A COMPARISON OF THE EFFECT OF LETHAL AND DETRIMENTAL CHROMOSOMES FROM DROSOPHILA POPULATIONS[1]

RAYLA GREENBERG AND JAMES F. CROW

Departments of Genetics and Medical Genetics, University of Wisconsin, Madison, Wisconsin

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THIS study was undertaken in an attempt to determine whether the effects of recurrent mutation on the population and the deleterious effects of inbreeding are due primarily to a small number of genes of major effect or to the cumulative activity of a number of genes with individually small effects. It has been known since the work of TIMOFEEFF-RESSOVSKY (1935) and KERKIS (1938) that X-ray-induced mutations, and presumably spontaneous mutations as well, consist not only of lethals and near-lethals but include many with minor effects. It is likely that such a study would underestimate the number of minor genes for two reasons: (1) X-ray-induced changes include more chromosome breakages which are likely to be more drastic in their effect, and (2) mutants whose effects are very small would not be detected in such an experiment for lack of statistical resolving power.

This experiment consisted of the extraction of 465 second chromosomes from wild and from long-continued laboratory populations and the measurement of their effect on viability when homozygous and in random heterozygous combinations. In this way we obtain the distribution of homozygous deleterious effects among these chromosomes. This can be compared with similar data on newly induced mutations, to yield information on the relative persistance in the population of mutants of different homozygous viability classes.

Genes with a very slight detrimental effect cannot be detected individually, but their over-all influence can be assessed by applying the concept of the genetic load (MORTON, CROW, and MULLER 1956). The genetic load due to, say, mutation is the extent to which the population fitness (or whatever is being measured) is reduced in comparison to what it would be if mutation were not occurring. In addition to the mutation load there are the segregation load, due to segregation of homozygotes at loci where a heterozygote is favored, and other loads (CROW 1958).

In order to maximize the effect of mutations with small effects, the measurements were made on natural populations or on long continued cage populations which had been maintained long enough to be somewhere near equilibrium between selection and mutation. Similar measurements have been made by others (TH. DOBZHANSKY, HOLZ, and SPASSKY 1942; TH. DOBZHANSKY and SPASSKY 1953, 1954; DUBININ 1946; PAVAN, CORDEIRO, N. DOBZHANSKY, TH. DOBZHANSKY, MALOGOLOWKIN, SPASSKY, and WEDEL 1951; GOLDSCHMIDT

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1951; IVES 1945), though the method of analysis differed from that which we employ here. Studies on the distribution of lethals, sublethals, and detrimentals, among newly arising mutants have been made by TIMOFEEFF-RESSOVSKY (1935), KERKIS (1938), KÄFER (1952), FALK (1955), and BONNIER and JONSSON (1957).

EXPERIMENTAL METHODS

The chromosomes analyzed came from two sources: (1) a wild population collected in Madison, Wisconsin (Population I); and (2) a cage that had been maintained as a large population for several years by DR. BRUCE WALLACE as a control for his radiation experiments (IIa and IIb).

Males from these populations were crossed individually with a *Cy/cn bw* female (*Cy* = Curly wing, *cn* = cinnabar eyes, *bw* = brown eyes). A single Curly male (*Cy*/+) from each culture was crossed with *SM1*/bw^D female. (*SM1* is a chromosome with a pericentric inversion in addition to the usual Curly inversions, which prevent virtually all crossing over on the second chromosome. This chromosome produces the Curly wing phenotype in heterozygotes and is lethal when homozygous; bw^D is a dominant brown eye color.)

To produce flies homozygous for a second chromosome extracted from the population, *SM1* (*Cy*)/+ females from the above crosses were mated with their bw^D/+ brothers in individual pairs. There are four classes of progeny, expected in equal numbers, *Cy*/bw^D, *Cy*/+, bw^D/+, and +/+. The last class has two identical chromosomes derived from the same chromosome in the original male.

Flies carrying random combinations of wild chromosomes were obtained by following the same crossing procedure, except that the last mating was between flies derived from different wild males. The extracted chromosomes were cyclically permuted in the crosses, i.e., 1×2, 2×3, 3×4, . . . $(n-1) \times n$, $n \times 1$.

This system of crossing was designed so that each chromosome would be represented the same number of times in the heterozygous (random) combinations as in the homozygous (identical) combinations. This ideal was not quite fully achieved, for despite carrying duplicate cultures to meet such contingencies, some crosses were not completed due to sterility or inviability of the cultures. Heterozygous and homozygous combinations were made at the same time and run as nearly as possible under identical conditions, in order to minimize any confounding effect of secular fluctuations in the laboratory environment.

All cultures were kept in a constant temperature room at 24–26°C. The medium used was a standard corn meal, molasses, yeast, agar type sprayed with live yeast and with 0.5 percent propionic acid as a mold inhibitor.

Each pair of parents was transferred to a fresh vial, usually after four days. Counts were made on the 12th, 15th, and 18th day after mating. The values in Table 2 are the averages of the total number in both vials.

DATA

In Table 1 and Figure 1 are given the distributions of the viabilities of +/+ flies, the viabilities being expressed as the ratio of the +/+ class to the average

of the $Cy/+$ and $bw^D/+$ classes. The Cy/bw^D class was omitted from the calculations because of its reduced frequency in all experiments.

As in all other species of Drosophila examined, the distributions for the homozygotes are bimodal. One peak lies just below the normal viability, showing that most of the homozygous individuals are not as fit as heterozygotes. This must mean that in an equilibrium population there are many deleterious genes with very slight effects, bordering closely on the normal. The second peak of the distribution lies at the level of complete lethality. In addition to these chromosomes which kill all of the homozygotes, there are a few which allow a very small number of $+/+$ individuals to survive, usually less than ten percent of the normal number. Moreover, in many cultures of this type the $+/+$ flies emerged late. For these reasons it is convenient to include with the lethals those chromosomes which gave less than ten percent as many wild type as the control mean. The frequencies of lethals in the three tests were 26.0, 24.0, and 23.9 percent, in general agreement with values found by other investigators for second chromosomes of *Drosophila melanogaster*.

The mean numbers of flies per pair of parents are given in Table 2. An absence of any appreciable effect of crowding is indicated by the lack of any negative correlation between the numbers in the Cy/bw^D, $Cy/+$, and $bw^D/+$ classes and the $+/+$ class, despite absolute changes in the $+/+$ frequencies in homozygotes and heterozygotes. Differences in numbers of flies per culture among the three experiments may be accounted for by the tests having been performed under different food and laboratory conditions, in addition to having involved flies from different strains.

In Table 2 are also presented the mean viability ratios for heterozygotes (A), homozygotes (B), and nonlethal homozygotes (C). The mean ratios for all three experiments were obtained by weighting each value by the reciprocal of its variance.[2]

By observing the mean viability ratios, we see that a population suddenly made homozygous for all second chromosomes would retain, under our experimental conditions, only about 63 percent of the normal viability. Made homozygous for all second chromosomes excepting those carrying lethals, the population would retain about 84 percent normal viability. The reduction in viability in the latter case is due to a group of genes with relatively mild effect. Since the boundaries of these are not discrete and are definable only by a convention, it is best to group them all together as "detrimentals," which here include the "semilethals" mentioned by other authors. Estimation of the frequency of detrimentals poses a difficult problem since this group of mutants merges imperceptibly with the normals. Therefore, rather than establishing arbitrary limits to the detri-

[2] HALDANE (1956) has shown that estimates of viability such as A and B are biased. If n and N are the numbers of two classes, the viability estimate, $n/(N+1)$ is almost unbiased, and for this reason is preferable to the uncorrected estimate, n/N. However, we are interested primarily in the ratio (in fact, the log of the ratio) of two such viability estimates. The log of A/B is the same whether A and B are calculated as the average of $(n/N+1)/(m/M+1)$ or of $(n/N)/(m/M)$, provided that the numbers N and M are approximately the same magnitude. Since that is true in this study, the correction would have a negligible effect.

TABLE 1

Distribution of viability of homozygotes and heterozygotes for second chromosomes, expressed in viability ratios, i.e., $+/+ : \frac{1}{2}(\mathrm{Cy}/+ + \mathrm{bw^D}/+)$. *The figures indicate numbers of chromosomes that fall in the different viability classes*

	Viability ratios																		
Experiment	0	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	Total
I-homozygotes	58	2	3	3	5	8	8	16	32	34	25	16	9	5	5	1	1	..	231
IIa. homozygotes	23	6	1	2	4	3	2	10	13	17	16	13	8	2	1	..	..	..	121
IIb. homozygotes	25	2	3	0	0	2	2	5	13	21	23	13	1	3	..	..	..	..	113
Total	106	10	7	5	9	13	12	31	58	72	64	42	18	10	6	1	1	..	465
I-heterozygotes*	..	..	..	..	1	2	2	8	23	22	41	36	22	28	13	12	2	2	216
IIa. heterozygotes	..	..	..	..	..	..	..	3	9	20	26	27	17	11	2	2	0	1	118
IIb. heterozygotes	..	..	..	..	..	..	..	2	9	19	29	33	15	5	1	..	..	..	113
Total*	..	..	..	..	1	2	2	13	41	61	96	96	54	44	16	14	2	3	447

* Two outlying observations 2.18 and 2.50 omitted from this line.

TABLE 2

Mean genotype numbers and viability ratios in three experiments

	n	Cy/bw^D	$Cy/+$	$bw^D/+$	$+/+$	Viability ratio* $+/+ : \frac{1}{2}(Cy/+ + bw^D/+)$
			Experiment I (Madison)			
Heterozygotes	216	40.10	45.76	47.59	47.84	1.049 ± .018
Homozygotes	231	40.49	48.00	47.45	28.29	.614 ± .028
Nonlethal homozygotes	171	38.97	47.04	46.60	38.19	.829 ± .020
			Experiment IIa (Wallace)			
Heterozygotes	118	51.08	60.68	57.80	59.63	1.012 ± .016
Homozygotes	121	53.36	61.68	60.31	37.96	.641 ± .038
Nonlethal homozygotes	92	53.14	60.20	60.46	49.79	.841 ± .025
			Experiment IIb (Wallace)			
Heterozygotes	113	70.62	81.00	73.58	75.91	.985 ± .013
Homozygotes	113	68.36	78.81	74.01	48.74	.656 ± .038
Nonlethal homozygotes	86	65.71	76.90	72.17	63.88	.860 ± .022

* Viability ratio for all experiments.
Heterozygotes (A) 1.008±.0088
Homozygotes (B) .632±.0194
Nonlethal homozygotes (C) .842±.0127.

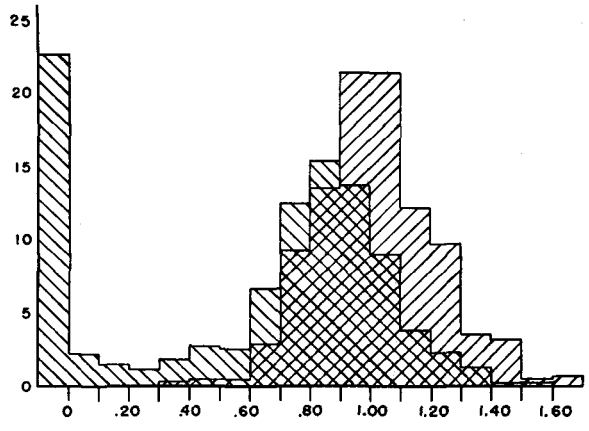

FIGURE 1.—Distribution of frequencies of viability ratios, $+/+ : \frac{1}{2}(+/Cy + bw^D/+)$. Abscissa: viability ratios. Ordinate: frequencies of chromosomes, in percent. ///// = heterozygotes. Reverse hatch = homozygotes. Crosshatched area is area of overlap.

mental class, it is desirable to have a method which takes into account even those detrimentals with extremely minute effect. This may be achieved by measuring the genetic load, which gives an estimate of the total effect of all grades of mutant genes upon a population.

METHODS OF ANALYSIS

The homozygous load may be estimated from the data in the following manner. Let us assume (1) a series of independently acting lethals with frequencies Q_i and probabilities of death S_i; (2) a series of independently acting detrimentals with frequencies q_i and probabilities of death s_i; (3) a series of independent heterozygous or environmental factors with frequencies X_i and probabilities of death H_i. This includes effects due to chance homozygosis of alleles in independent chromosomes.

On this model the probabilities of surviving the adverse effects of these factors are as follows:

The fraction of survivors among:

(1) heterozygotes $= A = (1 - X_1H_1)(1 - X_2H_2) \ldots\ldots$
$= \Pi\,(1 - X_iH_i)$.

If the number of factors is large and the separate probabilities small, this is approximately

$$e^{-\Sigma X_iH_i}$$

(2) homozygotes $= B = (1 - X_1H_1)(1 - X_2H_2) \ldots (1 - Q_1S_1)(1 - Q_2S_2) \ldots (1 - q_1s_1)(1 - q_2s_2) \ldots$

$$= \Pi\,(1 - X_iH_i)(1 - Q_iS_i)(1 - q_is_i).$$

$$\sim e^{-\Sigma X_iH_i - \Sigma Q_iS_i - \Sigma q_is_i}$$

(3) nonlethal homozygotes $= C = (1 - X_1H_1)\ (1 - X_2H_2) \ldots (1 - q_1s_1)(1 - q_2s_2)$

$$= \Pi\,(1 - X_iH_i)(1 - q_i\bar{s}_i)$$
$$- \Sigma X_iH_i - \Sigma q_is_i$$
$$\sim e$$

Let T = total load $= \Sigma q_is_i + \Sigma Q_iS_i$
D = detrimental load $= \Sigma q_is_i$
L = lethal load $= \Sigma Q_iS_i$

Then,

$$\frac{B}{A} = \frac{e^{-\Sigma X_iH_i - \Sigma q_is_i - \Sigma Q_iS_i}}{e^{-\Sigma X_iH_i}} = e^{-\Sigma q_is_i - \Sigma Q_iS_i} = e^{-T}$$

$$\frac{B}{C} = \frac{e^{-\Sigma X_iH_i - \Sigma q_is_i - \Sigma Q_iS_i}}{e^{-\Sigma X_iH_i - \Sigma q_is_i}} = e^{-\Sigma Q_iS_i} = e^{-L}$$

$$\frac{C}{A} = \frac{e^{-\Sigma X_iH_i - \Sigma q_is_i}}{e^{-\Sigma X_iH_i}} = e^{-\Sigma q_is_i} = e^{-D}$$

Thus, $T = ln\ A - ln\ B$
$L = ln\ C - ln\ B$
$D = ln\ A - ln\ C$

From Table 2 we see that the average value of T is .467. Thus we can say that the typical second chromosome in this population carries .467 lethal equivalents of which .287 are lethals and .180 are nonlethal detrimentals.

We are especially interested in computing the theoretical ratio of D to L for various specific models of gene action, and comparing these with observed results. In particular, the comparison of the D:L ratio in newly arisen mutations with that of chromosomes from natural populations is of interest.

The expectations may be derived as follows.

New mutations

In a group of new mutations, not yet exposed to selective elimination, the ratio of detrimentals to lethals will be determined solely by the total rates at which such mutants occur. To determine the load we weight each mutant by the amount of harmful effect it causes. Thus the D:L ratio is $\Sigma us/\Sigma US$, where u is the total rate of mutation to detrimental alleles at a locus and s is the average homozygous effect of detrimentals at this locus. U and S refer to the same quantities for lethals. The summations are over all relevant loci. If the same locus produces both detrimental and lethal alleles, the contribution may be thought of as divided proportionately between the two loads. If the mutation rate and the selective disadvantage are independent, the D:L ratio becomes $n\bar{u}\bar{s}/N\bar{U}\bar{S}$ or, since this

TABLE 3

The expected Detrimental:Lethal load ratio for various levels of dominance

	D:L Ratio		D:L Ratio (equilibrium)
	General	u and s independent	D:L Ratio (new mutants)
New mutants	$\Sigma us/\Sigma US$	$n\bar{s}/N\bar{S}$	
Equilibrium	$\Sigma(u/h)/\Sigma(U/H)$		
I. $h > 0$			
a. h constant	$\Sigma u/\Sigma U$	n/N	$\bar{S}/\bar{s}$
b. hs constant	$\Sigma us/\Sigma US$	$n\bar{s}/N\bar{S}$	1
c. h/s constant	$\Sigma(u/s)/\Sigma(U/S)$	$n\bar{S}/N\bar{s}$	$(\bar{S}/\bar{s})^2$
II. $h = 0$	$\Sigma\sqrt{us}/\Sigma\sqrt{US}$	$n\sqrt{\bar{s}}/N\sqrt{\bar{S}}$	$(\bar{S}/\bar{s})^{1/2}$
III. $h < 0$			
a. k alleles	$\Sigma\tilde{s}/\Sigma\tilde{S}$	$n\tilde{s}/N\tilde{S}$	1
b. 2 alleles $s_1 >> s_2 = \text{const.}$		n/N	$\bar{S}/\bar{s}$

u,U—mutation rate (total rate per locus to all mutant alleles).
s, S—proportional reduction in fitness of the homozygote.
h, H—a measure of dominance, ranges from one for completely dominant mutant, through zero for completely recessive, to negative values for overdominant locus.
n, N—number of relevant loci.
$\tilde{s}$ indicates the harmonic mean of s.
Lower case letters refer to detrimentals, upper case to lethals.

independence implies that $\bar{u} = \bar{U}$, $n\bar{s}/N\bar{S}$. N and n are the number of lethal- and detrimental-producing loci, and $\bar{S}$ and $\bar{s}$ are the average disadvantage caused by lethals and detrimentals, respectively.

Equilibrium population

Model I.—Incomplete dominance, h > 0:

Consider the following model (WRIGHT 1931):

Genotype	AA	Aa	aa
Frequency	$(1\text{-}q)^2$	$2q(1\text{-}q)$	q^2
Relative fitness	1	$1 - hs$	$1 - s$

We let q stand for the frequency of the mutant allele under consideration, or if there are multiple alleles, q is the sum of all their frequencies. Let u be the total rate of mutation to all mutant alleles at this locus, and s the weighted average selective disadvantage of all mutant alleles. This model is justified as a first approximation because recessive or nearly recessive mutant alleles frequently show absence of dominance in combination with each other.

At equilibrium, $q \sim u/hs$, unless h is nearly zero (WRIGHT 1931). The genetic load due to this locus in a homozygous population is then qs, or u/h (MORTON, CROW, and MULLER 1956; CROW 1958). The D:L ratio is $\Sigma(u/h)/\Sigma(U/H)$ where, as before, the capital letters refer to lethals and the lower case to detrimentals.

For $0 < h < 1$, we consider three special cases:

Ia.—h = H = *a constant:* The D:L ratio reduces to $\Sigma u/\Sigma U = n\bar{u}/N\bar{U}$, or if the average mutation rate is the same for lethals and detrimentals, n/N, where n and N are the number of loci giving rise to detrimentals and lethals. (As before, if a locus is giving rise to both, it is divided proportionately between the numerator and denominator.)[3]

Ib.—hs *constant:* The D:L *ratio is* $\Sigma us/\Sigma US$, or if mutation rate and selective value are independent, is $n\bar{s}/N\bar{S}$.

Ic.—h/s *constant:* The ratio is $\Sigma(u/s)/\Sigma(U/S)$, which, if mutation rate and selection value are independent, is $n\bar{S}/N\bar{s}$.

Model II.—Mutant gene completely recessive, h = 0:

The equilibrium frequency of the mutant gene or genes is $\sqrt{u/s}$. The homozygous load for this locus is qs, or $\sqrt{us}$. The D:L ratio is $\Sigma\sqrt{us}/\Sigma\sqrt{US}$. If the mutation rate is independent of the selective disadvantage, this becomes $n\sqrt{s}/N\sqrt{S}$.

Model III.—Overdominance, h < 0:

At a locus where heterozygotes are favored over all homozygotes a balanced polymorphism exists (FISHER 1930). Unless the mutation rate is of a magnitude comparable to that of selective differences between genotypes (which must rarely, if ever, be the case) the bulk of the load is not due to mutation, but to

[3] The condition that $h = H =$ a constant is unnecessarily restrictive. It is only necessary that the harmonic mean of h and H be the same and that dominance be independent of mutation rate. The condition of Models Ib and Ic may be similarly relaxed.

homozygotes that arise by segregation from the favored heterozygotes. This has been called the segregation load (CROW 1958).

We assume the fitness and frequencies to be as follows. No restriction is placed on the number of alleles, but all heterozygotes are assumed to be equal in fitness. A_i and A_j represent any two alleles, and q_i and q_j their frequencies.

Genotype	A_iA_i	A_iA_j
Frequency	q_i^2	$2q_iq_j$
Relative fitness	$1-s_i$	1

At equilibrium, $q_i = 1/s_i\Sigma(1/s_i)$ (WRIGHT 1949).[4]

In a randomly mating population the segregation load is $\Sigma s_i q_i^2 = 1/\Sigma(1/s_i) = \tilde{s}/k$, where $\tilde{s}$ is the harmonic mean of s, and k is the number of alleles at the locus. In a homozygous population, the load is $\Sigma q_i s_i = \bar{s}$. It is interesting to note that the contribution of each allele to the homozygous load is the same, i.e., $q_i s_i = \tilde{s}/k$, and that this is the same as the total load from all homozygous alleles in a randomly mating population.

IIIa.—Multiple alleles: The D:L ratio in a homozygous population is $\Sigma\tilde{s}/\Sigma\tilde{S}$, where the sums are over all overdominant loci maintaining detrimental or lethal alleles. This may be written $n\bar{\tilde{s}}/N\bar{\tilde{S}}$, where s is the arithmetic mean of a series of harmonic means. Notice that this is practically the same as Ib.

IIIb.—One pair of alleles, s_2 *constant and much less than* s_1*:*

With this model the load due to the more drastic allele is $\tilde{s}/k$, where k is two, and may be written $s_1s_2/(s_1 + s_2)$, which if $s_1 >> s_2$ is approximately s_2. Assuming s_2 is constant and the same for loci where s_1 is lethal as for those where it is detrimental, the D:L ratio is simply n/N. Note that this is the same as Ia.

All these models and their consequences are summarized in Table 3.

If detrimentals and lethals have the same dominance (Ia), the D:L ratio at equilibrium is larger than that in new mutants by a factor $\bar{S}/\bar{s}$. For example, if $\bar{S}$ is 1.0 and $\bar{s}$ is 0.1, the D:L ratio would be ten times as large in an equilibrium population as initially. On the other hand, if minor genes were more dominant than lethals in such a way that hs is constant (Ib), the initial D:L ratio is the same as the equilibrium value. Conversely, if minor mutants are less dominant (Ic) the equilibrium load is greatly increased over the initial.

It should be emphasized that these particular models (Ia, Ib, and Ic) are completely arbitrary and were chosen as algebraically convenient expressions for three contrasting situations. Model Ib is unrealistic for small values of s, for it admits of values of h greater than unity. However, the available data hardly justify more refined models, although others having the property of increasing dominance with decreasing s are readily devised.

Likewise many other models for overdominance could be introduced, but the

[4] This is easily seen by noting that one way of specifying the equilibrium condition is that, since the gene frequencies must remain constant, the fraction eliminated by selection has to be the same for all alleles. Thus, $s_i q_i^2/q_i = s_i q_i$ is constant. Letting $s_i q_i = C$, $q_i = C/s_i$. Since $\Sigma q_i = 1$, $C = 1/\Sigma(1/s_i)$, and $q_i = 1/s_i\Sigma(1/s_i)$.

two, IIIa and IIIb, were chosen to illustrate the fact that overdominant models exist such that the $D:L$ ratios are the same as for other models where $0 < h < 1$. Therefore, the $D:L$ ratio *by itself* cannot distinguish between partial dominance and overdominance.

COMPARISON OF THE PREDICTIONS FROM VARIOUS MODELS WITH OBSERVATIONS

Table 4 shows the total load (T), the detrimental load (D), the lethal load (L), and the $D:L$ ratio for each of the populations analyzed in this study. Similar data have been collected by other workers for natural populations as well as for new mutations induced by radiation.

The calculations given in Table 5 are based on published data of various authors who studied natural populations of several Drosophila species. Especially voluminous and useful for this analysis are the data of DOBZHANSKY and his associates.

TABLE 4

Genetic load, expressed in lethal equivalents, for the second chromosome in equilibrium populations of D. melanogaster

Population	n	Detrimental	Lethal	Total	$D:L$
Madison	231	.235 ± .029	.300 ± .051	.536 ± .049	.783
Wallace (a)	121	.185 ± .034	.272 ± .066	.457 ± .061	.680
Wallace (b)	113	.136 ± .029	.271 ± .063	.406 ± .060	.502
From weighted means	465	.180 ± .017	.287 ± .034	.467 ± .032	.627

TABLE 5

Genetic load, expressed in lethal equivalents, for several natural populations of Drosophila

Species	Chromosome	Number tested	Load: Detrimental (D)	Load: Lethal (L)	Load: Total (T)	$D:L$	Author
D. melanogaster	II	243	.345	.341	.686	1.012	GOLDSCHMIDT 1951
D. prosaltans	II	304	.179	.305	.484	.587	DOBZHANSKY and SPASSKY 1954
	III	284	.066	.061	.127	1.082	
D. willistoni	II	2004	.303	.380	.683	.797	PAVAN *et al.* 1951
	III	1166	.246	.271	.517	.908	
D. persimilis	II	106	.260	.186	.446	1.398	DOBZHANSKY and SPASSKY 1953
	III	172	.303	.194	.496	1.562	
	IV	140	.448	.175	.623	2.560	
D. pseudoobscura	II	326	.123	.157	.280	.783	DOBZHANSKY, HOLZ, and SPASSKY 1942
	IV	352	.268	.174	.442	1.540	
D. pseudoobscura	II	109	.516	.214	.730	2.411	DOBZHANSKY and SPASSKY 1953
	III	116	.421	.193	.614	2.181	
	IV	108	.346	.108	.454	3.204	
Weighted mean		...	.271	.280	.551	1.072	
Unweighted mean		...	.294	.212	.506	1.540	

The data were not always presented in a way that is adapted to our form of calculation, so that it has been necessary to make several assumptions and approximations. Firstly, 0–10 percent normal viability was made the criterion for lethality to conform with our procedure. In addition, our data are in the form of the ratio of two genotypes. However, some of the papers give the data as the proportion of the relevant class among the total, which overestimates the detrimental viabilities. If we let R stand for the ratio a/b, and P for the proportion $a/(a+2b)$, then $R=2P/(1-P)$. All the data originally given in proportions were corrected by this formula.

The $D{:}L$ ratio in our data is .627, whereas the weighted average for the other studies is 1.072. The data are variable, but in general the other species appear to have a $D{:}L$ ratio higher than we found.[5] However, the difference is not large.

These results imply that either (1) mutants with small effects occur with no greater frequency than lethals, or (2) they have more dominance than lethals and hence are eliminated relatively more rapidly as heterozygotes. It should be possible to distinguish between these two alternatives by examination of data on the $D{:}L$ ratio in newly occurring mutations. However, the data from various authors are not in agreement, as shown in Table 6.

In the TIMOFEEFF-RESSOVSKY (1935) data the $D{:}L$ ratio is .711 or 1.053, depending on the environmental conditions. An independent study on a smaller scale was done by FALK (1955), in which fitness was measured in terms of hatchability. For 56 chromosomes tested there were 3.5 times as many detrimentals

TABLE 6

Detrimental and lethal frequencies and the respective loads for newly induced mutations in D. melanogaster

Author	Chromosome	Number tested	Detrimentals		Load		$D{:}L$
			Lethals	Detrimental	Lethal	Total	
TIMOFEEFF-	I	432 (uncrowded)	1.9	.106	.149	.254	.711
RESSOVSKY (1935)	I	436 (crowded)	2.3	.140	.133	.273	1.053
KERKIS (1938)	I	134 (uncrowded)	2.7	...	...	...	...
		143 (crowded)	3.3	...	...	...	...
FALK (1955)	III	approx. 56	3.5	.092	.182	.274	.505
KÄFER (1952)	II	500 D_1	..	.036	.161	.197	.224
		500 D_2	..	.105	.398	.504	.264
		500 D_3	..	.166	.463	.629	.359
	I	500 D_1	..	.032	.063	.095	.508
		500 D_2	..	.013	.126	.138	.103
		500 D_3	..	.053	.195	.248	.272
	Mean	3000	.75	.068	.234	.302	.288
BONNIER and JONSSON (1955)	II	173	.82	.037	.172	.209	.215

[5] The data of GOLDSCHMIDT on *D. melanogaster*, which were gathered for another purpose, are not well suited for this type of calculation since there were no control experiments; so the value 1.012 should not be given undue weight.

as lethals, which is not significantly different from the 2–3 times obtained by TIMOFEEFF-RESSOVSKY and also by KERKIS. This is a minimum estimate, since mutants with very small effects would not have been detected. A conflicting result is represented in the work of KÄFER (1952), who examined 500 second and X chromosomes for each of three X-ray doses. The $D:L$ ratio averaged .288. Statistically detectable detrimentals were 0.75 as frequent as lethals. The results of BONNIER and JONSSON (1957) indicate slightly less detrimental effect giving a $D:L$ ratio of .215.

Assume that h is positive, ignoring for the moment the possibility of overdominance. If h is constant (Model Ia) the $D:L$ equilibrium *load* ratio is n/N, which is the same as the initial ratio of detrimental to lethal mutation *frequencies*. According to TIMOFEEFF-RESSOVSKY, KERKIS, and FALK, this is at least 2–3, perhaps considerably higher if there are a large number of mutants with minute effects. This value is much larger than the average of the $D:L$ ratios observed for natural populations.

On the other hand, if the value of h increases as s decreases the initial and equilibrium load ratios will be more similar. In fact, with the particular model (Ib) where hs is constant, they are the same. The observed $D:L$ ratios in our population (.627), which were obtained from uncrowded cultures, agree with the initial value from TIMOFEEFF-RESSOVSKY's uncrowded cultures (.711). This suggests that detrimentals are more dominant than lethals, and are eliminated *relatively* more rapidly.

This result is also in agreement with FALK's (1955) data, but his numbers are too small to be more than suggestive. ALLEN P. JAMES (unpublished) has also found evidence, in comparison of haploid and diploid yeasts, that mildly deleterious mutants are more dominant than those with drastic effects.

Nevertheless, in the light of KÄFER's results we must refrain from drawing any definite conclusions at this time. If it develops on subsequent investigation that the array of newly occurring mutations as reported by KÄFER is the more typical one, a different interpretation of the present results would ensue. Her observation of 75 percent as many detrimentals as lethals agrees well with our observed equilibrium $D:L$ ratios, giving support for Model Ia. To whatever extent her method failed to detect mutants of small effect there would be a departure in the direction of Model Ib.

At the moment there does not seem to be any way to distinguish between these two models until the discrepancy between the data on newly arising mutants can be clarified. However, there is no support from this analysis for model Ic (h/s constant). Our results imply that mutants with small homozygous effect have at least as much dominance as those of more drastic effect, and if the data of KERKIS and TIMOFEEFF-RESSOVSKY are relevant, considerably more dominance.

Possibly some of the difference in different experiments lies in the density of crowding in the cultures. Both TIMOFEEFF-RESSOVSKY and KERKIS showed that the ratio of detrimentals to lethals increased when the cultures were crowded. Possibly the nutrition was greater in quality or amount in KÄFER's experiments.

This may be an explanation of the slight discrepancy between our results and those of some others who studied wild populations. Our cultures were uncrowded, as evidenced by absence of negative correlations between different classes in a culture, and this may not have been true of other studies.

All the foregoing discussion has assumed that h is positive. It is necessary to consider the possibility that a large number of loci are overdominant. As stated earlier, the D:L ratio alone cannot distinguish between these two possibilities. However, other considerations make it seem unlikely that any appreciable fraction of loci are overdominant, if our data are correct.

MULLER and CAMPBELL (unpublished) and STERN, CARSON, KINST, NOVITSKI, and UPHOFF (1952) have published data on the amount of dominance of "recessive" lethals. In each case the heterozygotes had a viability reduction of about four or five percent, relative to nonlethal-containing controls. Since this is an average value, the possibility that some lethals are heterotic is not ruled out. HIRAIZUMI and CROW (1960) have carried out a similar study, but on lethals extracted from natural populations. These results also show a decreased viability of heterozygotes, the amount being about 2.5 percent. Furthermore, there were very few instances in which the same lethal was found more than once and none where it was present more than twice. A lethal that is heterotic, or even neutral in the heterozygous condition would accumulate in a much higher frequency than one that is disadvantageous in the heterozygote, so that this test of a natural population is much more stringent than a study of new mutants. Similar conclusions were drawn by CORDEIRO (1952) and DA CUNHA, DE TOLEDO, PAVAN, DE SOUZA, PIRES DE CAMARGO, and DE MELLO (1958). Therefore, we can conclude that the great majority of lethal genes in a population are "classical" and not heterotic, and in fact, have an appreciable heterozygous disadvantage, enough that this is the major factor in determining their frequency in the population.

If lethals are representative of mutants in general, then there are very few heterotic loci. However, the possibility remains that mutants with small homozygous effects are more frequently overdominant. Yet our data offer no support for this interpretation since the D:L ratio is low. If lethal mutations were partially dominant, but there were an appreciable number of overdominant loci with small homozygous effects, they would accumulate in the population in large numbers and inflate the D:L value. Since this value is already small, there is not room for very such many loci. For as their number increases, we must postulate still greater dominance or still smaller initial numbers of mildly deleterious mutants with $h > 0$. The most probable conclusion from our data is that heterotic loci make a relatively small contribution to the homozygous genetic load.

The suggestion that a major fraction of all loci may be heterotic has been made by WALLACE (1958) on the basis of a study of radiation induced mutations in which he finds that flies with a pair of chromosomes that are identical except for the fact that one has been radiated have a higher viability than flies with the same chromosomes without radiation. Presumably such radiation-induced mutants would be deleterious to some extent if made homozygous. Why do these not show up as a contribution to the homozygous load in our analysis?

The answer is not clear. As stated earlier, there is no evidence in these data that overdominant loci are making any appreciable contribution to the homozygous load. Since each locus of this type would make a disproportionately large individual contribution to D, this argues that the fraction of such loci must be very small. A population in which there is an overdominant locus where the harmonic mean homozygote viability reduction ($\bar{s}$) is .01 will have a homozygous load of one percent irrespective of the number of alleles. This follows from the formulae given under Model III. It would require 50 "classical" loci with $u = 10^{-5}$ and $h = .05$ to produce the same homozygous load, since this is u/h for each locus. This means that a very small minority of heterotic loci could cause a substantial reduction in homozygous viability. Furthermore, unless the number of alleles is very large, they would also cause a large reduction in viability of a randomly mating population. Of course, there may be many overdominant loci with extremely minute s values which would be impossible to detect by these methods. The question then becomes the number of nearly neutral alleles that can be maintained against random fixation.

The argument that only a small fraction of loci are overdominant does not imply that these are not important in a randomly mating population. On the contrary, a small minority could still be a major factor in determining the mean and variance of the population fitness.

Our analytical procedure is based on the assumption that different mutants act independently. There may well be appreciable interaction, particularly synergism such that multiple mutants are eliminated at a faster rate than if each one caused an independent probability of death. TH. DOBZHANSKY, LEVENE, B. SPASSKY, and N. SPASSKY (1959 and several earlier papers) have shown evidence for homozygous epistatic effects revealed by recombination between chromosomes from natural populations, but the data do not permit any estimate of the magnitude and direction of such interactions. More relevant to our problem are existing data on the linearity of inbreeding decline in yield of corn (NEAL 1935), various characters in guinea pigs (WRIGHT 1922), and litter size and weight in swine (DICKERSON, BLUNN, CHAPMAN, KOTTMAN, KRIDER, WARWICK, and WHATLEY 1954), which argue against such synergistic effects being large. On the other hand, STRINGFIELD (1950) reported that with certain maize hybrids backcrosses showed higher yields than F_2 populations, indicating some complementary interaction. The point could be tested further by comparison of homozygous and heterozygous chromosomes with different levels of background heterozygosity in the other chromosomes, or tests of combinations of mutants. Pending more explicit evidence that synergistic effects are large, the assumption of independence seems to us to be a reasonable first approximation.

SUMMARY

Four hundred and sixty-five second chromosomes were extracted from a natural population of *Drosophila melanogaster* and from a large population cage that had been maintained for several years. A comparison of the viability of homozygous

chromosomes and random nonidentical pairs revealed that the homozygotes had a viability reduction or genetic load of 47 percent. The ratio of the load due to mildly detrimental mutants (D) to that for lethals and near lethals (L) was .627. These results imply that either (1) mutants with small effects occur with no greater frequency than lethals, or (2) they have more dominance than lethals and hence are eliminated relatively more rapidly as heterozygotes.

Comparison of the D:L ratio in these populations, assumed to be somewhere near equilibrium, with that for newly induced mutations can give information on the comparative rate of elimination of lethals and detrimentals. The data are insufficient to distinguish between greater dominance for genes of small effect and the same average dominance for lethals and detrimentals. However, the data offer no support for the idea that mildly detrimental genes are less dominant, or for any substantial contribution to the homozygous load from overdominant loci.

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