBZHANSKY, PAVLOVSKY, SPASSKY, and SPASSKY (1955). The available data remain, nevertheless, fragmentary and unsatisfactory in several ways.

(1) All the experiments were made with the aid of a technique which involved comparisons of the viability of individuals homozygous for a given wild chromo-

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some with individuals heterozygous for the same chromosome. This is a method for the detection of recessive genetic variants. A superior method was evolved by WALLACE (1956); it makes all the test cultures contain a class of flies which do not carry the chromosome being tested at all. This class serves as a common denominator, and its presence permits detection of dominant effects of the tested chromosomes.

(2) The old experiments have shown that the viability for homozygotes for many wild chromosomes is extremely sensitive to environmental variations. We do not, however, know how the frequencies of the different viability classes (lethals, semilethals, subvital, and supervital chromosomes) would compare if tested under more stringent and at more optimal environmental conditions.

(3) Some data have suggested that, at least in Drosophila, individuals homozygous for wild chromosomes are less homeostatic (i.e., more sensitive to environmental changes) than individuals whose two chromosomes of a pair are derived from not closely related parents. This has been questioned by some writers, and misinterpreted and misconstrued by others; further data are obviously needed.

(4) How a chromosome behaves in homozygotes may not matter greatly in outbred populations in which the probability of any one chromosome being present in duplicate in any individual is slight. More important is what a chromosome does in heterozygotes (see above, point 1). It is desirable to know whether individuals carrying different combinations of chromosomes are phenotypically uniform or heterogeneous, and if heterogeneous, whether the heterogeneity is correlated with the effects of the component chromosomes in homozygotes. The experiments described in this, and on the following articles, were designed to test these points.

MATERIAL AND EXPERIMENTAL PROCEDURE

A sample of the natural population of *D. pseudoobscura* from the vicinity of Austin, Texas, was collected in March, 1957 by MR. T. G. GREGG. The population at Mather, California, was sampled in June of the same year by one of us (TH. DOBZHANSKY). The analysis of the samples was carried out in the laboratory in New York.

For analysis of the second chromosomes, wild males, or sons of wild females, were outcrossed to females having the dominant gene Bare (*Ba*, short bristles) and the recessive glass (*gl*, disarranged eye facets) in one of the second chromosomes, and the dominant Delta (Δ, expanded wing veins) in the other second chromosome. The *Ba gl* chromosome had also an inversion which suppressed most of the crossing over. In the F_1, a single *Ba gl*/+ male was taken, and crossed to *Ba gl*/Δ females from the stock culture. In the F_2, groups of eight *Ba gl*/+ females and like numbers of Δ/+ males were selected and transferred to fresh culture bottles six times at one- to two-day intervals, at room temperatures. Half of the resulting bottles with eggs deposited in them were placed in a constant temperature room at 25°C, and the other bottles in a similar room at 15°-16°C. When the progenies hatched, they were counted four times at three-day intervals

in the bottles at 25°C and at five-day intervals at 15°C. The progenies should contain four types of flies, in equal numbers, as follow: wild (+/+), Bare (*Ba gl*/+), Delta (Δ/+), Bare-Delta (*Ba gl*/Δ).

The wild type flies carry in duplicate (are homozygous for) the wild second chromosome under test; the Bare and Delta flies carry the same chromosome in heterozygous condition; the Bare-Delta flies do not carry this chromosome at all.

For the analysis of the third chromosomes, an analogous series of crosses was made. Wild males, or sons of wild females, were crossed to females having the dominant Lobe (*L*, eye shape), the recessive orange (*or*, eye color), and an inversion (Santa Cruz) in one third chromosome, and the dominant Blade (*Bl*, wing shape), Scute (*Sc*, some bristles absent), and the recessive purple (*pr*, eye color) in the other third chromosome. A single F_1 *Bl Sc*/+ male from each cross was mated to *L or*/*Bl Sc pr* females from the stock culture; in the next generation groups of eight *L or*/+ females and *Bl Sc pr*/+ males were taken and allowed to oviposit consecutively in six culture bottles. As with the second chromosomes, half of the bottles were then kept at 25° and half at 15°C. The progenies contained four classes of offspring, namely: wild (+/+). Blade-Scute (*Bl Sc pr*/+), Lobe (*L or*/+), Blade-Scute-Lobe (*Bl Sc pr*/*L or*).

The wild type flies are homozygous for the wild third chromosomes which are being tested; the Blade-Scute and the Lobe flies carry these wild chromosomes in single dose; the Blade-Scute-Lobe flies are free of these chromosomes. These four classes should be equally frequent among the zygotes formed, but among the adult flies their frequencies are quite variable because of the variations in the viability. The homozygous class, +/+, varies from complete lethality to normality and to supervitality.

RESULTS AND DISCUSSION

The normal viability standards: The average viability in a given environment of flies which carry the two chromosomes of a pair taken at random from a given population is regarded as "normal" for that population. Since the chromosomes derived from not closely related parents will usually differ somewhat in their gene contents, the "normal" flies will be mostly heterozygous. They will be referred to below as "heterozygotes", to distinguish them from the "homozygotes" which carry certain chromosomes in duplicate. Operationally, the standards of normal viability can be arrived at by means of rather simple control experiments.

For the control of second chromosomes, *Ba gl*/+ females and Δ/+ males were taken from the progenies of two *different* wild parents, and intercrossed. As in the main experiments, six bottles were obtained with eggs deposited by the same group of eight females and eight males; three bottles were allowed to develop at 25°C and the three others at 15°C. The +/+ class obtained in the progeny carries, then, the two + chromosomes derived from different ancestors. The average viability of the flies of this class is normal by definition. The control experiments for the third chromosomes involved, analogously, crosses of eight *L or*/+

females × eight *Bl Sc pr*/+ males from *different* cultures. Again six cultures were raised for tests of each pair of chromosomes, three of them at 25° and three at 15°C. The counts in the control cultures were made exactly as in the cultures of the main experiment dealing with the homozygotes.

Table 1 reports a summary of the results separately for the second and the third chromosomes, for the two temperatures, and for the chromosomes of Texas and of California origins. The frequencies of the +/+ class, the average viability of which is normal by definition, may be calculated in two ways. First, the frequencies may be expressed in percentages of the total numbers of all the flies counted in the test cultures. Secondly, we may calculate the ratios of the numbers of +/+ flies to Bare-Delta (or respectively, Blade-Scute-Lobe) flies in the same cultures. Table 1 reports both the percentages and the ratios.

Table 1 shows that the frequency of the +/+ is invariably higher in the experiments involving chromosomes of California origin than in those with chromosomes of Texas origin. The crosses are such that the Y chromosome comes from the wild population, the X from the laboratory stock, and on the average ¾ of the gene content of the uncontrolled autosome comes from the laboratory stock. The higher frequency of +/+ when the chromosomes are of California origin may be due either to a direct effect of these chromosomes on this background under laboratory conditions or to a different effect on viability of the dominant mutant genes used as markers in the experiments (i.e., *Ba*, Δ, *Bl*, *Sc*, and *L*) when the wild component of the genetic background is of California rather than Texas origin.

The normal viability is defined as the *average* viability of the +/+ class in the control experiments. Different pairs of wild chromosomes yield, however, somewhat different percentages of +/+ flies, as reported in Figure 2. It will be shown below that these differences are greater than could be accounted for by sampling errors and environmental fluctuations.

Viability of the homozygotes: The frequencies of the +/+ flies in the cultures in which this class of flies was homozygous for different wild chromosomes are summarized in Figure 1, which shows the numbers of chromosomes yielding various percentages of +/+ flies among the flies counted in the test cultures.

TABLE 1

The "normal" viability standards. Mean percentages and mean ratios of the heterozygous +/+ class in the control experiments

Chromosome	t°C	Locality	Percentages Mean	σ	Ratios Mean	σ	Chromosome combinations
Second	25°	Texas	26.03±0.31	2.20	1.103±0.023	0.160	49
Second	25°	Calif.	27.12±0.49	2.85	1.179±0.031	0.189	34
Second	15°	Texas	26.31±0.36	2.56	1.108±0.027	0.196	52
Second	15°	Calif.	27.14±0.46	2.66	1.234±0.033	0.191	33
Third	25°	Texas	24.68±0.31	2.30	1.010±0.021	0.156	56
Third	25°	Calif.	28.12±0.45	2.29	1.288±0.039	0.199	26
Third	15°	Texas	24.27±0.40	2.87	1.006±0.028	0.205	52
Third	15°	Calif.	26.31±0.37	1.88	1.088±0.029	0.146	26

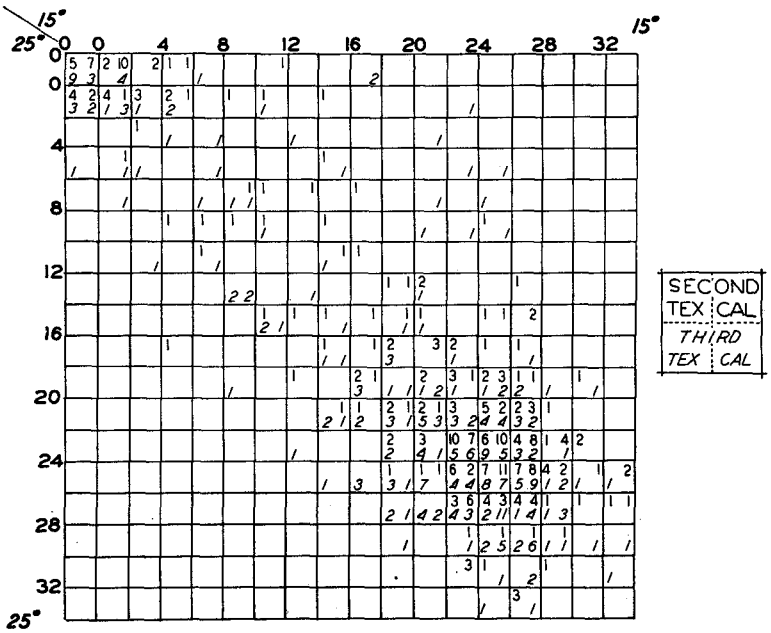

FIGURE 1.—Viability of the homozygotes for different chromosomes, as measured by the percentages of the wild type class observed in the cultures raised at 25°C (ordinates) and at 15°C (abscissae). The figures for the second and the third chromosomes of Texas and of California origins are placed as shown in the diagram on the right.

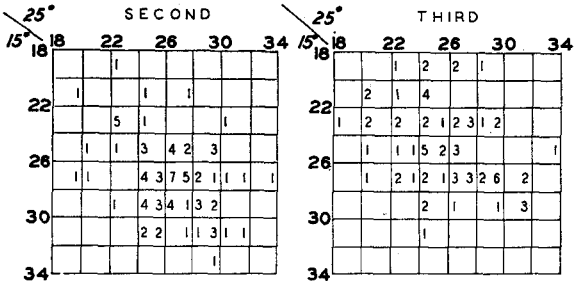

FIGURE 2.—Viability of the heterozygotes for different combinations of chromosomes, as measured by the percentages of the wild type class observed in the cultures raised to 25°C (abscissae) and at 15°C (ordinates). The figures on the left in the squares are for the Texas chromosomes, and on the right for California chromosomes.

These frequencies could be also expressed as ratios of +/+ to Bare-Delta, or to Blade-Scute-Lobe, flies (as was done in Table 1); the two representations give the same picture, but the ratios are more variable. Figure 1 is constructed like a correlation table; the entries in each cell show the numbers of the chromosomes which yielded certain percentages of +/+ homozygotes in test cultures at the two temperatures, 25° and 15°C. In order to represent the data for the second and

for the third chromosomes of Texas and of California origins in the same figure, the numbers in each cell are placed as shown in the scheme on the right. The mean viabilities of the homozygotes for the chromosomes studied (i.e., the mean percentages of the +/+ in the cultures) are shown in Table 2; these figures may be compared with the mean viabilities of the heterozygotes shown in Table 1.

Comparison of Tables 1 and 2 shows that the mean viability of the homozygotes is consistently lower than that of the heterozygotes. If the populations studied suddenly became homozygous for their second chromosomes, the resulting loss of fitness, expressed in percentages of the normal viability would be:

Texas, at 25°C—24.8 percent	California, at 25°C—26.2 percent
Texas, at 15°C—31.8 percent	California, at 15°C—31.9 percent

Homozygosis for third chromosomes would result in a somewhat smaller loss of viability, namely:

Texas, at 25°C—22.9 percent	California, at 25°C—26.5 percent
Texas, at 15°C—20.9 percent	California, at 15°C—23.9 percent

Since the second chromosome is cytologically longer, and presumably carries more genes than the third, this difference between the chromosomes is not unexpected. It should be noted that the loss of fitness in the relatively more stringent environment at 25°C is not much, if any, greater than in the more favorable environment at 15°C. Table 2 also shows that the values for Texas are not significantly, but consistently, lower than for California chromosomes. Since the same has been observed in the control experiment (see above), the inference that the marking genes used interact less favorably with the California than with the Texas genotype is strengthened.

Chromosomes which yield no +/+ flies in the test cultures (the zero class in Figure 1) are lethal when homozygous. Semilethal chromosomes reduce the viability of the homozygotes to less than one half of normal. The normal survival rates are shown by the control figures in Table 1. The percentages of lethal and semilethal chromosomes combined turn out to be as follows:

	Second chromosome	Third chromosome
Texas, 25°C	25.6 ± 3.5	20.3 ± 3.2
Texas, 15°C	22.1 ± 3.3	20.4 ± 3.3
California, 25°C	22.7 ± 3.6	22.9 ± 3.6
California, 15°C	21.5 ± 3.6	16.4 ± 3.1

There is no significant difference in the incidence of lethal and semilethal chromosomes between the Texas and California populations studied. Combining the data for the two localities, we have:

	Percentage	Chromosomes examined
Second chromosomes, 25°C	24.3 ± 2.5	288
Second chromosomes, 15°C	21.8 ± 2.5	284
Third chromosomes, 25°C	21.5 ± 2.4	293
Third chromosomes, 15°C	18.5 ± 2.3	292

TABLE 2

The viability of homozygotes for the chromosomes studied, expressed in mean percentages of the +/+ class in the cultures

Chromosome	t°C	Locality	Mean	σ	Chromosomes studied
Second	25°	Texas	19.58±0.73	9.06	156
Second	25°	Calif.	20.03±0.88	10.08	132
Second	15°	Texas	17.95±0.74	9.18	154
Second	15°	Calif.	18.46±0.88	10.01	130
Third	25°	Texas	19.00±0.68	8.47	153
Third	25°	Calif.	20.66±0.71	8.44	140
Third	15°	Texas	19.19±0.72	8.82	152
Third	15°	Calif.	20.03±0.81	9.60	140

The incidence of lethals is slightly, but again not significantly higher at 25° than at 15°C. It is interesting to compare the incidence in the present material with that observed in older experiments, all of which were conducted at 25°C. The relevant figures are as follows:

	Second chromosomes	Third chromosomes
Mather, California, 1957	22.7 ± 3.6	22.9 ± 3.6
Mather, California, 1951	33.0 ± 4.5	25.0 ± 4.0
San Jacinto, Calif., 1940–41	21.3 ± 1.8	13.9 ± 1.0
Death Valley, Calif., 1937	.....	17.0 ± 1.3
Austin, Texas 1957	25.6 ± 3.5	20.3 ± 3.2
Austin, Texas 1953	31.5 ± 3.5	.....
Mexico and Guatemala, 1938	.....	30.0 ± 4.2

The genetic loads are clearly greater in some geographic populations of the species than in others. It may also be that the load does not remain constant with time in the same population. Thus, the incidence of lethal second chromosomes at Mather, California, decreased by 10.3 ± 5.7 percent between 1951 and 1957. This does not reach the conventional level of statistical significance. However, IVES (1954) did observe changes with time in the genetic load of the Massachusetts population of *D. melanogaster*.

Subvital and normal chromosomes: Disregarding the chromosomes which are lethal or semilethal in homozygotes, we are left with an array of quasi-normal chromosomes. The mean viabilities of the homozygotes for quasi-normal chromosomes are shown in Table 3. Comparing the data in this table with those in Table 1 and 2, it can be seen that in every instance the mean viability of the quasi-normal homozygotes is intermediate between that of the heterozygotes and that of random homozygotes (i.e., including the lethals and semilethals). Some of the quasi-normal chromosomes are subvital when homozygous.

The conventional boundary between the subvital and semilethal chromosomes lies at 50 percent of normal viability. It is more difficult to delimit the subvital from the normal and the rare supervital chromosomes; this is because the via-

TABLE 3

The viability of quasi-normal homozygotes, expressed in mean percentages of the +/+ class in the cultures

Chromosome	t°C	Locality	Mean	σ	Chromosomes studied
Second	25°	Texas	23.04±0.34	3.61	110
Second	25°	Calif.	23.55±0.35	3.52	100
Second	15°	Texas	24.24±0.33	3.49	110
Second	15°	Calif.	25.14±0.31	3.12	100
Third	25°	Texas	23.44±0.34	3.66	115
Third	25°	Calif.	24.91±0.35	3.57	105
Third	15°	Texas	22.90±0.34	3.62	115
Third	15°	Calif.	24.41±0.30	3.03	105

bilities of the quasi-normal chromosomes form a bell-shaped distribution, the mean and the mode of which lie clearly below those for heterozygotes (cf. Tables 1 and 3). A method of estimation of the incidence of subvital chromosomes was evolved by WALLACE and MADDEN (1953; see also DOBZHANSKY and SPASSKY 1953, 1954). A brief statement will suffice here. We have defined the normal viability as the mean of the viabilities of random heterozygotes. However, the observed frequencies of the +/+ class in the cultures in which this class is heterozygous are variable. The observed, or total, variance (σ^2_{ges}) is due in part to sampling errors (σ^2_s), in part to environmental fluctuations (σ^2_e), and in part to genetic differences among the heterozygotes (σ^2_g). All these values are calculable from our data (see the papers referred to above for detail). Subvital chromosomes may, then, be defined as those yielding homozygotes with viability more than two standard deviations ($2\sigma_g$) below the normal mean, but higher than semilethal chromosomes (i.e., higher than 50 percent of the normal viability).

The estimates of the total variance, and of its components, for the homozygotes and the heterozygotes obtained in our experiments are summarized in Table 4.

TABLE 4

Observed variance (σ^2_{ges}), variance due to environment (σ^2_e), to sampling errors (σ^2_s), and the genetic variance (σ^2_g) among homozygotes for quasi-normal chromosomes and among heterozygotes

Chromosomes		t°C	Homozygotes				Heterozygotes			
			σ^2_{ges}	σ^2_e	σ^2_s	σ^2_g	σ^2_{ges}	σ^2_e	σ^2_s	σ^2_g
Second,	Texas,	25°	16.00	4.18	3.68	8.14	5.11	3.16	3.26	—1.31
Second,	Texas,	15°	15.58	1.99	3.00	10.59	6.30	2.06	3.27	0.97
Second,	Calif.,	25°	13.30	2.96	3.39	6.95	8.44	3.24	3.48	1.72
Second,	Calif.,	15°	11.25	2.51	3.28	5.46	7.23	3.52	3.59	0.12
Third,	Texas,	25°	11.73	4.07	4.14	3.52	5.44	2.01	3.90	—0.47
Third,	Texas,	15°	12.88	4.74	3.27	4.87	9.23	3.27	4.24	1.72
Third,	Calif.,	25°	15.45	4.32	3.42	7.71	5.43	0.61	3.52	1.30
Third,	Calif.,	15°	10.65	3.04	3.27	4.34	3.72	1.00	3.25	—0.53

It can be seen that the sampling variances (σ^2_s) are about the same for the homozygotes and the heterozygotes. This means only that the numbers of the flies per culture were nearly the same in homozygous and heterozygous cultures (see below). The environmental variances (σ^2_e) are higher on the average for the homozygotes, although the data contain three ostensible exceptions to this rule. This situation will also be considered in more detail below. What is important here is that the genetic variances (σ^2_g) are strikingly and consistently greater in homozygotes. In fact, some of the estimates of σ^2_g for heterozygotes are negative, which only means that the observed total variance (σ^2_{ges}) was in these cases smaller than could be accounted for by sampling errors and environmental fluctuations. Using, then, the WALLACE-MADDEN method, we arrive at the following estimates of the percentages of quasi-normal chromosomes which are subvital when homozygous:

		Second chromosomes	Third chromosomes
Texas	25°C	89.6 percent	81.1 percent
Texas	15°C	56.0 percent	29.5 percent
California	25°C	69.1 percent	68.0 percent
California	15°C	81.6 percent	86.7 percent

The only comparable estimates in the literature are those of DOBZHANSKY and SPASSKY (1953) for subvital chromosomes in the Mather, California, population in 1951. These estimates are 93.5 percent for the second and 41.3 percent for the third chromosomes. It is unfortunately difficult to assign any confidence ranges for these estimates. The experimental errors involved are undoubtedly considerable. Averaging up all the available figures, we obtain 78 percent for the second and 61 percent for the third chromosomes; these are probably the best estimates of the frequencies of subvital chromosomes in natural population of *Drosophila pseudoobscura* now obtainable. Estimates of the frequencies of supervital chromosomes would be even less reliable.

Modification of the viability by temperature change: As stated above in the description of the experimental procedure, we have examined the viability of the homozygotes for certain chromosomes and of the heterozygotes for certain combinations of chromosomes by raising three replicate test cultures at each of the two temperatures, 25° and 15°C. We shall now inquire as to what extent the viability is modifiable by environmental changes. The differences between the cultures kept at 25° and at 15°C may be referred to as "macroenvironmental" and those between the cultures kept at the same temperature as "microenvironmental". The data for the macroenvironmental variations are summarized in the form of correlation tables in Figure 1 for the homozygotes, and in Figure 2 for the heterozygotes.

With the homozygotes it is obvious, at a glance at Figure 1, that the performances of a chromosome at the two temperatures are strongly correlated; the chromosomes which are lethal at one temperature are mostly lethal also at the other; the chromosomes which are quasi normal at 25°C are mostly quasi normal at 15°C. However, some striking modifications of the viability record are observed.

Thus, 22 of the chromosomes which were lethal at 25°C (0 on the vertical axis) were semilethal at 15°C (0 to 14 percent on the horizontal axis), and two were even subvital (16 to 18 percent). Of the chromosomes which were lethal at 15°C, only one became definitely semilethal (4–6 percent), and 11 produced up to two percent of the +/+ class. Some of the chromosomes which were semilethal at 25°C became subvital or even normally viable at 15°C; transformation from semilethality at 15°C to subvitality at 25°C seems to be less frequent. No such sharp changes occur among heterozygotes (Figure 2). The viability is evidently most variable among those homozygotes which are lethal or semilethal at least at one of the temperatures used in our experiments.

It is less evident, and therefore important to prove, that there is a real diversity also among the quasi-normal chromosomes. The same problem arises with respect to the diversity of the heterozygous chromosome combinations. We have, therefore, computed the correlation coefficients from the data summarized in Figures 1 and 2. In making the calculations, we have chosen to consider only those homozygotes which yielded no less than 14 percent of +/+ flies in the tests at both temperatures. (In so doing, we have ignored a few chromosomes which behaved as quasi normals at one temperature but as extreme subvitals or semilethals at the other). All the heterozygous combinations which were tested at both temperatures were utilized. The correlation coefficients (r) obtained are as follows:

	Second chromosomes	Third chromosomes
Texas, homozygotes	$+0.46 \pm 0.08$	$+0.23 \pm 0.09$
California, homozygotes	$+0.27 \pm 0.09$	$+0.31 \pm 0.09$
Texas, heterozygotes	$+0.39 \pm 0.12$	$+0.16 \pm 0.14$
California, heterozygotes	$+0.32 \pm 0.16$	$+0.25 \pm 0.18$

The correlation coefficients are in every instance positive, although some of them do not attain the conventional level of statistical significance. We conclude that the homozygotes for different quasi-normal chromosomes as well as the "normal" heterozygotes, are not uniform in viability, and, moreover, that their viabilities at the two temperatures are positively correlated.

Macroenvironmental variability in homozygotes and heterozygotes: The problem which now logically arises concerns the relationships between the viability of homozygotes, or of heterozygotes, for certain chromosomes and their modifiability by environmental influences. Does sensitivity to environmental changes usually go together with low fitness? And do genotypes which show high fitness in one environment tend to preserve this fitness over a range of environments?

We shall examine this problem both in connection with the macroenvironmental and with the microenvironmental variations. To start with the former, as shown in Figures 1 and 2, we have data on the viabilities at 25° and 15°C, as measured by the percentages of the +/+ class of flies produced in the test cultures, of an array of homozygotes and of heterozygotes for certain chromosomes. Let P_{25} and P_{15} be the percentages of +/+ observed at the two temperatures in the test cultures for a given chromosome. We now compute $(P_{25} + P_{15})/2$, which is the unweighted mean percentage of the +/+ class at the two temperatures. Let

this value be called the mean viability, P_m, of a given homozygote or heterozygote. The crude variance of the mean viability is evidently $(P_{25} - P_{15})^2/2$. A part of this variance is certainly due to sampling errors. The sampling variance is easily estimated for P_{25} and for P_{15} from the numbers of the flies counted in the cultures. Subtracting the sum of the sampling variances from the crude variance, we obtain an estimate of the macroenvironmental variance. From this value we derive, further, a coefficient of variation, C_{ma}; this is, evidently, the square root of the macroenvironmental variance, multiplied by 100, and divided by P_m.

The coefficients of variation, C_{ma}, are plotted against the mean viabilities, P_m, in Figures 3 and 4, for homozygotes and heterozygotes respectively. In preparing these diagrams, we ignored the homozygotes which produced fewer than five percent of +/+ flies in the cultures. Where the estimates of C_{ma} had negative

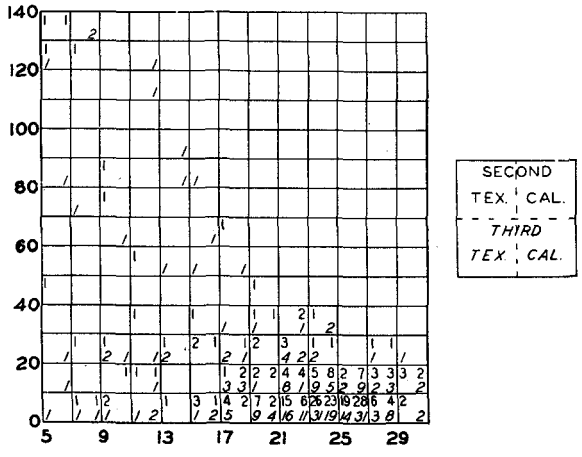

FIGURE 3.—Correlating the viability (abscissae) and the macroenvironmental variability (ordinates) of the homozygotes for different chromosomes. The viability is measured in mean percentages of the wild type class obtained in the cultures raised at 15°C and at 25°C. The figures for the second and the third chromosomes of Texas and of California origins are placed as indicated in the diagram on the right.

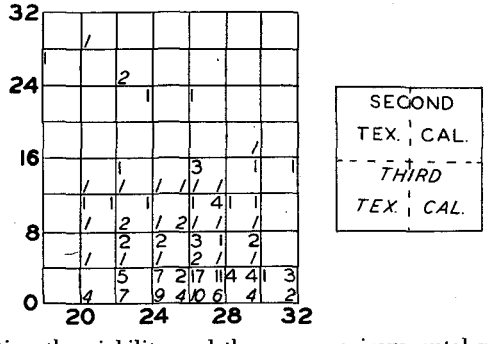

FIGURE 4.—Correlating the viability and the macroenvironmental variability of the heterozygotes for different combinations of chromosomes. This figure is constructed like Figure 3.

signs, they were taken to be zero; (such negative values arise when the crude variance happens to be smaller than the sampling variance). From the data in Figures 3 and 4 we have computed the correlation coefficients, r, which are reported in Table 5. The correlations are negative in every instance. This means that the genotypes which yield high average viabilities tend to be less sensitive to environmental modifications than genotypes which yield lower average fitness. In other words, a genotype which produces good viability at one temperature usually does so, also, at the other temperature; a genotype which is poorly viable at one temperature may be as bad, or better, or worse at the other temperature.

Since none of the heterozygotes in our material are lethal, semilethal, or low subvital, it is reasonable that the correlation coefficients for the heterozygotes are lower than those for the homozygotes. In fact, some of the former are not even significantly different from zero (Table 5). To obtain estimates for homozygotes comparable with those for heterozygotes, we ignored all chromosomes which yielded fewer than 20 percent of +/+ at either temperature, 25° or 15°C. We have thus rejected not only lethal and semilethal but also a number of quasi-normal chromosomes which happen to be clearly subvital at at least one temperature. The correlation coefficients for the remainder of the quasi-normal chromosomes are given in the middle column of Table 5. They are again all negative, although none of them taken separately are high enough to be statistically significant. We conclude that low viability is correlated, both in homozygotes and in heterozygotes, with a sensitivity to temperature changes, and high viability with a capacity to live well at different temperatures.

Microenvironmental variability in homozygotes and heterozygotes: With only few accidental exceptions, the homozygotes for every chromosome, and every heterozygous combination of two chromosomes, were tested in three replicate cultures. The percentages of the +/+ class observed in the replicate cultures vary because of the sampling errors, and also because of the minor variations in the environmental conditions in these cultures (degree of crowding, amount of food, accidental contamination with different microorganisms, etc.). We wish to examine and to compare the microenvironmental variances in the cultures in which the +/+ class consisted of homozygotes and in those where this class was heterozygous for pairs of chromosomes.

The treatment of the microenvironmental variance is quite analogous to that described above for the macroenvironmental variance. First, the observed, or

TABLE 5

Correlations between the mean viability, P_m, and the coefficient of macroenvironmental variation, C_{ma}. (Further explanation in text)

Chromosome	Locality	All homozygotes	Quasi-normal homozygotes	Heterozygotes
Second	Texas	−0.60±0.06	−0.12±0.10	−0.24±0.13
Second	Calif.	−0.45±0.08	−0.18±0.10	−0.33±0.16
Third	Texas	−0.47±0.07	−0.16±0.10	−0.25±0.14
Third	Calif.	−0.61±0.06	−0.16±0.10	−0.10±0.20

crude, variance is computed for each chromosome homozygote or heterozygote. Then the sampling component is estimated from the numbers of the flies counted in the cultures. Subtracting the sampling variance from the crude variance gives an estimate of the environmental variance. Where this estimate comes out negative, it is considered to equal zero. The standard deviations and the coefficients of microenvironmental variance, C_{mi}, are then calculated. In Figures 5 and 6 these coefficients are plotted against the mean viabilities (P_{25} or P_{15}), as measured by the percentages of the +/+ class among the flies counted in a given group of three replicate cultures.

The correlation coefficients, r, computed from the data reported in Figures 5 and 6, are presented in Table 6. For the homozygotes, the eight r's are all negative, and all but one are statistically significant. It is evident that, as with the macroenvironmental variability, the microenvironmental variability tends to be relatively greater in poorly viable homozygotes than in homozygotes of better viability. The situation is not so clear for the heterozygotes, since here three of the eight r's are ostensibly positive, and only one (negative) r is significantly

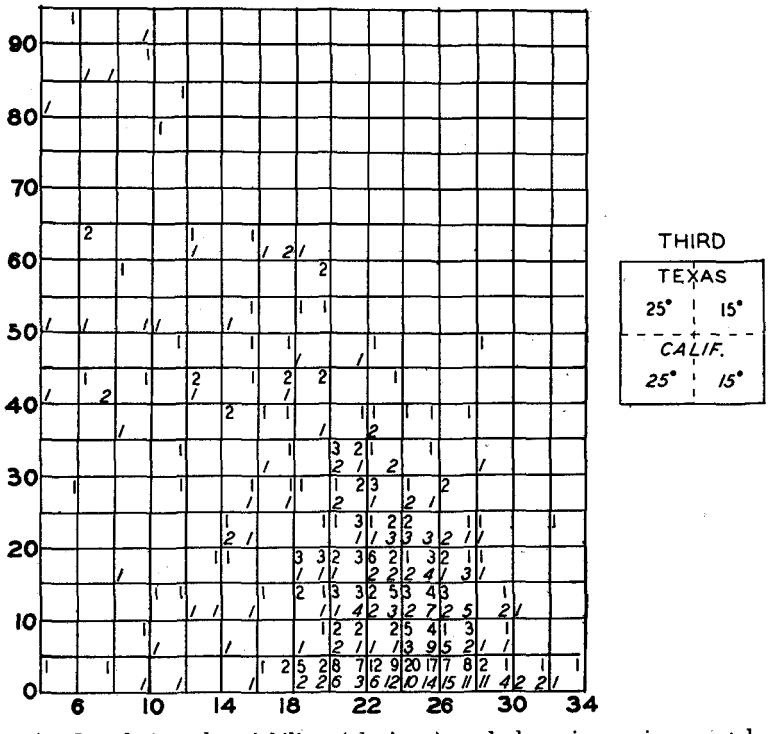

FIGURE 5.—Correlating the viability (abscissae) and the microenvironmental variability (ordinates) of the homozygotes for different third chromosomes. The viability is measured by the mean percentages of the wild type class observed in the cultures, and the variability by the coefficients of variation between the replicated cultures raised at the same temperature. The figures for the chromosomes of different geographic origins are placed as shown in the diagram on the right.

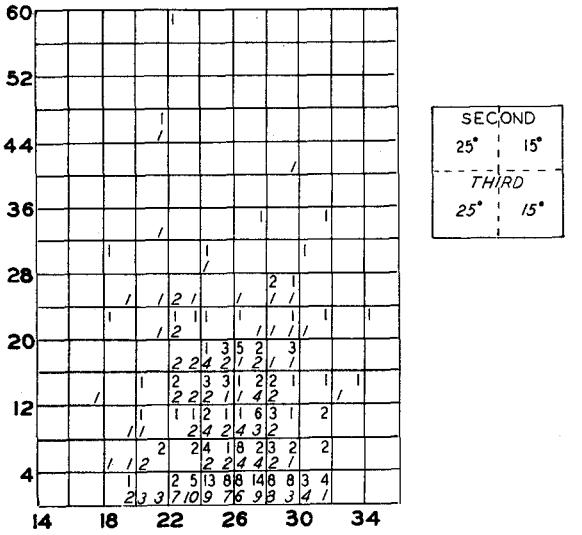

FIGURE 6.—Correlating the viability and the microenviromental variability of the heterozygotes for different chromosomes. This figure is constructed like Figure 5.

TABLE 6

Coefficients of correlation between the mean variability at a given temperature (P_{25} or P_{15}) and the coefficient of microenvironmental variation, C_{mi}. (Further explanation in text)

Chromosome	Locality	t°C	All homozygotes	Quasi-normal homozygotes	Heterozygotes
Second	Texas	25°	—0.11	—0.08	—0.19
Second	Texas	15°	—0.36	+0.01	—0.10
Second	Calif.	25°	—0.49	—0.25	—0.14
Second	Calif.	15°	—0.25	—0.10	—0.00
Third	Texas	25°	—0.66	+0.02	—0.30
Third	Texas	15°	—0.59	—0.26	+0.18
Third	Calif.	25°	—0.49	—0.24	+0.06
Third	Calif.	15°	—0.59	—0.25	+0.00

different from zero. To obtain comparable data, we have computed the correlation coefficients only for those quasi-normal homozygotes which yielded more than 20 percent of +/+ flies in the sum of the three replicated cultures. The figures are given in the column second from the right in Table 6. Two of the eight r's are positive (but neither of them is significantly different from zero), and six are negative (four of them close to the conventional significance level).

It may, then, be that the microenvironmental variability tends to be lower in heterozygotes than in homozygotes of comparable mean viability. To test this inference further we have calculated the mean coefficients of microenvironmental variability for the quasi-normal homozygotes (yielding more than 20 percent of +/+ in the cultures), and for the heterozygotes. The data are summarized in Table 7. It can be seen that the mean C_{mi} is ostensibly higher for the quasi-normal

TABLE 7

Mean coefficients of microenvironmental variation (C_{mi}) for the quasi-normal homozygotes and for the heterozygotes

Chromosome	Locality	t°C	Quasi-normal homozygotes	Heterozygotes
Second	Texas	25°	12.17	8.92
Second	Texas	15°	8.14	6.96
Second	Calif.	25°	9.53	8.13
Second	Calif.	15°	10.30	9.06
Third	Texas	25°	11.40	8.22
Third	Texas	15°	10.35	10.16
Third	Calif.	25°	9.55	5.54
Third	Calif.	15°	9.51	6.84

homozygotes than for the heterozygotes in six out of the eight comparisons. However, the difference is significant only in two comparisons (the third chromosomes tested at 25°C, in both of which the homozygotes are more variable. Taking all the data together, the mean C_{mi} for the quasi-normal homozygotes turns out to be 9.813 and for the heterozygotes, 8.259; the difference between these two figures is 1.554 ± 0.624, which, by the ordinary t test, is significant between the 0.02 and 0.01 probability levels. We conclude that chromosome homozygotes show, on the average, greater microenvironmental variabilities than the heterozygotes of similar viability.

Are heterozygous flies phenotypically uniform?: Drosophila pseudoobscura is a widespread and common species often forming populations of rather large genetically effective sizes (DOBZHANSKY and WRIGHT 1941, 1947, and other works). In populations of this sort, individuals homozygous for any one chromosome may be expected to occur only rarely; a great majority of the wild flies are heterozygotes. How the homozygosis for a given chromosome affects the fitness of its carriers is, then, of relatively minor importance in determining the fate of this chromosome, and the fate of the genes which it carries, under natural selection. On the contrary, the variations in fitness, even minor ones, which a chromosome might produce in its heterozygous carriers will be quite important determinants of its retention in, or elimination from, the gene pool of the population. One looks, therefore, for evidence of variation in fitness among the heterozygotes which carry different chromosomes.

Relevant evidence has been presented in Table 4. If the viability of the wild type heterozygotes were uniform, then the variance of the percentages of the +/+ class observed in the control experiments would be entirely accountable for by environmental variations and sampling errors; the genetic variance, σ_g^2, would be zero. Indeed, Table 4 shows that three of the eight estimates of σ_g^2 are negative, but the mean of the eight is still positive, +0.44. This may not be significantly different from zero; the average σ_g^2 for homozygotes is about 15 times larger and quite significant. Nevertheless, we have good reasons to think that the σ_g^2 for heterozygotes is greater than zero. In Figures 1 and 2 we have plotted the per-

centages of the +/+ class which a given chromosome or chromosome combination yielded at the two temperatures, 25° and 15°C. (The correlation coefficients computed from such data have been presented on a preceding page.) These correlation coefficients are positive and vary from $+0.16 \pm 0.14$ to $+0.31 \pm 0.09$, two of them being statistically significant even when considered in isolation from the others. Now, if the variance of the percentages of the +/+ class obtained at either temperature were due wholly to sampling errors and to microenvironmental variations, then no correlation between the results at the two temperatures would be expected. In reality, this correlation is not much weaker for the heterozygotes than it is for the quasi-normal homozygotes. We conclude that genetically conditioned variations in the viability do exist among the "wild type" heterozygotes as they do, to a much greater extent, among the homozygotes.

Comparing the productivity of the cultures in which homozygotes and heterozygotes are born: The data presented above show that the viability of individuals homozygous for second and third chromosomes extracted from natural populations is lower than that of the "normal" heterozygotes. We wish to inquire whether the productivity of (the number of flies hatching in) experimental test cultures which produce about one third or less of the progeny consisting of homozygotes falls below that of the cultures which produce heterozygotes.

Two facts must be kept in mind in this connection. First, the "wild type" heterozygotes and homozygotes are both produced in cultures in which a majority of the flies are heterozygous for a wild chromosome and for a chromosome with mutant marker genes. Secondly, our experimental cultures are deliberately somewhat overpopulated in order to help bring out the viability variations. Some mortality doubtless takes place in these cultures, and only a part of the eggs deposited survive to become adult flies. It would not be very surprising, therefore, if we were to find that even the cultures in which the homozygous class is eliminated by lethality would not suffer any diminution of the productivity. The data summarized in Table 8 show, however, that such a diminution does occur.

Excepting the California second chromosomes tested at 15°C, every one of the

TABLE 8

Number of flies in test cultures in which the +/+ class is homozygous or heterozygous for certain chromosomes

			Homozygotes			Heterozygotes		
Chromosome	Locality	t°C	Chromosomes tested	Total flies	Flies per chromosome	Combinations tested	Total flies	Flies per combination
Second	Texas	25°	156	87,103	558.4	50	31,106	622.1
Second	Texas	15°	154	99,785	648.0	52	35,306	679.0
Second	Calif.	25°	132	76,035	576.0	34	20,531	603.9
Second	Calif.	15°	130	77,395	595.3	33	19,615	594.4
Third	Texas	25°	153	77,600	507.2	56	32,847	586.5
Third	Texas	15°	152	87,474	575.5	51	29,953	587.3
Third	Calif.	25°	140	78,341	559.6	26	16,722	643.2
Third	Calif.	15°	140	82,129	586.6	26	15,506	596.4
Total			1,157	665,862	575.5	328	201,586	614.6

seven possible comparisons in Table 8 show that the cultures in which the homozygotes are produced contain fewer flies than do cultures in which the "wild type" class is heterozygous. The mean numbers of flies per tested chromosome, or chromosome combination, are 575.5 for the homozygotes and 614.6 for the heterozygote-producing tests. Since every test involved raising three replicate cultures (see above), the average numbers of flies per culture were 191.8 and 204.9, respectively. This means a loss of about 6.4 percent of the productivity of the cultures, owing to the deficient viability of the homozygous class.

SUMMARY

The aim of the present work has been to estimate the magnitude of the genetic loads carried in the second and third chromosomes of *Drosophila pseudoobscura*. Samples of 156 second and 153 third chromosomes from the population living near Austin, Texas, and 132 second and 140 third chromosomes from Mather, California, have been studied. The effects of homozygosis for these chromosomes were examined in flies raised at 25° and at 15°C.

Homozygosis for second chromosomes results in a loss of, on the average, between one quarter and one third of the viability of homozygotes (compared to an average heterozygote). The average viability loss in homozygotes for third chromosomes is slightly smaller, 21 to 26 percent. There is no great difference in the magnitudes of the losses at the near-optimal temperature 15°C and at the less favorable 25°C.

Between 21.5 and 25.6 percent of the second, and between 16.4 and 22.9 percent of the third chromosomes in our samples were lethal or semilethal when in double dose. Among the chromosomes free of lethals and semilethals, roughly 78 percent of the second and 61 percent of the third chromosomes produced subvital homozygotes.

The effects of the chromosomes on the viability of the homozygotes are often different in the cultures raised at 25° and at 15°C. There is, nevertheless, a fairly strong correlation between the viability records at the two temperatures (Figure 1).

It is convenient for our purposes to distinguish between macro- and microenvironmental variability. The former concerns the performance of the chromosomes at the two temperatures, 15° and 25°C, and the latter refers to variations observed in replicate cultures at the same temperature. Both the macro- and the microenvironmental variabilities, as measured by the coefficients of variation, are greater in the less viable than in the more viable phenotypes. In other words, there is a negative correlation between the viability and the variability. This is true both for homozygotes and for heterozygotes. However, the mean variation coefficients are somewhat lower for heterozygotes than for homozygotes of about equal viability (Table 7). A superior viability record in one environment is, thus, usually accompanied by good records in other environments. Highly viable homozygotes and heterozygotes are those which possess superior homeostatic properties, and the homeostatic capacities are on the average higher in heterozygotes than in homozygotes.

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