CHROMOSOME INTERACTIONS IN *DROSOPHILA MELANOGASTER*. I. VIABILITY STUDIES

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ABSTRACT

The nature of fitness interactions is an important, yet unsolved, question in population genetics. We compare the egg-to-adult viability of individuals homozygous for either a second or a third chromosome with the viability of individuals homozygous for both chromosomes simultaneously. On the average, the viability of the two-chromosome homozygotes is somewhat greater than expected assuming that the fitnesses of the single-chromosome homozygotes interact in a multiplicative fashion. This result differs from previous observations that indicate either no significant deviations from the expectation or lower-than-expected average fitnesses for the double homozygotes.

THE nature of the fitness function remains one of the fundamental problems in population genetics. Population genetics models often assume that different loci have independent fitness effects. Biological knowledge suggests that such independence is unlikely to be general since the products of different genes interact in various ways in the organism. The question, then, is whether the assumption that gene loci have independent fitness effects is acceptable to a first approximation.

Experimental investigation of the fitness function would ideally proceed by measuring first the fitness of individuals with known genotypes at each of two or more individual loci, and then the fitness effects of the loci in combination. But it is almost, if not completely, impossible to separate experimentally the fitness effects of given loci from those of closely linked loci with which they may be associated in linkage disequilibrium. An alternative procedure is to measure the inbreeding depression. If different gene loci have independent (multiplicative) fitness effects, then the relationship between the inbreeding coefficient, F, and the concomitant fitness depression should be linear. Two different experimental approaches are possible. One approach consists of varying the amount of inbreeding over the whole genome by mating relatives with different degrees of relatedness; this approach is possible in all sorts of organisms. Studies of this kind have been conducted mostly with Drosophila (e.g., LATTER and ROBERTSON 1962; DOBZHANSKY, SPASSKY and TIDWELL 1963; TORROJA 1964; MALOGOLOWKIN-COHEN *et al.* 1964; KOSUDA 1972), but also with Tribolium (LEVENE *et al.* 1965). LATTER and ROBERTSON (1962) found in *D.*

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melanogaster no departure from linearity with inbreeding levels up to $F = 0.90$. But most other studies indicate that when the coefficient of inbreeding is large ($F > 0.25$) the reduction in fitness is greater than would be expected by linear extrapolation from low inbreeding levels.

The second approach to measure the inbreeding depression consists in comparing the fitness of individuals fully homozygous for one or another chromosome with the fitness of individuals simultaneously homozygous for both nonhomologous chromosomes; at present, this approach is only possible with the few Drosophila species in which there exist "balancer" stocks that permit one to obtain individuals homozygous for whole chromosomes. This approach has two advantages: (1) the relative degree of homozygosis is precisely known, and (2) the magnitude as well as the sign of the interaction can be calculated. Three such studies have been published, which deal with only one fitness component, namely egg-to-adult viability. Moreover, the results are inconsistent. SPASSKY, DOBZHANSKY and ANDERSON (1965) in *D. pseudoobscura* and KOSUDA (1971) in *D. melanogaster* found that the fitness of two-chromosome homozygotes was, on the average, significantly lower than expected from the single-chromosome homozygotes. However, TEMIN *et al.* (1969) found in *D. melanogaster* that the average interaction between chromosomes was not significantly different from zero.

We have conducted two experiments with chromosome homozygotes of *D. melanogaster* in order to provide further insight into the nature of the fitness function. The first is, like the previous studies, an investigation of egg-to-adult viability. The results are reported in the present paper. The second study, reported in a companion paper (SEAGER, AYALA and MARKS 1982), investigates total fitness, using the method developed by SVED and AYALA (1970).

MATERIALS AND METHODS

Several hundred *Drosophila melanogaster* flies were collected at MacDonald Ranch, Napa County, California, in October and November 1974. Females were individually placed in vials and allowed to lay eggs. Wild-caught males and F_1 males from the wild-caught females were crossed to females from a laboratory stock, following the scheme outlined in Figure 1.

The laboratory stock combines balancer chromosomes for both the second and the third chromosomes. The second-chromosome balancer is SM5, marked with Curly (*Cy*), whereas the homolog is marked with Bristle (*Bl*) and Lobe (L^2). The third-chromosome balancer is TM3 marked with Stubble (*Sb*), Serrate (*Ser*) and ebony (e^s), whereas the homolog is marked with ebony (e^{11}). Each balancer chromosome has multiple inversions that suppress the appearance of recombinant progeny. The dominant morphological alleles (*Cy*, *Bl*, L^2, *Sb*, and *Ser*) behave as recessive lethals. Additional details concerning the mutants as well as the balancer chromosomes can be found in LINDSEY and GRELL (1968).

Males were individually crossed to females of the balancer stock (P generation, in Figure 1). From each progeny, one F_1 male, marked with *Cy*, *Sb*, and *Ser*, was again crossed to the balancer stock. The use of a single male at this stage insures that all second and third wild chromosomes within a line are identical by descent (and that the second and third wild chromosomes derive from the same wild male). In the second generation, males and females marked with *Cy*, *Sb*, and *Ser* were intercrossed. Because the balancer chromosomes are recessive lethals, only four kinds of flies are expected in the F_3 generation: (A) homozygotes for both the second and the third chromosome; (B) homozygotes for the third, but not the second chromosome; (C) homozygotes for the second, but not the third chromosome; (D) heterozygotes for the second as well as for the third chromosome. No meiotic drive is driven to occur in the F_2 flies; hence, if the four kinds of F_3 individuals have

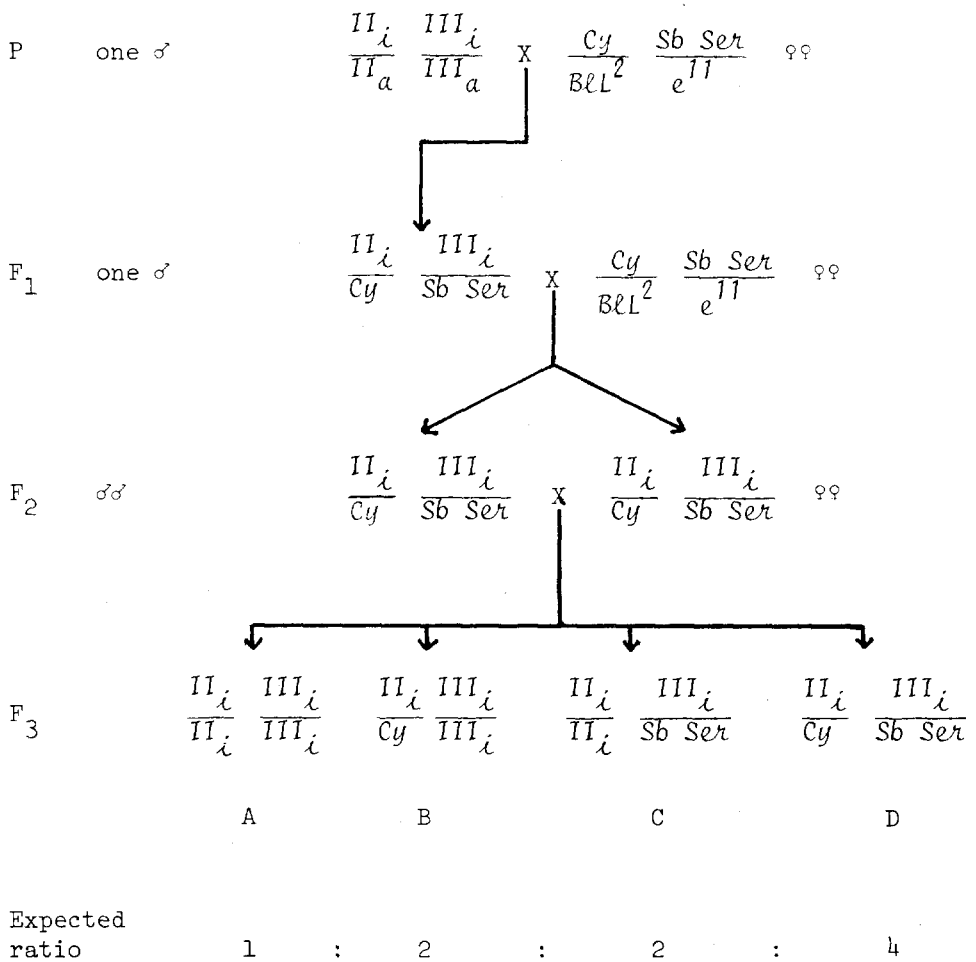

FIGURE 1.—Mating scheme used to extract wild second and third chromosomes simultaneously. The subscripts *i*, *a* indicate wild-type chromosomes. *Cy* and *Sb Ser* indicate balancer chromosomes which prevent viable recombinant progeny. *BlL*^{2} and e^{11} indicate marker chromosomes.

identical egg-to-adult viability, the four F_3 genotypes are expected in the proportions 1:2:2:4 (see Figure 1). Deviations from these expected proportions allow one to estimate the relative viability of the four kinds of individuals.

Control (outbred) crosses were made by crossing F_2 males and females from two different lines. These crosses are also expected to yield four kinds of progeny in the F_3 generation in the proportions 1:2:2:4. However, in contrast to the experimental (inbred) crosses, the files with a wild phenotype for one or both chromosomes are not homozygous for whole chromosomes, but rather carry two homologs derived from different wild flies. Hence, the completely wild-type flies in these progenies have effectively the *same genetic constitution* as flies in nature with respect to the second and third chromosomes. The relative viabilities of the four types of individuals in the control crosses are also calculated from the deviations between the observed and expected number of flies of each kind.

It should be noted that the X chromosomes of the F_3 flies (Figure 1) are all derived from the balancer stock, whereas the small fourth chromosomes are a mixture of wild and balancer-stock chromosomes. The second and third chromosome jointly account for about 80% of the genome of *D. melanogaster*.

We studied the results of 154 experimental crosses. The minimum number of F_3 flies scored per cross depended on the number of phenotypic classes recovered. At least 200 flies were scored when only one class was recovered (*i.e.*, the double-heterozygous parental class); at least 275 flies when two classes were recovered; at least 375 when three classes were recovered; and at least 500 when all four progeny types were present among the F_3 adults.

In addition, we made 50 control crosses, by crossing in the F_2 generation (see Figure 1) males from one line with females from a different line. These crosses involved 50 of the 154 experimental lines, with each line crossed to two different ones. Both types of reciprocal crosses were made between each two lines. At least 250 F_3 flies were scored from each reciprocal cross; *i.e.*, no fewer than 500 flies for each of the 50 control crosses. 42,167 F_3 flies were scored for all 50 control crosses.

The experimental as well as the control crosses were made under conditions that minimize larval competition. Five pairs of flies were crossed in a given half-pint culture. Moreover, the egg-laying flies were transferred to a fresh culture every 2 or 3 days and the progenies were also scored every 2 or 3 days, starting soon after emergence. We used a standard cornmeal-molasses-agar culture medium and conducted the experiments in an air-conditioned room maintained at about 23°C.

Viability was estimated as follows (see SEAGER 1979 for additional details). First, we obtained for any given line the viability, v_{BAL}, of the homozygotes relative to the double-balancer heterozygote. Thus, for example, the viability of flies homozgyous for the second chromosome (C in Figure 1) was estimated relative to the double-balancer heterozygotes (D in Figure 1) by their departure from the expected ratio 2:4 (*i.e.*, the number of C flies was multiplied by two and divided by the number of D flies). Second, the average viability of the wild-type heterozygotes relative to the double-balancer heterozygotes, v_{HET}, was calculated for the control crosses. Thus, the average viability of flies heterozygous for two different wild second chromosomes was calculated relative to the double-balancer heterozygotes for the control crosses (*i.e.*, for each control cross we did as before—the number of C flies was multiplied by two and divided by the number of D flies—and then obtained

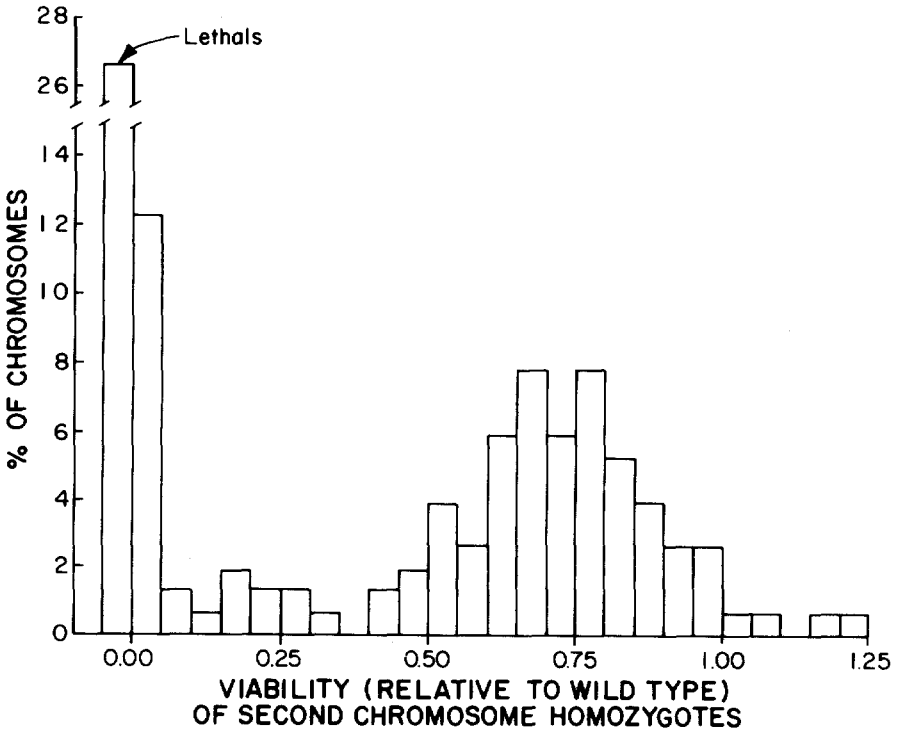

FIGURE 2.—Viability distribution of second chromosome homozygotes. N = 154 chromosomes.

the average for all control crosses). The viability, v, of the chromosome homozygotes relative to wild-type heterozygotes was calculated simply as $v = v_{BAL}/v_{HET}$.

Notice that the estimation procedure compares the viability of flies that differ only in the genotype for the chromosome under consideration. That is, the viability of the homozygotes for the second chromosome (II_i/II_i, C in Figure 1) is being estimated relative to flies that are wild-type heterozygous for the second chromosome (II_i/II_j, where i and j represent two different second chromosomes sampled from the natural population), but which are similar for the other chromosomes (*i.e.*, in the third chromosomes the two types of flies are both heterozygous for the balancer, $III_i/Sb\ Ser$). This procedure assumes that the viability of the double-balancer heterozygotes (D in Figure 1) is the same in the experimental as in the control crosses. KATZ and CARDELLINO (1978) and COCKERHAM and MUKAI (1978) have shown that the viability of heterozygotes for a balancer (*SM1*) and a wild-type chromosome depend on the particular wild-type chromosome. We submit that this may not be a major problem in our (or similar) experiments. The phenomenon will affect the viability estimates of individual chromosomes, but is not likely to affect the overall mean viability values whenever a large number of wild-type chromosomes is examined.

RESULTS

The viability distributions of the single-chromosome homozygotes are shown in Figure 2 for the second chromosome and in Figure 3 for the third chromosome. These distributions are similar to those obtained in other studies (for a review, see DOBZHANSKY 1970). The distributions are bimodal, with the highest mode at lethality, whereas most other chromosomes exhibit "quasi-normal" viability and have a mode near 0.75 viability. A relatively small number of chromosomes

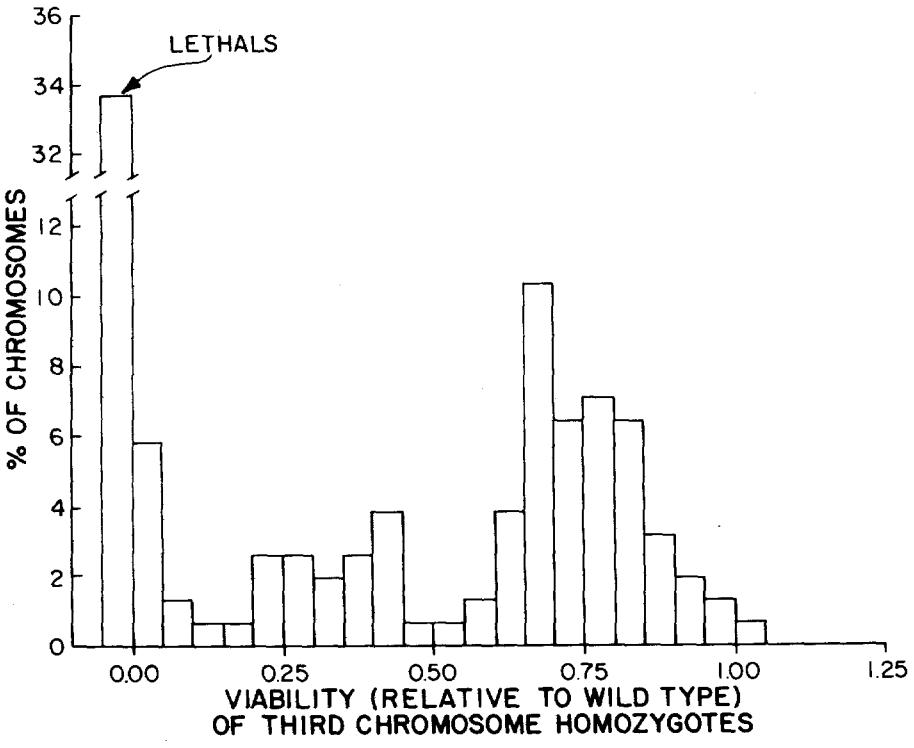

FIGURE 3.—Viability distribution of third chromosome homozygotes. N = 154 chromosomes.

are semilethal (viability between 0 and 0.50). The mean viability is 0.411 ± 0.030 for the second-chromosome homozygotes and 0.380 ± 0.038 for the third-chromosome homozygotes.

Figure 4 gives the viability distribution of the double-chromosome homozygotes. There is a high peak at lethality, but no well defined mode in the quasi-normal region; actually, only 15% of the double homozygotes have fitness greater than 0.50. The mean viability for the double-chromosome homozygotes is 0.167 ± 0.021.

The observed frequency of double-chromosome *lethals* approximately agrees with the expected frequency calculated from the single-chromosome lethal frequencies. The observed number of lethals is 41 (0.266) for the second-chromosome homozygotes and 52 (0.338) for the third-chromosome homozygotes. Hence, the expected frequency of double-chromosome lethals is $1 - (0.734 \times 0.662) = 0.514$, or 79.2 out of 154 lines. The observed number of double-chromosome lethals is 84.

There is, however, evidence of interactions between second and third chromosomes with respect to lethality. The most extreme instance involves a case of "cured" lethality. This situation, when the *deleterious effects* are less than expected (*i.e.*, when the *fitness* of the double homozygotes is greater than expected), is called negative synergism. The third-chromosome homozygotes of

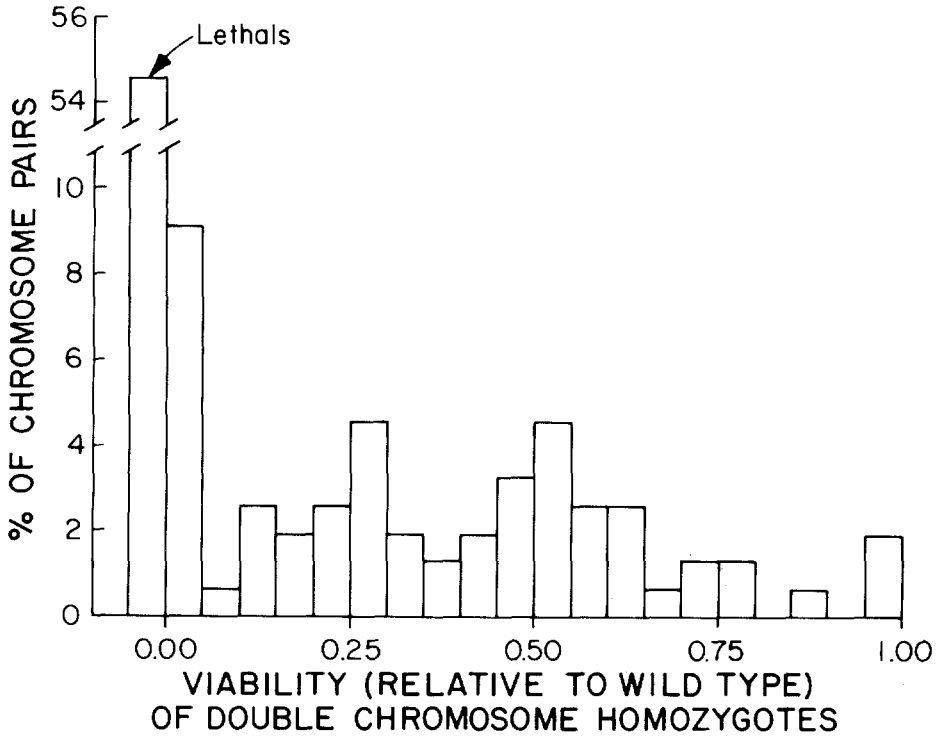

FIGURE 4.—Viability distribution of double chromosome homozygotes. N = 154 chromosome pairs.

line 123 are lethal; yet the viability of the double-chromosome homozygotes is 0.215. A case of cured lethality was reported in *D. pseudoobscura* by DOBZHANSKY, SPASSKY and ANDERSON (1965).

There may also be instances of positive synergism resulting in lethality. There is positive synergism when the *deleterious effects* produced by homozygosis for both chromosomes are enhanced (i.e., when the *fitness* of the double homozygotes is less than expected). In 7 of the 154 lines, no double-chromosome homozygotes were observed in the F_3, although both kinds of single-homozygotes were present. However, the viability of one or both single-chromosome homozygotes is very low in every one of the seven lines. The expected viability of the double-chromosome homozygotes is no greater than 0.04 in any one of these lines, and the expected number of double-homozygous individuals is three or fewer. Therefore, the absence of double-homozygotes among the F_3 flies might result from sampling errors rather than to positive synergistic interactions.

All four types of progeny appeared in the F_3 of 60 lines. The fitness interaction between the second and the third chromosome can be quantified in these lines. For this purpose, previous workers (DOBZHANSKY, SPASSKY and ANDERSON 1965; TEMIN *et al.* 1969; KOSUDA 1971) have used the i statistic, defined as follows:

$$i = 1 - \frac{\text{observed viability of the double homozygote}}{\text{expected viability of the double homozygote}},$$

where the expected viability for the multiplicative model is the product of the viabilities of the single homozygotes. One undesirable property of i is that it is not symmetric around zero ($-\infty \leq i \leq 1$). We introduce here the statistic k, kindly suggested by PROFESSOR JACK L. KING, which is simply the correlation between heterozygosity and homozygosity for the two chromosomes in the F_3 (with the sign changed so that, like i, it is negative for negative synergism and positive for positive synergism). k has better properties than i because it is symmetric around zero ($-1 \leq k \leq 1$) and because it is less sensitive to random fluctuations in the sampling of progeny (see APPENDIX). We will use both statistics in order to compare our i values with those of previous workers.

Table 1 gives the observed and expected viability of the double homozygotes for the 60 lines for which all four types of F_3 progeny were observed. The expected viability is calculated assuming a multiplicative model. The mean observed viability for this subset of all lines is 0.415 ± 0.033, whereas the mean expected viability is 0.374 ± 0.029, suggesting that negative synergistic interactions prevail on the average. This is confirmed by examining the k values. The mean value of the k statistic is -0.022 ± 0.011, which is just significantly negative. Moreover, 38 of the 60 values of k are negative and only 22 positive. If the actual mean k is not different from zero, then equal numbers of positive and negative k's are expected. If the mean k is zero the exact probability of observing 38 or more negative values is

$$\sum_{i=38}^{60} \binom{60}{i} (1/2)^{60} = 0.026,$$

which is significant. The two-tailed test (probability of either more than 37

negative values or more than 37 negative values) gives P = 0.052, which is at the edge of significance.

In order to facilitate comparison between our results and those of other investigators, the values of the i statistic for each line are shown in the last column of Table 1. The mean value is $\bar{i} = -0.320 \pm 0.154$, which is significantly negative. This significant negative effect might be attributed to the disproportionate contribution of a few large negative values, given that the lower bound of i is $-\infty$. In each of four lines, the value of i is in fact less than -1.0 (this will occur whenever the observed viability is more than twice as large as the expected viability). The values of i for these four lines are -8.45 (line 037), -1.03 (line 092), -1.63 (line 122) and -2.84 (line 199). If these values are conservatively set at $i = -1.0$, the mean value for all 60 lines becomes $\bar{i} = -0.155 \pm 0.053$, which is also significantly negative. The median value of i is between -0.124 and -0.135.

In summary, the various ways of analyzing the results indicate that, on the average, the interactions between the second and third chromosome are significantly negative *i.e.*, that the fitness of the double homozygotes is greater than predicted on the basis of a multiplicative model.

DISCUSSION

An organism is an integrated whole whose component elements act together as a unit. It has long been known that interactions between genes at different loci exist; in fact, most genetics texts discuss the topic of epistatic gene interactions. Molecular biology provides an understanding of the kinds of processes that account for gene interactions. Examples of such processes are gene regulation and the successive action of different gene products along a biochemical pathway.

The question that we raise is whether gene interactions might modify the fitness effects of individual gene loci. In a classical study of fitness interactions between chromosomes, SPASSKY, DOBZHANSKY and ANDERSON (1965) used an analogy to illustrate how fitness interactions might occur. "Suppose that two diseases, A and B, respectively, cause 40 and 50% mortality. The chances of survival are, consequently 0.6 and 0.5, respectively; what are the chances of death and survival of individuals afflicted with A and B simultaneously? If the changes are independent, A and B together will cause death in 70% of individuals, while 30% will survive. However, the occurrence of one disease may weaken the organism so that it would be more susceptible to the effects of the other disease. This is positive synergism. Conversely, one disease may lighten or eliminate the other; this is negative synergism, perhaps exemplified by the interaction of malaria and syphilis in man. How a given pair of pathological conditions will interact could be predicted only on the basis of a detailed knowledge of their etiologies and their effects on the organism."

The implications of the last sentence for population genetics may be excessively pessimistic. Whether detailed knowledge of the mode of action of each gene is necessary before we can predict the joint fitness effects of two or more genes is a question to which we shall turn in a moment. There is, however, one

TABLE 1

Observed and expected double homozygote viabilities and viability interaction coefficients (k *and* i)

Line No.	Double homozygote viabilities		k	i
	Observed	Expected		
004	0.717	0.408	−0.138	−0.756
007	0.969	0.879	−0.024	−0.103
010	0.642	0.503	−0.060	−0.275
011	0.028	0.039	0.035	0.285
012	0.602	0.470	−0.060	−0.282
016	0.784	0.578	−0.076	−0.357
017	0.246	0.217	−0.026	−0.132
018	0.429	0.589	0.075	0.271
020	0.733	0.386	−0.155	−0.899
021	0.241	0.191	−0.049	−0.261
026	0.177	0.290	0.101	0.389
033	0.147	0.194	0.056	0.244
035	0.341	0.184	−0.138	−0.852
037	0.191	0.020	−0.244	−8.445[a]
040	0.034	0.174	0.208	0.805
041	0.349	0.326	−0.015	−0.069
044	0.160	0.143	−0.022	−0.119
047	0.491	0.355	−0.078	−0.381
048	0.494	0.329	−0.097	−0.500
054	0.077	0.047	−0.063	−0.637
055	0.762	0.654	−0.038	−0.165
072	0.625	0.566	−0.024	−0.103
076	0.578	0.696	0.046	0.170
082	0.264	0.574	0.172	0.540
087	0.257	0.225	−0.027	−0.144
092	0.375	0.185	−0.160	−1.026[a]
095	0.543	0.432	−0.056	−0.258
096	0.541	0.563	0.010	0.039
097	0.997	0.697	−0.089	−0.431
102	0.882	0.723	−0.050	−0.220
103	0.520	0.438	−0.042	−0.188
109	0.009	0.007	−0.015	−0.329
113	0.406	0.539	0.067	0.246
117	0.535	0.737	0.077	0.274
122	0.376	0.143	−0.209	−1.626[a]
126	0.134	0.072	−0.096	−0.856
128	0.435	0.513	0.039	0.152
132	0.591	0.497	−0.042	−0.189
134	0.985	0.651	−0.103	−0.512
146	0.525	0.659	0.055	0.203
150	0.011	0.018	0.041	0.405
151	0.274	0.289	0.011	0.051
166	0.291	0.289	−0.001	−0.006
168	0.642	0.704	0.022	0.088
169	0.471	0.450	−0.011	−0.047
171	0.514	0.442	−0.037	−0.162
177	0.594	0.761	0.060	0.219

TABLE 1 *(continued)*

Line No.	Double homozygote viabilities		k	i
	Observed	Expected		
178	0.471	0.344	−0.073	−0.367
180	0.690	0.595	−0.037	−0.160
182	0.138	0.215	0.087	0.358
185	0.304	0.440	0.084	0.308
187	0.267	0.214	−0.047	−0.248
188	0.481	0.470	−0.006	−0.023
191	0.201	0.205	0.004	0.021
199	0.034	0.009	−0.085	−2.835[a]
201	0.599	0.461	−0.064	−0.298
209	0.033	0.042	0.025	0.221
210	0.138	0.142	0.005	0.027
211	0.275	0.210	−0.057	−0.307
214	0.280	0.292	0.009	0.042
Mean ± S.E.	0.415 ± 0.033	0.375 ± 0.029	−0.022 ± 0.011	−0.320 ± 0.154

[a] If these four values of $i < -1.0$ are conservatively set at $i = -1.0$, mean $i = -0.155 \pm 0.053$.

special reason why we have used that quotation, namely the definitions of positive and negative synergism. SPASSKY, DOBZHANSKY and ANDERSON (1965) define positive synergism as an enhancement of the *deleterious* effects of genes, not an enhancement of fitness. Conversely, negative synergism is defined as a reduction of the deleterious effects. These definitions are reflected in the sign of the statistic, i, used to measure fitness interactions—the value of i is positive when the interactions decrease fitness and negative when the interactions increase fitness. Although one might be inclined to interpret positive synergism as implying an enhancement of fitness rather than an enhancement of deleterious effects, we have followed the conventions of SPASSKY, DOBZHANSKY and ANDERSON (1965) in order to avoid further confusion. Consequently, we have defined k so that it takes a positive value when fitness interactions result in a fitness decrease, and a negative value when fitness is increased.

Fitness interactions between genes are likely to be present in many cases. But is the situation as hopeless as the last sentence quoted above intimates? If it is, the task of the population geneticists would be practically impossible, because it would not be possible to extrapolate from the study of one or a few genes to the whole genome nor, for example, to estimate the number of genes responsible for the fitness effect observed when a whole chromosome, or the whole genome, is tested. On the other hand, it might be the case that gene interactions can be ignored, at least to a first approximation, because they tend to cancel each other out or because they are small compared to the main effects due to each individual gene.

Population geneticists use two models when considering the simultaneous effects of individual genes. The *multiplicative model* assumes that *fitnesses* are multiplicative; for example, if two genotypes, *aa* and *bb*, have each fitness of

0.99, the fitness of the *aa bb* genotype is assumed to be $0.99 \times 0.99 = 0.9801$. The *additive model* assumes that the *selection coefficients* are additive; for the example given, the selection coefficient of the *aa bb* genotype would be $0.01 + 0.01 = 0.02$ and, correspondingly, the fitness would be 0.9800. The additive model often simplifies the mathematical treatment. Moreover, when the selection coefficients are small, the additive and the multiplicative models yield fitnesses that are not very different from each other, as illustrated by the example given. However, when the selection coefficients are large, the additive model becomes nonsensical. Consider, for example, two genotypes *cc* and *dd* each with a fitness of 0.40. According to the additive model, the genotype *cc dd* would have a selection coefficient of $0.60 + 0.60 = 1.20$ and a fitness of $1 - 1.20 = -0.20$; but fitness cannot be smaller than zero. The selection coefficients of homozygotes for whole chromosomes are often quite large. Therefore, we only consider the multiplicative model in the present case.

The results of a number of studies indicate that fitness interactions between individual genes are not rare. Examples include visible mutants in *D. melanogaster* (WILSON 1968, 1972), allozymes in *D. pseudoobscura* (MARINKOVIĆ and AYALA 1975a, b), and newly arisen viability mutants in *D. melanogaster* (MUKAI 1969). In *D. melanogaster*, the effects of "recessive" lethals in heterozygous condition is modified by the presence of other nonallelic lethals (KITAGAWA 1967) and by the genetic background in general (MUKAI and YAMAGUCHI 1974).

Previous studies of fitness interactions over the genome as a whole or between full chromosomes have yielded conflicting results, but the overall evidence suggests that positive synergism prevails. The studies of fitness interactions between full chromosomes have all been concerned with egg-to-adult viability—one in *D. pseudoobscura* (SPASSKY, DOBZHANSKY and ANDERSON 1965) and two in *D. melanogaster* (TEMIN *et al.* 1969; KOSUDA 1971); all three experiments gave positive average values of i that were significantly greater than zero in two cases ($\bar{i} = 0.094 \pm 0.016$ in SPASSKY *et al.* 1965, and 0.075 ± 0.025 in KOSUDA 1971), but not in a third (0.021 ± 0.018 in TEMIN *et al.* 1969). Our results differ from previous studies in that the average effects are negatively synergist ($\bar{i} = -0.320 \pm 0.154$; $k = -0.022 \pm 0.011$).

It is, however, informative to examine individually the chromosome combinations. PROFESSOR H. LEVENE has derived a formula to calculate the 95% confidence interval of i (SPASSKY *et al.* 1965). In the study of SPASSKY *et al.* (1965), 180 out of 219 (82%) combinations have confidence intervals that include zero, whereas 35 (16%) had only positive limits and 4 (2%) had only negative limits. We have calculated the 95% confidence limits of i for our data with the following results: 50 out of 60 (83%) intervals include zero, 2 (3%) have only positive bounds, whereas 8 (13%) have only negative bounds. Thus, the results of both studies agree in that in a majority of cases (82–83%) there are no synergistic interactions (or they are too small to be statistically significant). The difference between the two studies concerns the relatively small number of cases of significant synergism, which are predominantly positive (16 *vs.* 2%) in the study of SPASSKY, DOBZHANSKY and ANDERSON (1965), but predominantly negative (13 *vs.* 3%) in our experiments.

One important observation is that this paper, as well as all the previous studies of fitness interactions between full chromosomes, have only examined egg-to-adult viability effects and only under near-optimal conditions. Yet, the existing evidence indicates that viability may have a relatively unimportant effect on overall fitness whereas other fitness components, such as fertility and differential mating success, may be much more important (SVED and AYALA 1970; PROUT 1971; BUNDGAARD and CHRISTIANSEN 1972; TRACEY and AYALA 1974; MARINKOVIĆ and AYALA 1975a, b). The results of an experiment conceived in parallel to the present one show that, when overall fitness is measured, negative synergistic interactions predominate overwhelmingly (SEAGER, AYALA and MARKS 1982). This suggests that the fitness independence often assumed in population genetics may not be warranted. It also implies that the concealed genetic load present in natural populations of *D. melanogaster* can account for the selective maintenance of a greater number of polymorphisms than had previously been estimated.

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APPENDIX

DERIVATION AND ERROR ANALYSIS OF INTERACTION STATISTICS

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I. DERIVATION

SPASSKY, DOBZHANSKY and ANDERSON (1965) introduced the statistic *i* that can

be used to quantify fitness interactions between chromosomes in crosses yielding single homozygotes for each of two chromosomes, double homozygotes and double heterozygotes. The selection coefficients, s and t, of the single homozygotes and i, the interaction coefficient, are related in the following model:

Genotype		$\frac{II}{Cy}\ \frac{III}{SbSer}$	$\frac{II}{II}\ \frac{III}{SbSer}$	$\frac{II}{Cy}\ \frac{III}{III}$	$\frac{II}{II}\ \frac{III}{III}$	Total No.
Observed numbers	Outbred	D	C	B	A	N
	Inbred	d	c	b	a	n
Expected numbers	Outbred	$N\delta$	$N\gamma$	$N\beta$	$N\alpha$	N
	Inbred	$N\delta$	$N\gamma\,(1-s)$	$N\beta\,(1-t)$	$N\alpha\,(1-s)\,(1-t)\,(1-i)$	n

where α, β, γ, δ are the fitnesses of the four genotypes.

The maximum-likelihood estimate of i may be obtained by forming cross-products and setting observed numbers equal to expected numbers, yielding $i = 1 - \frac{aBCd}{AbcD}$. When the chromosomes are independent in effect, *i.e.*, there is no interaction, $i = 0$. When the double chromosome homozygote fitness is smaller than expected under independence, $i > 0$. A larger-than-expected fitness for the double chromosome homozygote gives $i < 0$. The range of i is asymmetrical about zero, $-\infty < i < 1$.

It is convenient to express i directly in terms of fitnesses, particularly when overall fitness, and not just the viability component, is being measured. The fitnesses of the single and double homozygotes are estimated as $(1 - s) = Dc/dC$; $(1 - t) = Db/dB$; $(1 - s)(1 - t)(1 - i) = Da/dA$; and i may be rewritten as i = 1 − observed fitness of the double homozygote/expected fitness of the double homozygote.

We introduce a new statistic, k, to quantify such interactions. k is the correlation between heterozygosity and homozygosity for two chromosomes. The range of k is symmetrical about zero, the point of no interaction, $-1 \leq k \leq 1$; k always has the same sign as i.

k is derived as follows. In Table A1 below we represent a chromosomal homozygote by *IIi/IIi* or *IIIi/IIIi*, and a chromosomal heterozygote by *IIi/IIj* or *IIIi/IIIj*. We let the X variable of the correlation coefficient represent the second chromosome, let Y represent the third chromosome, and assign the arbitrary values 1 for homozygosis and 0 for heterozygosis; any arbitrary values will give the same results, but 0 and 1 are the easiest to work with.

The fitnesses can be thought of as the genotypic frequencies after one generation of selection in a population begun with equal frequencies of the four genotypes. The correlation between second and third chromosomes for homozygosity or heterozygosity, with sign reversed to give it the same sign as i, is

$$-r = k = -\frac{\overline{XY} - \overline{X}\,\overline{Y}}{\sqrt{(\overline{X}^2 - \overline{X}^2)(\overline{Y}^2 - \overline{Y}^2)}} = -\frac{e - (e+f)(e+g)}{\sqrt{[e+f-(e+f)^2][e+g-(e+g)^2]}}$$

If the effects on fitness of homozygosity for one chromosome do not depend

TABLE A1

Genotype	Fitness of this combination	Heterozygosis-homozygosis value: II X	III Y	X^2	Y^2	XY
IIi/IIIi IIi/IIIi	e	1	1	1	1	1
IIi/IIIi IIi/IIIj	f	1	0	1	0	0
IIi/IIIi IIj/IIIi	g	0	1	0	1	0
IIi/IIIi IIj/IIIj	h	0	0	0	0	0
Sum or mean	1	$e+f$	$e+g$	$e+f$	$e+g$	e

on whether the other chromosome is homozygous or heterozygous, there will be no correlation. If the chromosomes interact, *i.e.*, there is an association of fitness effects for homozygosity of the two chromosomes, there will be a correlation, the magnitude of which is a measure of the strength of the fitness interaction.

In order to estimate viability interactions, the viabilities of the double homozygote and of each of the single homozygotes are calculated, following the method of R. D. SEAGER and F. J. AYALA (see above). By this method, the viability of the double heterozygote is 1.0. These relative viabilities are then normalized so that they add up to 1.0, yielding *e*, *f*, g and *h*.

II. ERROR ANALYSIS

One advantage of *k* over *i* is that its range is bounded symmetrically about zero, the point of no interaction. The sampling properties of the two statistics were investigated by computer simulation. Five hundred draws were taken from a [0, 1] uniform distribution using the IMSL uniform random number generator on a CDC 74 computer and appropriately converted into a multinominal vector of the four fitness classes, forming a simulated data set. The sample size of 500 is the minimum number of progeny scored in the actual experiments. The parameters *i* and *k* were then estimated from 100 simulated data sets. Next we calculated the variance of each statistic and the difference beween the input parameter and the mean of the simulations, as a measure of bias; we also tested the normality, skewness and kurtosis of the sampling distribution of each statistic. We did this entire procedure for five fitness sets chosen to cover the range of possible interactions: one large and one small negative interaction, one large and one small positive interaction, and one with no interaction. For the fitness sets giving small negative and small positive interactions, two additional runs of 100 samples were made. Normality was tested by dividing the observed range of parameter values into 25 equal intervals, and comparing observed and expected numbers in the intervals with a chi-square test. The results are shown in Table A2.

In general, *k* is less biased than *i*, although the opposite is found in a few cases. A less biased *k* is particularly evident when the coefficient values are

TABLE A2

Error analysis of the interaction statistics i *and* k

Run	Statistic	Fitness set [a]	Expected value	Bias[b]	Variance	Tests for normality: χ^2 for fit	Skew	Kurtosis
1	*i*	0.18, 0.22	0.00000	−0.00062	0.02866	P > 0.50	**	
	k	0.27, 0.33	0.00000	0.00308	0.00160	P > 0.50		
2[c]	*i*	0.17, 0.24	0.36022	−0.00027	0.01715	P > 0.75	**	**
	k	0.31, 0.28	0.10907	0.00428	0.00224	P > 0.40		
2′[c]	*i*	0.17, 0.24	0.36022	−0.00934	0.01726	P > 0.25	**	
	k	0.31, 0.28	0.10907	0.00075	0.00228	P > 0.50		
3	*i*	0.091, 0.409	0.91687	−0.00097	0.00031	P > 0.10		
	k	0.364, 0.136	0.54822	−0.00046	0.00122	P > 0.25		
4[c]	*i*	0.26, 0.21	−0.88662	−0.03535	0.11700	0.1 > P > 0.05		
	k	0.21, 0.32	−0.15697	0.00011	0.00186	*		
4′[c]	*i*	0.26, 0.21	−0.88662	−0.04518	0.13271	0.1 > P > 0.05	*	
	k	0.21, 0.32	−0.15697	−0.00081	0.00201	P > 0.50		
5	*i*	0.375, 0.125	−4.98204	−0.05605	1.25085	P > 0.10	*	
	k	0.167, 0.333	−0.41748	0.00317	0.00142	P > 0.75		

* $P < 0.05$; ** $P < 0.01$. All significant skews are negative (to the left) and the significant kurtosis is positive (leptokurtic).

[a] Fitness of the double homozygote listed first, followed by the two single homozygotes and then wild type. Fitnesses sum to 1.0.

[b] Bias = observed − expected.

[c] Runs 2 and 2′; 4 and 4′ are replicates.

negative. More importantly, k has a much smaller sampling variance than i, usually by one or two orders of magnitude. The only exception occurs when i is very close to its maximum value of 1.0, as in run 3. Moreover, the sampling variance of k is fairly stable over the wide range of possible interactions studied, ranging from 0.00122 to 0.00228. The sampling variance of i is very variable, ranging from 0.00031 to 1.25085.

The distribution of k is significantly non-normal is one instance, that of i lies on the edge of significance for the same fitness set (run 4). When this fitness set was rerun using a different random number seed, the distribution of k did not differ significantly from normality (run 4′). For five of the seven runs i is significantly skewed to the left. In one case it is both skewed and leptokurtic.

Over small ranges of i and k, such as those generated from a given fitness set, i and k are highly correlated ($r = 0.99$, 98 d.f.). Over the entire ranges of i and k, however, there is no one to one correspondence. Different fitness sets that give the same value of i may give different values of k and *vice versa*.

Although it is tedious to compute the asymptotic sampling variance for i or k, the asymptotic sampling variance for a logarithmic transformation of i denoted $i' = -\ln(1 - i)$, is easily written in compact form following HALDANE (1956). We

TABLE A3

Sampling variance for i′ with n = 500 *vs. the theoretical sampling variance*

Run[a]	Variance	
	Observed in simulations	Theoretical
1	0.02713	0.03367
2	0.03867	0.03369
2′	0.03888	
3	0.04401	0.04707
4	0.03160	0.03299
4′	0.03431	
5	0.03366	0.03932

[a] The fitness sets are shown for each run in Table A2.

calculated the sampling variance of i' from the stimulated data sets generated with the fitness sets shown in Table A2. These values, along with the theoretical values, are shown in Table A3. The observed and theoretical variances do not differ significantly. Thus if i' is used as an index of interaction, we are probably justified in using the formula for the asymptotic variance to set confidence limits about the estimates of interaction.

The effect of decreasing sample size on the variance of the interaction statistics was also studied. For three of the fitness sets (interactions zero and small negative and positive) we made runs with sample sizes of 300 and 100. The sampling variance for i increases slightly faster as sample size decreases than the sampling variance for k. For $n = 300$, sampling variance is from 1.2 to 2.3 times larger for i, and from 1.2 to 2.1 times larger for k. For $n = 100$, sampling variance is 5.6 to 9.9 times larger for i, and 4.5 to 6.5 times larger for k.

Our simulations show the sampling properties of k make it a better interaction statistic than i, in the sense that it is less sensitive to random fluctuations in the sampling of progeny. The sample variance of k is usually smaller than that of i, and is much more stable over a wide range of interactions.