THE GENETIC VARIABILITY OF THIRD CHROMOSOMES IN A LOCAL POPULATION OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

Five hundred and two third chromosomes were extracted from a large cage population of *Drosophila melanogaster* initiated two months after collection of the progenitors near Raleigh, North Carolina in 1970.—Salivary gland chromosomes of 489 chromosome lines were examined and 54 chromosomes were found to carry inversions. The inversions were classified into three polymorphic types [*In(3L)P*, *In (3R)P*, and *In(3R)C*] and two unique types. The polymorphic inversions were found in frequencies of 0.012, 0.088, and 0.010, respectively.—Viabilities of homozygotes and heterozygotes were examined. Chromosomes with lethals occurred with a frequency of 0.495: 0.537 in the group of inversion-carrying chromosomes and 0.490 in the group of inversion-free chromosomes. The average homozygote viability computed on the basis of an average heterozygote viability of 1.0000 was 0.3235 if lethal lines were included and 0.6290 if they were excluded. The detrimental load to lethal load ratio (D:L ratio) was 0.70 (=0.4636–0.6650). The average viability of lethal heterozygotes was significantly larger than that of lethal-free heterozygotes. It appears, however, that lethal genes in heterozygotes have deleterious effects on fitness as a whole.—The average degree of dominance for viability polygenes was estimated to be about 0.3–0.4 in lethal-free individuals and nearly zero in lethal heterozygotes. Overdominance or some form of balancing selection was suggested at some loci. The difference between the values obtained for average degree of dominance due to genetic backgrounds and superior *vibaility* of lethal heterozygotes (but not fitness as a whole) suggests that some epistasis or coadaptation occurs.—The results described above are similar to those obtained for the second chromosomes (MUKAI and YAMAGUCHI 1974).

ALTHOUGH the genetic variability of second chromosomes from natural populations of *Drosophila melanogaster* has been extensively investigated (e.g. GREENBERG and CROW 1960; OSHIMA and KITAGAWA 1961; MUKAI and YAMAGUCHI 1974), few results have been reported for the third chromosomes. However, the frequencies of lethal-carrying third chromosomes have been estimated by several investigators (SPIESS and ALLEN 1961; BAND and IVES 1963; PAIK 1966; ALLEN 1966 and others) for the purpose of making comparisons with

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the frequencies obtained for lethal-carrying second chromosomes. It was concluded that the frequency of lethal-carrying third chromosomes is equal to or slightly larger than that for the second chromosomes. However, the studies on the mildly deleterious genes with respect to genetic load are scanty (cf. TEMIN *et al.* 1969).

Recently, MUKAI and YAMAGUCHI (1974) reported on an extensive analysis of the second chromosomes extracted from a Raleigh, North Carolina population. Significant findings in this investigation were: (1) The frequency of lethal-carrying chromosomes in this population was greater than in a northern population (Madison, Wisconsin). (2) The estimates of the average degree of dominance for lethal genes ($H_L = 0.01-0.02$) and viability polygenes ($\overline{h}_p = 0.2-0.3$), homozygous detrimental load ($D = 0.33$) and lethal load ($L = 0.50$) were mutually consistent in that mutation rates estimated for polygenes and lethal genes on the basis of the values obtained for the above genetic parameters agreed well with a reliable independent estimate (MUKAI 1964; MUKAI *et al.* 1972). (3) The existence of overdominance or some form of balancing selection at some loci and a little epistasis in viability were indicated. These findings are significant for an understanding of the mechanisms for maintenance of large numbers of isozyme polymorphisms discovered recently in many species (HUBBY and LEWONTIN 1966; O'BRIEN and MACINTYRE 1969 and others).

The main purpose of the present investigation is to determine if results similar to those obtained from the second chromosomes (MUKAI and YAMAGUCHI 1974) can be obtained from the study of the third chromosomes extracted from the same population. Special attention was paid to polymorphic inversions, as was done by MUKAI and YAMAGUCHI (1974) for the second chromosomes.

MATERIALS AND METHODS

Extraction of third chromosomes: A cage population was constructed with 3960 flies from 660 isofemale lines which had been kept at 25° for 2 months after collection from a state park near Raleigh, North Carolina in summer of 1970. Males and females were sampled from the cage twice in December, 1970 (447 lines) and once in May, 1971 (55 lines). They were individually mated to 3 *Sb/Pr* (*Sb*: Stubble, which is included in *In(3LR)TM3*. This chromosome suppresses crossing over along the whole chromosome; *Pr*: Prickly, see LINDSLEY and GRELL 1967).

A single *Sb* male fly from each cross was mated again with 3 *Sb/Pr* females, and 3 *Sb* females were crosses to 3 *Sb* males in the next generation. Thus, 502 third chromosomes were extracted. These chromosomes were maintained at 25° as lines balanced with the *TM3(Sb)* chromosomes. Genetic backgrounds (X, II, IV chromosomes and cytoplasm) of these lines were made up of random chromosomes and cytoplasm from the same population, since the genetic backgrounds of *Sb/Pr* flies had been substituted with chromosomes and cytoplasm from the same population. Cytological examination of salivary gland chromosomes was made for each chromosome line after making a cross to a standard wild type (W507).

Estimation of relative viability: Homozygote and heterozygote relative viabilities were estimated as follows: Crosses were made between 5 $Sb/+_i$ females and 5 $Sb/+_i$ males with 4 simultaneous replications in each chromosome line, where i indicates line number. In the offspring, $Sb/+_i$ and $+_i/+_i$ flies segregate at an expected ratio of 2 : 1. The relative viabilities of random heterozygotes were estimated in a way similar to the above, combining two successively numbered lines, i.e., $Sb/+_i \times Sb/+_{i+1}$ in order to secure random combination of

different chromosome lines. As in the case of homozygotes, five-pair matings were conducted with four simultaneous replications. In both cases, four days after the crosses were made, all 10 flies in a vial were transferred to a second vial. Four days after the transfer, all flies were discarded. In both vials, the original and the transferred, all flies were counted 3 or 4 times until the 18th day after the cross or transfer was made. *Sb* flies and wild-type flies from the pair of vials were respectively pooled and considered as a single observation. Relative viability was expressed as the ratio of (the number of wild-type flies) to (the number of *Sb* flies +1) (cf. HALDANE 1956). It was also expressed in terms of natural logarithms for estimating the average degree of dominance of viability polygenes or mildly deleterious genes.

Ten sets of crosses (10 replications) were made at 10 different times and 45 to 56 lines were employed per replication. Homozygote and heterozygote relative viabilities within replications were estimated at the same time. Before the analyses, all relative viabilities were standardized by replication to the average heterozygote relative viability. This experiment is referred to as the RD experiment.

Average degree of dominance of viability polygenes: Average degree of dominance of viability polygenes was estimated by the regression method (MUKAI and YAMAGUCHI 1974; see also MUKAI *et al.* 1972). The theoretical basis for this estimation procedure rests on the Wrightian fitness model with two alleles, A and a. Let p represent the frequency of A, q $(=1-p)$ denote the frequency of a, h be the degree of dominance, and s stand for the selection coefficient against mutant homozygote with respect to *viability*. Then, the following relationships can be obtained for a locus.

Genotype	AA	Aa	aa
Frequency	p^2	$2pq$	q^2
Deleterious viability effect (Y)	0	hs	s
Corresponding homozygotes	$AA+AA$	$AA+aa$	$aa+aa$
Sum of viability effects (X)	0	s	$2s$

Based upon the above, the variance of X [Var(X)] and Y [Var(Y)], and covariance between X and Y [Cov (X,Y)] can be calculated as follows:

$$\mathrm{Var}(X) = 2pqs^2 \tag{1}$$

$$\mathrm{Var}\ (Y) = pq[2(1-2pq)h^2 - 4q^2h + q\ (1+q)]s^2 \tag{2}$$

$$\mathrm{Cov}\ (X,Y) = 2pqs^2[h + q\ (1-2h)] \tag{3}$$

(A) *Partially recessive polygenes only*: Under the condition of $h' >> \sqrt{\mu/s'}$, where μ is the mutation rate from A to a, h' is the degree of dominance and s' is the selection coefficient against mutant homozygote (aa) with respect to *fitness* as a whole, the gene frequency for an equilibrium population, considering fertility and other fitness components, may be approximated as $\hat{q}_i \doteq \mu_i/c_i h_i s_i$ (at locus i). The $h_i s_i$ is concerned with *viability* alone while $c_i h_i s_i$ represents the selection coefficient against the heterozygote for *fitness as a whole*. Under the condition that $\mu_i s_i/c_i$ is not correlated with h_i in an equilibrium population, the regression coefficient of Y on X ($\beta_{Y \cdot X}$) on a chromosome basis approximates the harmonic mean of the degrees of dominance for newly arisen mutants $[1/\overline{(1/h)}]$ (the frequencies of these genes were proportional to mutation rate alone). This value is approximately the average degree of dominance for viability polygenes in equilibrium populations ($\bar{h}_E$) (*cf.* MORTON, CROW and MULLER 1956; HIRAIZUMI and CROW 1960). That is

$$\beta_{Y \cdot X} \doteq 1/\left(\overline{\frac{1}{h}}\right) \doteq \bar{h}_E\ . \tag{4}$$

It should be noted here that the gene frequency in an equilibrium population is a function of mutation rate and *selection coefficient* in contrast to the case of newly arisen mutations. Even when one of the homologous chromosomes carries a recessive lethal gene(s), this approximation holds.

The regression coefficient of X on Y ($\beta_{X \cdot Y}$) approximates the inverse of the average degree of dominance of newly arisen mutant viability polygenes ($\bar{h}_N$):

$$\beta_{X.Y} \doteq 1/\bar{h}_N \tag{5}$$

When one of the component homologous chromosomes carries a recessive lethal gene(s),

$$\beta_{X.Y} \doteq 1/(2\bar{h}_N) \ . \tag{6}$$

(B) *Partially recessive polygenes and overdominant polygenes*: If there are partially recessive and overdominant polygenes, $\beta_{Y.X}$ and $\beta_{X.Y}$ can be expressed as

$$\beta_{Y.X} = \frac{\mathrm{Cov}(X,Y) + \mathrm{Cov}(X,Y)'}{\mathrm{Var}(X) + \mathrm{Var}(X)'} \tag{7}$$

$$1/\beta_{X.Y} = \frac{\mathrm{Var}(Y) + \mathrm{Var}(Y)'}{\mathrm{Cov}(X,Y) + \mathrm{Cov}(X,Y)'} \tag{8}$$

where $\mathrm{Var}(X)'$, $\mathrm{Var}(Y)'$, and $\mathrm{Cov}(X,Y)'$ are for overdominant loci.

The derivations of these formulae were made under several reasonable assumptions, all described in MUKAI and YAMAGUCHI (1974).

In an equilibrium population, $\mathrm{Cov}(X,Y)' = 0$ with respect to fitness as a whole, and $\beta_{Y \cdot X}$ and $1/\beta_{X \cdot Y}$ can be expressed as follows:

$$\beta_{Y.X} = \bar{h}_E \left[1 - \frac{\mathrm{Var}(X)'}{\mathrm{Var}(X) + \mathrm{Var}(X)'} \right] \tag{7}'$$

$$1/\beta_{X.Y} = \bar{h}_N \left[1 + \frac{\mathrm{Var}(Y)'}{\mathrm{Var}(Y)} \right] \tag{8}'$$

Thus, if overdominant loci are present in addition to partially recessive loci, $\beta_{Y \cdot X}$ and $1/\beta_{X \cdot Y}$ will underestimate and overestimate $\bar{h}_E$ and $\bar{h}_N$, respectively. The bias for $\bar{h}_N$ can be much greater than that for $\bar{h}_E$. A large value of $1/\beta_{X \cdot Y}$ for an equilibrium population, then, indicate the presence of overdominance under this model. Although $\mathrm{Cov}(X,Y)'$ with respect to *viability* is not always zero in an equilibrium population, it has been numerically shown that the above conclusion holds (cf. MUKAI and YAMAGUCHI 1974).

Ninety chromosome lines with viability indices estimated to be larger than 0.6 were used in a viability experiment to estimate the average degree of dominance of viability polygenes. Cyclic matings were made for the estimation of heterozygote viabilities. Simultaneously homozygous viabilities of these lines were carried out. Only 70 out of the 90 total number of chromosome lines were used in the analysis, since 20 of the lines were suspected to carry recessive semi-lethals. The reason is that their homozygous viabilities were less than 0.5. As a result, the number of crosses used was 55. The number of simultaneous replications per cross was 8. This experiment is labeled the TK experiment.

In addition, 48 random heterozygotes in the RD experiment and their constituent homozygotes were used to estimate the average degree of dominance of viability polygenes. The constituent chromosomes in these heterozygotes had viability indices larger than 0.6 in homozygous condition. Furthermore, 121 random heterozygotes were also employed that had one chromosome with lethal genes and the other with a viability index larger than 0.6.

Estimation of mutation rates: Using a modification of the formulae given by GREENBERG and CROW (1960), MUKAI and YAMAGUCHI (1974) estimated mutation rates for polygenes affecting viability and for lethal genes. However, their formulae cannot be directly used for data from the present experiment since the lethal heterozygotes were found to have a better average viability than the lethal-free heterozygotes. Thus, it was necessary to use a modification of the formulae of MUKAI and YAMAGUCHI (1974). The Wrightian fitness model with two alleles A

and a was used. The following parameters are represented (cf. MUKAI and YAMAGUCHI 1974):

M_L, M_{SL}, M_p = Mutation rate per chromosome of major genic recessive lethals, semi-lethals, and viability polygenes, respectively.
$k = M_{SL}/M_L$.
H_L, H_{SL}, h_p = The average degree of dominance of major genic lethals, semi-lethals, and mutant polygenes, respectively, in an equilibrium population, or the harmonic mean of the degrees of dominance of the newly arisen mutant genes for viability.
$u = H_{SL}/H_L$.
c_p, c_{SL} = c value of polygenes and major genic semi-lethals, respectively. $\bar{c}_p$ and $\bar{c}_{SL}$ are the harmonic means of c_p's and c_{SL}'s, respectively. It is assumed that $\bar{c}_p = \bar{c}_{SL} = c$.
D' = the detrimental load due to incomplete recessive genes.
H'_L = the average degree of dominance of major genic lethals for fitness as a whole.
B = the average relative viability of all homozygotes.
C = the average relative viability of non-lethal homozygotes.

Using these parameters, the following modified formulae can be obtained:

$$M_p = \frac{h_p}{1-2h_p}\left[cD' + H'_L\left\{ln\,(C/B)\right\}\left\{2\left(k + \frac{cH_L}{H'_L}\right) - \frac{k}{uH_L}\right\}\right] \tag{9}$$

$$M_L = H'_L\ ln\,(C/B) \tag{10}$$

M_p and M_L were estimated from the results obtained in the RD experiment.

RESULTS AND ANALYSES

Distributions of relative viabilities in homozygotes and heterozygotes: A total of 502 third chromosomes were extracted and all the homozygote and 498 heterozygote relative viabilities were examined. The distribution patterns of these relative viabilities are given in Table 1. The number of lethal-carrying chromosomes, which showed viability indices lower than 0.1 of the average heterozygote viability (cf. GREENBERG and CROW 1960), was 246 and the relative frequency of these chromosomes was 0.49. Only four chromosomes (relative frequency was 0.008) had higher viabilities than the average viability for heterozygotes. Among these, no line was *significantly* above the average viability for heterozygotes. This result is probably due to the small number of observations for each line ($n = 4$). For one line (line no. 238), higher than the average heterozygote relative viability was recorded for all 4 observations (mean: 1.1025 *vs.* 1.0000). Thus, it appears that the best homozygote had greater viability than the average heterozygote.

MUKAI and YAMAGUCHI (1974) used the following formula for cases where the best homozygote had greater *fitness* than the average heterozygote *fitness*:

$$m/n > \left(\frac{\overline{t_1^2}}{t_1 + t_2}\right)/2\bar{\mu}\ , \tag{11}$$

where m is the number of partially recessive loci; n is the number of overdominant loci; t_1 and t_2 are selection coefficients against normal and mutant homozygotes, respectively, in the two-allele overdominance model; μ is the average mutation rate per locus.

TABLE 1

Distributions of homozygote and heterozygote viabilities

Range of viability	Homozygotes			Heterozygotes		
	Total	Inversion-carrying	Inversion-free	Total	Inversion-carrying	Inversion-free
0 –0.05	236 (4)	28	204			
0.05–0.15	21 (1)	3	17			
0.15–0.25	15	1	14			
0.25–0.35	14 (2)	5	7			
0.35–0.45	19	1	18			
0.45–0.55	20 (2)	1	17			
0.55–0.65	34	3	31			
0.65–0.75	45 (2)	5	38	3		3
0.75–0.85	57	5	52	27 (3)	5	19
0.85–0.95	31 (2)	2	27	135 (7)	27	101
0.95–1.05	8		8	192 (10)	37	145
1.05–1.15	2		2	99 (8)	15	76
1.15–1.25				34 (2)	8	24
1.25–1.35				6 (1)	3	2
1.35–1.45				2	1	1
Total	502 (13)	54	435	498 (31)	96	371

Figures in the parentheses are chromosome lines lost before cytological test.

In this experiment, we did not find evidence that the fitness of the best homozygote is greater than the average fitness of heterozygotes. However, the results from this viability experiment and for the second chromosomes (cf. MUKAI and YAMAGUCHI 1974) suggest that formula (11) holds true for the third chromosomes. Thus, if $t_1 = t_2 = t$, and $\mu = 5 \times 10^{-5}$, then $t_1^2/(t_1 + t_2)\,(= t/2)$ is 0.05 for $t = 0.1$, 0.005 for $t = 0.01$, and 0.0005 for $t = 0.001$. Then $m/n > 500$, 50, and 5 for $t = 0.1$, 0.01, and 0.001, respectively. These results suggest that many overdominant loci cannot be expected when the selection coefficient is large. For example, if $t = 0.01$, the number of overdominant loci is less than 50 (cf. MUKAI and YAMAGUCHI 1974), assuming that the number of cistrons is the same as the number of bands in salivary gland chromosomes (JUDD, SHEN and KAUFMAN 1972).

Out of 502 chromosome lines 489 were cytologically examined. The other 13 were lost before the examination. There were 3 polymorphic inversions [*In(3L)P*, *In(3R)P*, *In(3R)C*] in addition to unique inversions. Frequencies of the polymorphic inversions were 0.012 (7/489), 0.088 (43/489) and 0.010 (5/489), respectively. One chromosome carried *In(3L)P* and *In(3R)P*, and another chromosome had one unique inversion and *In(3R)P*. The distributions of inversion-carrying and inversion-free lines are tabulated in Table 1 and graphically presented in Figure 1. The frequency of lethal-carrying chromosomes was 0.537 (29/54) for those with inversions and 0.490 (= 213/435) for those without inversions. The average relative viability of the lethal-free homozygotes

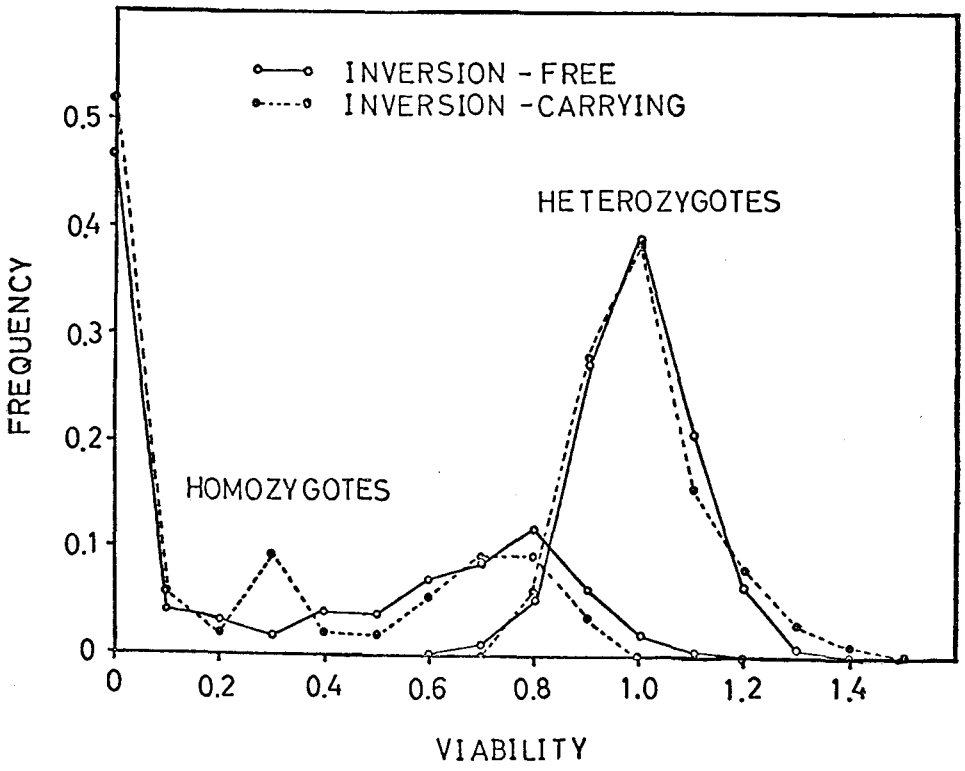

FIGURE 1.—Frequency distributions of homozygote and heterozygote viabilities for the third chromosomes. The average viability of the heterozygotes is 1.0000.

TABLE 2

Average viabilities in homozygotes and heterozygotes

	Homozygotes			
	Lethal and lethal-free		Lethal-free	
Type of chromosome	*N**	Average viability	*N**	Average viability
Inversion	54	0.2618	25	0.5602 ± 0.0279
Standard	435	0.3296	222	0.6401 ± 0.0091
Inversion and standard	502	0.3235	256	0.6290 ± 0.0086

	Heterozygotes	
Genotype	*N**	Average viability
Inversion heterozygotes	96	1.0100 ± 0.0131
Inversion-free heterozygotes	371	0.9975 ± 0.0071
All heterozygotes	498	1

N stands for the number of chromosome lines or the number of crosses.

* In the number of lines or crosses (*N*), the sum of the first two rows is not equal to the number in the third row, since some lines were lost after the viability tests were done, but before the examination of their salivary gland chromosomes was completed.

was 0.5602 ± 0.0279 in the inversion-carrying chromosomes and 0.6401 ± 0.0091 in the inversion-free chromosomes (see Table 2). They are significantly different at the 1% level. After chromosome lines were classified into 4 groups according to their relative viabilities ($0 \leq v \leq 0.1$,$0.1 < v \leq 0.25$, $0.25 < v \leq 0.50$, $0.5 < v \leq 0.75$, and $0.75 < v$), a heterogeneity test with respect to the distribution patterns was performed between the inversion-carrying and the inversion-free chromosome groups, but no significant heterogeneity was detected ($\chi^2_{df=3} = 2.146$, $0.5 < P$).

The distributions of heterozygote relative viabilities, inversion-carrying and inversion-free, are also shown in Table 1 and Figure 1. The average relative viability of inversion-free heterozygotes was 0.9975 ($N = 371$) and that of inversion-carrying heterozygotes was 1.0100 ($N = 96$), as shown in Table 2. These two values are not significantly different. The average relative viability of heterozygotes whose constituent chromosomes included the 25 inversion-carrying, lethal-free chromosomes was 0.9962 ± 0.0160 ($N = 48$). This value is almost the same as the average heterozygote relative viability for the inversion heterozygotes, although the average homozygous relative viability for the inversion-carrying, lethal free chromosomes was significantly smaller than that observed for the inversion-free chromosomes (Table 2). This finding suggests the presence of epistasis or co-adaptation within the inversion-carrying chromosomes.

Detrimental load to lethal load ratio (D:L *ratio*): The average relative viability of all homozygote lines (B) was 0.3235 and that of homozygous lethal-

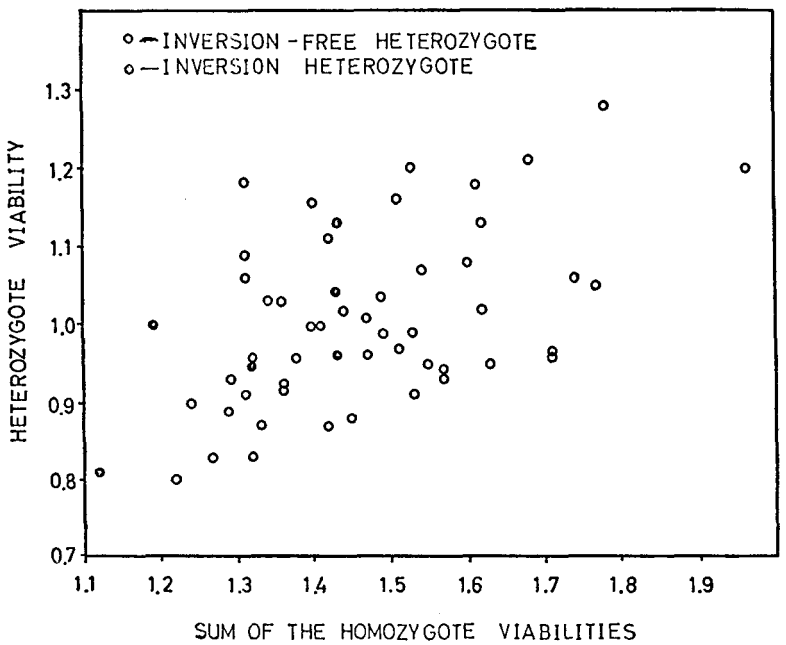

FIGURE 2.—The relationship between heterozygote viability and the sum of the viabilities of component homozygotes with viability indices greater than 0.5 (TK).

free lines (C) was 0.6290). The average relative viability of random heterozygotes (A) was standardized to 1 (Table 2). Using the formulae given by GREENBERG and CROW (1960), the total homozygous load (T), detrimental load (D) and lethal load (L) can be calculated as follows:

$$T = ln\, A - ln\, B = 1.1286$$
$$D = ln\, A - ln\, C = 0.4636$$
$$L = ln\, C - ln\, B = 0.6650.$$

The D:L ratio is 0.697. This figure agrees very well with the result obtained by MUKAI and YAMAGUCHI (1974) for the second chromosomes of the same natural population (0.667).

These D:L ratios were calculated with respect to the average viability of the population. Assuming the 'classical model', the random genetic load is approximately 2 × total mutation rate per haploid set of chromosomes. MUKAI (1964) estimated that a minimum total mutation rate for the second chromosome in *D. melanogaster* is about 0.14. If this mutation rate is applicable for the third chromosome, and if $\bar{c}$ is 2.0, then the D:L ratio with respect to the optimum genotype in the population becomes $(D{:}L)_0 \doteq (0.4636 + 0.14)/0.6650 = 0.91$.

Recently, MUKAI (manuscript in preparation) estimated the homozygous load for overdominant loci as approximately 0.10 for the second chromosome. Assuming that this value can be used for the third chromosome, the D:L ratio due to non-overdominant loci becomes 0.76, which is rather smaller than 1.03 obtained by MUKAI and YAMAGUCHI (1974) for the second chromosome. The D:L ratio of newly arisen mutations on the third chromosome is unknown, but this ratio for the second chromosome is about 1 [0.975 estimated by MUKAI and YAMAZAKI (1968) and 0.954 estimated by MUKAI, *et al.* (1972)]. These figures are very close to the present estimate for the third chromosomes of the natural population. GREENBERG and CROW (1960) have shown that this agreement can be expected if *hs* is approximately constant over the range from lethal and semi-lethal to mildly deleterious viability genes.

Lethal heterozygote relative viability: Out of 496 random heterogygotes, there are 133 lethal-free heterozygotes, 240 'single-lethal' heterozygotes, and 123 'double-lethal' heterozygotes. 'Single-lethal' heterozygote indicates that one of the homologous chromosomes carried at least one recessive lethal gene, and 'double-lethal' heterozygote means that each of the homologous chromosomes carries at least one recessive lethal gene. Table 3 represents the results. Single lethal heterozygotes had the highest viability (1.0120 ± 0.0069) and lethal-free heterozygotes had the lowest (0.9873 ± 0.0084). A significant difference (5% level) was detected between the viabilities of lethal-free individuals and the single-lethal heterozygotes. The genetic variance in relative viability was 0.004821 ± 0.001200 for lethal-free, 0.006823 ±0.001099 for single-lethal and 0.006989 ± 0.001654 for double-lethal heterozygotes. These values are not significantly different.

Average homozygous viabilities of constituent lethal-free chromosomes were compared to obtain information about apparent lethal-heterozygote superiority.

TABLE 3

Average viabilities of lethal-free, single-lethal and double-lethal heterozygotes

Genotype	All crosses		Inversion-carrying		Inversion-free	
	N	Average viability	*N*	Average viability	*N*	Average viability
Non-lethal/Non-lethal'	133	0.9873 ± 0.0084	24	0.9968 ± 0.0231	100	0.9873 ± 0.0093
Non-lethal/Lethal	240	1.0120 ± 0.0069 *	44	1.0191 ± 0.0190	181	1.0105 ± 0.0075
Lethal/Lethal'	123	0.9914 ± 0.0100	28	1.0155 ± 0.0217	90	0.9827 ± 0.0115

* The difference between these two viabilities is significant at the 5% level.

The average homozygous viabilities of the lethal-free chromosomes which were constituents of lethal heterozygotes and which were constituents of lethal-free heterozygotes were 0.6348 ± 0.0152 and 0.6335 ± 0.0143, respectively. These values were almost the same. This result indicates that the superiority of the single-lethal heterozygotes to lethal-free heterozygotes was due to epistasis and/or linkage disequilibrium.

The effect of the inversions on the relative viability of lethal heterozygotes is also shown in Table 3. There were 371 inversion-free heterozygotes, of which 100 were lethal-free, 181 were single-lethal, and 90 were double-lethal. The single-lethal heterozygotes showed the highest relative viability, but these values were not significantly different. On the other hand, out of 96 inversion-carrying heterozygotes, 24 were lethal-free, 44 were single-lethal, and 28 were double-lethal. Average relative viability of the single-lethal heterozygotes was the highest among the three classes. Since the number of crosses was small, no significant differences were detected (but the tendency was the same). Thus, the effects of inversions were not found on the lethal heterozygote viabilities. On the contrary, effects were detected for the second chromosome (MUKAI and YAMAGUCHI 1974).

An average disadvantage in viability of lethal heterozygotes was estimated by linear regression to be −0.0134 per lethal-carrying chromosome. This figure is equivalent to −0.0098 per lethal gene since the average number of lethal genes per lethal-carrying chromosome is 1.37, assuming that lethal genes were distributed on the chromosomes according to a Poisson distribution.

In order to estimate the heterozygous effects of lethal genes on male and female productivities, the average numbers of progenies per pair of vials (see MATERIALS AND METHODS) from *Sb*/+ and *Sb*/*l* males (females were pooled) and females (males were pooled) were estimated, respectively. The results are presented in Table 4. These values were standardized so as to make the average productivity of *Sb*/+ be 1.0000, considering the difference in viability among the progenies in the same vials. The standardized values are presented in the parentheses in the same table. In both cases, lethal genes showed significant deleterious effects in the heterozygous condition. The average $\bar{H}_L$ values per lethal gene are 0.0244 for female productivity and 0.0188 for male productivity, when the average number

TABLE 4

Average numbers of progenies per pair of vials from Sb/+ *and* Sb/l *males and females, respectively*

	N	*Sb*/+ Productivity	*N*	*Sb*/+ Productivity
Female	1012	287.18 ± 1.97 1	972	278.47 ± 2.16 (0.9665 ± 0.0100)
Male	1012	286.54 ± 2.10 1	972	279.14 ± 2.03 (0.9742 ± 0.0100)

N stands for the number of pairs of vials. The figure in parenthesis is the relative value after approximate adjustment considering the difference in viabilities of progenies.

of lethal genes per lethal-carrying chromosome is considered. These values might be different from the actual values in natural populations since the genetic backgrounds were interpopulational. However, we make a compromise and use them for the further analysis.

Under the assumption that relative fitness of a genotype may be approximately expressed as the product of viability, male productivity and female productivity, the relative fitness of lethal heterozygotes was estimated to be 0.9666 with a $\bar{H}'_L$ value thus being 0.0334. It should be noted here that, even if the lethal heterozygotes showed superior viability, they were inferior to lethal-free heterozygotes with respect to fitness as a whole. WALLACE (1962) reported superior viability of lethal heterozygotes on the average, but the situation he observed might have been the same as found in the present case.

Average degree of dominance of viability polygenes: Newly arisen viability mutations may be classified into three categories excluding dominant lethal genes: lethal, major genic semi-lethal, and mildly deleterious genes (or viability polygenes). The homozygous effects of these three types of genes do not appear continuous [lethal genes, selection coefficient (s) $\doteq$ 1.0; semi-lethal genes $\bar{s} \doteq$ 0.5; mildly deleterious genes, $\bar{s} = 0.02$–0.03; see MUKAI (1964)], and furthermore, the h value of semi-lethal genes is much smaller than the value for viability polygenes. Thus, if major genic semilethals are included in the chromosomes for which the regression coefficient for heterozygote relative viability on the sum of the component homozygote relative viabilities is estimated, the regression coefficient will underestimate the average degree of dominance of viability polygenes for an equilibrium population. In order to avoid underestimation, random heterozygotes that had constituent chromosomes with viability indices larger than 0.6 in the homozygous condition and the homozygotes containing these constituent chromosomes were used from the RD experiment. Forty-eight crosses were used. Furthermore, 121 lethal heterozygotes which satisfied the condition described earlier (relative viability of homozygote > 0.6) were employed from the RD experiment. Genetic regression coefficients for heterozygote relative viabilities on homozygote relative viabilities were calculated by removing nongenetic variations and covariations for the groups, including and excluding inversion-carrying individuals, respectively. Viability indices were expressed by

TABLE 5

Genetic regression coefficients for heterozygote viabilities on sums of the corresponding homozygote viabilities

	Including inversion-carrying chromosomes				Excluding inversion-carrying chromosomes			
	Lethal heterozygotes		Lethal-free heterozygotes		Lethal heterozygotes		Lethal-free heterozygotes	
Viability index	Ratio	Log	Ratio	Log	Ratio	Log	Ratio	Log
$v^* > 0.60$	−0.006	0.028	0.064	0.082	0.087	0.059	0.037	0.060
	±0.146	±0.130	±0.219	±0.183	±0.166	±0.141	±0.207	±0.185
	(121)	(121)	(48)	(48)	(95)	(95)	(38)	(38)
$v^* > 0.65$	−0.060	−0.008	0.276	0.352	0.040	−0.024	0.236	0.354
	±0.201	±0.173	±0.389	±0.340	±0.237	±0.206	±0.353	±0.352
	(105)	(105)	(39)	(39)	(80)	(80)	(30)	(30)
$v^* > 0.70$	−0.163	−0.146			−0.135	−0.251		
	±0.285	±0.277	——	——	±0.319	±0.299	——	——
	(85)	(85)			(65)	(65)		

* Range of the homozygote viabilities.
The figures in parentheses indicate the number of heterozygous crosses.

ratio and by natural logarithm. The results are presented in Table 5. Standard errors were calculated following TALLIS (1959). From this table, it is clear that the estimated genetic regression coefficients are smaller than the previous estimates (MUKAI *et al.* 1972; MUKAI and YAMAGUCHI 1974). This result may be due to the contamination of major genic semi-lethals. Thus, heterozygotes whose constituent chromosomes had viability indices greater than 0.65 in the homozygous condition were used to make the same calculations. The results are also given in Table 5.

It is seen in this table that the $\hat{h}_E$ values became larger and almost equal to the estimate obtained by MUKAI and YAMAGUCHI (1974) ($\hat{h}_E = 0.293$) for the case of lethal-free heterozygotes. However, the genetic regression coefficients did not change much for lethal-carrying individuals. Thus, the condition of homozygote relative viabilities was again changed to $v > 0.70$, and the regression coefficients were calculated only for lethal-carrying heterozygotes and their component lethal-free chromosomes. These results are also given in Table 5. Under these conditions the genetic regression coefficients are all negative, but not significantly different from 0.

Using the results from the TK experiment, the genetic regression coefficients were estimated. For this case the condition for homozygote relative viabilities was $v > 0.50$. The results are 0.28–0.41 and are consistent with the values obtained for the lethal-free group ($v > 0.65$). There is no significant difference between the results obtained on the basis of a ratio index and those for a logarithmic index.

The average degrees of dominance for newly arisen mutant viability polygenes were estimated by formula (5) using the results from the TK and RD ($v > 0.65$, lethal-free heterozygotes) experiments. The pooled results from these experi-

ments range from 0.82 to 1.09 and are much larger than the values obtained by direct estimate ($\bar{h}_N = 0.43 \pm 0.008$, MUKAI 1969b; MUKAI and YAMAZAKI 1964, 1968). These results suggest the existence of overdominance or some form of balancing selection, as was found for the second chromosomes. Genetic variances and genetic covariances necessary for estimation of the average degrees of dominance of viability polygenes are presented in Table 6 along with the estimates of genetic regression coefficients (average degree of dominance).

Estimation of mutation rates: We are not attempting to estimate mutation rates accurately using the present method, but instead are attempting to determine if the estimates of genetic parameters are mutually consistent. Lethal mutation rate was first estimated using formula (10) and the estimates of H'_L, B, and C ($\hat{H}'_L = 0.033$, $\hat{B} = 0.3235$, and $\hat{C} = 0.6290$). The result is $\hat{M}_L = 0.022$. This value is approximately 4 times larger than the standard rate (0.005–0.006, WALLACE 1968). This may be due to mutator factors, which were recently found in the Raleigh population at a high frequency (CARDELLINO and MUKAI 1975), and probably also due to overestimation of H'_L.

TABLE 6

Estimation of the average degrees of dominance for viability polygenes in an approximately equilibrium population ($\bar{h}_E$) *and of newly arisen mutant viability polygenes* ($\bar{h}_N$)

Chromosome	Including inversion-carrying chromosomes				Excluding inversion-carrying chromosomes			
Viability index	Ratio		Log		Ratio		Log	
Experiment	RD	TK	RD	TK	RD	TK	RD	TK
No. of crosses	39	55	39	55	30	49	30	49
No. of simultaneous replications	4	8	4	8	4	8	4	8
Variance (Y) $\times 10^5$	651.05	1024.66	821.19	952.88	805.22	1052.24	1069.64	948.04
Variance (X) $\times 10^5$	719.48	2477.58	1116.16	4001.00	946.20	2346.08	1298.34	3692.24
Covariance (X,Y) $\times 10^5$	198.60	1000.29	393.20	1177.64	222.82	967.46	459.91	1060.96
$\hat{\bar{h}}_E$	0.276 ±0.387	0.404 ±0.091	0.352 ±0.340	0.294 ±0.073	0.241 ±0.353	0.412 ±0.102	0.354 ±0.352	0.287 ±0.083
Pooled $\hat{\bar{h}}_E$	0.397 ± 0.089		0.297 ± 0.072		0.398 ± 0.098		0.290 ± 0.080	
$\hat{\bar{h}}_N$	3.278 ±3.837	1.024 ±0.232	2.089 ±1.660	0.809 ±0.201	3.614 ±5.164	1.088 ±0.270	2.326 ±2.122	0.894 ±0.258
Pooled $\hat{\bar{h}}_N$	1.032 ± 0.232		0.828 ± 0.200		1.094 ± 0.269		0.915 ± 0.256	

Variance (Y) stands for the variance of the heterozygote viabilities.
Variance (X) stands for the variance of the sums of the component homozygote viabilities.
Covariance (X,Y) is the covariance between X and Y.
$v > 0.65$ and RD and $v > 0.50$ in TK.

From the present experiment and previous data, it is assumed that $h_p = 0.2$ $\bar{c} \doteq 2$, $k = 1/8$ (cf. MUKAI 1964; MUKAI and YAMAGUCHI 1974), and $u \doteq 2$ (cf. GREENBERG and CROW 1960; MUKAI and YAMAZAKI 1968). The estimate of h_p in the present experiment was approximately 0.30 (logarithmic scale)—0.40 (ratio scale), but it should be considered that about 50% of the chromosomes carried lethal genes and the h_p values in the lethal heterozygotes were found to be nearly zero. The D' value has not been estimated for the third chromosomes, but it is thought to be between 0.25 and 0.35 [remember $\hat{D} = 0.4636$, and $\hat{D}$ (due to overdominant loci) $\doteq 0.10$ for the second chromosomes (MUKAI, manuscript in preparation)]. If $\hat{D}' = 0.25$ and $\overline{h}_p = 0.20$, then $M_p = 0.21$, which is consistent with the result of direct estimate for the second chromosomes (MUKAI 1964; MUKAI *et al.* 1972). If $\hat{D}' = 0.35$ and $\overline{h}_p = 0.20$, then $M_p = 0.27$.

Thus, it is concluded that if the D value is relatively large (about 0.2) due to loci where some forms of balancing selection including overdominance selection are operating, and $\overline{h}_p$ is approximately 0.2, the mutation rate estimate here agrees with the result of our previous direct estimation (MUKAI 1964; MUKAI *et al.* 1972). It should be noted that it is not clear whether the mutator factors found in the Raleigh population significantly increase the mutation rate of polygenes affecting viability (CARDELLINO and MUKAI 1975).

In conclusion, a high mutation rate for viability polygenes (or mildly deleterious genes) was supported and the average degree of dominance is much greater than that for lethal genes, at least in lethal-free individuals.

DISCUSSION

Nature of the cage population: As described above, the present experimental materials were extracted from the cage two and seven months after its initiation. We assume that the cage population approximately reflects the characteristics of the original Raleigh population. This assumption is supported by the following: The frequency of lethal-carrying chromosomes in the first sample was 0.49 (= 220/447) and that in the second sample, which was extracted 5 months after the first, was 0.47 (= 26/55). These values are not significantly different from each other.

Factors affecting the estimate of the average degree of dominance of viability polygenes: In the present experiment, it was assumed that the *TM3(Sb)* chromosomes completely suppresses the effect of deleterious genes in the homologous chromosomes (but it is not always true).

MUKAI (unpublished) studied the effect of incomplete dominance of marker chromosomes, such as *TM3(Sb)* and the *SM1(Cy)*, on the estimate of the average degree of dominance of viability polygenes. He let h represent the degree of dominance of mutant viability polygenes in intra-populational crosses of wild-type chromosomes and let k symbolize the degree of dominance in heterozygotes having marker and wild-type chromosomes.

For the case when there is no correlation between k and h, the following relationship can be obtained between the regression coefficient of heterozygote viabilities on the sums of the component homozygotes (β) and $\bar{h}_E$:

$$\bar{h}_E \doteq \frac{\beta(1-\bar{k})^2 + \sigma_k^2(\beta - 1/2) - \frac{1}{2}\bar{k}(\bar{k}-1)}{1-\bar{k}} \tag{12}$$

where σ_k^2 represents the variance of k. If $\hat{\beta} = 0.3$ and $\hat{\sigma}_k^2 = 0.044$ [this is an estimate of σ_k^2 (genetic variance of h) for newly arisen polygenic mutations (MUKAI 1969b)], then the $\bar{h}_E$ values are 0.27, 0.29, 0.31, 0.33, 0.35, and 0.37 for $\bar{k} = -0.1$, 0, 0.1, 0.2, 0.3, and 0.4, respectively. These values are not much different from 0.30. If $\bar{k} = 0$ [this assumption is supported by the results obtained by WALLACE and DOBZHANSKY (1962)], then $\bar{h}_E$ becomes

$$\bar{h}_E \doteq \beta + \sigma_k^2\left(\beta - \frac{1}{2}\right) . \tag{12}$$

Thus, insofar as β is less than 1/2, $\hat{\beta}$ is an overestimate of $\bar{h}_E$; but the magnitude of the error is extremely small, as shown above.

When there is complete correlation between k and h, or $k = ah$,

$$\bar{h}_E = \frac{\beta}{1 + a\{\beta(2 - a\bar{h}_N) + \bar{h}_N(1 - 0.5a) - 0.5\}} . \tag{13}$$

If $\hat{\beta} = 0.3$ and $\hat{\bar{h}}_N = 0.43$ (MUKAI 1969b), then $\hat{\bar{h}}_E$ values are 0.29, 0.32, 0.34, 0.35, and 0.40 for $a = -0.1$, 0.2, 0.4, 0.5, and 1.0, respectively. Thus, $\hat{\beta}$ becomes an underestimate of $\bar{h}_E$ if a is positive.

For both cases, the magnitude of bias is not large in comparison with the standard errors of $\bar{h}_E$. Therefore, results obtained with respect to the average degree of dominance of viability polygenes approximately hold even if the *TM3(Sb)* chromosome does not always suppress the effects of deleterious genes in homologous chromosomes.

It has been reported that there are synergistic interactions among viability polygenes in homozygous conditions (MUKAI 1969a; TEMIN *et al.* 1969; KOSUDA 1971), but no experimental evidence for such interactions have been reported for the heterozygous condition. MUKAI (unpublished) conducted computer simulations and found that synergistic interactions in the heterozygous condition cause an overestimation of $\bar{h}_E$ values if the regression method of estimation is used. In this work, the model $y = 1-h(ax+bx^2)$ was employed, where y represents heterozygote viability, a and b designate positive constants, x is the number of heterozygous loci, and h stands for the degree of dominance (cf. MUKAI 1969a). There probably is a small amount of synergistic interaction in the heterozygous condition, since $\bar{h}_E$ was estimated to be slightly larger than the expected value (0.30–0.40 *vs.* 0.17–0.27, cf. MUKAI 1969b). However, the difference between the estimated and expected values is not large.

Recently, MUKAI *et al.* (1974) have reported that at some loci alleles showing inferiority in viability may show superior fertility to their alternate alleles. In such cases, the gene frequencies become intermediate and the regression coefficients of heterozygote viabilities on the sums of the homozygous viabilities of the component chromosomes become larger.

Comparison of the second and the third chromosomes:

(1) *Inversions*: The frequencies of chromosomes carrying polymorphic inversions were 0.168 (= 116/691) and 0.108 (= 53/489) for the second and the third chromosomes, respectively. On the other hand, the frequency of chromosomes with unique inversions was 0.020 (= 14/691) for the second chromosomes and 0.004 (= 2/489) for the third chromosomes. The differences between the second and third chromosomes are significant for both frequencies ($\chi^2_{df=1} = 8.26$, $P < 0.005$ for the former comparison and $\chi^2_{df=1} = 5.60$, $P < 0.025$ for the latter). These frequencies are negatively correlated with the frequencies of lethal-carrying chromosomes. The generality of this phenomenon should be examined in a future investigation.

A significant difference in homozygous relative viability was found between third chromosomes that were lethal-free inversion carriers, and those that were lethal- and inversion-free (see Table 2). However, heterozygote relative viabilities for these two types are almost equal. A difference in average homozygote relative viabilities between these two types of chromosomes was not found for the second chromosomes (MUKAI and YAMAGUCHI 1974). This difference between the second and third chromosomes might indicate that the genes in the third chromosome inversions are more highly coadapted than those in the second chromosomes, but that their frequency in the population studied was much lower than the frequency of the inversions of the second chromosomes.

(2) *Frequency of lethal-carrying chromosomes*: The frequencies of lethal-carrying chromosomes involving all chromosomes examined were 0.40 (= 275/691) and 0.49 (= 246/502) for the second and the third chromosomes, respectively. These two figures are significantly different ($\chi^2_{df=1} = 10.02$, $P < 0.005$). However, the frequencies in inversion-carrying chromosomes were almost the same [0.538 (= 70/130) for the second chromosomes and 0.537 (= 29/54) for the third chromosomes], whereas a large difference was detected in the inversion-free chromosomes [0.365 (= 205/561) for the second and 0.490 (= 213/435) for the third chromosomes; $\chi^2_{df=1} = 15.63$, $P < 0.0005$)]. At present, the consistency of these results over many populations is unknown. SPIESS and ALLEN (1961) and others reported that the frequency of lethal-carrying chromosomes was higher in the third chromosomes than in the second chromosomes. However, WALLACE, ZOUROS and KRIMBAS (1966) and others found that these frequencies were not different. In fact, the third chromosome is approximately 1.1 times longer than the second chromosome.

(3) *Average degree of dominance*: Lethal heterozygotes were found to have a greater average relative viability than the lethal-free individuals. In order to examine whether this phenomenon also holds for semi-lethal chromosomes, the relative average viabilities of D/D', D/N, and N/N' were compared. Here, D

stands for a drastic chromosome [homozygous viability smaller than one-half the average heterozygote viability (WALLACE 1962)], and N for a non-drastic chromosome. The results are as follows:

$$D/D' : 0.9893 \pm 0.0083 \quad (n = 191)$$
$$D/N : 1.0132 \pm 0.0067 \quad (n = 236)$$
$$N/N' : 0.9865 \pm 0.0010 \quad (n = 69)$$

where n is the number of pairs of vials. The average viability of D/N is significantly greater than that of N/N'. This is clear evidence for coadaptation or epistasis with respect to *viability*. A similar result was obtained by WALLACE (1962) for the second chromosomes, but was not detected in the second chromosomes extracted from the Raleigh population (MUKAI and YAMAGUCHI 1974). However, we cannot say that lethal (or semi-lethal) genes show heterosis or overdominance with respect to *fitness*. The main reason is as follows: MUKAI and YAMAGUCHI (1974) predicted the frequency of lethal-carrying chromosomes to be 0.7 using WRIGHT's (1937) formula under the assumptions that (1) the lethal genes are completely recessive, (2) the lethal mutation rate is 10^{-5}/locus/generation, (3) the number of loci where lethal mutations can occur is 500 (cf. IVES 1945; WALLACE 1950), and (4) the effective size of the population is 2×10^4 (MUKAI and YAMAGUCHI 1974). The observed frequency was significantly lower than the predicted value. Indeed, the estimate of the $\bar{H}'_L$ value for fitness as a whole was 0.033, although there is some question about the method of estimation and the genetic background when fertility was estimated. Thus, it appears that there is almost no difference in relative fitness as a whole between lethal heterozygotes of the second and the third chromosomes, although a difference was detected in viability.

An interesting phenomenon was detected in the third chromosomes with respect to the average degree of dominance of viability polygenes. In contrast to the second chromosomes, an average h_E value of viability polygenes in lethal heterozygotes of the third chromosomes was nearly zero. This value is about 0.3–0.4 in lethal-free heterozygotes, although the difference is not significant. This phenomenon is consistent with the fact that the average viability of D/N is better than that of N/N'. It is difficult to find a role of inversion-carrying chromosomes with lethals to explain this peculiar phenomenon (Table 5), since the same tendencies can be seen for those with and without inversion-carrying chromosomes. However, these viability polygenes must show relatively large h values on the average, since the magnitudes of the detrimental and the lethal loads for the third chromosomes are not much larger than those for the second chromosomes and thus the D:L ratios are almost equal to each other (or the ratio for the third chromosomes is slightly smaller than that for the second chromosomes). In fact, the h_E value for the second chromosome is 0.2–0.3 (MUKAI and YAMAGUCHI 1974).

Finally, it should be pointed out that in both the second and the third chromosomes, a small degree of overdominance or some form of balancing selection was detected by means of the genetic regression coefficient ($\beta_{X \cdot Y}$).

General consideration: The genetic variability at the majority of loci in the third chromosomes may have been maintained by the balance between mutation and selection pressures. The decisive evidence is that the *D*:*L* ratio in this supposedly equilibrium population is the same as or slightly smaller than that of newly arisen mutations. We have shown in the present experiment that lethal genes are deleterious in heterozygous condition with respect to fitness as a whole, although some heterotic effect was seen in viability. Thus, if deleterious genes showed heterosis, their gene frequencies should have been increased in the equilibrium population and the *D*:*L* ratio should be extremely large (cf. GREENBERG and CROW 1960). The experimental results are inconsistent with this prediction. The consistency among the estimated genetic parameters under the assumptions of "classical" multiplicative gene action was *roughly* shown in the estimation of mutation rates.

However, the existence of some form of balancing selection including overdominance cannot be neglected since a large estimate of $\overline{h}_N$ was obtained. The presence of epistasis is also indicated by (1) the difference in estimated values of the average degree of dominance of viability polygenes between lethal heterozygotes and lethal-free heterozygotes and by (2) the approximately equal average relative viabilities of inversion heterozygotes and inversion-free heterozygotes in spite of the fact that the average viabilities of inversion-carrying and inversion-free chromosomes were significantly different. However, the contribution of epistasis to the maintenance of genetic variability appears to be small.

It is important to clarify the nature of balancing selection.

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LITERATURE CITED

ALLEN, A. C., 1966 The effects of recombination on quasinormal second and third chromosomes of *Drosophila melanogaster* from a natural population. Genetics **54**: 1409–1422.

BAND, H. T. and P. T. IVES, 1963 Comparison of lethal + semi-lethal frequencies in second and third chromosomes from a natural population of *Drosophila melanogaster*. Can. J. Genet. Cytol. **5**: 351–357.

CARDELLINO, R. A. and T. MUKAI, 1975 Mutator factors and genetic variance components of viability in *Drosophila melanogaster*. Genetics **80**: 567–583.

GREENBERG, R. and J. F. CROW, 1960 A comparison of the effect of lethal and detrimental chromosomes from Drosophila populations. Genetics **45**: 1154–1168.

HALDANE, J. B. S., 1956 Estimation of viabilities. J. Genet. **54**: 294–296.

HIRAIZUMI, Y. and J. F. CROW, 1960 Heterozygous effects on viability, fertility, rate of development and longevity of Drosophila chromosomes that are lethal when homozygous. Genetics **45**: 1071–1083.

HUBBY, J. L. and R. C. LEWONTIN, 1966 A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. Genetics **54**: 577–594.

IVES, P. T., 1945 The genetic structure of American populations of *Drosophila melanogaster*. Genetics **30**: 167–196.

JUDD, B. H., M. W. SHEN and T. C. KAUFMAN, 1972 The anatomy and function of a segment of the *X* chromosome of *Drosophila melanogaster*. Genetics **71**: 139–156.

KOSUDA, K., 1971 Synergistic interaction between second and third chromosomes on viability of *Drosophila melanogaster*. Japan. J. Genet. **46**: 41–52.

LINDSLEY, D. L. and E. H. GRELL, 1967 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. **627**.

MORTON, N. E., J. F. CROW and H. J. MULLER, 1956 An estimate of the mutational damage in man from data on consanguineous marriages. Proc. Natl. Acad. Sci. U.S. **42**: 855–863.

MUKAI, T., 1964 The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. Genetics **50**: 1–19. ——, 1969a The genetic structure of natural populations of *Drosophila melanogaster*. VII. Synergistic interaction of spontaneous mutant polygenes controlling viability. Genetics **61**: 749–761. ——, 1969b The genetic structure of natural populations of *Drosophila melanogaster*. VIII. Natural selection on the degree of dominance of viability polygenes. Genetics **63**: 467–478.

MUKAI, T., R. A. CARDELLINO, T. K. WATANABE and J. F. CROW, 1974 The genetic variance for viability and its components in a local population of *Drosophila melanogaster*. Genetics **78**: 1195–1208.

MUKAI, T., S. I. CHIGUSA, L. E. METTLER and J. F. CROW, 1972 Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. Genetics **72**: 335–355.

MUKAI, T. and T. MARUYAMA, 1971 The genetic structure of natural populations of *Drosophila melanogaster*. IX. A prediction of genetic equilibrium. Genetics **68**: 105–126.

MUKAI, T., L. E. METTLER and S. I. CHIGUSA, 1971 Linkage disequilibrium in a local population of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **68**: 1065–1069.

MUKAI, T. and O. YAMAGUCHI, 1974 The genetic structure of natural populations of *Drosophila melanogaster*. XI. Genetic variability in a local population. Genetics **76**: 339–366.

MUKAI, T. and T. YAMAZAKI, 1964 Position effect of spontaneous mutant polygenes controlling viability in *Drosophila melanogaster*. Proc. Japan Acad. **40**: 840–845. ——, 1968 The genetic structure of natural populations of *Drosophila melanogaster*. V. Coupling-repulsion effect of spontaneous mutant polygenes controlling viability. Genetics **59**: 513–535.

O'BRIEN, S. J. and R. J. MACINTYRE, 1969 An analysis of gene enzyme variability in natural populations of *Drosophila melanogaster* and *D. simulans*. Am. Naturalist **103**: 97–113.

OSHIMA, C. and O. KITAGAWA, 1961 The persistence of deleterious genes in natural populations of *Drosophila melanogaster*. Proc. Japan Acad. **37**: 158–162.

PAIK, Y. K., 1966 Genetic variabilities in second and third chromosomes from Korean populations of *Drosophila melanogaster*. Japan. J. Genet. **41**: 325–333.

SPIESS, E. B. and A. C. ALLEN, 1961 Release of genetic variability through recombination. VII. Second and third chromosomes of *Drosophila melanogaster*. Genetics **46**: 1531–1553.

TALLIS, G. M., 1959 Sampling errors of genetic correlation coefficients calculated from analysis of variance and covariance. Aust. J. Stat. **1**: 35–43.

TEMIN, R. G., H. U. MEYER, P. S. DAWSON and J. F. CROW, 1969 The influence of epistasis on homozygous viability depression in *Drosophila melanogaster*. Genetics **61**: 497–519.

WALLACE, B., 1950 Allelism of second chromosome lethals in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **36**: 654–657. ——, 1962 Temporal changes in the roles of lethal and semi-lethal chromosomes within populations of *Drosophila melanogaster*. Am. Naturalist **96**: 247–256. ——, 1968 Mutation rates for autosomal lethals in *Drosophila melanogaster*. Genetics **60**: 389–393.

WALLACE, B. and TH. DOBZHANSKY, 1962 Experimental proof of balanced genetic loads in Drosophila. Genetics **47**: 1027–1042.

WALLACE, B., E. ZOUROS and C. B. KRIMBAS, 1966 Frequencies of second and third chromosome lethals in a tropical populations of *Drosophila melanogaster*. Am. Naturalist **100**: 245–251.

WRIGHT, S., 1937 The distribution of gene frequencies in populations. Proc. Natl. Acad. Sci. U.S. **23**: 307–320.

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