GENETICS OF NATURAL POPULATIONS. XXXII. INBREEDING AND THE MUTATIONAL AND BALANCED GENETIC LOADS IN NATURAL POPULATIONS OF DROSOPHILA PSEUDOOBSCURA[1]

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Received October 25, 1962

CASTLE, CARPENTER, CLARK, MAST and BARROWS (1906) were the first to use Drosophila as a laboratory animal, and discovered it was suitable for genetic experimentation; they explored the effects of inbreeding on the fertility of the flies. They found that inbreeding resulted in no decrease of fertility in strains in which a selection for fertility was practiced in each generation. The attention of geneticists working with Drosophila then shifted to other problems; little accurate information concerning the consequences of inbreeding and outbreeding in Drosophila is available.

Meanwhile, starting with the pioneering work of CHETVERIKOV (1927), it was discovered that natural populations of Drosophila carry enormous concealed genetic loads. Few, if any, of the "normal" flies found in nature do not carry one or more chromosomes which would, if present in double dose (i.e., in homozygous condition), cause lethality, semilethality, subvitality, sterility, or physiological or morphological aberrations of various sorts. The health, vigor, and adaptedness of the "wild-type" flies is due mainly to the load being concealed in heterozygous condition. Moreover, the genetic load contains several components of disparate biological significance. The mutational and balanced loads are of particular interest. Genes and gene complexes which are deleterious when homozygous, and also deleterious, or at least neutral, when heterozygous, constitute the mutational load. Those which are deleterious when homozygous but enhance the adaptive value of the heterozygous carriers compose the balanced load. Attempts to estimate the relative magnitudes of the mutational and balanced loads have so far given ambiguous results (DOBZHANSKY, KRIMBAS and KRIMBAS 1960, WALLACE and DOBZHANSKY 1962, GREENBERG and CROW 1960).

Homozygosis is relatively more and heterozygosis relatively less frequent in inbred than in outbred populations; inbreeding of normally outbred populations, therefore, results in uncovering a part of the normally concealed genetic load. This is true of the mutational as well as of the balanced fractions of the load (only completely dominant deleterious mutants, if such exist, would constitute an exception). However, CROW (1952, 1958) and MORTON, CROW and MULLER

[1] The work reported here was carried out under Contracts Nos. AT-(30–1)-1151 and AT-(30–1)-3096, U. S. Atomic Energy Commission, with Columbia University and the Rockefeller Institute respectively.

(1956) pointed out that (granting certain assumptions) the reduction of the fitness resulting from inbreeding will be relatively greater with mutational than with balanced loads. Investigation of inbreeding effects is, consequently, a way of evaluating these components of the genetic loads. Genetically well studied species of Drosophila are most favorable material for such investigation. In Drosophila, and at present in it alone, the genetic load can be measured both through examination of the consequences of inbreeding, and by making a census of the chromosomes in a given population which produce various kinds of effects in double dose. An attempt to obtain such a measurement by these two independent techniques is reported in the following pages.

MATERIAL AND TECHNIQUES

Samples of the populations of *Drosophila pseudoobscura* were captured at Mather, Tuolumne County, California, on June 4th and 5th; at Rustler Park, Chiricahua Mountains, Arizona, on June 15th, and the Southwestern Research Station, Cave Creek, Chiricahua Mountains, on June 14th and again on September 5th, all in 1961. These four samples will be referred to below as M, A, B, and C respectively.

The flies collected were brought to the laboratory at the Southwestern Research Station (samples M, A, and B), or at Columbia University (Sample C); the females were placed in individual cultures, and allowed to produce offspring. Groups of six to ten virgin females and males were taken from each culture and intercrossed with flies from other cultures of the same sample; i.e., females from culture A with males from B, females from B with males from C, etc. The progeny of these "unrelated" crosses is as outbred as flies in the natural population from which the sample came; they may be said to have an inbreeding coefficient F=0. Matings of similar groups of siblings from the same culture give brother-sister inbred progenies with inbreeding coefficient F=0.25. When the progenies of unrelated crosses hatched, groups of female and male siblings from each culture were inbred; the degree of inbreeding thus obtained is, on the average, the same as would result from matings of half-sibs, with an inbreeding coefficient F=0.125. (Professor Howard Levene finds that this may be a slight underestimate, because with six to ten pairs of siblings taken from each progeny, not all possible genotypes will be included. An estimate of F between 0.1325 and 0.1390 will be more accurate. It happens, however, that the underestimate of F reinforces the arguments that are made in the present article.)

To study the influence of the inbreeding on the viability of progeny, samples of 50 eggs were taken from each mating, and the numbers of the adult flies obtained from each sample were recorded. The following technical details should be noted. The flies oviposited on a culture medium darkened by admixture of carbon black and poured on paper spoons. In the Arizona laboratory the oviposition took place at room temperature (sample M and parts of A and B), until the daily temperature rose to 30°C and above in the middle of the day and began to injure the eggs. Thereafter the oviposition was in an incubator at 25°C. The eggs

were counted always less than 24 hours after the deposition (i.e., before any larvae hatched), and a portion of the medium with 50 eggs was lifted from the spoon and transferred to a culture bottle with a cornmeal-molasses-agar medium (in Arizona) or cream-of-wheat-molasses medium (in New York). The development took place at 25°C. In Arizona the medium sometimes dried excessively, and was reconditioned by addition of a weak yeast suspension. All in all, the cultures in Arizona had a more variable environment than those raised in New York. In the series M, A, and B only one sample of 50 eggs was taken from each cross. In C, two samples of 50 eggs were made from eggs deposited on different spoons and at least one day apart. The percentages of the eggs giving rise to adults in all series are shown in Table 1. Since all samples numbered 50 eggs, the numbers of flies obtained are exactly half of the percentage (except in sample C where two samples from each progeny were taken).

The material for the census of the chromosomes producing lethal, semilethal, etc., effects when in double dose, came from sample C, and all the experiments were carried out in New York. The techniques have been described in DOBZHANSKY, HOLZ and SPASSKY (1942), DOBZHANSKY and SPASSKY (1955), and in other publications, and need not be detailed here. In all cases a chromosome with suitable gene markers was used, which permitted obtaining individuals either homozygous or heterozygous for the chromosomes to be tested from the natural population, together with individuals carrying the gene markers and the same wild chromosomes in heterozygous condition. In all, 113 second, 104 third, and 115 fourth chromosomes were analyzed in homozygotes, together with 90 heterozygous combinations for second, 90 for third, and 108 for fourth chromosomes. This leaves out of account the tiny fifth chromosome, which is not yet under genetic control, and the X chromosome, the genetic load in which is a separate problem.

RESULTS

Egg to adult survival in inbred and outbred progenies: Table 1 shows that complete survival—i.e., 50 eggs giving 50 adults—is infrequent even in outbred progenies, and rare in inbred ones. The average percentages of survival, calculated from the data in Table 1, are shown in Table 2.

Inbred progenies give a lower survival than outbred ones, and stronger inbreeding (F=0.25) depresses the survival more than does moderate inbreeding (F=0.125). The M sample is an apparent exception, the lowest score having been obtained after the moderate inbreeding; the difference is not statistically significant, and the cultures with the moderate inbreeding might have been injured by heat. Data on survival and mortality with and without inbreeding may be analyzed in terms of the A and B statistics suggested by MORTON, CROW and MULLER (1956). The A value is the fraction of the zygotes dying owing to genetic and environmental causes in a randomly mating population (F=0). B is "the average number of lethal equivalents in the zygote that would result from doubling the chromosomes" of a haploid set, a lethal equivalent being "a group of mutant genes of such number that, if dispersed in different individuals, they would cause

on the average one death." The A and B values are estimated by calculating the regression slopes from the observed survival, or mortality, data with different degrees of inbreeding. They are shown in Table 3.

The B value is highest in the M sample and lowest in C. This may be related to the fact that the M sample was tested under more stringent and C under more nearly optimal conditions; however, as shown in Table 2, M and C gave statistically significant differences in survival only in inbred, but not in outbred cul-

TABLE 1

Numbers of cultures with different percentages (from 34 to 100) of survival from egg to adult stage. The degrees of inbreeding are F = 0, 0.125, 0.250

Sample	34 36	38 40	42 44	46 48	50 52	54 56	58 60	62 64	66 68	70 72	74 76	78 80	82 84	86 88	90 92	94 96	98 100	N
F = 0																		
M	..	..	..	..	1	1	..	1	1	3	1	4	7	5	7	6	21	58
A	1	..	1	1	..	1	4	3	4	7	5	6	6	12	7	9	8	75
B	..	..	..	..	2	2	1	1	1	2	6	7	14	12	17	26	52	143
C	..	..	..	1	..	1	1	2	2	5	19	19	31	38	54	62	32	268
F = 0.125																		
M	..	..	3	..	1	4	5	7	5	4	6	6	5	2	2	4	1	55
A	..	..	1	..	1	3	2	7	2	8	4	3	8	3	6	4	7	59
B	..	..	..	1	3	3	2	6	7	12	11	13	12	5	10	10	7	102
C	..	1	..	1	1	2	1	7	9	19	14	36	44	51	41	24	7	258
F = 0.250																		
M	1	1	1	1	6	3	2	3	3	4	6	4	3	11	4	4	2	59
A	..	1	6	3	9	4	3	9	3	8	6	5	6	5	4	3	..	75
B	..	2	2	3	3	5	5	8	10	12	20	13	13	14	12	8	6	136
C	..	..	..	1	1	7	4	8	13	19	29	28	22	18	20	12	2	184

TABLE 2

Percentages of eggs developing to the adult stage in outbred and inbred progenies

Sample	F=0	F=0.125	F=0.25
M	88.66 ± 1.52	71.44 ± 1.90	73.64 ± 2.12
A	80.60 ± 1.60	77.98 ± 1.88	67.42 ± 1.80
B	90.32 ± 0.92	78.60 ± 1.28	75.92 ± 1.70
C	87.98 ± 0.58	81.92 ± 0.60	78.16 ± 0.80

TABLE 3

Estimates of the A and B values and of the B:A ratios obtained from the observed mortality rates in outbred and inbred progenies

Sample	A	B	B:A
M	0.1614	0.7424	4.60
A	0.1968	0.7154	3.63
B	0.1192	0.6946	5.82
C	0.1321	0.4723	3.57

tures. The genetic load in the populations studied, estimated by the method suggested by MORTON, CROW and MULLER, is then between 0.47 (the C sample) and 0.74 (the M sample) lethal equivalents per gamete. The B:A ratios are, considering the observational errors involved, quite uniform, varying only from 3.6 to 5.8. The significance of these values will be discussed below.

Genetic and environmental variables in survival: The question that naturally presents itself at this point is to what extent the survival and mortality in inbred and outbred progenies is attributable to genetic and environmental causes. No precise quantitative answer can be given, but our data do permit certain inferences to be made.

In the first place, the viability of eggs may be a function of the health of the mother producing them. The variability due to this cause we tried as far as possible to minimize. Every sample of 50 eggs used came from cultures with six to ten females; of course, some of these females contributed probably more eggs than others, but it is unlikely that the whole batch ever came from only one mother. All the parents were themselves as outbred as the flies in the natural population from which their ancestors came, regardless of whether the progenies they produced in our experimental cultures had the F coefficient equal to zero or 0.12 or 0.25. However, the parents in each culture were the descendants of either one (in case of brother-sister progenies) or of two (in the other progenies) females collected in the wild and inseminated, presumably, by a single wild male. The wild progenitors and, consequently, their offspring which were used for inbreeding or outbreeding, may well have been genetically variable. One way to test this possibility is opened by the fact that the outbred progenies were obtained by crosses according to the scheme A × B, B × C, C × D . . . . Z × A, so that most cultures were used in two crosses each. (This scheme was not strictly adhered to, so that some cultures were used in only one, and some in more than two crosses.) We have looked for correlations between the proportions of survival of the progeny in pairs of crosses in which the same culture participated. In no sample has a significant correlation been found.

As stated in the description of techniques above, with the C sample each test has been made in duplicate. As a result, we have data on the proportions of survival in two batches of 50 eggs each, deposited by the same group of parents on different days. These proportions proved to be positively correlated, the correlation coefficients, r being as follows:

F = 0.000	$r = 0.28 \pm 0.05$
F = 0.125	$r = 0.35 \pm 0.06$
F = 0.250	$r = 0.37 \pm 0.06$

This correlation may be due to genetic as well as to environmental causes. A somewhat better insight into this matter is afforded by an analysis of the variance. We have examined the egg to adult survival in 268 outbred progenies and in 258 and 184 inbred ones with F coefficients 0.125 and 0.250 respectively. For each progeny two batches of 50 eggs each were taken. The variance between the

progenies and between the batches within the progenies are as follows (mean squares):

	F = 0	F = 0.125	F = 0.250
Between Progenies	89.08	94.56	115.04
Within Progenies	42.64	42.68	49.50

The variance between the batches of eggs within a progeny is due almost entirely to sampling errors and to environmental fluctuations; the only possible genetic component here might come from unequal contributions that the different mothers make to the egg samples collected on different days. These mothers, and their mates, are always siblings, but, of course, they are not genetically identical. The corresponding component in the variance between progenies is one-half of the variance within progenies, the remainder being genetic and due to environmental fluctuations in the cultures in which the parents were raised. The influence of the environments of the parents on the viability of their offspring may not in general be negligible, but in our particular experiments with the C sample it could hardly have been important. The frequencies of survival in the different progenies are, thus, dependent on the genetic constitution of the parents. This is, of course, not unexpected, since some of the parents were heterozygous for recessive lethal or semilethal genes or gene complexes, while others were free of them (see below).

It may be useful to mention here another source of error—namely, the possibility that Drosophila females caught in their natural habitats may have been inseminated by more than one male. The method used to appraise the importance of this error is as follows. In many natural populations of *D. pseudoobscura* some X chromosomes carry a triple inversion which is associated with the so-called "sex-ratio" condition (DOBZHANSKY 1944, Plate 4); a male carrying such an X chromosome usually produces only daughters and no sons, and therefore transmits his X chromosome to his entire progeny. In 1957, 1959, and 1961, using population samples collected in Arizona and in California, we endeavored to identify by cytological examination the progenies of the wild females inseminated in nature by sex-ratio males. Such progenies should consist of female larvae only, and all these larvae should be heterozygous for the sex-ratio inversions in their X-chromosomes. If a female mates with two males, one of them sex-ratio and the other normal, some of her progeny will be daughters not carrying the sex-ratio X, and some will be males also not carrying the sex-ratio X. In all, we examined the progenies of 98 non-sex-ratio females mated in nature to sex-ratio males, studying the chromosomes of at least 15 larvae in each progeny. In only two cases could the results be interpreted as indicating a double insemination. Be it noted that this does not necessarily mean that almost all wild-caught females have mated with only one male; an equally valid interpretation is that the sperms of different males do not rapidly mix in female sperm receptacles, and, therefore, the progeny which a female produces in a culture bottle comes almost always from a single father.

The genetic load in the second, third and fourth chromosomes: In species of

Drosophila in which mutant gene markers and inversions are available, the genetic load may be estimated by a method involving a very special kind of inbreeding by means of a series of crosses designed to obtain zygotes carrying in duplicate certain chromosomes sampled from a natural population. Crosses are arranged according to the scheme D/wild-1 ♀ × D/wild-1 ♂, where D is a dominant mutant marker and wild-1 is a chromosome from a natural population. If D is lethal when homozygous, the cross should produce 66.7 percent D/wild-1 and 33.3 percent wild-1/wild-1 zygotes, the latter being homozygous for the chromosome wild-1. As a control, crosses are made of D/wild-1 ♀ × D/wild-2 ♂, D/wild-2 ♀ × D/wild-3 ♂, etc.; the zygotes produced are, then 66.7 percent D/wild-1 and D/wild-2, and 33.3 percent wild-1/wild-2, the class not carrying D being now heterozygous for two different wild chromosomes.

We have tested by the above method the viability of homozygotes for 113 wild second, 104 third, and 115 fourth chromosomes from the C sample of the Arizona population, and also of 90 heterozygotes for pairs of second, 90 for pairs of third, and 108 for pairs of fourth chromosomes from the same population. The gene markers used (symbolized above as D) were the mutants Bare (*Ba*) in the second, Blade and Scute (*Bl* and *Sc*) in the third, and Curly (*Cy*) in the fourth chromosomes. The results obtained are summarized in Table 4, which shows the numbers of chromosomes and chromosome combinations which give various percentages of the wild/wild class in the cultures.

The mean percentages of the wild/wild flies in the cultures of all categories are shown in Table 5. For the heterozygotes, these percentages are close to the ideal 33.3; for the homozygotes, they are much lower. This lowering is evidently the consequence of the genetic load concealed in the natural population being uncovered in the experimental cultures. The average viability of the flies carrying the two chromosomes of a pair taken at random from a given population is considered to be "normal." The fact that the proportions of the wild/wild heterozygotes in the test cultures are close to the ideal, signifies, then, that the mutant markers used in the experiments do not appreciably harm the flies in the experimental environments (in fact, the *Cy* marker ostensibly improves the viability of its carriers!). Some cultures have produced zero percent of wild/wild flies; the chromosomes involved in these cultures are lethal when homozygous. The cultures with fewer than 16 percent but more than zero percent of wild/wild flies, contain chromosomes that are semilethal when homozygous. The cultures with more than 16 per cent wild/wild flies are quasinormal; it can be seen at a glance in Tables 4 and 5 that the mean viability of quasinormal homozygotes is lower than that of the heterozygotes; this indicates that some of the quasinormal chromosomes are subvital when homozygous.

Several populations of *D. pseudoobscura* have been studied for frequencies of lethal chromosomes, but only DOBZHANSKY and SPASSKY (1953) have made a census of lethal and semilethal second, third, and fourth chromosomes in the same sample, taken at Mather, California, in 1951. A comparison of the figures

TABLE 4

Numbers of chromosomes or chromosome combinations giving various percentages of wild type (flies free of mutant markers) in test cultures

Chromosome	0%	>0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	>40%	N
2nd, Homo.	17	4	.	1	1	.	2	1	1	2	2	5	5	7	12	16	16	7	11	2	1	.		113
2nd, Hetero.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	8	9	19	27	12	8	4	.		90
3rd, Homo.	15	5	1	1	.	.	1	2	1	3	.	6	8	9	10	13	13	6	6	1	1	2		104
3rd, Hetero.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	5	11	21	14	17	14	4	2		90
4th, Homo.	18	8	4	1	5	1	.	.	.	4	3	3	9	7	12	19	15	4	2	.	.	.		115
4th Hetero.	.	.	.	.	.	.	.	.	.	.	.	.	1	4	10	27	29	20	11	5	1	.		108

TABLE 5

Mean viabilities, in percentages of wild/wild flies in the test cultures, and in viability ratios

Chromosome	All chromosomes	Quasinormal	Viability ratios
2nd, Homo.	22.36 ± 1.17	29.12 ± 0.52	0.642 ± 0.035
2nd, Hetero.	32.24 ± 0.36	.....	0.955 ± 0.011
3rd, Homo.	21.65 ± 1.22	26.68 ± 0.54	0.618 ± 0.038
3rd, Hetero.	32.98 ± 0.32	.....	0.978 ± 0.017
4th, Homo.	18.81 ± 1.18	27.08 ± 0.50	0.520 ± 0.034
4th, Hetero.	30.96 ± 0.29	.....	0.923 ± 0.016

for that California sample with our Arizona sample is as follows (in percentages):

	Second	*Third*	*Fourth*
California	33.0 ± 4.5	25.0 ± 4.0	25.9 ± 4.2
Arizona	23.9 ± 4.0	25.0 ± 4.3	32.2 ± 4.4

Arizona second chromosomes appear to have fewer, and fourth chromosomes more, lethals and semilethals than the California ones, but the differences do not reach the conventional significance level. (For more comparative data, see DOBZHANSKY, HUNTER, PAVLOVSKY, SPASSKY and WALLACE 1963). The mean viabilities of the homozygotes for Arizona chromosomes are shown in Table 5. The viabilities are calculated in three ways: (1) the mean percentage of the wild/wild flies in all cultures, (2) the mean percentage of wild/wild in quasinormal cultures (i.e., excluding those containing lethals and semilethals), and (3) the viability ratios. These latter are calculated by dividing the numbers of flies not showing gene markers (i.e., wild/wild) by half the number of those with these markers (i.e., *D*/wild). All these viability estimates are close to those obtained by DOBZHANSKY and SPASSKY (1953) for California and by SPASSKY, SPASSKY, PAVLOVSKY, KRIMBAS, KRIMBAS and DOBZHANSKY (1960) for California and for Texas populations. The genetic loads in the California (our sample M) and the Arizona populations (our samples A, B, C) are about equally large.

GREENBERG and CROW (1960) have described a method for the estimation of the genetic load, in lethal equivalents, from data of the same type as those in Table 5. The number of lethal equivalents in the total load, T, is equal to the natural logarithm of the viability ratio for the heterozygotes, minus the natural logarithm of the viability ratio for the homozygotes. (They use for these viability ratios the same symbols, A and B, which are used by MORTON, CROW and MULLER 1956, although the values involved are not the same.) The T values, i.e., the lethal equivalents carried in the three chromosomes, are as follows:

2nd — 0.394
3rd — 0.461
4th — 0.574
Total — 1.429

The figure obtained, 1.429, is almost exactly three times as large as that obtained from the inbreeding data by the MORTON, CROW and MULLER method, which is 0.47 (see below).

DISCUSSION

Several attempts to evaluate the magnitude and the nature of the genetic loads in man and in other animals through studies on inbreeding effects have been made in recent years. MORTON, CROW and MULLER (1956) analyzed data on childhood mortality in progenies of cousin marriages and in families in which the parents were not known to be related. From three sets of such data, collected by investigators in France and in America, they concluded that the genetic loads in the populations concerned amounted to 1.5 to 2.5 lethal equivalents per

gamete, or 3 to 5 lethal equivalents per "average person." The B/A ratios varied from 7.9 to 24.4, from which the authors concluded that the balanced genetic load in man does not make "any substantial contribution to B, and that the genetic damage we are measuring is mutational." This conclusion was reiterated by CROW (1958), who recognized, however, that it would not be valid if the "load is based on loci with a large number of alleles, maintained by balanced polymorphism." The same conclusion was urged by MORTON (1960, 1961 and other publications); he found B/A ratios as high as 226 and 442 for recessive muscular dystrophy and deaf-mutism in man, values certainly too high to be explained by multiple allelism.

The approach to the problem of genetic loads suggested by MORTON, CROW and MULLER is certainly very ingenious; it is a legitimate and fruitful working hypothesis. The problem is, however, one of great complexity, requiring careful scrutiny of the explicit and implicit assumptions and the sources of error involved. The genetic load is quite unlikely to be either wholly mutational or wholly balanced. NEEL and SCHULL (1962) have shown that a B/A ratio as high as 10 in the expressed loads can arise with anywhere from 33 to 91 percent of the load being of balanced origin, the balanced loci having only two to five alleles giving heterosis in heterozygotes. These authors have analyzed by far the best existing data on inbreeding effects in man, collected in two cities in Japan, Hiroshima and Nagasaki; the B/A ratios turned out to be 4.63 and 4.48. In addition, their estimates of B are substantially lower than those found by MORTON, CROW and MULLER, and the main component of B is mortality in Hiroshima and morbidity in Nagasaki. NEEL and SCHULL concluded that the balanced "component in genetically determined death and disease is greater than has appeared to be the case from studies on inbreeding in Caucasian populations." PISANI and KERR (1961) utilized data on inbreeding effects in domestic animals, and found the B/A ratios in different breeds of chickens to vary from −2.8 to 6.8, and in cattle from −9 to 7.3. They conclude that "overdominance is important in chickens and perhaps in swine."

The B/A ratios we have observed in *Drosophila pseudoobscura* vary from 4.5 to 5.8. They resemble those found by NEEL and SCHULL in the human populations of Japan. If we were to suppose that the genetic load in Drosophila is entirely of balanced origin, we would be forced to make the unlikely assumption that the variable genes in our Drosophila population all had four to six alleles, all giving equally heterotic combinations. No such assumption is, however, called for; as shown by NEEL and SCHULL, the results are compatible with a variety of proportions of balanced and mutational components, including a decided predominance of the former.

Another fact which needs explanation is that the analysis of the genetic loads in separate chromosomes leads to an estimate of the total load of 1.43 lethal equivalents, while the estimate in the same population sample obtained by observations on the egg to adult survival in outbred and inbred progenies is only 0.47 lethal equivalents. Even in the M sample (from Mather, California) the estimate

obtained by the latter method is only 0.74; i.e., only one half of what the chromosome analysis seems to reveal. It is arguable that the environment is more stringent in the cultures used for tests of chromosomal homozygotes than in those in which the egg survival is measured, but the difference is certainly not drastic enough to account for results so widely different.

It is in a different direction that we should look for an explanation. The highest degree of inbreeding in the experiments on egg to adult survival is F=0.25. To calculate the genetic load, we extrapolate from the data on F=0, F=0.125 and F=0.25 to compute the survival with complete homozygosis, F=1. The extrapolation is based on the assumption (explicitly stated by MORTON, CROW and MULLER 1956) that the effects of different harmful genes are additive, and the mortality should increase linearly with increasing degrees of inbreeding. In the experiments in which we obtain chromosome homozygotes by using crosses with genetic markers, we obtain complete homozygosis (inbreeding coefficient F=1) for a given chromosome. Our own data on inbreeding ranging from F=0 to F=0.25 give no indication of significant departures from linearity within this range of the F's. These data are, of course, subject to considerable sampling errors, and anyway, it is quite possible and even probable that higher degrees of inbreeding would result in disproportionally high mortalities. To put it differently, the adverse effects of the components of the genetic load uncovered by inbreeding tend to be "synergistic," rather than simply additive, at least at higher degrees of inbreeding. Low inbreeding leads, thus, to lower estimates of the genetic load than does complete homozygosis.

The assumption of "synergistic" (epistatic) interactions of the components of the genetic loads is not an *ad hoc* hypothesis invented to explain the data reported in the present article. KRIMBAS, LEVENE, SPASSKY, SPIESS, and the present authors (see SPIESS 1958, DOBZHANSKY and SPASSKY 1960, and KRIMBAS 1960 for further references) have made a systematic study of the release of genetic variance through recombination in four species of Drosophila: *D. pseudoobscura*, *D. persimilis*, *D. prosaltans* and *D. willistoni*. Chromosomes were selected which in homozygous condition yielded normal or only slightly subnormal viability; heterozygotes were obtained carrying various pairs of such chromosomes; recombination by crossing over resulted in formation of chromosomes which, when homozygous, exhibited the whole range of viabilities, from lethal and semilethal to normal and supervital. Statistical analysis showed that an appreciable part of the recombination effects was due to epistatic interactions of the polygenes carried in the chromosomes, which before the recombination behaved as though they were more or less identical.

In conclusion, it should be pointed out that the discrepancies between the estimates of the genetic load obtained with the aid of different methods do not necessarily invalidate these methods. In population genetics the determination of even the order of magnitude of certain genetic parameters, such as the genetic load, is at present desirable. It should, however, be kept in mind that the usefulness of the oversimplified assumptions which we are forced to make in our work in order

to do any work at all may only be temporary. These assumptions become dangerous when they are not taken for what they are, and when attempts are made to retain them in the face of contradictory evidence.

SUMMARY

Samples of natural populations of *Drosophila pseudoobscura* were taken in certain localities in California and in Arizona. The laboratory-bred progeny of the wild flies were utilized to make crosses, the offspring of which had inbreeding coefficients of F=0, 0.125 and 0.250. Batches of 50 eggs (in one of the experiments with replications) were taken from each cross, and the numbers of adults developing from these eggs were recorded. As expected, the percent survival was lower in inbred than in outbred progenies. The A and B statistics were computed; the B/A ratios varied from 4.5 to 5.8. The estimates of the genetic loads turned out be no less than 0.5 and no more than 0.9 lethal equivalents per average gamete.

Series of crosses utilizing appropriate genetic markers in the second, third, and fourth chromosomes were then made in order to estimate the proportions of the lethal and semilethal chromosomes and the mean viabilities of the homozygotes and heterozygotes for these chromosomes. The estimate of the genetic load derived with the aid of this method comes to about 1.4 lethal equivalents per average gamete.

It is suggested that an appreciable fraction of the genetic load is balanced, and that the components of the genetic load tend to act synergistically when brought to manifestation in homozygous condition.

ACKNOWLEDGMENTS

We are greatly indebted to Drs. Howard Levene, I. M. Lerner, and Bruce Wallace for many enlightening discussions and for advice concerning the statistical treatment of the data; to the American Museum of Natural History for the hospitality at the Southwestern Research Station, Chiricahua Mountains, Arizona; and to the Department of Plant Biology, Carnegie Institution of Washington, for the hospitality at Mather, California. Professor J. F. Crow has contributed some remarks, the value of which is enhanced rather than diminished by the fact that our evaluation of the results presented above is not in agreement on all points.

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* This paper is mistakenly numbered in the Journal as XXIX.

† This paper is misnumbered in the Journal as XXVIII.