THE INFLUENCE OF EPISTASIS ON HOMOZYGOUS VIABILITY DEPRESSION IN *DROSOPHILA MELANOGASTER**

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THIS study was carried out in an attempt to assess the importance of epistasis in the deleterious effects of homozygosity. The experimental procedure utilized mating systems, which have now become standard, whereby chromosomes from natural populations of *Drosophila melanogaster* are extracted and made homozygous. Interaction between chromosomes was measured by comparison of the viability of flies simultaneously homozygous for the two major autosomes with flies homozygous for one or the other. At the same time, intrachromosome interactions were studied by comparison of three levels of inbreeding in a particular chromosome: 100% homozygosity due to identity by descent ($F = 1$); 50% reduction in heterozygosity ($F = .5$); and random combinations ($F = 0$).

The interaction of homozygous lethals and severe detrimentals is of minor consequence to the population because these mutants are of such low individual frequency that simultaneous homozygosity for two or more is a very unusual event in non-inbred populations. On the other hand, mildly deleterious genes are common enough as a group that their homozygous interactions may possibly have appreciable population consequences. Furthermore, their homozygous effects could be of the same general magnitude as their heterozygous effects and those of more drastic chromosomes, and it is as heterozygotes that most mutants probably have their major effect on the population. For these reasons, we have concentrated mainly on the interactions of mildly deleterious chromosomes.

The results to be presented here show no statistically significant systematic interaction between mildly deleterious homozygotes on the two major autosomes. Within a chromosome there is a barely significant synergistic interaction. Our results will be compared with those from a similar experiment by SPASSKY, DOBZHANSKY, and ANDERSON (1965).

EXPERIMENTAL METHODS

Natural populations of *Drosophila melanogaster* at two locations in Madison, Wisconsin provided the material for the study. Flies for Experiments I, III, and IV were collected during the early fall seasons of 1962, 1963, and 1965, and those for Experiment II were collected in August and September of 1963 at another location approximately a mile distant.

Males and females from the populations were mated in single pairs to generate wild-type

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daughters, designated as generation 1. Ultimately only one progeny female from each original mating was used for analysis, thus making it possible to have virgin females that are equivalent to a random sample of the wild population, regardless of whether or not the original female had been inseminated before capture.

The essential plan was to extract second and third chromosomes from the generation-1 females and measure their viability effects when homozygous, separately and jointly, and when heterozygous. By a series of matings shown in Figure 1 and described in detail below, these autosomes were balanced over the dominant, inversion-associated markers *Cy* (Curly wings) and *Me* (Moire eyes) on chromosomes *2* and *3*, respectively. With "+" indicating the appropriate chromosome (*II* or *III*) from nature, the cross $+_{II}/Cy$; $+_{III}/Me \times +_{II}/Cy$; $+_{III}/Me$ gives a theoretical ratio of

$$\begin{aligned} &4\ +_{II}/Cy;\ +_{III}/Me \\ &2\ +_{II}/+_{II};\ +_{III}/Me \\ &2\ +_{II}/Cy;\ +_{III}/+_{III} \\ &1\ +_{II}/+_{II};\ +_{III}/+_{III} \end{aligned}$$

The "+" homologues in a progeny fly can be descended from a) the same chromosome in a generation-1 female, b) the same generation-1 female, or c) independent generation-1 females,

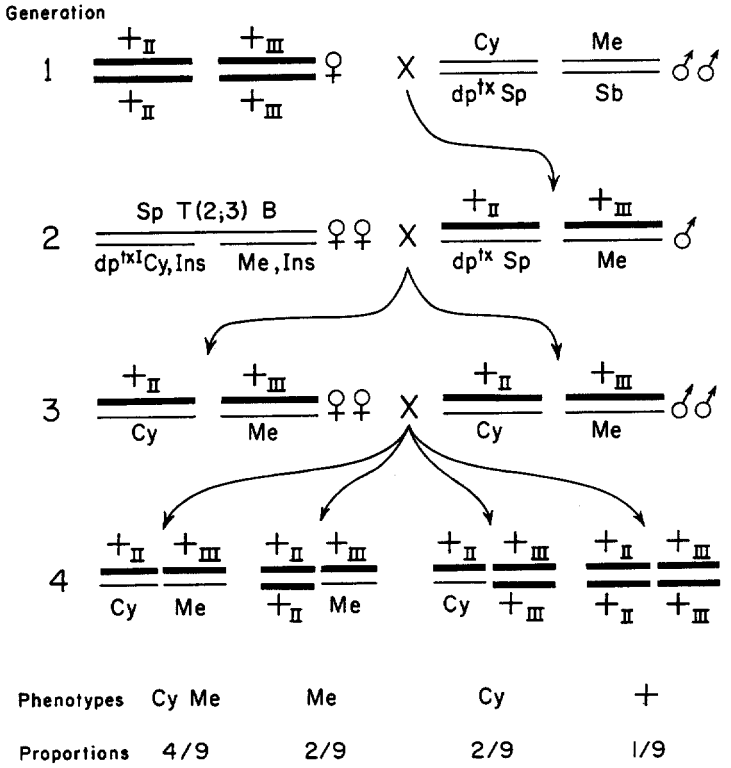

FIGURE 1.—The mating scheme used to produce and identify flies homozygous ($F = 1$) for the second, third, or both chromosomes. Chromosomes from nature are indicated by thick lines. The genotypes of the laboratory stocks are abbreviated to show only the pertinent markers. This figure shows the descendants of a single generation-2 male (e.g., 1A in Figure 2). The entire mating scheme, including modifications to produce F values of 0 and .5, is shown in Figure 2.

to correspond to inbreeding coefficients of 1, .5, or 0 for each autosome. The relative frequencies of the four classes at the different inbreeding levels give a measure of the effect of homozygosity or semi-homozygosity on viability.

Two special stocks synthesized by H. J. MULLER were used to facilitate the collection of virgin females for the final cross. These carried several balanced lethals, dominant markers, and inversion systems to suppress crossing over. One of the stocks contained, in addition, a translocation between chromosomes *2* and *3* which had the labor saving effect of eliminating through aneuploidy certain unnecessary progeny types in generation 3. The relevant marker genes, all of which were lethal when homozygous, were, for chromosome *2*, dp^{tx} and dp^{txI} (lethal dumpy-thoraxate alleles), *Sp* (Sternopleural bristles), and *Cy* associated with inversions. For chromosome *3* they were *Me*, *Sb* (Stubble bristles), and Sb^{l}, a recessive lethal allele of Stubble. The stocks contained *Cy* with either the inversions *Ins(2L + 2R)Cy* or the more viable and complex *Cy,InsO5* (OSTER 1956), along with one or both *Me* inversions as indicated in the formulae below. In addition one stock carried the inversions *In(3R)C* and *sep,In(3LR)*, named for separated wings. A few other mutants not of direct relevance to these experiments were also present. For a detailed description of all symbols the reader is referred to BRIDGES and BREHME (1944), MULLER (1916, 1955), or MULLER and OSTER (1963).

Experiments I, II, and III, using uncrowded cultures, were carried out as follows (see Figure 1). Each generation-1 wild-type virgin female (numbered consecutively from 1, 2, . . . n) was crossed singly with a few males from the first stock: dp^{tx} *Sp cn/Cy,Ins(2L + 2R)Cy* cn^{2} sp^{2}; *In(3L)Me In(3R)C e l(3)e/ sep,In(3LR) ri* p^{p} *Sb*. From each mating, *two* generation-2 sons of the composition dp^{tx} *Sp cn/*$+_{II}$; *Me . . ./*$+_{III}$ were collected and crossed individually to several virgin females from the second stock: *S Sp T(2;3)B bw/*dp^{txI} *Cy,InsO5 pr* cn^{2}; *Me,Ins ri* Sb^{l}. From the generation-3 progeny of each such cross six males and six virgin females of the composition $+_{II}/Cy$; $+_{III}/Me$ were collected and distributed for three types of matings, two pairs of parents per vial (or in a very few cases, one or two females × one male, depending upon availability).

As previously stated, the mating system, diagrammed in Figure 2, was designed to provide three levels of inbreeding for genes on the second and third chromosomes from nature. Indicated by *F*, WRIGHT's "Coefficient of Inbreeding" (1922), they are:

(1) $F = 1$. Here the mating in generation 3 was between full sibs, descended from the same generation-2 male and, therefore, necessarily carrying identical second and third chromosomes. Using the descendants of generation-1 female line number 1 as an example, the generation-2 males are designated 1A and 1B. In generation 3 a cross was performed between the sons and daughters of 1A, symbolized as 1A × 1A. Similarly there was a cross among the offspring of 1B, that is 1B × 1B. In generation 4, progeny appear which are completely homozygous for chromosomes 2 or 3 or both.

(2) $F = .5$. Here the mating in generation 3 was between males and females descended from the same generation-1 female but different generation-2 sons. Using line 1 as an example again, the matings are symbolized as 1A × 1B and its reciprocal, 1B × 1A. Since, for any particular locus, there was a 50% probability of the generation-2 sons inheriting the identical allele from their mother, the wild-type progeny in generation 4 have an average inbreeding coefficient of 50% for the genes from nature.

(3) $F = 0$. In this series the mating in generation 3 was between males and females descended from different generation-1 females. For example, the combinations were: 1A × 2B, 1B × 2A, and so on, where the lines were cyclically permuted in a series (i.e., 2A × 3B etc.). This set, with its random combinations of chromosomes, is sometimes referred to as the "heterozygous" group. It served as the control, against which the viability reductions were measured.

This system was designed to insure equal representation of each line in each of the three inbreeding sets. For any particular line the three sets were processed simultaneously and the flies reared under the same laboratory conditions. At generation 3 the parents were transferred to fresh food after four days and then removed after another four, giving two four-day broods. Generation-4 progeny were counted on the 12th and 18th days after the start of each brood; that

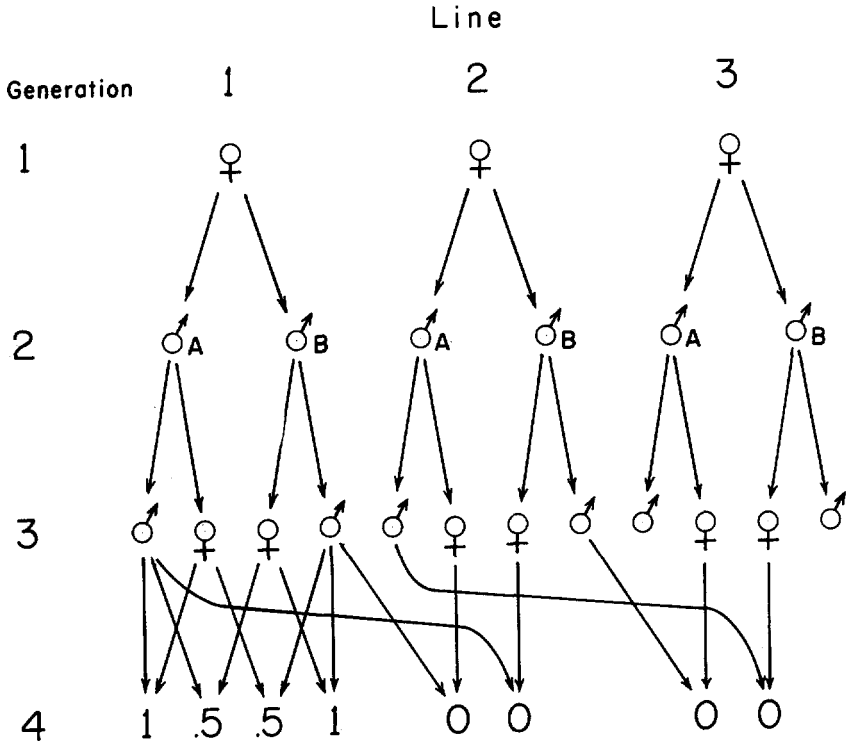

FIGURE 2.—The pedigree for obtaining chromosomes with three degrees of homozygosity, indicated by the inbreeding coefficients given in the bottom row. The matings shown in generation 3 descending from female line 1 are repeated in all the lines. Crosses between independent lines are cyclical: 1A♂ × 2B♀, 1B♂ × 2A♀, 2A♂ ×3B♀, 2B♂ × 3A♀, . . . nA♂ × 1B♀, nB♂ × 1A♀. Thus each chromosome appears the same number of times at each inbreeding level.

is, all progeny emerging through the 18th day were recorded. The average number of progeny per pair of vials was 100 at $F = 0$, 91 at $F = .5$, and 84 at $F = 1$ (see Table 2).

Approximately 800 generation-1 female lines (or about 1600 generation-2 males) were analyzed in this way over a period of many months, with 25–30 lines processed at a time. Since the original collections from nature were seasonally restricted, it was necessary to store many of the lines, either as generation-1 females or as generation-2 males, at 19°C for various lengths of time before processing.

In Experiment IV the genetic techniques and stocks were the same as described above and in Figure 1. Since the intention here was to observe the relationship between genetic load and environmental conditions, generation 4 in this experiment was raised in cultures that were more crowded than the above. In particular, we were interested in seeing whether more rigorous laboratory conditions, which might be more representative of the situation in nature, would influence the ratio of the detrimental to the lethal load or the interaction. The modifications from Experiments I–III are as follows:

(1) In generation 1 the mating in Experiment IV is the reciprocal of that in Figure 1, i.e., a wild-type *son* of a female collected in nature was mated to the first marked stock. The reason for using a female in the uncrowded series had been to allow for free recombination of genes appearing in her sons in generation 2, whose progeny were then mated to produce an average inbreeding coefficient of .5. In Experiment IV only the $F = 1$ and $F = 0$ sets were constructed.

(2) In generation 2, only one son was tested per line, again because there was no analysis of

$F = .5$. He was crossed with 10 virgin females from the translocation stock in order to produce a large number of progeny.

(3) In generation 3, 20 $+_{II}/Cy$; $+_{III}/Me$ virgin females and 20 males of the same genotype were collected from each cross and used in the $F = 1$ and $F = 0$ matings as described previously, but here with 10 pairs of parents (or in a few cases 9 pairs) per vial. When it was not possible to obtain nearly 20 virgin females of the required type, $F = 1$ was given preference and $F = 0$ omitted. However, for every group of $F = 1$ matings completed (277 in all), there was a contemporary sample of $F = 0$ crosses (113 in all). The parents were transferred to new food after six days and removed after another six. There were usually three progeny counts for each of the two six-day broods. The mean total numbers of progeny per pair of vials were 202 at $F = 1$ and 231 at $F = 0$.

A small subsidiary study pertaining to the crowding method was performed to test the necessary assumption that the number of eggs deposited by each female was independent of the density of the culture. For this investigation, $+_{II}/Cy$; $+_{III}/Me$ flies were collected from generation 3 during Experiment IV and mated in vials as in the $F = 0$ series, in pairs of two or of ten. After three days they were transferred to a second set of vials, allowed to lay eggs for three more days, and then discarded. All vials were randomized to eliminate possible bias. Eggs deposited in the second set of vials were allowed to develop there, whereas the food from the original vials, with the eggs on it, had been carefully removed and transferred, immediately after the parents were emptied, to a large bottle containing its usual amount of fresh medium. The mean numbers of progeny recovered in two such small experiments are shown in Table 1. With ten pairs of parents more progeny developed when a larger amount of food was available, in the bottles, but this did not occur with two pairs. There was an approximate five-fold increase in mean progeny number in comparing two pairs of parents in vials or bottles, about 25, with ten pairs in bottles, 126.4, supporting the initial assumption.

METHODS OF ANALYSIS

The homozygous viability effects have been estimated from the data according to the basic method developed in detail by GREENBERG and CROW (1960). As extended to inter-chromosomal interactions, the model may be outlined as follows (see also CROW 1968):

Let the *observed* numbers (or ratios) in the four phenotypic classes in generation 4 be:

	Cy Me	+ *Me*	*Cy* +	+ +
$F = 0$	W	X	Y	Z
$F = 1$	w	x	y	z

The *expected* ratios are:

$F = 0$	W	X	Y	Z
$F = 1$	W	XS	YT	$ZSTI$

where

S = viability of homozygotes for the second chromosome relative to that of heterozygotes for this chromosome

T = viability of homozygotes for the third chromosome relative to that of heterozygotes for this chromosome

I = a measure of interaction.

If the viability effects of the two homozygous chromosomes are independent, then the ratio of survivors in the double homozygotes will be the product of those for the single homozygotes, and $I = 1$. When I is less than 1, there is positive or reinforcing epistasis. Alternatively stated, there is a synergistic deleterious effect of the two pairs of homozygous chromosomes. When I is greater than 1, there is negative or opposing epistasis. For synthetic lethals, S and T would be about 1 and I would be 0. It is, of course, not possible on this model to measure I for classical lethals, whose separate viability values are each zero.

Consistent estimates of the viability parameters may be obtained by equating the expectations to the observations. To neutralize the effect of the background genotype and the marker genes, this is done in terms of the ratios between the appropriate classes and the *Cy Me* class. Specifi-

TABLE 1

Mean total numbers of progeny in experiments on density effects

Experiment	2 pairs of parents			10 pairs of parents		
	N	Vials	Bottles	*N*	Vials	Bottles
1	9	33.2	27.7	10	96.4	147.3
2	10	21.8	21.7	12	79.2	117.3
Pooled	19	27.2	24.5	22	87.0	126.4

N = number of replications.

cally, for S we focus on the + *Me* class, where the effect of chromosome *2* is manifested. The observed ratio of + *Me* to *Cy Me* among the homozygotes to the same ratio among the heterozygotes is $(x/w)/(X/W)$. In terms of expected ratios this is $(XS/W)/X/W) = S$. Equating the observed to the expected, we have the estimating equation

$$S = xW/Xw$$

Similarly,

$$T = yW/Yw$$
$$I = XYwx/xyWZ$$

The S and T viabilities are each based on only two phenotypic classes. It would also be possible to estimate S from Yz/yZ and average this with the above estimate, thereby reaching the greater precision inherent in more data. Likewise T could be estimated using Xz/xZ. However, this necessitates that I be 1, an assumption we wish to avoid.

The homozygous effects may be alternatively expressed as loads, which give the total *reduction* in viability which would occur if each of the mutant genes involved were made homozygous in a different individual (MORTON, CROW and MULLER 1956). Assuming a series of independently acting genes, the probability of surviving the adverse effects of all these genes is given approximately by the first term of the Poisson distribution. Thus, $S = e^{-s}$, $T = e^{-t}$, and $I = e^{-i}$, where s and t are the mean homozygous loads on the second and third chromosomes, respectively, and i is the mean interaction load. The loads are additive rather than multiplicative; for example, in the double homozygote the load is $(s + t + i)$ whereas the relative survival is STI. If the second and third chromosomes are independent in their effects, $i = 0$. Expression in terms of loads corrects for cases of more than one mutant on a chromosome, so that s, for example, is larger than $1\text{-}S$. The loads are computed from the data by the formulae:

$$s = \ln(Xw/xW)$$
$$t = \ln(Yw/yW)$$
$$i = \ln(xyWZ/XYwz)$$

The equivalent survivals and loads at $F = .5$ are obtained by substituting the observed numbers of w, x, y, and z at that inbreeding level.

In the experiments the lethal chromosomes are readily identifiable, permitting a subdivision of each homozygous load into lethal (L) and detrimental (D) portions. Because of the observation of occasional survivors, whose sporadic appearance is sensitive to environmental conditions, time of emergence, and other variables, the results are more reproducible if all cultures with less than 10% of the expected frequency are classified as lethal (GREENBERG and CROW 1960). The detrimental load itself is further subdivided into two components, the severe detrimentals (D_s), with 10–50% of the control viability, and the mild detrimentals (D_m), with more than 50%. D_s is approximately comparable to the semilethals described by several authors, and D_m approximately to quasinormals or subvitals.

To isolate the viability or load components associated with mild detrimentals, severe detrimentals, and lethals, we extend the methods of GREENBERG and CROW (1960). Let A, B, C, and D, stand for observed viabilities as follows:

A = the viability of heterozygotes for the chromosome under consideration (always measured relative to the *Cy Me* class)
B = the viability of all homozygotes
C = the viability of nonlethal homozygotes (>10%)
D = the viability of nonlethal, non-severe-detrimental homozygotes (>50%)

Now, let h be the fraction by which average viability is reduced due to heterozygous effects, l the average reduction by lethals, s by severe detrimentals, and m by mild detrimentals—each class assumed to act independently of the others. (Note that the use of D and s as just defined immediately above is to be restricted to this section on analytical methods only. These are to be distinguished from D for detrimental load and s for second chromosome load as used elsewhere throughout the paper.) Then the expected viabilities are:

$$\text{Exp } (B) = K(1\text{-}h)(1\text{-}l)(1\text{-}s)(1\text{-}m) = K V_h V_l V_s V_m$$
$$\text{Exp } (C) = K(1\text{-}h)(1\text{-}s)(1\text{-}m) = K V_h V_s V_m$$
$$\text{Exp } (D) = K(1\text{-}h)(1\text{-}m) = K V_h V_m$$
$$\text{Exp } (A) = K(1\text{-}h) = K V_h$$

where K is a proportionality constant. V_m, V_s, V_l, and V_h are the relative viability components associated respectively with mild detrimentals, severe detrimentals, lethals, and the heterozygous effects of all of these. Note that the viabilities A-D are directly observed, whereas the V series is derived. Thus V_m is what the average relative viability of homozygotes would be if all lethals and severe detrimentals could somehow be removed, leaving only the mild detrimentals. Likewise, V_l is the relative viability of homozygotes containing only lethals, and V_s, only severe detrimentals. $V_m V_s$ $(= V_d)$ would apply to homozygotes containing both kinds of detrimentals, but no lethals. We make no attempt here to estimate V_h.

From the relations above, the components are obtained as follows:

$$V_m = 1\text{-}m = D/A$$
$$V_s = 1\text{-}s = C/D$$
$$V_l = 1\text{-}l = B/C$$
$$V_d = V_m V_s = C/A$$

Notice that $V_m V_s V_l = (D/A)(C/D)(B/C) = (B/A)$, as it should, showing that the viability of all homozygotes is the product of the component viabilities.

This compartmentalization of viabilities may be applied to the second chromosome (S), third (T), or interaction (I), as defined earlier. As an example, to calculate $V_m = D/A$ for the second chromosome, we compute xW/Xw using only homozygotes with greater than 50% viability and, of course, heterozygotes. The same calculation using only homozygotes with viability greater than 10% gives $V_d = C/A$. The ratio of these is $(C/A)/(D/A) = C/D$, which is an estimate of V_s. Similarly V_l for chromosome 2 is estimated by the ratio of xW/Xw for all homozygotes divided by the same quantity for nonlethal homozygotes. For T we use the appropriate yW/Yw ratios.

The corresponding loads are given by the negative natural logarithms, which may be applied similarly to get s, t, and i. In general, the loads for mild detrimentals, severe detrimentals, and lethals are, respectively:

$$D_m = -\ln(D/A)$$
$$D_s = -\ln(C/D)$$
$$L = -\ln(B/C)$$

The load for all homozygotes is $-\ln(B/A)$, the sum of the component loads.

The loads computed this way give the full effect each independent component would have if it could be measured in the absence of the others. For example, D_m measures the load caused by mild detrimentals, including those that are hidden by being on the same chromosome as a lethal.

There are two sources of error not corrected by this method. One is caused by possible misclassification. This would happen, for example, if a chromosome with several detrimentals has its viability so reduced that it is classified as a lethal, leading to a possible overestimate of the lethal frequency. However, this is not likely to be an appreciable error because of the relatively

small number of chromosomes with homozygous viabilities in the range from 10–60% (TEMIN 1966). On the other hand, there is a greater possibility of underestimating the effect of the mildly detrimental class by erroneously classifying some of the chromosomes as carrying severe detrimentals. The other source of error has to do with interactions between the classes. If the interaction is reinforcing, as later data in this paper suggest, there would again be a tendency to overestimate the severe detrimental effect at the expense of the mild detrimentals. Therefore, for both reasons, it is quite possible that the D_m load is slightly underestimated.

The loads were obtained from means calculated by four methods, using the CDC 1604 computer: (1) Weighted means, using the natural logarithms of the total numbers of flies in the appropriate classes in all cultures combined. (2) Unweighted means of the natural logarithms of ratios between appropriate classes. For example, to obtain the load on chromosome 2, $\ln(x/w)$ was computed for each culture. (3) Weighted means of natural logarithms of appropriate ratios for each culture, where the weighting factor was proportional to the harmonic mean of the number in the relevant classes. (4) Same as 3, but the weighting factor was proportional to the arithmetic instead of the harmonic mean.

Notice that method (1) simply uses the pooled data from all cultures, thus giving each fly equal weight. It is also equivalent to obtaining the weighted mean of the ratio of the class of interest to the *Cy Me* class, the weight being the number of *Cy Me* flies in the culture. For example, by method (1)

$$s = \ln \frac{\Sigma X}{\Sigma W} - \ln \frac{\Sigma x}{\Sigma w}$$

where x, w, X, and W stand for the number of flies in the appropriate classes per culture. But this can also be written

$$s = \ln \frac{\Sigma W\left(\frac{X}{W}\right)}{\Sigma W} - \ln \frac{\Sigma w\left(\frac{x}{w}\right)}{\Sigma w}$$

showing that these are weighted averages of the ratios.

The four methods gave quite similar load values. We have used method (1) to present the main loads in this paper, as being probably the most precise and least biased.

However, method (1) is not suitable for measuring interactions. Spurious negative interactions can enter if the homozygous values are heterogenous, as the following simple hypothetical example shows.

Culture	w	x	y	z	wz/xy
1	100	90	90	81	1
2	100	80	90	72	1
3	100	80	80	64	1
4	100	70	70	49	1
5	100	20	20	4	1
6	100	10	10	1	1
Total	600	350	360	271	1.3

Even though there is no interaction in any single culture, there is in the total. This suggests averaging wz/xy for each culture, but that quantity is highly skewed. Therefore, we have averaged the natural logarithms of these quantities. The amount of information per culture with respect to $\ln(wz/xy)$, if the underlying distribution is multinomial, is

$$\left(\frac{1}{w}+\frac{1}{x}+\frac{1}{y}+\frac{1}{z}\right)^{-1}$$

so the interaction value for each culture was weighted by the harmonic mean of the four quantities (method 3).

RESULTS

Genetic loads: The total numbers of flies in the four classes in generation 4 are given in Table 2 for each experiment. Any pair of cultures with fewer than 5

TABLE 2

Total numbers of flies in four phenotypic classes

Experiment	N	*Cy Me*	+ *Me*	*Cy* +	+ +
		$F = 1$			
I	366	18,863	6,305	6,765	2,178
II	892	39,051	12,528	15,578	5,024
III	320	13,234	4,722	5,637	2,148
IV	277	31,187	9,873	11,203	3,591
		Means			
I-III	1578	45.09	14.93	17.73	5.93
IV	277	112.59	35.64	40.44	12.96
		$F = .5$			
I	350	17,726	7,520	7,973	3,401
II	877	36,160	14,635	17,788	7,342
III	282	11,011	4,675	5,975	2,464
		Means			
I-III	1509	43.01	17.78	21.03	8.75
		$F = 0$			
I	360	17,900	8,735	9,688	4,762
II	871	35,826	17,406	21,352	10,471
III	312	11,517	5,917	7,328	3,468
IV	113	11,479	5,477	6,139	3,050
		Means			
I-III	1543	42.28	20.78	24.87	12.12
IV	113	101.58	48.47	54.33	26.99

Mean numbers per line are given for Experiments I-III combined and for Experiment IV. F = inbreeding coefficient; N = number of lines.

Cy Me is omitted. If the descendants of each male in generation 2 are regarded as a separate line, there were 1855 lines in the entire study, with a total of 505,658 flies counted. The observed ratio in the $F = 0$ group differs significantly from the theoretical 4:2:2:1 ratio, due to the consistently lower viability of the third chromosome carrying the marker *Me*.

Table 2 also gives the mean number of flies per line in each class. The increasing mean numbers in the + *Me*, *Cy* +, and + + classes with decreasing inbreeding is, of course, expected. The accompanying decrease in *Cy Me* shows a slight replacement effect, also reported in earlier work (TEMIN 1966). The data are pooled for Experiments I, II, and III where two pairs of parents were used, and compared with the results for Experiment IV, using ten pairs. While the number

of parents increased 5-fold, the number of progeny increased a little less than 2.5-fold, indicating some crowding and a smaller proportion of survival to adulthood.

The data of Table 1 support the assumption that in the density range of these experiments the number of eggs deposited per female was independent of crowding. With two pairs of parents there was sufficient food in the vials for all potentially emergent flies to develop, but with ten pairs the maximum emergence was not realized because of competition within vials, as shown by increased emergence with greater food supply. The data to follow show, however, that this level of crowding was not enough to influence the homozygous loads.

Table 3 gives the frequencies of lethals on chromosomes *2* and *3* appearing separately and in combination. The Chi-square values are conventional tests of independence in a 2×2 contingency table. The agreement with independence is very close at both inbreeding levels. As expected, the population is in gametic phase equilibrium with regard to lethals on these autosomes.

The mean frequency of lethals on the second chromosome, .230, confirms the value of .231 found earlier (TEMIN 1966). The frequency on the third chromosome, .225, is close to what would be predicted from the approximately equal lengths of the two autosomes.

Table 4 presents the loads at $F = 1$ obtained by method (1), as described above. The total load is divided into its components caused by lethals (L), severe detrimentals (D_s), and mild detrimentals (D_m). There is no systematic difference

TABLE 3

The independence of lethal frequencies on the second and third chromosomes

Experiment	N	Observed lethals			Expected lethals	χ_1^2	P
		2	*3*	*2* and *3*	*2* and *3*		
			$F = 1$				
I	366	86	87	25	20.4	1.742	.19
II	892	226	203	46	51.4	.995	.32
III	320	63	76	17	15.0	.453	.50
IV	277	51	52	9	9.6	.052	.82
Total number	1855	426	418	97	96.0	.018	.90
Proportion		.230	.225	.052	.052		
			$F = .5$				
I	350	30	40	5	3.4	.889	.31
II	877	119	104	16	14.1	.332	.56
III	282	30	38	0	4.0	4.015	.05
Total number	1509	179	182	21	21.6	.021	.91
Proportion		.119	.121	.014	.014		

$F = 1$: Total χ^2 for 4 df = 3.242. Heterogeneity $\chi^2_3 = 3.224$. P = .37

$F = .5$: Total χ^2 for 3 df = 5.236. Heterogeneity $\chi^2_2 = 5.215$. P = .07

The expected are computed on the basis of independence. The list under "*2*" gives the number of lines with chromosome *2* lethals whether or not accompanied by lethals on chromosome *3*; likewise for "*3*". N = total number of lines.

TABLE 4

Homozygous loads at F = *1*

Experiment	Total load	D_m	D_s	L	D_m/L	D/L
			Second Chromosome			
I	.378	.090	.048	.240	.375	.575
II	.415	.079	.053	.283	.279	.466
III	.365	.113	.070	.182	.621	1 005
IV	.410	.099	.076	.235	.421	.745
Total	.404	.095	.062	.247	.385	.636
TEMIN '66	.409	D = .147		.262		.563
			Third Chromosome			
I	.412	.067	.069	.276	.243	.493
II	.401	.105	.047	.249	.422	.610
III	.401	.153	.038	.211	.725	.905
IV	.398	.120	.056	.221	.543	.796
Total	.415	.122	.053	.240	.508	.729

Load components are D_m for mild detrimentals (viability $>$ .5), D_s for severe detrimentals (.1 — .5), and L for lethals ($<$ .1). $D = D_m + D_s$.

between Experiment IV, where the cultures were crowded, and the uncrowded Experiments I-III in either the loads or the D/L ratios. The crowding might have been expected to convert some normals into mild detrimentals, some mild into severe detrimentals, and the latter into lethals. Not only could the total load possibly be increased in this way but since there are more mild than severe detrimentals (TEMIN 1966) this might inflate the D value more than the L value, leading to a larger D/L ratio. However, none of this was observed; greater crowding may be needed to demonstrate such a possibility.

For all experiments combined the total load on chromosome *2* was .404, of which .157 is caused by detrimentals and .247 by lethals. This is in excellent agreement with the respective values of .409, .147, and .262 reported earlier by TEMIN (1966), using different tester stocks. The loads on chromosome *3* are similar. For both chromosomes the mild detrimentals as a group make a larger contribution than the severe, despite the much smaller effect per gene. The total genetic load due to hidden viability factors on the major autosomes is .819 lethal equivalents per gamete, or 1.64 per zygote, mostly due to lethals.

Table 5 shows corresponding data for an inbreeding coefficient of .5 per tested chromosome, confirming that a substantial fraction of the load is due to lethals. The lethal and severe detrimental loads are just about half as large as for $F = 1$, as expected. The D_m class appears to be somewhat less than expected; this will be considered later.

The loads are translated into viability coefficients in Table 6, by the formulae $S = e^{-s}$ and $T = e^{-t}$. A population made completely homozygous for all second chromosomes would have about 67% of the mean viability of a randomly mating population (or for the third chromosomes, 66%). Homozygosis for all second

TABLE 5

Homozygous loads at F = .5

Experiment	Total load	D_m	D_s	L	D_m/L	D/L
			Second Chromosome			
I	.140	.032	.024	.084	.381	.667
II	.183	.012	.032	.139	.086	.317
III	.191	.070	.030	.090	.778	1.111
Total	.173	.028	.029	.115	.243	.496
			Third Chromosome			
I	.185	.031	.037	.117	.265	.581
II	.192	.032	.028	.132	.242	.455
III	.159	.024	.029	.106	.226	.500
Total	.184	.030	.031	.124	.242	.492

TABLE 6

Mean viabilities of homozygotes at two breeding levels, expressed as a fraction of heterozygous viabilities

	$F = 1$			$F = .5$		
	S	T	I	S	T	I
V_m	.91	.89	.97	.97	.97	.98
V_s	.94	.95	. .	.97	.97	. .
V_d $(= V_m V_s)$	.85	.84	. .	.94	.94	. .
V_l	.78	.79	. .	.89	.88	. .
V_{ho} $(= V_d V_l)$	.67	.66	. .	.84	.83	. .

All experiments are pooled. S = second chromosome viability, T = third chromosome viability, I = interaction. V_d = all detrimentals, V_m = mild detrimentals, V_s = severe detrimentals, V_l = lethals, V_{ho} = all homozygotes.

chromosomes except those with lethals would give about 85% of the normal viability, the reduction being due to detrimentals. A group of second chromosomes homozygous for mild detrimentals only would have 91% of normal viability. These values are multiplicative; for example, for the second chromosome .91 × .94 × .78 = .67.

Intra-chromosome interactions: Table 7 compares the loads at $F = 1$ and $F = .5$ for Experiments I-III. The loads for the second (s) and third (t) chromosomes have been summed, and the loads at $F = .5$ have been doubled. The total load is more than twice as large for $F = 1$ as for $F = .5$. Examination of the components separately reveals that this non-linearity of inbreeding effect lies almost entirely within the D_m group. (This type of analysis can be done separately for chromosomes *2* and *3*, by comparison of Tables 4 and 5, and shows the effect to be consistent in the two autosomes.) That there is no interaction in the lethal group is, of course, implicit in the method; two lethals have the same mortality as one. There is also no evidence for interactions in the D_s group, for these genes are rare

TABLE 7

Comparison of loads at F = *1 and* F = *.5 in Experiments I–III*

Experiment	Total load		D_m		D_s		L	
	F_1	$2F_{.5}$	F_1	$2F_{.5}$	F_1	$2F_{.5}$	F_1	$2F_{.5}$
I	.790	.650	.157	.126	.117	.122	.516	.402
II	.816	.750	.184	.088	.100	.120	.532	.542
III	.766	.700	.266	.188	.108	.118	.393	.392
Total	.796	.714	.190	.116	.106	.120	.500	.478

The loads for second and third chromosomes are combined. F_1 = load at $F = 1$; $F_{.5}$ = load at $F = .5$. In the absence of interaction $F_1 = 2F_{.5}$.

enough (see TEMIN 1966 for frequency distribution) that the occurrence of two of them on a single chromosome is unlikely.

The apparent difference between the mild detrimental load for $F = 1$ and twice that for $F = .5$ is at the borderline of statistical significance. The calculations in Table 7 are based on method (1) which does not lend itself to an empirical estimation of the variance. Using data from method (3), which gave almost identical loads, there is a difference between the two values of .0674 ± .033.

This positive interaction effect shown in the D_m group is minimal in the respect that the method does not measure interactions for very closely linked genes. The chromosomes from the original generation-1 female were allowed to undergo only one generation of recombination, so that closely linked genes would remain in the same linkage phase and not show any differential effect at the two values of F.

Inter-chromosome interactions: For interactions between chromosomes we also concentrate on the D_m group. The lethals, by definition, do not interact, and the D_s genes are near-lethal and extremely rare in the population. Thus, the i value for the D_s mutants is not of great significance in population dynamics, since these mutants would be eliminated primarly through their main effects on the individual homozygotes (if indeed they are eliminated as homozygotes at all), and only very rarely through joint effects. On the other hand, the i value for the more common mild detrimentals could be an important factor in selection against these genes.

Table 8 gives the harmonically weighted mean values of ln (XY/WZ), calculated by method (3), for each inbreeding level for lines which are mildly detrimental on both chromosomes *2* and *3*. For all experiments the weighted mean of −.009 at $F = 0$ is very close to the expected value of zero for independence, although there was considerable variation among experiments. Differences between the means at $F = 0$ and at $F = 1$ or $F = .5$, respectively, give the i loads. The mean i is .021 ± .018 at $F = 1$ and .020 ± .018 at $F = .5$. (The latter i was obtained from the mean ln for Experiments I-III only, not shown for $F = 0$ in the table.) Thus, the interactions are small and not significantly different from zero. Moreover, in Experiments I-III the variance is underestimated due to the non-independence of the two lines (A and B, see Figure 2) descending from each genera-

TABLE 8

Interactions between chromosomes containing mild detrimentals

Experiment	$F=1$		$F=.5$		$F=0$		$F=1$	$F=.5$
	n	$\overline{ln}$	n	$\overline{ln}$	n	$\overline{ln}$	i	i
I	173	.056	240	.019	357	—.016	.073±.041	.036±.035
II	403	.001	579	.006	865	—.021	.022±.027	.028±.024
III	157	—.049	188	.042	310	.066	—.116±.045	—.024±.045
IV	149	.030	...	...	113	—.043	.073±.038	... ...
Total	882	.011	1007	.016	1645	—.009	.021±.018	.020±.018
Total*	896	.028	1016	.032	1656	.001	.027	.024
Total flies	113,800		107,204		180,515			
SPASSKY *et al.*	189	.045				—.080	.125	
Total flies	141,827				122,701			

$W = Cy\ Me$, $X = +\ Me$, $Y = Cy\ +$, $Z = +\ +$. The designation $\overline{\ln}$ is the mean of $\ln(XY/WZ)$ for each line, weighted by the harmonic mean of W, X, Y, and Z. Other symbols are: n = number of D_m lines, i = interaction load. The second total (Total*) for each F was computed by doing a single calculation of $\ln (XY/WZ)$ from the total numbers of flies in all experiments. The corresponding figures from SPASSKY *et al.* (1965), computed by the second procedure, are given for comparison.

tion-1 female. However if taken at face value the results do suggest a small positive epistasis between autosomes for mild detrimentals, in agreement with the intrachromosome situation. Interactions obtained from our data by method (1), using a single computation for each F of the $\ln (XY/WZ)$ of the total numbers of flies in all experiments, are given in the line labelled Total*. The results agree with the above, but show a slight positive deviation for the following reason. In method (3), using weighted logarithms per culture, any cultures which would otherwise have been included in the D_m category but in which the ++ class was entirely absent, had to be omitted from the analysis because of the zero term in the denominator. Such cultures would have a positive interaction, so that their omission has led to a slight underestimate of i with method (3). This does not affect the result in any substantial way because, as noted from the table, there were only 14, 9, and 11 such cultures at the three inbreeding levels, respectively. Table 8 also gives corresponding figures for SPASSKY *et al.* (1965) calculated by method (1) from their totals in the four classes in quasinormal cultures in an interaction experiment on *D. pseudoobscura*. The i value is substantially greater than ours but most of this comes from the heterozygote interaction of —.080 compared to our value of .001. The means for the homozygotes themselves agree more closely between the two works.

This interaction component in the heterozygotes in the *D. pseudoosbcura* experiment is expressed in another way in Table 9. In the data of SPASSKY *et al.* (1965) the effect of the mutant chromosome depends on the background, although there were, in fact, no main viability effects of the markers *Ba* and *L*; the observed mean percentages in the control were 44.31 : 22.05 : 21.85 : 11.79. On the other hand, the present data on *D. melanogaster* show an appreciable effect of the *Cy*

TABLE 9

Comparison of effects of mutant markers in different backgrounds, as shown by the ratios of mean numbers of flies at F = 0

D. melanogaster Experiment	Total flies	+ vs. *Me*		+ vs. *Cy*	
		++:+ *Me*	*Cy* +:*Cy Me*	++:*Cy* +	+ *Me*:*Cy Me*
I–III	154,370	.583	.588	.487	.491
IV	26,145	.557	.535	.497	.477
D. pseudoobscura		+ vs. *L*		+ vs. *Ba*	
	Total flies	++:+ *L*	*Ba* +:*Ba L*	++:*Ba* +	+ *L*:*Ba L*
	122,701	.539	.498	.535	.493

Ratios for *D. melanogaster* are based on Table 2, this paper and for *D. pseudoobscura* on Table 2, SPASSKY *et al.* (1965).

and *Me* chromosomes but very little interaction. The *Me* class is consistently lower than *Cy* (see Table 2) but this effect is the same whether or not *Cy* is in the background, and vice versa. Of course, there are many known cases of gene interactions where markers have differing effects according to the background. The analytical method chosen for obtaining the loads, by comparing inbred with random crosses and not with the theoretical 4:2:2:1 ratio, is designed to eliminate such background effects and bring various experiments into alignment. If the loads are translated into terms of I, by the formula $I = e^{-i}$, the values are .97 for the present data (see Table 6) and .88 for SPASSKY *et al.* (1965). Considering all the possible sources of variation between the experiments in these two species, including the heterozygous interaction just described for the latter, this may not be a significant difference. The total numbers of flies in the two studies are similar, with the present work having fewer data per genome but a larger sample of genomes, which perhaps may also account for some of the difference.

Variance in degree of epistasis: The suggestion of an average positive epistasis between as well as within chromosomes raises the question of the variation among genomes in this regard. Are there large interactions of opposite direction which effectively cancel each other out, leaving a small net positive epistasis, or are the interactions uniformly small and positive? To approach this question, an analysis of variance of the interactions among the mild detrimental lines was performed, using the two broods per line as replicas to arrive at an estimate of the error variance. Lines from Experiments I-III were included in this analysis if they met the following criteria: each brood contained a minimum of 25 flies; the combined broods met the 50% criteria of viability on chromosomes *2* and *3* each; in each brood X and Y had to be greater than zero, or else Z had to be greater than zero if either X or Y were zero. (There were very few, if any, of the latter type of replicate, but the standard had to be applied for numerical purposes.) The measure of interaction for this analysis, chosen for its symmetry and limited range, was $(XY—WZ/XY+WZ)$. This quantity will be zero with no interaction and will always be between -1 and $+1$. It will be $+1$ for a synthetic lethal culture

TABLE 10

Analysis of variance of interactions of mild detrimentals in n *lines, 2 broods per line, with Experiments I–III included*

	Mean interaction values		
	$F = 1$	$F = .5$	$F = 0$
n	522	706	1019
brood 1	.038	.007	.018
brood 2	.034	.042	.027
Total	.036	.025	.023

Analysis of Variance

Source of variation	Parameter estimated by m.s.	$F = 1$ df	$F = 1$ m.s.	$F = .5$ df	$F = .5$ m.s.	$F = 0$ df	$F = 0$ m.s.
Total		1043	.102	1411	.099	2037	.094
Between	$\sigma^2 + 2\sigma^2_m$	521	.114	705	.099	1018	.096
Within	σ^2	522	.090	706	.100	1019	.091
Variance ratio		1.259*		.992		1.058	
σ^2		.090		.100		.091	
σ^2_m		.012		—.0004		.003	

The measure of interaction is $(XY—WZ)/(XY+WZ)$; m.s. = mean square; df = degrees of freedom; F = inbreeding coefficient.

with no flies in the Z class, or -1 for extreme epistasis in the opposite direction. The mean values of this quantity at each F are given in the upper half of Table 10 for each replica separately, and for the two combined. The means are all positive, in agreement with the results obtained with the earlier methods, although here we are not interested in the absolute value itself but rather in its direction and variation. The analysis of variance presented in the lower half of Table 10 shows that the within line variation, σ^2, generally consistent from one inbreeding level to another, is .090 — .100. The estimate of the variation from one mild detrimental line to another, σ^2_m, greatest in the complete homozygotes, and near zero in the other series, is just a small fraction of that. Considering all of the factors which would contribute to the variance among lines, including the differences among experiments, evident from Table 8, and the differences among the sub-groups of lines processed by any one time, this suggests a *relative* uniformity among the mild detrimentals for this particular measure of interaction. Thus, while the result for the complete homozygotes is at the border of significance, there does not appear to be any evidence here for large differences in magnitude and direction of the interactions.

Synthetic lethals: The data were also screened for possible synthetic lethals involving components on the second and third chromosomes, that is, cases in which two normal, or nearly normal chromosomes gave a lethal effect in combination. In Table 11 is a list of lines from the $F = 1$ series which are neither lethal nor severely detrimental on chromosomes *2* and *3*, by virtue of having more than 50% of the expected number of each single homozygote, but which are lethal in the double homozygote by the 10% criterion. Only those cultures with 20 or

TABLE 11

A list of progeny counts in nine presumptive synthetic lethal lines, defined as having more than 50% of the expected numbers in the single homozygotes and less than 10% of the expected in the double homozygote

Original tests					Further tests				
Cy Me	*+ Me*	*Cy +*	*++*	Total	*Cy Me*	*+ Me*	*Cy +*	*++*	Total
36	11	22	1	70	....	...	...	..	....
56	17	21	0	94	1078	455	561	49	2143*
20	11	11	0	42	1133	397	544	27	2101*
59	36	38	0	133	125	30	52	0	207
45	15	19	1	80	....	...	...	..	....
53	16	23	0	92	....	...	...	..	....
69	34	41	0	144	....	...	...	..	....
36	15	13	1	65	....	...	...	..	....
37	15	23	0	75	....	...	...	..	....
Totals									
411	170	211	3	795	....	...	...	..	....

Comparison of Viabilities

	+ Me:*Cy Me*	*Cy +*:*Cy Me*	*S*	*T*	*I*
Presumptive synthetic lethals	.414	.513	.85	.88	.03
All mild detrimentals	.445	.516	.91	.89	.98
All heterozygotes	.489	.580	..	..	..

In "Further tests"* indicates the sum of 20 replications.

more *Cy Me* are listed. In the $F = 1$ series, there were only 9 such cases out of the total 1855, or a frequency of .005. In the $F = .5$ series there were 3 cases (not shown, since they may duplicate some already listed); in $F = 0$, none, as expected. Thus, although they are occasionally found, there is no evidence here that synthetic lethals are frequent enough to make an important contribution to the inbreeding effect. Some of the lines were retested for confirmation; progeny counts on these are given in the right hand section of the table. One of the presumptive synthetic lethals upon retesting becomes more of a synthetic "detrimental" than lethal in the sense that it has more than 10% of the expected double homozygotes. Further testing of the other cultures would have been desirable, but was not feasible in the midst of the large main experiment. A summary of the data, excluding the re-tests, is given at the bottom of the table. The ratios of *+ Me*: *Cy Me* and of *Cy +*:*Cy Me* for the presumptive synthetic lethals compared with the mild detrimental homozygotes and the random heterozygotes indicate that the synthetic lethals are, in fact, only very slightly reduced in viability on chromosomes *2* and *3*. The second chromosome homozygotes have 85% and the third, 88% of the viability under random mating, whereas the double homozygotes have only 3%.

Is there extreme negative interaction? A search was also made for the opposite type of interaction, that is, where chromosomes were lethal in a single homozygote but non-lethal in the double homozygote. One instance was found where only

the *Cy Me* and ++ phenotypes were present, suggesting some type of negative epistasis or a translocation. Both of these explanations were, in fact, ruled out. Instead, the agent responsible was a recessive gene on the third chromosome which suppressed the *Cy* phenotype, causing the third chromosome homozygotes to be classified as wild type. The second chromosome homozygotes were missing due to an independent recessive lethal on chromosome *2*. Therefore, the suppressor, combined with the lethal, meant that the wild-type flies were, in fact, of the composition *Cy*/lethal-2; suppressor-*Cy*/suppressor-*Cy*. Details of the supporting data are given by MEYER and TEMIN (1965).

Visible mutants: While not within the main scope of the experiment, recessive visible mutations on the second and third chromosomes from nature were recorded during the progeny counts. For our purpose, a visible was loosely defined as a change in the external phenotype of the majority of the homozygous flies that was obvious to both of the investigators making the observations. Characters observed involved changes primarily in the eyes, wings, bristles, abdominal segmentation pattern, and general body shape and color. A few of the visible mutations occurred among the occasional survivors in an otherwise lethal group (omitted from the calculation below) as well as among detrimental genotypes. Based on the total number of nonlethal cultures, the frequency of visibles among nonlethals for the second chromosome was 53/1203 or .043 and for the third was 48/1212 or .040. The ratio of visibles to lethals was thus about .04:.23. There was one case, among the approximate 1200, of a joint occurrence of visibles on the second and third chromosomes, which is slightly less than the expected frequency of .0017.

DISCUSSION

The D_m/L *ratio and dominance of mild detrimentals:* GREENBERG and CROW (1960) suggested that a comparison of the *D*:*L* ratio in equilibrium populations with the same ratio for newly arising mutations can yield information on the comparative rates of elimination of lethals and detrimentals. The predicted relation between new and equilibrium *D*:*L* ratios was computed for several models of heterozygous effects, ranging from partial dominance through complete recessivity to overdominance. Those theoretical ratios were for loads measured from an optimum genotype, whereas the experiments measure the loads as departures from the mean viability of a randomly mating population. TEMIN (1966) and CROW (1968) have given the appropriate corrections to correspond to the collected data. Using such an approach with published data on new mutations induced by irradiation, the earlier analyses suggested that detrimentals have at least the same, if not greater dominance than lethals, with dominance defined as the selective disadvantage of the mutant heterozygote relative to that of the mutant homozygote. However, there was disagreement among the available results on radiation-induced mutations and moreover, the X-ray studies probably underestimate the *D*:*L* ratio for natural mutations. Now with MUKAI's studies (1964, 1968) on spontaneously arising mutations controlling viability, there is further support for the above suggestion. These mutations are mildly deleterious

with viability greater than 50% of "normal", with normal being the homozygous viability of the original chromosome from which all lines in the experiment were descended. This is essentially equivalent to our D_m class except that it is measured with respect to an original high viability chromosome whereas our reference point is the average viability of the population. The D_m/L ratios for new mutants on chromosome *2* estimated from two independent experiments by MUKAI were .65 and .70, or an average of .675 (see CROW 1968). This is on the same order of magnitude as the mean D_m/L ratio of .46 for chromosomes *2* and *3* in the present study. If mild detrimentals were more completely recessive than lethals they would persist in the population much longer, accumulate to a greater degree, and result in an equilibrium D_m/L ratio which is much higher than for new mutants; and if lethals were partially dominant and detrimentals overdominant, the difference would be even greater. Even with the same average dominance of lethals and mild detrimentals the equilibrium ratio would still be higher than for new mutations, by the ratio $\bar{S}/\bar{s}$, where $\bar{S}$ and $\bar{s}$ are the mean homozygous effects of lethals and detrimentals, respectively (GREENBERG and CROW 1960). From MUKAI's estimate of .02–.03 for $\bar{s}$ of mild detrimentals ($\bar{S}$, of course is 1), this would be about 30–50 times. The observations, therefore, fit the model of considerably greater dominance for mild detrimentals. If, as calculated by CROW and TEMIN (1964), the mean dominance of lethals at approximate equilibrium frequencies in a natural population is about .015, then that for mild detrimentals is estimated to be at least .25, by a consideration of MUKAI's work and the present results. The same general conclusion may be reached without involving any assumption about the dominance of lethals, leading to a figure of about .15 for mean dominance of mild detrimentals. (More detailed computations taking into account the different reference points are given in CROW 1968).

Other support for this conclusion comes from results such as those of WILLS (1966) on *D. pseudoobscura* showing a significant positive correlation between the viability of homozygotes and heterozygotes when complete lethals are omitted from the analysis. WILLS concludes that recessive lethals have little or no effect on heterozygous viability, although the magnitude of the standard errors does not rule out a small real difference of 1 or 2%, and that subvitals may have an estimated dominance of .18 or .3, as calculated from two different experiments.

Interactions for mild detrimentals: The combined results on Drosophila from several authors indicate that to the extent that interactions occur, they are in the direction of synergism. Estimates of the magnitude vary, and apart from the present work and that of SPASSKY *et al.* (1965), few direct measurements have been made. Our observed interchromosomal interaction of about 2%, which was not significantly different from zero, and much smaller than the main effects, would not have much impact on the population unless many mutants occurred together in the same homozygote. The intrachromosomal interaction for mild detrimentals was also small and in the positive direction. In SPASSKY *et al.* (1965), the epistasis is stronger, but as discussed earlier the many other sources of difference between the two works may mean that this is not a difference of significance.

MUKAI (1968) has reported a synergistic interaction between spontaneous mutant polygenes based on a nonlinearity between generation number and average viability of second chromosomes accumulating these mutants. In another type of study on Drosophila species by DOBZHANSKY, SPASSKY and TIDWELL (1963) and by MALOGOLOWKIN-COHEN, LEVENE, DOBZHANSKY, and SIMMONS (1964) the evidence for synergism is based on an indirect extrapolation: the load at complete inbreeding obtained from a direct analysis of the homozygous viability loads on individual chromosomes, using markers, was higher than predicted from measurements of egg to adult survival at low inbreeding coefficients. This is suggestive, but the different criteria of fitness, different environments, and so on may be enough to account for the discrepancy. In Tribolium, LEVENE *et al.* (1965) found evidence for a mean positive synergism, by nonlinearity of inbreeding effect at the F levels 0, 1/8, to 1/4 in one species studied although not in another.

Putting all studies together there seems to be some overall synergistic epistasis. To the extent that these studies, as a group, can be generalized, the often observed linearity of inbreeding effects in various organisms is not necessarily in disagreement with this, but may be based on these factors: (1) A large portion of the inbreeding depression is caused by lethals, which are necessarily non-interacting. (2) The average degree of epistasis for genes of small effect, as the mild detrimentals, is not very large. (3) Most non-Drosophila studies are at relatively low values of F whereas appreciable interactions may be revealed primarily at the high inbreeding levels. More data are needed to find out the range where the greatest nonlinearity begins and occurs. In the present study the i values at $F = 1$ and $F = .5$ were each about 2%, but the standard errors were large.

The evidence discussed previously for the partial dominance of mild detrimentals suggests that, if the dominance is large, interactions between heterozygous effects, in fact, may have an impact on the population, even if interactions of homozygous mutants may not be frequent enough to be influential. The extent of such interactions between heterozygous mild detrimentals has not, to our knowledge, been measured directly. However, for lethals, KITAGAWA (1967) has reported a significant deviation from linearity in multiple heterozygotes, using up to four lethal genes per second chromosome.

Synthetic lethals: Also bearing on the nature and direction of interactions is the question of synthetic lethals. Our study revealed a very low frequency, about .005, of true bichromosomal synthetic lethals, and no cases of the extreme opposite type of negative epistasis. The data in the literature show a great deal of variation on this question. DOBZHANSKY, SPASSKY, and ANDERSON (1965) report on 3 pairs of chromosomes, out of 120, which are synthetic semilethals. However, the single homozygotes in these cases often have reduced viabilities, some in the semilethal range themselves. The populational consequences of two deleterious chromosomes which interact to produce a double homozygote with viability somewhat lower than expected on the basis of independence are not appreciable; such mutants would be eliminated mainly by their single effects.

Another type of experiment has been done on several species of Drosophila to find intrachromosomal synthetic lethals arising by recombination between normal

or mild subvital chromosomes (SPASSKY, SPASSKY, LEVENE, and DOBZHANSKY 1958; SPIESS 1959; DOBZHANSKY, LEVENE, SPASSKY, and SPASSKY 1959; KRIMBAS 1961; SPIESS and ALLEN 1961). In this series there was no evidence for synthetic lethals in *D. melanogaster* or *D. persimilis*. In *D. pseudoobscura, D. prosaltans,* and *D. willistoni* the frequency of lethals arising was somewhat higher than expected from the mutation rate, but there were no male control tests to get the exact frequency of point mutations in these particular experiments and no mapping tests to locate the lethals; the evidence for some of them being synthetic, although suggestive, was indirect. In *D. pseudoobscura* 10 of 12 of the lethals arising in the above tests and then subjected to desynthesis could not be ascribed to single loci. (DOBZHANSKY and SPASSKY 1960).

In *D. melanogster* HILDRETH (1956), using normal chromosomes, found no synthetic lethals on the *X* or third. WALLACE *et al.* (1953) found 2.96% lethals from recombinant chromosomes, higher than expected from the mutation rate, but some of the chromosomes chosen had low homozygous viability to start with, and these were responsible for a large number of the lethals which arose. WALLACE, ZOUROS and KRIMBAS (1966) found no interchromosomal synthetic lethals in 119 tests on second and third chromosomes from nature.

Thus the apparent discrepancies in the literature on the frequency of synthetic lethals may perhaps depend on the nature of the chromosomes from which the lethals originate. In those studies where lethals arose by recombination, chromosomes of mild subvital or low viability were included in the starting material, whereas in those where none or very few occurred, only normal chromosomes were used.

SUMMARY

Viability effects of the second and third chromosomes at three inbreeding levels, and the role of inter- and intra-chromosomal interactions were measured in 1855 lines derived from wild populations of *Drosophila melanogaster*. At complete homozygosis ($F = 1$) the mean load per major autosome, that is the viability reduction compared with random heterozygotes ($F = 0$), was .41 lethal equivalents. Of this the largest portion, .24, was due to lethals (L), .11 to mild detrimentals (D_m), and .06 to severe detrimentals (D_s).—For reasons of its possible importance in the selection of these genes in populations and the greater feasibility of measuring it, the interaction for mild detrimentals was singled out for attention. The data suggest a slight positive or reinforcing epistasis between chromosomes, which, however, was not significantly different from zero. The double homozygote with mild detrimentals on chromosomes *2* and *3* had about 98% of the viability expected if the effects of the two single homozygotes were independent. An analysis of variance gave no evidence for large differences in magnitude or direction of the interactions. Synthetic lethals occurred with a frequency of only .005 and there were no cases of extreme negative or opposing epistasis.—For interactions within a chromosome the data similarly show a small positive but barely significant epistasis, from the observation that the load due to mild detrimentals at $F = 1$ was more than twice that at $F = .5$.—Comparison of viabil-

ity loads in progeny raised from two *vs.* ten pairs of parents showed no systematic differences between these crowding levels.

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