SYNERGISTIC INTERACTION BETWEEN SECOND AND THIRD CHROMOSOMES ON VIABILITY OF *DROSOPHILA MELANOGASTER*

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Under the existence of synergistic interaction the mutational load is theoretically shown with a quadratic model to be approximately one half that in the case of no interaction (Kimura 1961; Kimura and Maruyama 1966). Considerable evidence for synergistic interaction among deleterious genes within chromosomes has accumulated in recent years (Dobzhansky *et al.* 1963; Malogolowkin-Cohen *et al.* 1964; Kitagawa 1967; Temin *et al.* 1969; Mukai 1969), whereas information on epistatic interaction between chromosomes is sparse. Matsudaira (1963) reported positive epistatic interaction between irradiated second and third chromosomes of *Drosophila melanogaster*. Spassky *et al.* (1965) and Temin *et al.* (1969) have presented evidence for synergism between chromosomes derived from natural populations of *D. pseudoobscura* and *D. melanogaster*, respectively. Temin *et al.* (1969) has also shown a low frequency of "interchromosomal synthetic lethals". The problem of "interchromosomal synthetic lethals" is very interesting, as Kosuda and Moriwaki (1968, 1971) presented evidence for production of "intrachromosomal synthetic lethals" by synergistic interaction of deleterious genes on the second chromosome of *D. melanogaster*.

The main purpose of the present study is to investigate interchromosomal epistasis as expressed through the homozygous viability effects of second and third chromosomes of *D. melanogaster*, and to clarify the extent of genetic load which the natural population carries.

MATERIALS AND METHODS

Drosophila melanogaster flies provided for this experiment were collected from a natural population in Yamanashi, Japan, in the autumn of 1968 (Kosuda *et al.* 1969). Single pairs of second and third chromosomes were simultaneously extracted from each collected male fly and made homozygous with a complete marked inversion technique. Then their homozygous preadult viabilities, separately and jointly, were measured using the mating scheme of Fig. 1. Heterozygous ones were also calculated, applying the same procedure.

Collected male flies were individually mated with females of *Cy/Pm; Ubx¹³⁰e^s/Sb* strain, whose X chromosomes were previously replaced by those of the natural population. In the next generation, randomly selected males showing the *Cy, Ubx* phenotype were singly backcrossed to *Cy/Pm; Ubx¹³⁰e^s/Sb* females. In generation 3, several pairs

Fig. 1. Mating scheme for measuring homozygous viabilities of second and third chromosomes, separately and jointly.

of flies showing both *Cy* and *Ubx* were selected from the progeny of each cross and sib-mated. These flies were transferred a few times to fresh vials at two-day intervals. All flies that emerged from the culture vials were classified and counted in generation 4. Usually, flies recovered from the first and second replicates were counted, while the third and fourth replicates were used if necessary. There were no chromosome pairs with less than 100 *Cy Ubx*. As seen in Fig. 1, flies showing both *Cy* and *Ubx* are doubly heterozygous for two autosomes from nature. *Ubx* or *Cy* flies are single homozygotes for either the second or third chromosome, whereas wild type flies are doubly homozygous for both chromosomes. Since *Cy* and *Ubx* chromosomes are both homozygously lethal, the ratio of the four classes in the final generation, *Cy Ubx*, *Ubx*, *Cy*, and wild type should be 4 : 2 : 2 : 1 in the absence of any viability differences. Both *Cy* and *Ubx*¹³⁰ are effective crossover suppressor, so recombination between wild and marker chromosomes in generation 3 females is minimal. The ratios of the numbers of *Ubx*, *Cy*, and wild type flies give the measurements of viability of homozygotes for the second chromosome, for the third chromosome, and for both of the second and third chromosomes, respectively. Thus homozygous viabilities ($F=1$) for 289 pairs of wild second and third chromosomes were measured.

In order to obtain heterozygous viabilities ($F=0$) for second and third chromosomes, the females and males derived from different G₁ males were utilized in generation 3 (see Fig. 1). The measurement, however, was made in exactly the same way as that for homozygous viabilities, based on 47 combinations.

Throughout the experiments the temperature was kept at $25 \pm 1^\circ\text{C}$, and a culture medium containing 20% boiled yeast was used.

RESULTS

Genetic loads

Homozygous viabilities for pairs of second and third chromosomes simultaneously

isolated from wild flies are summarized in Table 1 and shown graphically in Fig. 2. Homozygous viabilities were classified into five classes, comparing with heterozygous

Table 1. Viabilities of second and third chromosomes

III-chr. II-chr.	Lethal	Semilethal	Subvital	Normal	Supervital	Total
Lethal	12	3	9	21	1	46 (15.92%)
Semilethal	4	1	4	4	0	13 (4.50%)
Subvital	16	0	6	16	0	38 (13.15%)
Normal	41	8	40	87	3	179 (61.94%)
Supervital	4	1	0	6	2	13 (4.50%)
Total	77 (26.66%)	13 (4.50%)	59 (20.42%)	134 (46.37%)	6 (2.08%)	289

Fig. 2. Frequency distribution of second and third chromosomes with different viabilities.

viabilities:

- Lethal: Viability less than 10% of that of heterozygotes.
- Semilethal: Viability 10 to 50% of that of heterozygotes.
- Subvital: Viability 50 to 75% of that of heterozygotes.
- Normal: Viability 75 to 125% of that of heterozygotes.
- Supervital: Viability greater than 125% of that of heterozygotes.

The usual criterion of ten per cent (Greenberg and Crow 1960) was adopted for lethality, because it was very difficult to distinguish between complete lethals and those with a few survivors. Such incomplete lethals would have almost no chance of surviving in nature.

As shown in Fig. 2, the viability distribution of single homozygotes for both chromosomes is clearly bimodal, having peaks at lethality and somewhat below normal viability. The lethal frequency of the second chromosome was 15.92%. This is in good agreement with the value, 15.42%, found previously in the same natural population, although the method is not the same (Kosuda *et al.* 1969). The frequency of deleterious genes, especially lethals, is considerably higher in the third chromosome than in the second chromosome in this population, despite the similar length of the two autosomes. In contrast, there is no significant difference in the contents of lethals between two autosomes in U.S.A. populations in general (Band and Ives 1961, 1963, Spiess and Allen 1961; Allen 1964; Wallace *et al.* 1966; Temin *et al.* 1969), though Allen (1968) reported a higher lethal frequency in the third chromosome than in the second.

A test of independence in a 3×3 contingency table (Lethal+Semilethal, Subvital, and Normal+Supervital) made from Table 1 was performed to examine whether pairs of second and third chromosomes simultaneously extracted from wild males combine at random or not. There was no significant departure from random association ($\chi^2=3.49$, d. f.=4, $0.3 < P < 0.4$). Temin *et al.* (1969) and Tsuno (1970) also found randomness of combination between the two autosomes of *D. melanogaster*.

Table 2. Segregation in four classes both at F=0 and F=1

		<i>Cy Ubx</i>	<i>+ Ubx</i>	<i>Cy +</i>	<i>+ +</i>	Total
F=0	No. of flies Ratio	13818 1	7098 .514	6837 .495	3410 .247	33363
F=1	No. of flies Ratio	39724 1	15258 .384	12269 .309	4186 .105	71437

Table 2 gives segregation in the four classes in generation 4 both at F=0 and F=1. The observed ratio in the outbred group (F=0) is close to the expected ratio 4 : 2 : 2 : 1 ($\chi^2=6.16$, d.f.=3, $0.1 < P < 0.2$). Lower ratios of both *Ubx* and *Cy* classes at F=1 in comparison with those at F=0 indicate a viability depression due to homozygosis for second and third chromosomes.

The relative viabilities of the single homozygotes for second and third chromosomes, *S* and *T*, respectively, were estimated by the method of Crow (1968) (Table 3). The relative viability of the homozygotes for the nearly normal (Subvital, Normal and Supervital) second chromosome, 0.95, is larger than that (0.85) for the third, as also shown

Table 3. The relative viabilities of single homozygotes to random heterozygotes

	S	T
<i>V_m</i>	.95	.85
<i>V_s</i>	.97	.96
<i>V_d</i> (= <i>V_m</i> <i>V_s</i>)	.91	.81
<i>V_l</i>	.85	.73
<i>V_{ho}</i> (= <i>V_d</i> <i>V_s</i>)	.77	.59

S: second chromosome viability, T: third chromosome viability, *V_m*: mild detrimental (viability > .5), *V_s*: severe detrimental (.1-.5), *V_l*: lethals, *V_d*: all detrimental, *V_{ho}*: all homozygotes.

Table 4. Genetic loads at F=1

	Total load	<i>D_m</i>	<i>D_s</i>	<i>L</i>	<i>D_m/L</i>	<i>D/L</i>
II-chr.	.263	.058	.039	.166	.349	.584
III-chr.	.528	.174	.039	.315	.552	.676
Both chr.	.791	.232	.078	.481	.472	.644
Temin <i>et al.</i> (1969)						
II-chr.	.404	.095	.062	.247	.385	.636
III-chr.	.415	.122	.053	.240	.508	.729
Both chr.	.819	.217	.115	.487	.446	.682

Load components are *D_m* for mild detrimental (viability > .5), *D_s* for severe detrimental (.1-.5), and *L* for lethals (<.1). The corresponding figures from Temin *et al.* (1969) are given for comparison.

graphically in Fig. 2.

Genetic loads at F=1 for both chromosomes were obtained by the formulae $s = -\ln S$ and $t = -\ln T$, respectively (Table 4). The detrimental load (*D*) was partitioned into severe detrimental load (*D_s*) and mild detrimental load (*D_m*). The mild detrimental load, especially in the third chromosome, is larger than the severe detrimental one, suggesting a greater number of mildly deleterious mutant genes or polygenes on both chromosomes since individual effects of such mutants are much smaller. The lethal load as well as total load in the third chromosome is considerably larger than that in the second chromosome. This situation is different from the Wisconsin population (Temin 1966; Temin *et al.* 1969). However, the total genetic load for both chromosomes, 0.791 lethal equivalents per gamete, is in very good agreement with that reported by Temin *et al.* (1969). *D*:*L* ratio for second and third chromosomes are 0.584 and 0.676, respectively. These values are almost the same as those reported by Temin (1966) and Temin *et al.* (1969). Table 4 also shows that the majority of the total load is attributable to lethals.

Epistasis between chromosomes

For the calculation of the magnitude of the epistatic interaction between second and third chromosomes, 166 chromosome pairs were used out of 289. These chromosome pairs were chosen where either the observed or the expected number was greater

than 40% of the expected number of random heterozygotes. Coefficients of interaction (I) for individual pairs were estimated according to Crow (1968). The coefficient of interaction is unity if no epistatic interaction exists, that is, if the effects of two major autosomes are independent. Coefficients of interaction smaller than 1 mean that the positive synergistic interaction exists, *i.e.*, the viability of a double homozygotes is smaller than the products of the two single homozygote viabilities. The distribution of I is given in the left half of Table 5.

Table 5. Distribution of coefficients of interaction (I)

I	n	
	Nat.	Art.
0.0—0.09		2
0.1—0.19		3
0.2—0.29	1	3
0.3—0.39	3	3
0.4—0.49	8	7
0.5—0.59	14	8
0.6—0.69	19	6
0.7—0.79	25	8
0.8—0.89	23	17
0.9—0.99	16	12
1.0—1.09	16	5
1.1—1.19	13	3
1.2—1.29	4	13
1.3—1.39	10	4
1.4—1.49	5	0
1.5—1.59	4	2
1.6—1.69	4	3
1.7—1.79	1	1
1.8—1.89		0
1.9—1.99		1
Total	166	101
mean I	.925±.025	.898±.042

As shown in the table, the coefficients of interaction were distributed normally and their mean value was 0.925, which is significantly different from unity. Thus the results mean that positive synergism exists between chromosomes on the average.

On the other hand, second and third chromosomes both of which had been isolated from different wild flies were combined artificially with the appropriate matings (Fig. 3). These artificially combined pairs of chromosomes were then made homozygous and the homozygous viabilities for two autosomes were measured, separately and jointly, in the same way as before. In the right hand section of Table 5, are presented coefficients of interaction which were gotten from artificially combined chromosome pairs. Although the numbers of artificially combined chromosome pairs are smaller than the natural pairs, the range of I in the former is wider than the latter. Actually the variance of I in the former is significantly larger than that in the latter ($F=1.40$, d.f.=109, 166, $P<0.01$), although nearly the same numbers of flies were counted for natural and artificial pairs (298.4 and 295.8 per pair, respectively). However, mean I is not significantly different between the two. The explanation of this difference in variance is obscure. Here again, the mean coefficient of interaction is significantly

smaller than unity. This is indicative of the synergistic interaction between mildly deleterious genes on the two different chromosomes.

Moreover, only in the artificially combined pairs of major autosomes, there were two cases out of 101 where I was smaller than 0.1, as presented in Table 5. They may be referred to as "interchromosomal synthetic lethals" in a sense that the observed viability of double homozygotes is less than ten per cent of the expected viability. It may be interesting to list the five smallest I's among the chromosome pairs combined artificially, including these two cases (Table 6). There were none, however, in the natural pairs of second and third chromosomes. Conversely, no case of extreme nega-

Fig. 3. Mating scheme for measuring homozygous viabilities of artificial pairs of second and third chromosomes.

Table 6. Cases with five smallest coefficients of interaction (I).

	<i>Cy Ubx</i>	<i>Ubx</i>	<i>Cy</i>	<i>+</i>	I
a	120 (150)	51 54	56 52	0 2	0 0.01)
b	147 (478)	64 176	49 143	2 4	0.09 0.08)
c	129 (718)	40 42	57 57	2 4	0.11 0.30)
d	133	46	68	3	0.13
e	101	54	53	4	0.14

The results of further tests are presented in parenthesis.

tive epistasis were found. That is, double homozygotes for the second and third chromosomes were lethal all the time, when one of major chromosomes carried lethal genes.

Cy-suppressor

Two third chromosomes which suppress the *Cy* phenotype in double dose were detected from 291 wild chromosome pairs in the course of this investigation. In these two cases, only the *Cy* class was absent in generation 4 in Fig. 1. These pairs of chromosomes had no chromosomal aberrations such as translocations. Further tests ruled out the possibility of extremely negative epistasis and recombination tests revealed that such third chromosomes had a recessive mutant gene near *se*, which suppress the *Cy* phenotype. These genes may be identical with "suppressor-*Cy*" reported by Meyer and Temin (1965) in a U. S. A. population. It is interesting that such curious mutant genes were found in two remote natural populations, and two chromosomes out of 291 carried such a gene.

DISCUSSION

The series of studies on release of genetic variability through recombination showed the importance of intrachromosomal epistatic interaction on viability, since many recombinant chromosomes with various viabilities, lethal to normal, were produced by recombination within chromosomes with normal or mildly deleterious viability (Spassky *et al.* 1958; Spiess 1959; Dobzhansky *et al.* 1959; Krimbas 1961; Spiess and Allen 1961; Allen 1966; Kosuda and Moriwaki 1971).

The synergistic nature of epistasis within chromosomes in homozygous condition was indirectly suggested from the discrepancy of two values of homozygous genetic load estimated by different methods in *D. pseudoobscura* and *D. willistoni* (Dobzhansky *et al.* 1963; Malogolowkin-Cohen *et al.* 1964). That is, total homozygous loads at $F=1$ in all chromosomes which were measured directly as in this study were higher than the one estimated by the Morton, Crow, and Muller method extrapolating F to 1. Also, Temin *et al.* (1969) confirmed synergism in *D. melanogaster*, based on non-linearity among loads at various level of inbreeding: relatively greater load was usually gained at higher F .

Mukai (1969) has found direct evidence for intrachromosomal synergistic interaction among spontaneous mutant polygenes for viability from a non-linearity between mean homozygous viability of the second chromosome and spontaneous mutant genes.

Kosuda and Moriwaki (1968, 1971) presented evidence for the production of "intra-chromosomal synthetic lethals" by synergistic interaction among deleterious genes or polygenes on the second chromosome of *D. melanogaster*.

The coefficient of interaction obtained in the present study was 0.90-0.93 at the mean value. This indicates the interchromosomal synergistic interaction of 7-10% towards the positive direction. Spassky *et al.* (1965) and Temin *et al.* (1969) whose analytical methods are the same as in this study, have reported coefficients of interaction of 0.88 in *D. pseudoobscura* and 0.97 in *D. melanogaster*, respectively. These studies plus the present study indicate consistent synergism between chromosomes, though the magnitude of the interaction is not so large.

Matsudaira (1963) and Tsuno (1970) reported positive epistasis between two major chromosomes of *D. melanogaster*, calculating the regression of the observed viability of double homozygotes on the viability expected on the basis of independence. They calculated regression coefficients smaller than 1, based on the homozygous viability for all pairs of second and third chromosomes, including severe detrimental and even lethals, and they presented the regression lines which almost pass through the origin, whereas chromosome pairs with insufficient viability were excluded from the estimate of interaction in the present study for the following reasons. For measuring viability and the coefficient of interaction, the sampling error is greater at the range of lower viability. And since no extreme negative epistasis exists, the pair of chromosomes, which has lethal genes, gives no interaction ($I=1$), as Crow (1968) pointed out. Therefore, the regression coefficient should be close to unity at the range of lower viability, and the frequency of lethals on both chromosomes in populations is higher, the closer the re-

gression coefficient approaches 1. Thus the magnitude of regression coefficients can be affected by the amount of lethals in populations, if severe derimentals and lethals are included. Furthermore, epistasis between severe detrimental has few effects on populations, since the frequency of these genes in natural populations is very low. Regression lines of the observed viability (Y) of double homozygotes on the expected one (X) for natural and artificial pairs of chromosomes are $\hat{Y}=0.496X+7.21$ and $\hat{Y}=0.439X+6.68$, respectively in the present study, and they agree very well (Fig. 4). It was shown that the intercept of an extension of the regression line on the Y-axis is at a considerable distance from the origin. The magnitude of epistasis could not be precisely compared based on the regression coefficient if the regression line dose not pass through the origin (Tsuno 1970).

Fig. 4. The regression of the observed viability of double homozygotes on the expected one.

Two cases of "interchromosomal synthetic lethals" out 101 artificially combined pairs of chromosomes were observed in this study. It is interesting that "interchromosomal synthetic lethals" appeared only in artificially combined chromosome pairs in which variance in coefficient of interaction was significantly larger than in natural pairs. Reasons for these differences between the artificially combined pairs and the natural ones must await further investigations.

Wallace *et al.* (1966) could not demonstrate "interchromosomal synthetic lethals" in 119 pairs of chromosomes in *D. melanogaster*; nor could the phenomenon be demonstrated in *D. pseudoobscura* (Dobzhansky *et al.* 1965; Spassky *et al.* 1965). The appearance of three lethals among the double homozygotes presented in Wallace *et al.*

(1966), referred to as "combination lethals" by them, is not unexpected under the multiplicative model of viability since either or both of two major chromosomes has a considerably lowered viability. Temin *et al.* (1969), however, has reported 9 such cases of "interchromosomal synthetic lethals" out of a total of 1855, a frequency of 0.005, although the number of counted flies in these cases was relatively small. Further testing seems necessary on this matter. Thus "synthetic lethals" between chromosomes seem to be rare if they exist, so they may not play any important role in natural populations. It is implied that interchromosomal epistasis may be smaller than intrachromosomal epistasis, for the frequency of "synthetic lethals" within chromosomes was higher than that between chromosomes (Kosuda and Moriwaki 1968, 1971).

Spassky *et al.* (1965) and Temin *et al.* (1969) as well as the present investigator could not detect any evidence of extreme negative epistasis between chromosomes in *D. pseudoobscura* and *D. melanogaster*. However, one case of complete negative epistasis is seen in Table 2 in Matsudaira (1963). It is questionable whether this case is, in fact, a result of negative epistasis due to the lack of further tests.

Kitagawa (1967) found synergistic interaction in heterozygous condition indicated by a significant deviation from linearity among fitness of multiple heterozygotes for lethal genes on the second chromosome of *D. melanogaster*. Further study of epistasis is needed especially in heterozygous condition for mildly deleterious genes, considering the greater dominance of these genes, although the tendency towards positive synergism may be generally accepted in homozygous condition.

SUMMARY

Homozygous viability effects of 289 pairs of second and third chromosomes simultaneously extracted from a natural population were measured, separately and jointly. Frequencies of lethal second and third chromosomes were 15.9% and 26.7%, respectively. Random association between chromosome pairs was confirmed. Total homozygous load on two major autosomes was calculated to be 0.791 lethal equivalents per gamete. This total load was partitioned into loads of 0.481, 0.232, and 0.078 due to lethals, mild detrimentals, and severe detrimentals, respectively. The *D/L* ratio for second and third chromosomes are 0.681 and 0.674. The results indicate that there exists about 7% interchromosomal synergistic interaction between mildly deleterious genes on the two chromosomes, and an interchromosomal epistasis of 10% towards the positive direction was also measured in the artificially combined pairs of two major autosomes. It is remarkable that two cases of "interchromosomal synthetic lethals" were found out of 101, and only in the artificially combined pairs of chromosomes in which variance in the magnitude of interaction was significantly larger than that in natural pairs of chromosomes.

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