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CHROMOSOME INTERACTIONS IN *DROSOPHILA MELANOGASTER*. II. TOTAL FITNESS

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ABSTRACT

In a large experiment, using nearly 200 population cages, we have measured the fitness of *Drosophila melanogaster* homozygous (1) for the second chromosome, (2) for the third chromosome, and (3) for both chromosomes. Twenty-four second chromosomes and 24 third chromosomes sampled from a natural population were tested. The mean fitness of the homozygous flies is 0.081 ± 0.014 for the second chromosome, 0.080 ± 0.017 for the third chromosome, and 0.079 ± 0.024 for both chromosomes simultaneously. Assuming that fitnesses are multiplicative (the additive fitness model makes no sense in the present case because of the large selection coefficients involved), the expected mean fitness of the homozygotes for both chromosomes is 0.0066; their observed fitness is more than ten times greater. Thus, it appears that synergistic interactions between loci are considerable; and that, consequently, the fitness function substantially departs from linearity. Two models are tentatively suggested for the fitness function: a "threshold" model and a "synergistic" model.——The experiments reported here confirm previous results showing that the concealed genetic load present in natural populations of Drosophila is sufficient to account for the selective maintenance of numerous polymorphisms (of the order of 1000).

IN the preceding paper, we raised the question of fitness interactions between sets of loci—how the fitness of an individual homozygous for two nonhomologous chromosomes relates to the fitness of the corresponding single-chromosome homozygotes. Previous studies, including our own, have considered one fitness component, *i.e.*, viability from zygote to adult. But other fitness components are important; for example, differences in fertility (female fecundity and male mating success) are known to have much greater fitness consequence than viability differences (SVED and AYALA 1970; PROUT 1971; BUNDGAARD and CHRISTIANSEN 1972; MARINKOVIĆ and AYALA 1975a, b; BRITTNACHER 1981).

We now investigate the nature of the fitness function taking into account all fitness components, *i.e.*, considering the totality of fitness effects as a single quantity. Moreover, fitness is studied under "natural" density conditions, *i.e.*, in cages where populations reach the carrying capacity of their environment, rather than in near-optimal density conditions as viability studies are usually

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performed. In our experiments, competition for limiting resources—food and living space—may contribute to fitness differences.

Our previous experiment has shown that the egg-to-adult viability of *Drosophila melanogaster* flies homozygous for both chromosomes *II* and *III* is, on the average, greater than is expected from the viability of the single-chromosome homozygotes on the assumption that different loci have independent fitness effects. When all stages of the life cycle are taken into account, we observe a larger departure from expectation but in the same direction—the fitness of the homozygotes for both chromosomes *II* and *III* is, on the average, about one order of magnitude greater than expected on the assumption of independent fitness effects. These results are of considerable import. First, because they show that there are fitness interactions between loci and, thus, that theoretical models assuming independence may not be valid. Second, because, if our results have general validity, the number of loci that can be maintained by overdominance and other forms of selection in natural populations may be much larger than has been previously estimated.

MATERIALS AND METHODS

Experimental

A sample of several hundred *Drosophila melanogaster* flies were collected at MacDonald Ranch, Napa County, California, in the fall of 1974. Whole wild second and third chromosomes were made homozygous by a series of crosses with a special balancer stock (SEAGER and AYALA 1982). Four genotypes are expected in the F_3 generation of such crosses: (1) *double homozygotes*, *i.e.*, flies homozygous for both a second and a third wild chromosome; (2) *single homozygotes* for the second wild chromosome, but heterozygotes for the third wild chromosome and a balancer; (3) *single homozygotes* for the third wild chromosome but heterozygotes for the second wild chromosome and a balancer; (4) *double balancers*, *i.e.*, heterozygotes for the wild and the balancer chromosomes at both the second and the third chromosomes. The expected ratios of the four genotypes are, respectively, 1:2:2:4, but these may be altered by viability differences among the genotypes.

One hundred fifty-four second and third wild chromosomes were extracted. In 94 of these lines, one or both wild chromosomes were lethal in homozygous condition, whereas for the other 60 lines all four kinds of genotypes were present in the F_3 generation of the crosses just mentioned. Twenty-four of these 60 nonlethal lines were selected for the present experiments. The 24 were chosen largely at random, but making sure that some with relatively low, and some with high, homozygous viability would be included. The mean egg-to-adult viabilities of the homozygotes relative to wild-type heterozygous flies are given in Table 1 for these 24 experimental lines. For purposes of comparison the mean viabilities of the homozygotes for all 60 nonlethal lines are also given in this table. It can be seen that the homozygotes for the 24 experimental lines have a somewhat greater-than-average viability, because we avoided using lines in which the viability of the homozygotes was close to zero.

In order to obtain flies homozygous for only the second or the third chromosome that would be heterozygous for *two wild-type chromosomes* at the other chromosome, we made crosses as shown in Figure 1. These crosses, which yield only two genotypes each, were made for each of the 24 experimental lines. For each of the 24 lines we thus ended with three sublines: the double-homozygote subline mentioned earlier with four genotypes (see also Figure 1 in SEAGER and AYALA 1982), and two single-homozygote sublines, one for the second and the other for the third chromosome.

Fitness was measured using population cages of the type described by AYALA (1968). Two replicate cages were started with each of the three sublines, that is six cages for each of the 24 lines, or a total of 144 experimental populations (contamination with mites or with flies made it occasionally necessary to start a third or even a fourth replicate). The single-homozygote cages

TABLE 1

Mean egg-to-adult viability relative to wild-type heterozygotes of the 24 experimental lines and of all 60 nonlethal lines in Drosophila melanogaster

	Double homozygotes	Second-chromosome homozygotes	Third-chromosome homozygotes
Experimental lines:			
Mean	0.554 ± 0.043	0.672 ± 0.034	0.676 ± 0.042
Range	0.241, 0.985	0.291, 0.954	0.215, 0.921
All 60 lines:			
Mean	0.415 ± 0.033	0.636 ± 0.034	0.611 ± 0.032
Range	0.009, 0.997	0.009, 1.160	0.021, 0.985

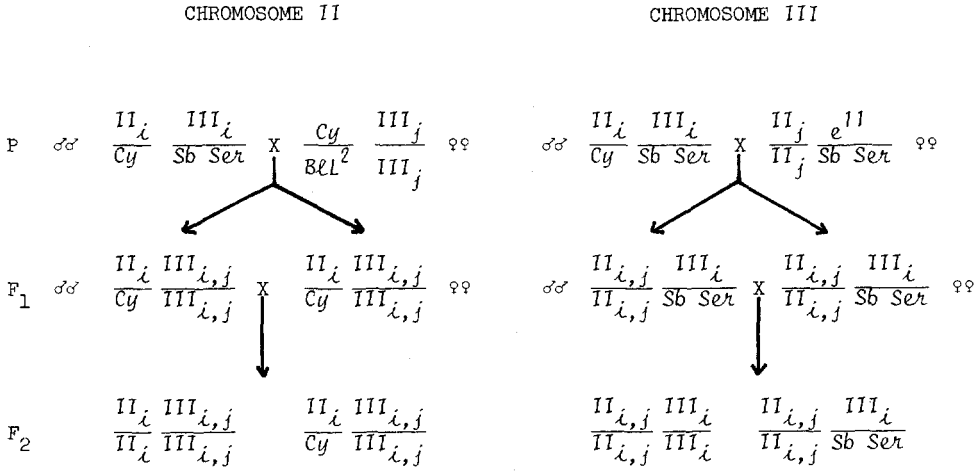

FIGURE 1.—Mating scheme for obtaining single-chromosome homozygotes of *Drosophila melanogaster* starting with males heterozygous for a wild and a balancer chromosome at both the second and the third chromosomes. *Cy* and *Sb Ser* represent balancer chromosomes that suppress recombination. BlL^2 and e^{11} represent marker chromosomes. The subscript *i* represents a given wild chromosome; *j* represents numerous wild chromosomes from laboratory stocks; *i,j* indicates that a given wild chromosome (*i*) has been added to the background of laboratory chromosomes. (For the mating scheme used to obtain the double-chromosome homozygotes, see SEAGER and AYALA 1982, Figure 1.)

make it possible to estimate the fitness of flies homozygous for either the second or the third chromosome relative to flies heterozygous for the corresponding wild and balancer chromosomes; the double-homozygote cages allow the estimation of the fitness of the double-chromosome homozygotes relative to heterozygotes for the two wild and the two balancer chromosomes.

We are, of course, interested in estimating the fitness of homozygous flies relative to flies heterozygous for wild chromosomes, *i.e.*, relative to flies carrying the same levels of heterozygosity as those living in natural populations. Control cages were established for this purpose, where the wild chromosomes from all 24 lines were introduced in each cage, together with the appropriate balancers. In these control cages, therefore, phenotypically wild flies have levels of heterozygosity comparable to those in nature. These cages make it possible to estimate the fitness of flies heterozygous for a wild and a balancer chromosome relative to wild flies. The fitness estimates of the homozygous flies relative to the balancer heterozygotes, obtained from the experimental cages, can thus be corrected by the fitness estimates of the balancer heterozygotes relative to wild flies,

obtained from the controls (see SVED and AYALA 1970; and TRACEY and AYALA 1974, for details). Ten control cages were established for the double-homozygote lines and eight for each one of the two single-homozygotes, giving a total of 26 control populations.

The two replicates of every experimental cage were started with the balancer chromosome in a frequency of either 20% or 45%. The control cages were started with the balancer chromosome in a frequency of 30% in half of them and 45% in the other half. (Because the balancer chromosome is lethal in homozygous condition, its maximum possible frequency is 50%.) Each cage was initiated with 500 flies, divided equally between the sexes. In the case of the control cages, all 24 wild chromosomes were equally represented in each cage.

Each population cage contained 10 food cups with ¾ oz. of a standard medium made of cornmeal, molasses and agar. A rolled up Kleenex tissue and 2 ml of a yeast solution were added before introducing the cup in the cage. The food cups were replaced on a rotating schedule, with each cup remaining in the cage for 21 days. The cages were maintained in an air-conditioned room at about 23°.

Adult frequencies were sampled once every 3 weeks or about once per generation (SEAGER 1982; TRACEY and AYALA 1974; CROW and CHUNG 1967) until there was evidence that the balancer chromosome had reached an equilibrium frequency or until it was eliminated. At least 300 flies were scored at each sample. All the food cups with the eggs, larvae and pupae, as well as the adults, were transferred to a clean cage after each sampling. Whenever the balancer reached an equilibrium frequency, egg samples were taken and reared under optimal conditions in order to obtain estimates of the zygote frequencies. The zygote frequencies are required for estimating fitnesses in the equilibrium populations (PROUT 1965). Each balancer chromosome reached nontrivial equilibrium frequencies in about 60% of the experimental cages, but was eliminated in the other 40%. In the control cages, the balancer chromosomes were always eliminated.

Estimation of fitness

The procedures used for fitness estimation were different for the equilibrium cages and for those in which the balancer was eliminated. Two different methods of estimating fitness were used in each situation.

When the balancer and wild chromosomes reached an equilibrium we used, as one method, that outlined by TRACEY and AYALA (1974). If h' is the zygote frequency of the balancer heterozygote, and $(1 - h')$ is the zygote frequency of the homozygote, the fitness of the homozygote relative to the balancer heterozygote is given by

$$w = \frac{2 - 3h'}{2 - 2h'} \tag{1}$$

Two fitness components, viability (V) and fertility (F) can also be estimated, using the formulae given by TRACEY and AYALA (1974).

The second method for estimating fitness in the equilibrium cages uses the formula given by ANDERSON (1969) for calculating fitness when sampling is done at the adult stage:

$$Q_t = \frac{W^t(W-1)Q_0}{W - 1 + (L - 2 + 2W)(W^t - 1)Q_0}, \tag{2}$$

where W is the fitness of the heterozygote relative to the homozygote (hence $w = 1/W$), Q_t is the equilibrium frequency, Q_0 is the initial frequency, W^t is the number of generations required to reach equilibrium, L is the late component of fitness (similar to fertility), E is the early component of fitness (similar to viability), and $W = E \cdot L$ (see PROUT 1965). Curve fitting to equation (2) yields maximum likelihood estimates for fitness (W), viability (E) and fertility (L). The maximum likelihood procedure is a modified version of ANDERSON'S (1969).

These two procedures give very similar fitness estimates for the equilibrium cages; the correlation between the two sets of fitnesses is $r = 0.98$ with 68 d.f. Given that the two estimation procedures are very different, the similarity of the two sets of values indicates that we are obtaining reliable fitness estimates.

When the balancer chromosome was eliminated from the populations, the first procedure used to estimate fitness was the maximum likelihood method just described, where Q_t is the last nonzero

frequency observed in the cages. Notice that this frequency is always very small, around 0.003, and thus it must be very close to the zygote frequency, certainly within the bounds of sampling error at such a low frequency.

The second procedure for estimating fitness in the cages where the balancer was eliminated is described by MOURÃO *et al.* (1972) and ANDERSON (1969). The formula is very similar to (2), except that the early and late components of fitness are not separated:

$$Q_t = \frac{W^t(W-1)Q_0}{W-1+(2W-1)(W^t-1)Q_0} \tag{3}$$

This is a polynomial of order $(t+1)$ in W, and can be solved by Newton's iterative method. Q_t is the last positive frequency observed; the other parameters are as for equation (2).

The fitnesses obtained by these two methods for the nonequilibrium populations do not agree as well as those obtained for the equilibrium populations; the correlation coefficient is $r = 0.69$ with 26 degrees of freedom for the experimental cages, and $r = 0.56$ with 14 degrees of freedom for the control cages. Using the two procedures given by equations (2) and (3), MOURÃO *et al.* (1972) obtained a correlation coefficient of $r = 0.91$ with 14 degrees of freedom, for nonequilibrium populations. For equilibrium populations, TRACEY and AYALA (1974) obtained $r = 0.96$ with 28 d.f. between fitness values obtained by equations (1) and (2).

In the double-balancer cages, there are four possible genotypes. The fitness of the double homozygotes was estimated in these cages relative to the double heterozygotes, using the two relevant methods in each case. The fitness of the single homozygotes was estimated using exclusively the single-balancer cages.

The fitness values obtained by the two appropriate methods (in the cages in which the balancer reached equilibrium as well as when it was eliminated) were averaged in order to obtain a fitness estimate for each cage. However, when one of the estimates was negative, this was ignored and only the other value was used. For additional details concerning the estimation of fitness, see SEAGER (1979).

Estimation of fitness interactions

Fitness values for multi-locus systems may depart from the values expected on the assumption that the fitnesses of the loci involved act independently. One statistic used to quantify departures from expectation is i (DOBZHANSKY *et al.* 1965), which may be defined as

$$i = 1 - \frac{\text{observed fitness}}{\text{expected fitness}}$$

For the multiplicative model, the expected fitness of the double homozygotes is obtained by multiplying the fitness of the corresponding single-chromosome homozygotes.

We have also used another statistic, k, which is a modified correlation coefficient (this statistic was suggested by PROFESSOR JACK L. KING; see SEAGER and AYALA 1982). k as well as i is negative when the observed fitness is greater than expected, and positive when it is smaller. k has the desirable property of being symmetric around zero ($-1 \leq k \leq 1$), where i is not ($-\infty < i \leq 1$); in addition, k is less sensitive than i to random fluctuations in progeny sampling.

RESULTS

Single homozygotes

The fitness of homozygous flies relative to the balancer heterozygotes can be estimated from the experimental cages. The control cages allow one to estimate the fitness of the balancer heterozygotes relative to wild-type flies (i.e., carrying two different wild chromosomes). Multiplication of the two fitness values gives an estimate of the fitness of homozygous flies relative to wild-type heterozygotes (SVED and AYALA 1970; TRACEY and AYALA 1974). These estimates are given in Table 2 for the flies homozygous for either the second or the third chromosome.

TABLE 2

Fitness of Drosophila melanogaster *homozygotes for either the second or the third chromosome relative to heterozygotes for two wild chromosomes*

Line	Second chromosome	Third chromosome
007	0.054	0.006
010	0.029	0.034
012	0.147	0.024
016	0.018	0.005
020	0.229	0.036
021	0.137	0.049
041	0.018	0.008
047	0.030	0.023
048	0.022	0.144
055	0.215	0.023
072	0.113	0.166
076	0.055	0.022
092	0.084	0.140
095	0.039	0.163
102	0.077	0.100
113	0.025	0.008
122	0.039	0.016
128	0.049	0.025
134	0.070	0.208
166	0.193	0.055
169	0.112	0.037
171	0.002	0.199
178	0.029	0.114
211	0.152	0.313
Mean	0.081	0.080
S.E.	0.014	0.017

The fitnesses of the balancer heterozygotes relative to the wild-type heterozygotes are given in Table 3.

Two replicate experimental populations were started for each wild chromosome, the initial frequency of the balancer chromosome being 0.20 in one, and 0.45 in the other replicate. The values given in Table 2 are the averages of the two replicates; the replicates have effectively identical fitness estimates, their correlation being, $r = 0.91$ (48 d.f., highly significant).

Chromosomes that are recessive lethal in the egg-to-adult viability test (SEAGER and AYALA 1982) have been excluded from the present experiment. Moreover, as noted above, the chromosomes used in the population cage experiment have somewhat greater than average egg-to-adult viability, even after the lethals are excluded (see Table 1). Yet, the present experiments show that the overall fitness of flies homozygous for either the second or the third chromosome is very low: the highest fitnesses observed are 0.229 (line 020) for the second chromosome and 0.313 (line 211) for the third chromosome, both in the semilethal range. The fitness value estimated for any one of the chromo-

TABLE 3

Fitness of heterozygotes for the balancer chromosomes relative to wild-type heterozygotes in the control populations

Cage	Second chromosome	Third chromosome	Second and third chromosomes
1	0.094[a]	0.120[a]	0.096[a]
2	0.070[a]	0.128[a]	0.067[a]
3	0.084[a]	0.172[a]	0.189[a]
4	0.136[a]	0.139[a]	0.087[a]
5	0.111	0.078	0.037[a]
6	0.225	0.081	0.055
7	0.086	0.100	0.042
8	0.113	0.158	0.045
9	—	—	0.181
10	—	—	0.140
Mean	0.115	0.122	0.094
SE	0.049	0.034	0.018

[a] Initial frequency of the balancer chromosome was 0.30; in all the other cages, it was 0.45.

somes is, of course, subject to experimental error, although the good agreement between replicates suggests that such error might not be too great. In any case, we are not interested in the fitness of particular chromosomes, but rather in the overall pattern and the average fitness decrease resulting from homozygosis for full chromosomes (SVED and AYALA 1970; SVED 1971; TRACEY and AYALA 1974).

The mean fitness for the second-chromosome homozygotes is 0.081 ± 0.014, somewhat lower than the values previously reported for this species. Previous estimates are 0.14 ± 0.04 (SVED 1971) and 0.112 ± 0.050 (TRACEY and AYALA 1974). (These means are calculated including negative fitness estimates; if the negative estimates are set equal to zero, the means are 0.17 ± 0.02 for SVED's data and 0.182 ± 0.029 for TRACEY and AYALA's.) Similarly, the mean fitness for the third-chromosome homozygotes is, in our experiment, 0.080 ± 0.017, slightly lower than SVED's (1975) mean of 0.10 ± 0.04 (0.12 ± 0.03, if negative values are converted to zeroes), and considerably lower than TRACEY and AYALA's (1974) mean of 0.318 ± 0.037. Considering that these experiments have been performed at different times and by different investigators, the consistency of the results is remarkable. (One factor that may have contributed to the relatively high mean fitness estimated by TRACEY and AYALA for the third chromosome homozygotes is that their egg-to-adult viability tests yielded an unusually high proportion of lethal and semilethal chromosomes—44% and 16%, respectively—which were all excluded from the fitness experiments.)

Two implications of these results deserve attention. First, that the inbreeding depression is substantially greater when total fitness is considered than when only egg-to-adult viability is taken into account, an observation already made by SVED and AYALA (1970). This indicates that fertility and related components of the life cycle have greater fitness consequences than egg-to-adult viability.

The second implication of our (and previous) results is that homozygosis for any one of the large autosomes effectively entails lethality or semilethality in *Drosophila melanogaster*.

Double homozygotes

The most significant new element of our experiments in relation to previous studies is the possibility of estimating fitness in flies simultaneously homozygous for the second and third chromosomes. These fitness estimates can, then, be compared with the fitness of the single-chromosome homozygotes for the same second and third chromosomes. Table 4 gives the estimated fitness of the double homozygotes, their expected fitness based on the multiplicative model, and the two coefficients, *k* and *i*, that measure fitness interactions between the two chromosomes. The fitness of the double-balancer heterozygotes relative to the wild flies, calculated from the control populations, are given in Table 3.

TABLE 4

Observed and expected fitness of the double homozygotes (relative to wild-type heterozygotes) and coefficients of interaction (k and i)[a]

Line	Observed fitness	Expected fitness	*k*	*i*
007	0.065 (0.004)	0.0003	−0.683	−216
010	0.023 (0.014)	0.001	−0.392	−22.0
012	0.010	0.004	−0.082	−1.86
016	0.158	0.0001	−0.922	−1579
020	0.045	0.008	−0.219	−4.49
021	0.015	0.007	−0.077	−1.24
041	0.000 (−0.050)	0.0001	0.012	1.00
047	0.000 (−0.022)	0.0007	0.026	1.00
048	0.090	0.003	−0.496	−27.1
055	0.095 (0.080)	0.005	−0.422	−18.4
072	0.016	0.019	0.016	0.149
076	0.012	0.001	−0.218	−9.00
092	0.080	0.012	−0.323	−5.78
095	0.094	0.006	−0.431	−13.7
102	0.208	0.008	−0.621	−26.0
113	0.004	0.0002	−0.184	−17.0
122	0.008 (−0.015)	0.0006	−0.214	−12.3
128	0.058	0.001	−0.581	−47.3
134	0.456	0.015	−0.657	−30.2
166	0.002	0.011	0.073	0.811
169	0.000 (−0.435)	0.004	0.060	1.00
171	0.042	0.0004	−0.369	−104
178	0.044 (0.010)	0.003	−0.354	−12.3
211	0.365	0.048	−0.436	−6.67
Mean	0.079 (0.073)		−0.312	−12.33[b]
SE	0.024 (0.024)		0.055	

[a] The values in parentheses are obtained using negative fitness estimates rather than converting them to zero.

[b] Median, rather than mean.

The "observed" fitness estimates given in Table 4 are the average values for the two replicate populations. (Exceptions are lines 048 and 169, for which only one population was available, and line 095, which is based on three replicates because two new ones were started when it was discovered that one of the original pair had become contaminated.) The procedures used to estimate fitness may yield negative estimates; these happened in eight populations. A negative fitness value can be treated in two ways. It may be set equal to zero and then averaged with the other replicate. Or it may be allowed to stand and be averaged with the other replicate; the values obtained by this second procedure are given in parentheses in Table 4. The use of negative fitness values can be justified on the grounds that, owing to experimental error, the estimates may be greater or lower than the "true" fitness values. Errors on the higher and on the lower side of the estimates would cancel out when means for all chromosomes are obtained. In any case, how negative estimates are treated is of little consequence in our experiments. The mean fitness of the double homozygotes is 0.073 ± 0.024 if negative values are used, and 0.079 ± 0.024 if they are set to zero. The difference is not statistically significant and does not affect in any way the conclusions reached below. Moreover, the fitness estimates for the two replicates are always in good agreement (their correlation is $r = 0.75$ using the negative values, and $r = 0.87$ if they are set to zero, 19 degrees of freedom in either case).

The results are surprising and remarkable. *The average fitness of flies homozygous for both the second and the third chromosomes is approximately the same as the average fitness of flies homozygous for only one or the other chromosome.* The values are 0.079 ± 0.024 (or 0.073 ± 0.024) for the double homozygotes, 0.081 ± 0.014 for the second chromosome homozygotes, and 0.080 ± 0.017 for the third chromosome homozygotes. If fitnesses are assumed to be multiplicative, the expected mean fitness of the double homozygotes should be about 0.0066; *the observed value is more than ten times greater.*

The range of fitness values for the double homozygotes extends from 0 (three populations) to 0.456 (population 134). The fitness of the double homozygotes in populations 134 and 211 is greater than the fitness of any one of the single homozygotes, although the third chromosome homozygotes exhibit relatively high fitness in both lines (0.208 and 0.313, respectively). In these two lines, fitness interactions between the second and third chromosomes ostensibly result in an increase of the fitness obtained when there is homozygosis for only one of the chromosomes. The fitness of the double homozygotes is higher than the fitness of at least one of the single-chromosome homozygotes in several other cases (e.g., lines 007, 016, 020, 048 and 055).

Table 4 gives the expected fitness for the double homozygotes on the assumption that fitnesses are multiplicative, *i.e.*, calculated simply by multiplying the fitnesses of the corresponding single chromosome homozygotes. The mean observed fitness is significantly greater than the mean expected fitness ($t = 3.22$, $P < 0.005$); this is hardly surprising since, as noted above, the mean observed fitness is one order of magnitude greater than the expected value. This result is confirmed when the individual lines are taken into consideration. If the null

hypothesis (*i.e.*, multiplicative independence) were correct, we would expect to observe equal numbers of greater-than-expected and smaller-than-expected fitnesses. But in 19 of the 24 lines, the observed fitness is greater than expected, a deviation that is statistically significant on the basis of a sign test ($P < 0.005$ if we use a one-sided test and $P < 0.01$ for a two-sided test).

Fitness interactions for the individual lines are quantified using two methods: the i statistic used by previous workers (DOBZHANSKY, SPASSKY and ANDERSON 1965; TEMIN *et al.* 1969; KOSUDA 1971) and the k statistic suggested by PROFESSOR J. L. KING. The mean value of k for all 24 populations is -0.312 ± 0.055 (or -0.263 ± 0.054 when negative values are used), which is significantly negative. Even if we exclude lines 134 and 211—for which the double homozygote fitness exceeds both single homozygote fitness—the mean value of k is -0.291 ± 0.057 (or -0.237 ± 0.055), also significantly negative. The results of the sign test are thus corroborated.

The values of the i statistic are given in the last column of Table 4. As expected, 19 of 24 values are negative (*i.e.*, for all the populations in which the observed fitness is greater than expected). Because i is not symmetrically distributed around 0, and many of the negative values are so large, it is not meaningful to calculate the mean value of i. If all the negative i's larger than one in absolute value are converted into -1.00, the mean value of i is -0.627 ± 0.155, which is significantly negative. More meaningful perhaps is to indicate that the medium value of i is -12.33. SEAGER and AYALA (1982) have calculated the 95% confidence intervals of the individual i values in their study of egg-to-adult viability. Unfortunately, no formula is available to calculate the 95% intervals of the individual i's using the population cage data. In any case, the results obtained using i confirm those obtained with the k statistic.

Fitness components

The formulae used to estimate fitness also yield estimates of two fitness components: "viability" and "fertility," or "early" and "late." (Viability in this case is measured under population cage conditions and thus is not the same as the egg-to-adult viability measured under near-optimal conditions in SEAGER and AYALA (1982).) These two estimates are not independent, because their product must yield the fitness estimate discussed in the previous sections. Hence, if the estimate for one of the components is very low (which may happen, owing to sampling errors), the other component must be correspondingly very high—a difficulty already noted by SVED and AYALA (1970). The individual estimates of fitness components are, therefore, potentially subject to considerable error and are, in any case, less reliable than the estimates for overall fitness. Nevertheless, the ensemble of fitness components estimates gives an idea of the relative contributions of viability and fertility to overall fitness.

The estimates of viability and fertility are given in Table 5 for the experimental populations and in Table 6 for the controls. The fertility of the homozygotes is smaller than their viability in most of the populations. This confirms previous results showing that fertility contributes to fitness variation considerably more

TABLE 5

Fitness components for the single-chromosome and double-chromosome homozygotes[a]

	Second chromosome		Third chromosome		Both chromosomes	
Line	V	F	V	F	V	F
007	0.694	0.274	0.798	0.618	2.827	0.039
010	0.953	0.069	0.537	0.214	0.260	0.086
012	1.199	0.179	0.316	0.359	0.677	0.006
016	0.491	0.172	0.149	0.110	0.749	0.685
020	9.133	0.056	0.332	0.279	0.254	0.152
021	0.335	0.590	0.296	0.392	0.034	0.318
041	0.235	0.181	0.249	0.181	0.065	0.000
047	1.362	0.005	0.711	0.033	0.004	0.000
048	0.381	0.107	0.439	0.573	0.033	1.342
055	1.535	0.314	0.485	0.336	3.576	0.043
072	0.489	0.422	1.138	0.308	8.081	0.001
076	0.486	0.292	0.365	0.298	0.402	0.019
092	0.288	0.792	0.385	0.570	32.796	0.002
095	0.300	0.416	0.475	1.496	0.477	0.586
102	1.072	0.634	0.930	0.899	2.486	0.075
113	0.400	0.144	0.190	0.212	0.014	0.158
122	1.306	0.269	1.402	0.018	0.091	0.328
128	0.530	0.472	0.897	0.136	0.333	0.066
134	0.317	0.710	0.397	1.496	2.711	0.146
166	0.892	0.319	10.825	0.361	0.002	0.598
169	1.139	0.186	0.281	0.272	4.098	0.000
171	0.258	0.053	0.201	1.925	0.043	1.718
178	0.175	0.906	0.295	1.612	0.134	0.640
211	0.572	0.403	0.300	3.710	0.747	0.235
Mean	1.023	0.332	0.933	0.684	2.537	0.302
S.E.	0.362	0.050	0.435	0.171	1.372	0.090

[a] V = viability; F = fertility.

than viability (SVED and AYALA 1970; see also PROUT 1971; BUNDGAARD and CHRISTIANSEN 1972; MARINKOVIĆ and AYALA 1975a, b; BRITTNACHER 1981).

Studies of the fitness reduction associated with the degree of homozygosity have been, for the most part, concerned with egg-to-adult viability under near-optimal conditions (see SEAGER and AYALA (1982) and references therein). The question arises whether the results of such studies allow one to make inferences concerning total fitness. We have investigated this question by correlating the egg-to-adult viabilities with the estimates for total fitness obtained from the population cages (Figure 2). In the case of flies homozygous for a single chromosome, the correlations are not significantly different from zero ($r = 0.176$ for the second chromosome and $r = -0.225$ for the third chromosome, with 22 d.f.); the correlation for the double homozygote is significantly positive, although quite low ($r = 0.461$, 22 d.f., $P < 0.05$). Because any one of the individual estimates used in the correlations is actually subject to some (unknown) degree

TABLE 6

Fitness components for flies heterozygous for the balancer chromosomes in the control populations

	Second chromosome		Third chromosome		Both chromosomes	
Cage	V	F	V	F	V	F
1	0.134	0.591	0.366	0.600	0.062	1.548
2	0.455	0.139	0.588	0.301	0.001	76.923
3	0.642	0.120	0.401	0.431	1.748	0.108
4	1.650	0.083	0.265	0.588	0.006	15.385
5	0.776	0.144	0.733	0.087	0.153	0.242
6	1.958	0.125	0.814	0.094	0.055	0.991
7	0.478	0.178	0.043	1.297	0.109	0.384
8	0.202	0.522	0.927	0.165	0.128	0.353
9	—	—	—	—	0.224	0.810
10	—	—	—	—	1.181	0.118
Mean	0.787	0.238	0.517	0.445	0.367	9.686
S.E.	0.236	0.070	0.106	0.141	0.189	7.616

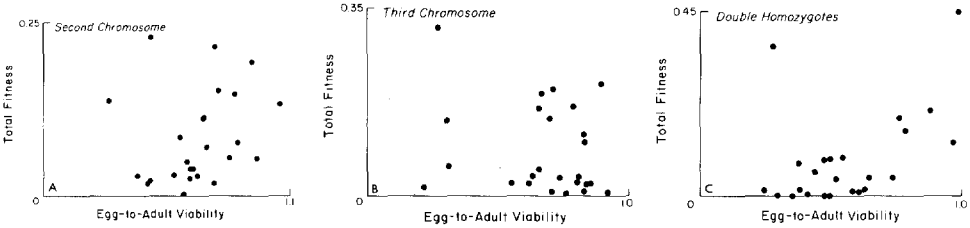

FIGURE 2.—Correlation between egg-to-adult viability and total fitness. (A), for flies homozygous for the second chromosome ($r = 0.176$, not significant); (B), for flies homozygous for the third chromosome ($r = 0.225$, not significant); (C), for flies homozygous for both the second and the third wild chromosome ($r = 0.461$, $P < 0.05$).

of experimental error, the r values given are greater than they would be if we knew the error around each value.

Similar correlations between egg-to-adult viability and total fitness can be calculated for the data of SVED (1971, 1975) and TRACEY and AYALA (1974) for either second or third chromosome homozygotes of *D. melanogaster*, and for the data of MOURÃO, AYALA and ANDERSON (1972) for second chromosome homozygotes of *D. willistoni*. None of these correlations is significantly different from zero. These results support SVED'S (1971, p. 103) contention that "considerable caution must be exercised in extrapolating from the results of viability ratio tests (*i.e.*, egg-to-adult viability estimates) to the overall fitness of Drosophila."

We have also calculated the correlations between the estimates of egg-to-adult viability and the estimates obtained for viability ("early" component of fitness) in the cages. None of these correlations is significantly different from zero ($r = -0.147$ for the second chromosome homozygotes, $r = -0.394$ for the

third chromosome homozygotes, and $r = -0.053$ for the double homozygotes, each with 22 d.f.). It appears that these two measurements consider attributes that are, in a substantial part, different.

DISCUSSION

Natural selection is the process postulated in order to account for the adaptive organization of living beings. One central aim of population genetics is to identify the mode of operation, and to measure the intensity, of natural selection. This latter task has proved elusive. There are few genes, and few traits, in very few organisms for which we have any reliable estimates of the strength of natural selection. Attempts to obtain a general understanding of the mode and intensity of natural selection have followed one of two approaches. One is indirect, based on the distribution of allelic frequencies in natural populations (usually for genes identified by the encoded enzymes and other proteins), or on the allelic differences between living species. The results of these studies remain controversial with respect to how much, if any, natural selection must be invoked. The other approach is direct: the study of the inbreeding depression. Inbreeding experiments deal with fractions of the genome as an ensemble rather than with specific genes, but this lack of specificity is an advantage because the results may than be applied to the "average" locus, rather than only to specific kinds of loci.

Inbreeding experiments have been performed with a few organisms, particularly with Tribolium and Drosophila (see SEAGER and AYALA 1982). In a few species of Drosophila, the existence of "balancer" chromosomes has made possible the construction of individuals homozygous for full chromosomes, with the advantage that the degree of homozygosis is precisely known. These studies have usually measured egg-to-adult viability, although other fitness components, such as rate of development, female fecundity and male fertility, have also been measured. In general, these studies have indicated a relatively small reduction in the fitness of the homozygotes (LEWONTIN 1974) and it was possible to attribute the fitness reduction mostly to a few genes—lethals and steriles—with large effects (TEMIN 1966). SVED and AYALA (1970) introduced a new technique, using population cages, that made possible to measure fitness as a whole in homozygous individuals. This technique has been used by various investigators in several species. The results of these studies greatly contrast with those from investigations of individual fitness components: the levels of natural selection uncovered are much higher than those previously observed. The mean fitness of homozygotes for a full chromosome is very low (around 0.15 or less) and no single chromosome appears as quasi normal—the maximum fitness of the individual chromosomes is always below 0.50—leading to the inference that many loci contribute to the fitness depression (SVED and AYALA 1970; MOURÃO *et al.* 1972; SVED 1971, 1975; TRACEY and AYALA 1974).

In order to obtain some notion of the number of loci involved and of their mode of operation, it is necessary, however, to know something about how loci interact with respect to fitness. The results presented in this paper are a first attempt in that direction. Interactions among loci at the molecular and organ-

ismic level are likely to be of many kinds, but the general question we are raising is whether gene interactions can be ignored with respect to total fitness; for example, because they are small or because they tend to cancel out. But if fitness interactions are not trivial, we shall be interested in the direction of the interactions, *i.e.*, whether fitness is greater or lesser than expected on the assumption of no interaction.

The results obtained are striking. We have confirmed previous results showing that, on the average, homozygotes for one whole chromosome have very low fitness and that no chromosome yields fitness that approaches normality. More important, we have observed that, on the average, the fitness of *D. melanogaster* homozygous for both the second and the third chromosomes (0.079 ± 0.024) is not much different from the fitness of flies homozygous for only the second (0.081 ± 0.014) or only the third chromosome (0.080 ± 0.017). If we assume that fitnesses are multiplicative, the average expected fitness of the double-chromosome homozygotes is 0.0066 ± 0.002; the observed value is more than ten times greater. (As pointed out by SEAGER and AYALA (1982) the additive model occasionally used in population genetics becomes nonsensical when the selection coefficients are, as in the present case, large.)

Our results have potentially important consequences with respect to two issues: (1) the number of polymorphisms that are maintained by natural selection, and (2) the nature of the fitness function. TRACEY and AYALA (1974) have calculated that if it is assumed that, at each polymorphic locus, the fitness of the homozygotes relative to the heterozygotes is reduced by 0.01, there could be in Drosophila populations as many as 1120 single-locus polymorphisms maintained by heterotic selection alone. But that assumes that single-locus fitnesses are multiplicative. The present experiments manifest large negative synergistic interactions. If we assume that similar synergistic interactions occur when there is homozygosis for less than one full chromosome, then the fitness depression observed in the homozygotes for one chromosome could account for a number of heterotic loci much greater than previously calculated. The question is, however, whether or not the kind of synergistic interactions herein observed between whole chromosomes, will also be the case for parts of chromosomes. This question can be explored experimentally, by obtaining individuals homozygous for partial chromosome segments and comparing their fitness with that of individuals homozygous for two or more of the segments. For the moment, however, it appears that the concealed genetic load present in natural populations may be sufficient to account for the selective maintenance of the numerous polymorphisms uncovered by electrophoresis and other methods (SIMMONS and CROW 1977).

The second issue raised is the nature of the fitness function. A variety of alternatives are possible. Here we shall consider three general models (Figure 3). One model assumes that there are no synergistic interactions between loci, but rather the fitness of a multilocus genotype is simply the product of the fitnesses of the corresponding single-locus genotypes (*multiplicative fitness model*). A second possible model is that there is some kind of threshold so that, beyond a certain degree of homozygosis, further increases in the number of

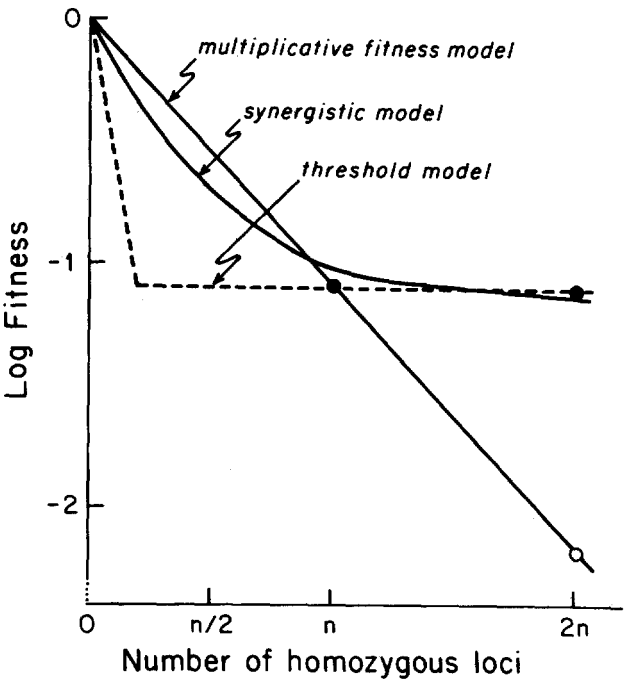

FIGURE 3.—Three models of fitness interactions. The abscissa represents the number of homozygous loci over the number characteristic of wild-type flies. The two black circles represent the experimental results. The average fitness of flies homozygous for either the second or the third chromosome (n loci) is about 0.081 (log = −1.09). If fitnesses are multiplicative, the fitness of the homozygotes for both chromosomes ($2n$ loci) should be 0.0066 (log = −2.18, open circle) rather than the observed 0.079. The slopes of the synergistic or the threshold models are arbitrary, since they cannot be guessed from the data. The validity of the threshold model might be established by measuring the fitness of individuals homozygous for a chromosomal segment of various lengths—half or one-quarter of the second chromosome (n/2 or n/4 loci), for example.

homozygous loci (each with a small effect on fitness) do not entail any additional decreases in fitness (*threshold model*; see SVED, REED and BODMER 1967). An intermediate alternative is that the fitness function is continuous, but that there are synergistic interactions between loci with small fitness effects so that as the number of homozygous loci increases the rate of decrease in fitness gradually decreases—with perhaps an asymptotic approach to some minimum fitness value (*synergistic model*).

Our results are, at face value, inconsistent with the first alternative, since the fitness of the double homozygotes is one order of magnitude greater than expected from the multiplication of the fitnesses of the single chromosome homozygotes. In fact, the fitness of the double homozygotes is not significantly different from the fitness of homozygotes for only one chromosome, suggesting that some threshold or asymptotic value may have already been reached when there is homozygosis for one full chromosome. But although the evidence for synergistic interactions appears unambiguous, the question whether or not a threshold or asymptotic minimum fitness boundary exists can only be resolved

with additional experiments. (We are speaking only of the cumulative effects of genes that each reduce fitness only slightly; lethal and sterile genes do, of course, exist.) Experiments of the type suggested above—namely, in which the fitness of individuals homozygous for one or another chromosomal segment is compared with the fitness of individuals homozygous for both chromosomal segments—can provide some light on this all-important question. We can only hope that such experiments will be done, in spite of the enormous amount of work required.

WILTON and SVED (1979), using an experimental approach similar to the one used in the present paper, have estimated the fitness of *D. melanogaster* homozygous for the X chromosome. The X chromosome was studied so as to provide evidence on the question of whether the chromosomal heterosis observed in experiments of this type is caused by heterozygous advantage at individual loci (overdominance) or to deleterious recessives (dominance). Deleterious recessive genes can be maintained at relatively high frequencies by modest mutation rates in the autosomes, but not in the X chromosome because they are subject to direct selection in the males. The frequency of X-linked deleterious recessives is expected to be only of the order of the mutation rate (LI 1955) and therefore such genes should contribute very little to X-chromosome heterosis.

The X chromosome of *D. melanogaster* is about half as long as either one of chromosomes *II* or *III*. The mean fitness of flies homozygous for the X chromosome is estimated by WILTON and SVED (1979) to be about 0.6, a result that seems to be inconsistent with the notion that most of the deleterious genes are maintained by dominance rather than overdominance. This possibility cannot, however, be excluded if it is assumed that many of the deleterious recessives are sex-limited, so that they affect only the females and not the males. However, WILTON and SVED calculate that the proportion of female-limited genes would have to be of the order of 50%, whereas the existing evidence (LINDSLEY and GRELL 1968; DRESCHER 1964) indicates that only about 10% or less of the X chromosome genes are female limited.

Overdominance, then, appears as the most likely explanation for the fitness reduction observed in the chromosome homozygotes. But, as WILTON AND SVED point out, the fitness reduction observed in the X-chromosome homozygotes is much too large even if overdominance is pervasive. Assuming that the average fitness of flies homozygous for either the second or the third chromosome is about 0.2 (which is an approximate average of the various experiments of this kind), then the expected fitness of flies homozygous for a chromosome half as long should be, assuming multiplicative fitness, $\sqrt{2} = 0.45$, which is not substantially different from the value of 0.6 observed for the X-chromosome homozygotes. However, the level of polymorphism that can be maintained by overdominance is much lower in the X chromosome than in the autosomes, because the heterozygote advantage can only exist in one sex, the females.

An important result obtained by WILTON and SVED (1979) is that hybrid dysgenesis (KIDWELL, KIDWELL and SVED 1977; BREGLIANO *et al.* 1980) is not a significant factor responsible for the results observed in experiments of the

present type. They used chromosomes extracted from two different natural populations. Flies from one population, but not the other, yield hybrid dysgenesis when crossed to some laboratory strains. Similar results were, nevertheless, obtained with both populations in the fitness studies.

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