THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*. XI. GENETIC VARIABILITY IN A LOCAL POPULATION[1]

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ABSTRACT

Six hundred and ninety-one second chromosomes were extracted from a Raleigh, North Carolina population, and the following experimental results were obtained: (1) Salivary gland chromosomes of all lines were observed and the number of inversion-carrying chromosomes was 130, among which 76 carried *In(2R)NS*, 36 carried *In(2L)t*, 4 carried *In(2L)t* and *In(2R)NS*, and 14 carried different kinds of rare inversions. (2) Viabilities of homozygotes and heterozygotes were examined. The frequency of lethal-carrying chromosomes was 275/691 (or 0.398):70/130 (or 0.538) in inversion-carrying chromosomes and 205/561 (or 0.365) in inversion-free chromosomes. The former is significantly higher than the latter. The average homozygote viability was 0.4342 including lethal lines and 0.7163 excluding those, the average heterozygote viability being 1.0000. The detrimental load to lethal load ratio (D:L ratio) was $0.334/0.501 = 0.67$. The average viability of lethal heterozygotes was less than that of lethal-free heterozygotes, significantly in inversion-free individuals but not significantly so in inversion-carrying individuals. Inversion heterozygotes seem to have slightly better viability than the inversion-free heterozygotes on the average, but not significantly so. (3) The average degree of dominance of viability polygenes was estimated to be 0.293 ± 0.071 for all heterozygotes whose component chromosomes had better viabilities than 0.6 of the average heterozygote viability, 0.177 ± 0.077 for inversion-free heterozygotes and 0.489 ± 0.082 for inversion heterozygotes. (4) Mutation rates of viability polygenes and lethal genes were estimated on the basis of genetic loads and average degrees of dominance of lethal genes and viability polygenes. Estimates were very close to those obtained by direct estimation. (5) Possible overdominance and epistasis were detected, but the magnitude must be very small. (6) The effective size of the population was estimated to be much greater than 10,000 by using the allelism rate of lethal-carrying chromosomes (0.0040) and their frequency.—On the basis of these findings and the comparison with the predicted result (MUKAI and MARUYAMA 1971), the mechanisms of the maintenance of genetic variability in the population are discussed.

GENETIC variability in natural populations with respect to viability has been studied for several species of Drosophila by many investigators (e.g., *D.*

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pseudoobscura: DOBZHANSKY and SPASSKY 1963; *D. willistoni:* KRIMBAS 1959; *D. melanogaster:* GREENBERG and CROW 1960), but their conclusions have not always agreed. Using an alternative method, MUKAI and his associates (MUKAI and YAMAZAKI 1971 and earlier) accumulated spontaneous mutations on second chromosmes of *D. melanogaster* and examined the nature of newly arisen mutant polygenes affecting viability. Main results were summarized in MUKAI and MARUYAMA (1971). In that paper, genetic equilibrium in random mating populations was predicted on the basis of the genetic parameters estimated. However, the prediction was made without considering DOBZHANSKY's (1952) co-adaptation theory.

Recently, a large number of polymorphisms of isozyme or allozyme genes have been discovered in random mating populations of many organisms, including man (cf. LEWONTIN and HUBBY 1966); and it has been generally recognized that most *D. melanogaster* populations carry rather high frequencies of inversions, some of which are polymorphic (cf. CHIGUSA, METTLER and MUKAI 1969; YANG, KOJIMA and KOVARIK 1971). This finding, together with seemingly permanent linkage disequilibria between these inversions and some isozyme genes (MUKAI, METTLER and CHIGUSA 1971), suggests the necessity for a detailed analysis of a population in approximate equilibrium where inversion polymorphisms exist. In recent years various kinds of environmental pollutants have been discussed as serious factors affecting natural populations. Clearly it would be useful to know the state of present populations so that they might be used as control populations in the estimation of the effects of polluting agents in the future. Thus, the Raleigh, North Carolina population was carefully analyzed once more (cf. CHIGUSA, METTLER and MUKAI 1969; MUKAI *et al.* 1972), paying attention to the polymorphic inversions.

In the present paper, the following will be attempted: (1) to measure genetic variability in terms of homozygous load and genetic variance; (2) to estimate the effective size of the population using the result of allelism tests for lethals; (3) to evaluate the approximate mutation rates of viability polygenes using the concept of the homozygous genetic load with respect to the average heterozygote viability of the population; (4) to estimate the effect of inversions on the average degree of dominance; (5) to examine overdominance and epistasis. Results obtained will be compared with the results predicted on the basis of newly arisen mutations. Furthermore, these detailed analyses will furnish additional information on the mechanisms of maintenance of isozyme polymorphisms in populations.

MATERIALS AND METHODS

Extraction of second chromosomes: Adult male and female flies were collected around the first of August (for one week), 1970, in a state park near Raleigh, North Carolina. Two sites, 1.4 miles apart, were chosen (Site A and Site B). Male flies were individually mated to 5 C160 females (C160:*In(2LR)SM1/In(2LR)Pm*, abbreviated as *Cy/Pm*. For a complete description, see MUKAI and BURDICK 1959). Female flies were individually mated to 5 *Cy/Pm* males. These female flies from nature were not always virgins, but further matings were expected with *Cy/Pm* males. Following the scheme described by WALLACE (1956), 324 second chromosomes were extracted from males and 367 from females. These chromosome types were maintained

as lines at 19°, balanced with *SM1*(*Cy*) chromosomes, which helps maintain less viable or lethal chromosome types. Cytological examination of the salivary gland chromosomes was made for each chromosome line after making a cross to a standard wild type (W507).

Experiment 1: Homozygote and heterozygote viabilities were estimated as in WALLACE (1956). Crosses were made between 5 $Cy/+_i$ females and 5 $Cy/+_i$ males with 4 simultaneous replications in each chromosome line, where i indicates line number. In the offspring, $Cy/+_i$ and $+_i/+_i$ flies segregate at an expected ratio of 2:1. The viabilities of random heterozygotes were estimated in a way similar to the above, combining two successively numbered lines, i.e., $Cy/+_i \times Cy/+_{i+1}$ 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 crosses were made, all 10 flies in a vial were transferred to a second vial. Four days after the transfer was made, all flies were discarded. In both vials, the original and transferred, all emerged flies were counted at 4 different times until the 18th day after the cross or transfer was made. *Cy* flies and wild-type flies from the pair of vials were respectively pooled and considered a single observation. The viability was expressed at first as the ratio of the number of wild-type flies to the number of *Cy* flies plus one (cf. HALDANE 1956). Crosses were made at 9 different times (9 replications). Seventy-two to 80 lines were employed per replication. The chromosomes for each replication were chosen at random from chromosomes that originated from males and females since line numbers had been assigned randomly. Homozygote and heterozygote viabilities were estimated at the same time within replications. Before the analyses were made all viabilities were standardized to the average viability of the heterozygotes within replications.

Experiment 2: Originally 55 chromosome lines that did not carry inversions, and showed larger viability indices than 0.6 of the average viability of random heterozygotes within replications in Experiment 1, were employed in this experiment to estimate the average degree of dominance of viability polygenes. Cyclic matings were conducted as in Experiment 1. However, it was found after the experiment that 6 lines out of 55 carried recessive lethals or their homozygous viability was less than 0.6 due to semi-lethal mutations and/or additional polygenic mutations or since the viabilities of these lines were overestimated previously in Experiment 1. Thus, crosses including these lines were omitted in the analysis. As a result, 38 crosses were employed. The total number of constituent chromosome lines was 49. The number of simultaneous replications per line or per cross was usually 8 but in some cases 5–7. The average was 7.26. In Experiment 2, the viability indices were expressed as $2 \times$ (number of wild-type flies)/(number of *Cy* flies $+$ 1.0).

Experiment 3: In order to estimate the effective size of the population, allelism tests were conducted using 153 lines carrying "recessive" lethal genes, i.e., crosses were made between Cy/l_i and Cy/l_j where l indicates a lethal-carrying chromosome, and i and j stand for line numbers. If the lethals that are located in the chromosomes of lines i and j are allelic, all the progenies of the above crosses show *Cy* phenotype; otherwise phenotypically *Cy* and wild-type flies segregate.

THEORETICAL CONSIDERATIONS

Estimation of the average degree of dominance of viability polygenes: In order to estimate the average degree of dominance, a model for a single locus is assumed at first where two alleles A ("normal" allele) and a (mutant allele) exist; the gene frequency of A is p and that of a is $q(=1-p)$; h is the degree of dominance of the mutant allele, and s is the selection coefficient against the aa homozygote (Wrightian fitness model). The following table can then be obtained with respect to the genotypic frequencies and the viability values under the condition of random combinations:

Genotype	AA	Aa	aa
Frequency	p^2	$2pq$	q^2
Relative viability (Y)	1	$1-hs$	$1-s$
Corresponding homozygotes	$AA + AA$	$AA + aa$	$aa + aa$
Sum of relative viability (X)	2	$2-s$	$2-2s$

Based upon the above, the variances of X and Y and covariance between X and Y can be calculated as follows (MUKAI *et al.* 1972):

$$\text{Variance of } X\text{: } V(X) = 2pqs^2 \tag{1}$$

$$\text{Variance of } Y\text{: } V(Y) = pq[2(1-2pq)h^2-4q^2h+q(1+q)]s^2 \tag{2}$$

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

1) Newly arising mutations: Mutations can be accumulated at a minimum pressure of natural selection using a multiple inversion technique (cf. MUKAI 1964). Using these mutant polygenes, it is possible to estimate the average degree of dominance of newly arising mutant viability polygenes. In a population consisting of individuals made by a random combination of chromosomes, the gene frequency of a at locus i may be expressed as $q_i = m\mu_i$ where m stands for the number of generations during which mutations were accumulated, μ_i being the mutation rate at locus i. In the following formulation, it is assumed that q_i is much smaller than 1 (or $p \doteq 1$), that there are n loci on the chromosome, and that there is additivity among loci.

The regression coefficient of Y on X ($\beta_{Y\cdot X}$)

$$= \frac{2\sum_{i=1}^{n} p_iq_i\,[h_i + q_i\,(1-2h_i)]s_i^2}{2\sum_{i=1}^{n} p_iq_is_i^2}$$

$$\doteq \frac{\sum_{i=1}^{n} m\,\mu_ih_is_i^2}{\sum_{i=1}^{n} m\,\mu_is_i^2} \text{ (assuming } |h_i| \gg q_i)$$

$$= \frac{\overline{\mu s^2}\,\Sigma h_i}{n\,\overline{\mu s^2}} \text{ (assuming } \mu_is_i^2 \text{ is not correlated with } h_i)$$

$$= \bar{h} \tag{4}$$

The regression coefficient of X on Y ($\beta_{X\cdot Y}$)

$$= \frac{2\sum_{i=1}^{n} p_iq_i\,[h_i + q_i\,(1-2h_i)]s_i^2}{\sum_{i=1}^{n} p_iq_i\,[2(1-2p_iq_i)h_i^2 - 4q_i^2\,h_i + q_i\,(1+q_i)]s_i^2}$$

$$\doteq \frac{\sum_{i=1}^{n} h_i}{\sum_{i=1}^{n} h_i^2} \text{ (assuming } |h_i| >> q_i \text{ and } s_i^2 \text{ is not correlated with } h_i \text{ and } h_i^2)$$

$$= \frac{1}{\bar{h} + \dfrac{\sigma_h^2}{\bar{h}}} \tag{5}$$

From (4) and (5)

$$\sigma_h^2 = \frac{\beta_{YX}}{\beta_{XY}} - \beta_{YX}^2 \tag{6}$$

The ratio of $V(Y)$ to $V(X)$ or R_N can be calculated as follows:

$$R_N = \frac{\sum_{i=1}^{n} p_i q_i [2(1-2p_iq_i)h_i^2 - 4q_i^2 h_i + q_i(1+q_i)]s_i^2}{2\sum_{i=1}^{n} p_i q_i s_i^2}$$

$$\doteq \frac{\sum_{i=1}^{n} m\mu_i s_i^2 h_i^2}{\sum_{i=1}^{n} m\mu_i s_i^2} \doteq \overline{h^2} \quad \text{(The same as above was assumed).}$$

$$= \bar{h}^2 + \sigma_h^2 \tag{7}$$

2) Mutant viability polygenes in an equilibrium population: We can extract chromosomes from an equilibrium population using a multiple inversion technique and random heterozygotes can be bred using these chromosomes. Thus, it is possible to estimate $\beta_{Y\cdot X}$ and $\beta_{X\cdot Y}$ on a chromosome basis.

A) Partially dominant polygenes ($h > 0$): Assuming partial dominance of mutant viability polygenes, or $h \gg \sqrt{\mu/s}$, the equilibrium gene frequency of a at locus i can be approximately expressed as $\hat{q}_i \doteq \frac{\mu_i}{c_i h_i s_i}$ where c_i is a factor (most probably greater than one) that relates viability reduction to selection on fitness. Then, the regression coefficient of Y on X ($\beta_{Y\cdot X}$) on a chromosome basis is:

$$\beta_{Y\cdot X} \doteq \frac{2\sum_{i=1}^{n} q_i h_i s_i^2}{2\sum_{i=1}^{n} q_i s_i^2}$$

$$= \frac{\sum_{i=1}^{n} \frac{\mu_i}{c_i h_i s_i} s_i^2 h_i}{\sum_{i=1}^{n} \frac{\mu_i}{c_i h_i s_i} s_i^2}$$

$$= \frac{\sum_{i=1}^{n} \left(\frac{\mu_i s_i}{c_i}\right)}{\sum_{i=1}^{n} \left(\frac{\mu_i s_i}{c_i}\right)\frac{1}{h_i}}$$

Assuming $\frac{\mu_i s_i}{c_i}$ is not correlated with $\frac{1}{h_i}$, then,

$$\beta_{Y\cdot X} \doteq \frac{1}{\overline{\left(\frac{1}{h}\right)}} \tag{8}$$

This is the harmonic mean of the h values of newly arisen mutations. MORTON, CROW and MULLER (1956) and HIRAIZUMI and CROW (1960) showed that under the partial dominance model, the harmonic mean of the h values of newly arisen mutations is approximately equal to the arithmetic mean of the h values in an equilibrium random mating population. Thus, $\beta_{Y\cdot X}$ gives an approximate average h value in an equilibrium random mating population, *even if* there are differential fertilities among genotypes.

Under the same assumptions as above, $\mu_i s_i / c_i$ is not correlated with h_i, $\beta_{X\cdot Y}$ can be calculated as follows:

$$\beta_{X.Y} \doteq \frac{1}{\bar{h}} \tag{9}$$

This is the inverse of the arithmetic mean of the h values for newly arising mutations.

From (8) and (9), the following formula can be derived, since $\beta_{Y.X} \cdot \beta_{X.Y} = r^2_G$:

$$\overline{h_N} \doteq \overline{h_E} \frac{1}{r^2_G} \tag{10}$$

where $\overline{h_N}$ and $\overline{h_E}$ are the average degrees of dominance of newly arising mutant genes and mutant genes in an equilibrium population, respectively, and r_G is the genetic correlation coefficient between the heterozygote viabilities and the sums of the corresponding homozygote viabilities.

The above method can be extended to lethal heterozygotes (Y) and the homozygotes for the lethal-free chromosomes (X) that are the constituents of the lethal heterozygotes. In this case, the contributions of lethal-carrying chromosomes to homozygotes are ignored and AA, $1/2\ AA + 1/2\ aa$, and aa, respectively, are the homozygotes corresponding to the heterozygous individuals. Accordingly, the X values are 1, $1 - 1/2\ s$, and $1 - s$ for these three homozygote groups. These considerations lead to the following formulae (11), (12), and (13):

$$\beta_{Y.X} \doteq \frac{1}{\overline{\left(\frac{1}{h_N}\right)}} \doteq \overline{h_E} \tag{11}$$

$$\beta_{X.Y} \doteq \frac{1}{2\,\overline{h_N}} \tag{12}$$

$$\overline{h_N} \doteq \overline{h_E} \frac{1}{2\,r^2_G} \tag{13}$$

The ratio of $V(Y)$ to $V(X)$ or R_E for lethal-free individuals is calculated from (8) and (9) as follows:

$$R_E \doteq \bar{h}\,\tilde{h} \tag{14}$$

where $\tilde{h}$ is the harmonic mean of h values.

For lethal heterozygotes, R_E can be calculated from (11) and (12) as follows:

$$R_E \doteq 2\,\bar{h}\,\tilde{h} \tag{14'}$$

B) Overdominant genes ($h < 0$): Using the Wrightian fitness model described above, the equilibrium gene frequency is a function of the degree of dominance (h). In the present case, h is defined as the degree of dominance with respect to viability. However, the equilibrium gene frequency is determined by fitness as a whole. Thus, a factor c_i that relates the degree of dominance of a viability gene to the degree of dominance of total fitness was introduced. The equilibrium gene frequency can then be expressed as $\hat{q} = c_i h_i/(2c_i h_i - 1)$. Then, the covariance between Y and X on a single -locus basis is:

$$\frac{2\ ch(ch-1)}{(2\ ch-1)^2}\ s^2 \left[\frac{h(c-1)}{2\ ch-1}\right] \tag{3'}$$

Since $ch < 0$, $\mathrm{Cov}(X, Y)$ is positive if $c > 1$, zero if $c = 1$ and negative if $c < 1$.

In the case of multiple loci, the situation becomes complicated, but insofar as $c = 1$, the following relation can be obtained:

$$\begin{aligned} \beta_{Y.X} &= 0 \\ \beta_{X.Y} &= 0 \end{aligned} \tag{15}$$

$\beta_{Y.X}$ and $\beta_{X.Y}$ may be either positive or negative according to the magnitude of c_i's.

Minimum ratio of the number of dominant loci to that of overdominant loci: The following genetic parameters are defined in addition to the above:

m = the number of partially dominant loci (only in this section)

n = the number of overdominant loci (only in this section)

t_1 and t_2 = selection coefficients as described in the following table. The following table shows the fitness relationship on a single-locus basis:

Model	AA	Aa	aa	$\bar{w}$	Best homozygote
Dominance	1	$1-h_i s_i$	$1-s_i$	$1-2\mu_i$	1
Overdominance	$1-t_{1j}$	1	$1-t_{2j}$	$1-\frac{t_{1j}t_{2j}}{t_{1j}+t_{2j}}$	$1-t_{1j}$

For the sake of simplicity, $\frac{t_{1j}t_{2j}}{t_{1j}+t_{2j}}$ is equated to sd_j.

Assuming that gene action is multiplicative and that the best homozygote fitness is better than the average fitness of heterozygotes, the following formula is obtained:

$$\frac{K \cdot 1^m \prod_{j=1}^{n} (1-t_{1j})}{K \cdot \prod_{i=1}^{m} (1-2\mu_i) \prod_{j=1}^{n} (1-sd_j)} > 1 \tag{16}$$

where K is a constant dependent upon the fitness index.

The following inequality can be obtained from (16) assuming that t, 2μ, and $\frac{t_{1j}\,t_{2j}}{t_{1j}+t_{2j}}$ are much less than 1:

$$m/n > \frac{\left(\overline{\frac{t_1^2}{t_1+t_2}}\right)}{2\bar{\mu}} \tag{17}$$

Estimation of mutation rates of viability genes from an equilibrium population: Spontaneous mutations affecting viability may be classified into three categories (excluding dominant lethals): (1) recessive lethal, (2) semi-lethal, and (3) polygenic (usually mildly deleterious). These three types of mutations appear to be discontinuous with respect to their individual homozygous effects (cf. MUKAI 1964; MUKAI *et al.* 1972). Using the concept of homozygous genetic loads in equilibrium populations, the estimation of polygenic and recessive lethal mutation rates has been attempted under the assumptions of multiplicative gene action and no overdominance. The model used by GREENBERG and CROW (1960) was modified slightly. The following parameters are defined:

A = average viability of heterozygotes
B = average viability of all homozygotes
C = average viability of non-lethal homozygotes
M_L = mutation rate of major genic recessive lethals per chromosome
M_{SL} = mutation rate of major genic semi-lethals per chromosome
M_p = mutation rate of viability polygenes per chromosome
$k = M_{SL}/M_L$
H_L = the average degree of dominance of recessive lethals in an equilibrium population, or harmonic mean of the degree of dominance of newly arisen lethal genes for viability
H_{SL} = the average degree of dominance of semi-lethals in an equilibrium population or harmonic mean of the degree of dominance of newly arisen semi-lethal genes for viability
$u = H_{SL}/H_L$
h_p = the average degree of dominance of mutant polygenes in an equilibrium population or harmonic mean of the degrees of dominance of newly arisen mutant viability polygenes for viability

We assume no overdominance in this model and $h \gg \sqrt{\mu/s}$ where h is the degree of dominance in general. Considering the effects of fertility, mating ability and so on, in addition to the effect

of viability, the gene frequency in an equilibrium population can be, as above, expressed approximately as $\hat{q}_i \doteq \frac{\mu_i}{c_i h_i s_i}$.

Homozygous lethal load (L) and detrimental load (D), measured as a deviation from the average heterozygote, were estimated by GREENBERG and CROW (1960) as follows:

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

Using the above notations, the following formulae can be obtained (cf. GREENBERG and CROW 1960; TEMIN 1966):

$$\frac{M_p}{\tilde{c}_p h_p} + \frac{M_{SL}}{\tilde{c}_{SL} H_{SL}} \doteq D + 2\left[\frac{M_p}{\tilde{c}_p} + \frac{M_{SL}}{\tilde{c}_{SL}} + \frac{M_L}{\tilde{c}_L}\right] \tag{18}$$

where $\tilde{c}_p$ is the harmonic mean of c_i's for polygenes, $\tilde{c}_{SL}$ for semi-lethal genes, and $\tilde{c}_L$ for lethal genes, and the term in brackets is the deviation of the average heterozygote from the mutation-free genotype.

$$\frac{M_L}{\tilde{c}_L H_L} \doteq L \tag{19}$$

It is assumed that (1) c_i and h_i are not correlated and (2) $\tilde{c}_p = \tilde{c}_L = \tilde{c}_{SL} = c$. Then, the following formulae are obtained:

$$M \doteq \frac{c\,h}{1-2\,h_p}\left[D + H_L\, ln\ (C/B)\left\{(2+2k) - \left(\frac{k}{u}\right)\frac{1}{H_L}\right\}\right] \tag{20}$$

$$M_L = c\,H_L\, ln\ (C/B) \tag{21}$$

Thus, it is possible to estimate the mutation rates of viability polygenes (and lethal genes) on the basis of homozygous loads, degrees of dominance and mutation rate ratio of lethals to semi-lethals.

RESULTS AND ANALYSES

Distribution of viabilities

In 9 different experiments, a total of 691 second chromosomes were extracted and their homozygote and heterozygote viabilities were examined. In one experiment 70–82 chromosome lines were used and their homozygote and heterozygote (random combination of the chromosomes) viabilities were estimated. There were 3 allelic lethal combinations in the 691 heterozygote crosses which were excluded from the analysis. Thus, 688 random heterozygotes were used for the present analysis. Special attention was paid to the effects of inversions on viability. It has been discovered that the Raleigh population of *D. melanogaster* carries a relatively high frequency of inversions (METTLER, CHIGUSA and MUKAI, unpublished; CHIGUSA, METTLER and MUKAI 1969). All the chromosome lines were cytolegically examined and 130 chromosome lines out of 691 were found to carry inversions. There were two polymorphic inversions, [*In(2L)t* and *In(2R)NS*], in addition to rare inversions: 76 chromosomes carried *In(2R)NS*, 36 carried *In(2L)t*, 4 carried *In(2L)t* and *In (2R)NS*, and 14 carried different kinds of rare inversions. The distribution patterns of homozygotes and heterozygotes are given in Table 1 and graphically presented in Figure 1.

Homozygotes: Distributions of inversion-carrying lines and of inversion-free lines were also tabulated in Table 1 and graphically presented in Figure 2 after standardization in order to make the total frequency in each category equal 1.00. By using the Kolmogorov-Smirnov non-parametric test, a significant difference

TABLE 1

Distributions of homozygote and heterozygote viabilities (both inversion-carrying and inversion-free individuals)

Range of viability	Homozygotes Total	Inversion-carrying	Inversion-free	Heterozygotes Total	Inversion-carrying	Inversion-free
0 –0.05	259	67	192	(3)	(3)	0
0.05–0.15	22	3	19	0	0	0
0.15–0.25	13	2	11	0	0	0
0.25–0.35	17	1	16	0	0	0
0.35–0.45	21	7	14	1	0	1
0.45–0.55	30	4	26	0	0	0
0.55–0.65	40	3	37	1	1	0
0.65–0.75	68	8	60	2	1	1
0.75–0.85	92	12	80	36	12	24
0.85–0.95	81	13	68	148	34	114
0.95–1.05	41	8	33	296	106	190
1.05–1.15	7	2	5	167	63	104
1.15–1.25	0	0	0	35	13	22
1.25–1.35	0	0	0	2	0	2
Total	691	130	561	688(691)	230(233)	458

was detected between the distribution patterns of the inversion-carrying lines and the inversion-free lines ($P < 0.005$). The characteristics of the differences were as follows: (1) The frequency of lethal-carrying chromosomes [in the present series of the papers, the lines showing viability indices lower than 0.1 of the average viability of random heterozygotes are classified as lethals following the conventional definition of GREENBERG and CROW (1969)] is much higher in the

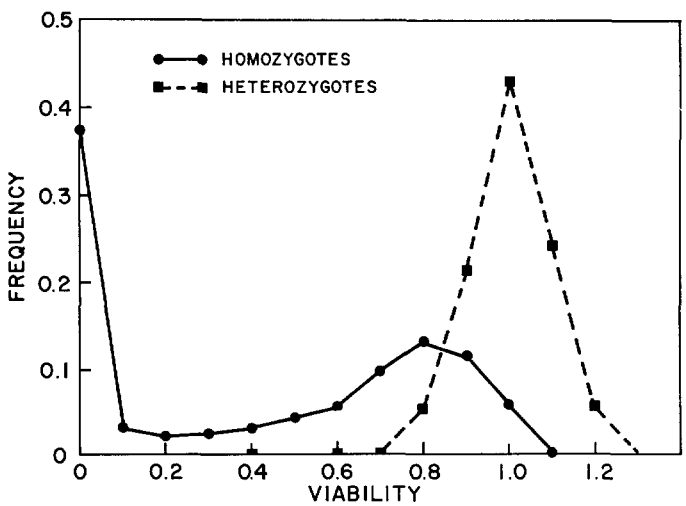

FIGURE 1.—Frequency distributions of homozygote and heterozygote viabilities of the second chromosomes. The average viability of heterozygotes is assumed to be 1.0000.

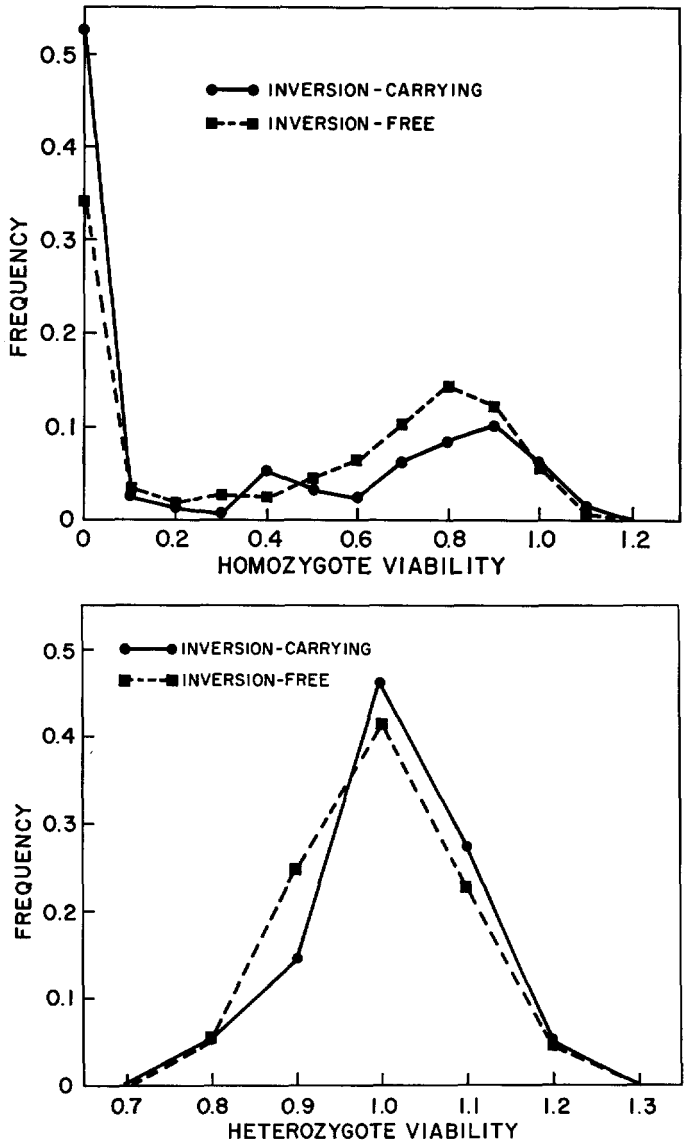

FIGURE 2.—distributions of inversion-carrying and inversion-free homozygotes and heterozygotes. upper figure: Homozygotes; lower figure: Heterozygotes.

inversion-carrying chromosomes than in the inversion-free chromosomes ($\hat{Q} = 0.54$ *vs.* 0.37, $\chi^2_{\text{d.f.}=1} = 13.19$, $P < 0.0005$). (2) Among the lethal-free chromosomes, those carrying inversions do not differ significantly from inversion-free chromosomes in average homozygous viability (Inversion-carrying chromosomes: 0.7253 ± 0.0296, number of lines (N) = 60; Inversion-free chromosomes: 0.7159 ± 0.0117, $N = 356$) or genotypic variance (Inversion-carrying chromosomes: 0.047046 ± 0.009693, d.f. $\doteq 47.1$; Inversion-free chromosome: $0.043016 \pm$

TABLE 2

Average viabilities of individuals carrying inversions

(A) Homozygotes	Lethal-carrying and lethal-free		Lethal-free	
Genotype	N	Average viability	N	Average viability
In(2R)NS	80	0.4307	30	0.7099 ± 0.0402
In(2L)t	40	0.3500	19	0.7136 ± 0.0612
Rare	14	0.6968	12	0.8108 ± 0.0570
All inversion-carrying	130	0.3401	60	0.7253 ± 0.0296
(All chromosomes sampled	691	0.4342	416	0.7163 ± 0.0108)

(B) Heterozygotes		
Genotype	N	Average viability
Rare	14	1.0113 ± 0.0205
In(2R)NS	144	1.0088 ± 0.0085
In(2L)t	75	1.0078 ± 0.0090
In(2R)NS and *In(2L)t**	13	1.0272 ± 0.0218
All inversion-heterozygotes	230	1.0083 ± 0.0064
(Inversion-free heterozygotes	458	0.9957 ± 0.0047)

* Included in both *In(2R)NS* and *In(2L)t* categories.
N = Number of lines or crosses.

0.003646, d.f. = 278.4). Thus, a linkage disequilibrium was detected only between inversions and lethal genes.

In order to obtain some information concerning the maintenance of polymorphic inversions, the average homozygote viabilities of respective inversions were tabulated in Table 2A. There were four chromosomes carrying both *In(2L)t* and *In(2R)NS* which were all lethal-carrying chromosomes, and are included in both categories [*In(2L)t* and *In(2R)NS*] in Table 2A. From this table, it is impossible to find any trends of inversion-carrying chromosomes.

Heterozygotes: The distribution of all heterozygotes, inversion-carrying heterozygotes, and inversion-free heterozygotes was tabulated in Table 1 and graphically shown in Figure 2 after standardization in order to make the total frequency in each category equal 1.00. The average viabilities of inversion-free and inversion-carrying heterozygotes are 0.9957 ± 0.0047 [Number of crosses (N) = 458] and 1.0083 ± 0.0064 ($N = 230$), respectively. They are not significantly different from each other. The genotypic variance of the former was estimated to be 0.005847 ± 0.000674 and the latter 0.005294 ± 0.000921. These two values are significantly different at the 1% level. However, in the inversion-free group there is one heterozygous cross of exceptionally low viability (viability index = 0.3599). When this cross was excluded, the average viability and the genotypic variance of this group was 0.9971 ± 0.0045 and 0.005002 ± 0.000619, respectively. Thus, on the average linkage disequilibrium between inversions and viability genes could not be detected except in the relationship between inversions and lethal genes. This does not necessarily exclude linkage disequilibrium between inversions and viability genes. The simplest interpretation must be that the magnitude is very small (cf. MUKAI, METTLER and CHIGUSA 1971).

The average viabilities of respective inversion heterozygotes are shown in Table 2B. The heterozygotes with respect to both *In(2R)NS* and *In(2L)t* are included in both of the categories of *In(2R)NS* and *In(2L)t*. By chance, homozygotes with respect to inversions were found, but the genes carried by these heterozygotes are not always homozygous. Indeed, their average viabilities are 1.0243 ± 0.0296 ($N = 14$) for *In (2R)NS*, and 0.9827 ($N = 1$) for *In(2L)t*. Thus, there is no evidence of appreciable inbreeding depression with respect to the change of gene arrangement alone. In general, inversion heterozygotes showed better average viabilities than the inversion-free individuals, although not significantly.

Detrimental load to lethal load ratio (D:L *ratio*): The average viability of all homozygote lines (B) is 0.4342 ± 0.0295 and that of homozygote lines excluding lethal-carrying lines (C) is 0.7163 ± 0.0108. The average viability of random heterozygotes (A) is 1.0000 ± 0.0038. 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:

$$T = lnA - lnB = 0.8343$$
$$D = lnA - lnC = 0.3336$$
$$L = lnC - lnB = 0.5007$$

The D:L ratio is 0.667. This figure agrees very well with the results for other populations obtained by different investigators: 0.636 by TEMIN *et al.* (1969), and 0.584 by KOSUDA (1971) in *D. melanogaster*, but is slightly smaller than the value obtained in *D. pseudoobscura* (D:$L = 0.855$) by WILLS (1966). 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 $2 \times$ total mutation rate per haploid set of chromosomes. MUKAI (1964) estimated the total mutation rate per second chromosome in *D. melanogaster* to be about 0.14. Thus, the D:L ratio with respect to the optimum genotype in the population becomes: $(D{:}L)_0 \doteq (0.3336 + 0.28)/0.5007 = 1.23$. Recently, the homozygous load due to overdominant loci was estimated to be about 0.10 (MUKAI, unpublished). If this amount is subtracted from the detrimental load, the D:L ratio becomes 1.03.

The D:L ratios in newly arisen mutations were estimated to be 0.975 (MUKAI and YAMAZAKI 1968) and 0.954 (MUKAI *et al.* 1972). These figures are very close to the estimate in the present experiment. GREENBERG and CROW (1960) have shown that this agreement can be expected if hs is approximately constant. The fact that the $\bar{h}$ value of mildly detrimental genes (or viability polygenes) is larger than $\bar{H}$ value of lethal genes was demonstrated in MUKAI *et al.* (1972) and will be shown in a later section of this paper.

Lethal heterozygote viability

All heterozygotes: In the present crossing scheme, individual lethal-carrying chromosomes were used twice, except for those chromosomes that contained lethals allelic to that in the homologous chromosomes (3 crosses). Out of 688 random heterozygotes, there are 241 lethal-free heterozygotes, 350 "single-lethal" heterozygotes, and 97 "double-lethal" heterozygotes. "Single-lethal" means that

TABLE 3

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

Genotype	All crosses N	All crosses Average viability	Inversion-carrying N	Inversion-carrying Average viability	Inversion-free N	Inversion-free Average viability
Non-lethal/Non-lethal'		1.0123 ± 0.0063		1.0118 ± 0.0115		1.0124 ± 0.0076
	241	(1.0000 ± 0.0062)*	68	(1.0000 ± 0.0114)	173	(1.0000 ± 0.0075)
Non-lethal/Lethal		0.9931 ± 0.0053		1.0059 ± 0.0090		0.9865 ± 0.0065
	350	(0.9810 ± 0.0052)	118	(0.9941 ± 0.0089)	232	(0.9744 ± 0.0065)
Letahl/Lethal'		0.9947 ± 0.0094		1.0098 ± 0.0151		0.9821 ± 0.0116
	97	(0.9826 ± 0.0093)	44	(0.9980 ± 0.0149)	53	(0.9701 ± 0.0115)
Average degree of dominance†		0.012		0.0026		0.018

N stands for the number of crosses.
* The figures in parentheses are the standardized values.
† Average degree of dominance on a locus basis.

only one of the homologous chromosomes carries at least one "recessive" lethal gene, and "double-lethal" represents individuals in which each of the homologous chromosomes carries at least one "recessive" lethal gene.

An analysis of variance was performed to detect differences in mean viabilities between lethal-free, single-lethal, and double-lethal heterozygotes, but no significant differences were detected as a whole. However, significant differences were found between lethal-free individuals and single-lethal heterozygotes, and between lethal-free individuals and double-lethal heterozygotes. The standardized mean values, assuming the average viability of lethal-free individuals is 1.00, are shown in Table 3. Since the frequency of lethal-carrying chromosomes is 0.398 (275 out of 691), the average number of lethal genes per lethal-carrying chromosome, assuming a Poisson distribution, is expected to be 1.275. Thus, the average degree of dominance of lethal genes per locus ($\bar{H}$) is estimated to be 0.012. If lethal genes were not completely recessive in the *Cy* heterozygotes but show the same amount of dominance as in the phenotypically wild-type individuals (intra-populational heterozygous genetic background), this $\bar{H}$ value becomes 0.024. However, the actual $\bar{H}$ value will be much closer to the former than the latter (cf. WALLACE and DOBZHANSKY 1962). These estimates are in agreement with the earlier direct estimates of HIRAIZUMI and CROW (1960) and CROW and TEMIN (1964).

In order to obtain some information of heterozygous effects of lethal genes on fecundity, the numbers of offspring per observation (a pair of vials) from *Cy/l* and *Cy/+* female parents were compared. The results are as follows:

Cy/+ females : 352.98 ± 1.58 (1.0000 ± 0.0045), $N = 2241$
Cy/l females : 350.61 ± 2.03 (0.9933 ± 0.0057), $N = 508$

where the figures in parentheses indicate standardized values and their standard errors and N is the number of observations. A significant difference could not be detected between them. This result does not necessarily indicate that lethal genes in the heterozygous condition have no effect on fecundity. There might be a dif-

ference between lethal-free and lethal heterozygotes in intra-populational heterozygous genetic background.

Inversion-free heterozygotes: There were 458 inversion-free heterozygotes, of which 173 were lethal-free heterozygotes, 232 were single-lethal and 53 were double-lethal heterozygotes.

As in the above case, an analysis of variance was conducted for examining whether or not there are differences between lethal-free, single-lethal, and double-lethal heterozygotes. Significant differences were detected in the data as a whole ($F_{2,455} = 4.08$, $P < 0.05$). The standardized mean values, assuming the average viability of lethal-free heterozygotes is 1.00, are shown in Table 3 together with the case of all heterozygotes. Significant differences were found between lethal-free heterozygotes and single-lethal heterozygotes, and between lethal-free heterozygotes and double-lethal heterozygotes.

In inversion-free chromosomes, the frequency of lethal-carrying chromosomes is 0.365 (205 out of 561) giving an expected average number of 1.245 lethal genes per lethal-carrying chromosomes. Thus, the average degree of dominance of lethal genes per locus is estimated to be 0.018. If lethal genes were not completely recessive in the *Cy* heterozygotes, but showed the same amount of dominance as in the phenotypically wild-type individuals, this $\bar{H}$ value becomes 0.035.

Inversion heterozygotes: In this category, 17 inversion homozygotes were included for the sake of convenience. There were 230 crosses that belong to this category. Among them, 68 were lethal-free heterozygotes, 118 were single-lethal heterozygotes and 44 were double-lethal heterozygotes. An analysis of variance was performed to detect differences between the mean viabilities of the above three types of heterozygotes; there were no differences. Pairwise comparisons were made between the three mean viabilities, but no significant differences were detected. The standardized mean values, assuming the average viability of lethal-free individuals is 1.00, are shown in Table 3 together with the above two cases.

The frequency of lethal-carrying chromosomes is 0.538 (70 out of 130) for inversion-carrying chromosomes. This indicates that, on the average, 1.436 lethal genes are expected per inversion-carrying lethal chromosome (cf. 1.254 per inversion-free lethal chromosome). Hence, the average degree of dominance of lethal genes per locus is estimated to be 0.0026, which is much smaller than the $\bar{H}$ value in inversion-free heterozygotes (0.018), and is not significantly different from zero. If the lethal genes show the same degree of dominance in *Cy* heterozygotes (interpopulationally heterozygous genetic background), the $\bar{H}$ value becomes 0.0053.

Average degree of dominance of viability polygenes

All the heterozygotes whose constituent chromosomes have both viability indices larger than 0.6 were chosen for the estimation of the average degree of dominance of viability polygenes. Formula (8) was employed for the estimation, which was already used by MUKAI and YAMAZAKI (1964, 1968) and MUKAI et al. (1972).

Lethal-free heterozygotes: In total, there were 130 heterozygotes which satisfied the above condition (homozygous viability > 0.6). The covariance between heterozygote viabilities and the sums of the corresponding homozygote viabilities, the variances of heterozygote viabilities and the sums of the corresponding homozygote viabilities are given in Table 4 together with the estimate of the average degree of dominance ($\bar{h}_E = 0.414 \pm 0.116$). This value is slightly larger than the previous estimate ($\bar{h}_E \doteq 0.21$, MUKAI *et al.* 1972) but not significantly so.

After excluding inversion-carrying heterozygotes from the above 130 crosses, there remained 96 crosses; the same calculation as above was done and the results are presented in Table 4. The $\bar{h}_E$ value becomes 0.255 ± 0.134, which is significantly larger than 0 and very similar to the previous estimate ($\bar{h}_E \doteq 0.21$).

The same calculation was done on the 34 inversion-carrying heterozygous crosses. The result was 0.807 ± 0.290 (Table 4).

The relationship between homozygote and heterozygote viabilities can be seen in Figure 3.

Lethal heterozygotes: Using heterozygotes in which one of the homologous chromosomes satisfies the above condition (viability > 0.6) and the other is a lethal-carrying chromosome, the estimation of the average degree of dominance of viability polygenes was made using formula (8). There were 192 lethal heterozygotes in which one of the homologous chromosomes showed a viability index

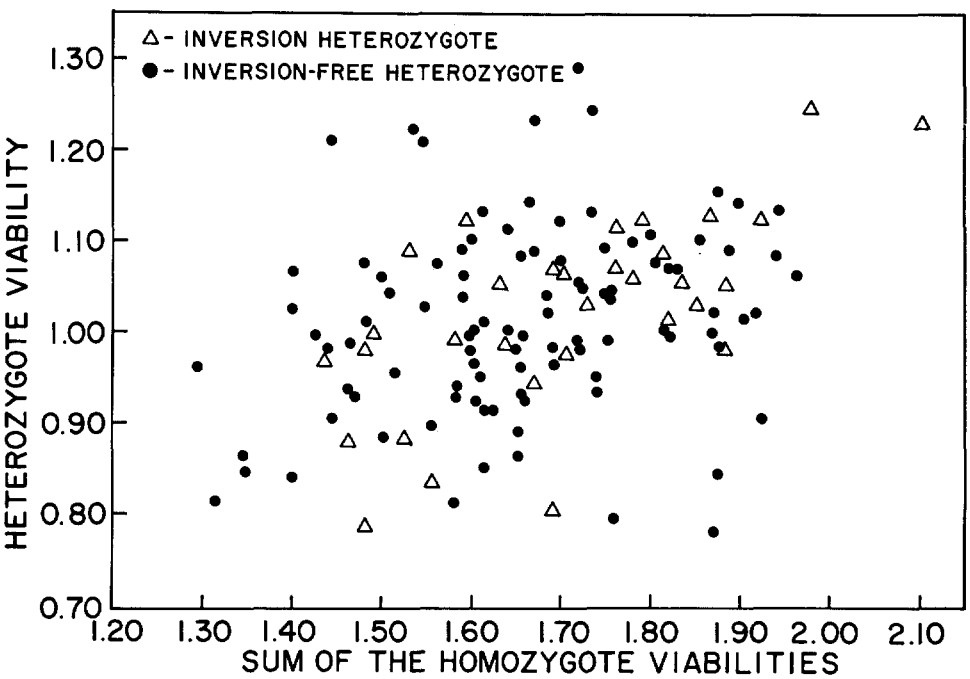

FIGURE 3.—The relationship between heterozygote viability and the sum of the corresponding homozygote viabilities.

TABLE 4

The results of the estimations of the average degree of dominance of chromosomes with viability indices greater than 0.6

	All heterozygotes		Inversion heterozygotes		Inversion-free heterozygotes		
	Lethal-carrying	Lethal-free	Lethal-carrying	Lethal-free	Lethal-carrying	Lethal-free	Lethal-free special
Number of heterozygous crosses	192	130	63	34	129	96	38
Number of simultaneous replications	4	4	4	4	4	4	7.26
Genetic covariance $\times 10^5$	185.767	560.391	374.250	1188.772	67.955	323.155	236.325
Genetic variance of sums of homozygotes $\times 10^5$	838.360	1355.080	914.804	1473.567	746.884	1266.791	1063.779
Genetic variance of heterozygotes $\times 10^5$	319.383	649.093	310.552	676.943	312.240	639.373	314.900
Average dominance ($\bar{h}_E$)	0.222 ± 0.089	0.414 ± 0.116	0.409 ± 0.145	0.807 ± 0.290	0.091 ± 0.118	0.255 ± 0.134	0.222 ± 0.159
Overall mean $\bar{h}_E$	0.293 ± 0.071		0.489 ± 0.082		0.177 ± 0.077	———	
Pooled results of 1969 and 1970†	0.21 ± 0.11		———		0.21 ± 0.15	0.210 ± 0.066	
Average dominance of newly arisen mutants ($\bar{h}_N$)*	0.860 ± 0.364	1.158 ± 0.310	0.415 ± 0.181	0.569 ± 0.163	2.297 ± 2.998	1.979 ± 1.026	1.332 ± 0.955
Genetic variance ratio‡	0.38	0.48	0.34	0.46	0.42	0.50	0.30

* Estimated by $\frac{\text{variance(Y)}}{\text{covariance(X,Y)}}$ for lethal-free individuals and by $\frac{\text{variance(Y)}}{2\,\text{covariance(X,Y)}}$ for lethal-carrying individuals.

‡ $\frac{\text{Genetic variance of heterozygotes}}{\text{Genetic variance of sums of homozygotes}}$

† From MUKAI *et al.* (1972).

larger than 0.6. Among them, 129 were inversion-free and the remaining 63 carried inversions. The $\bar{h}_E$ values were estimated for the above three cases—all heterozygotes: $\bar{h}_E = 0.222 \pm 0.089$; inversion-free: 0.091 ± 0.118; inversion heterozygotes: 0.409 ± 0.145. All the results are tabulated in Table 4.

Finally, an additional experiment was conducted for the estimation of $\bar{h}_E$ values using many simultaneous replications (Experiment 2). The same calculation as above was made. The results are shown in Table 4. The $\bar{h}_E = 0.222 \pm 0.159$. This value is very close to the estimate obtained by MUKAI *et al.* (1972).

From all the above experimental results, it might be concluded (1) that the average degree of dominance of viability polygenes is much higher than that of "recessive" lethal genes [The pooled values weighted by the inverses of the variances are 0.293 ± 0.071 for all heterozygotes, 0.177 ± 0.077 for inversion-free heterozygotes and 0.489 ± 0.082 for inversion heterozygotes] and (2) that inversion heterozygotes show an increased estimate of $\bar{h}_E$. A detailed discussion concerning this matter will be made in a future publication. The standard errors of the above pooled estimates are slightly underestimated since some inversion-free chromosomes were employed in both lethal-free heterozygous crosses and lethal-carrying heterozygous crosses.

As will be described below, if there is overdominance or any form of balancing selection at some loci in addition to partial dominance at the majority of loci, the above estimates of $\bar{h}_E$ are underestimates.

Estimation of mutation rates

As far as homozygous load with respect to the average heterozygote viability of the population is a function of mutation rates and degrees of dominance, it is possible to estimate the mutation rates as a function of genetic load and the degrees of dominance of mutant genes. The theory has been described above. The following parameters, which are necessary for using formulae (18) and (19), were estimated in the present experiment:
$B = 0.4342 \pm 0.0295$, $C = 0.7163 \pm 0.0108$, $D \doteq 0.3336$, $H_L \doteq 0.012$, and $h_p \doteq 0.20$. (There is a large range of h_p but conservatively we assume this value.) The ratio u may be estimated using an approximate relationship proposed by GREENBERG and CROW (1960): $hs \doteq$ constant. If we assume a selection coefficient of semi-lethals = 0.5, it may bereasonable to assume $H_{SL} = 2\ H_L$ or $u \doteq 2.0$. We have no accurate estimate of M_{SL}, but the experimental results of MUKAI (1964) and MUKAI *et al.* (1972) indicate that $k \doteq 1/8$. Thus, from formulae (18) and (19), mutation rates of viability polygenes and "recessive" lethals per second chromosome can be estimated as follows:

$$M_p = 0.104c$$
$$M_L = 0.006c$$

We do not know the exact c value, but it is definitely larger than 1.0. The value of $\hat{D}$ might be slightly overestimated since the present model neglects the contribution of overdominant loci. Indeed, the D value due to overdominant loci was estimated to be about 0.10 (MUKI, unpublished). Thus, $M_p = 0.0707c$. If fertility

has the same effect as viability for the maintenance of the present "viability" polygenes (as will be suggested later) M_p turns out to be 0.141, a value very close to the previous estimate (0.141, MUKAI 1964; 0.17, MUKAI *et al.* 1972). We do not know how much overdominant lethal genes and lethal genes showing associative overdominance contribute to the lethal load (L), but the amount seems to be very small since the allelism rate between lethals is small. Thus, M_L appears to be slightly overestimated, but its order of magnitude is the same as the independent estimate of M_L (0.005–0.006).

Overdominance and mild epistasis

It has been reported that some isozyme genes show seemingly permanent linkage disequilibria with polymorphic inversions (MUKAI, METTLER and CHIGUSA 1971). Synergistic interactions between the second and the third chromosomes and among genes within these chromosomes have been reported in homozygous condition using *D. melanogaster* (TEMIN *et al.* 1969). In the present experiment, it has been shown that the average degree of dominance of "recessive" lethal genes differs according to their genetic backgrounds. Thus, epistasis must be common in natural populations but perhaps small in magnitude (see DISCUSSION). We do not have much evidence for overdominance in natural populations. Three trials were made to detect or infer overdominance, or overdominance and epistasis.

Approximate ratio of the number of partially dominant loci and overdominant loci: From Figure 1, it can be seen that the viabilities of some homozygous lines are better than the average viability of heterozygotes. Actually, 19 lines out of 691 showed better viabilities than the average heterozygote. After viability tests were concluded, all lines were maintained in the stockroom as mixtures of $Cy/+$ and $+/+$ flies. After approximately 2 years, at least in 50 lines, $Cy/+$ flies were completely eliminated from the half-pint milk bottles. This implies that $\frac{+_i}{+_i}$ flies in some lines had better fitness than $Cy/+$ flies. Thus, it may be possible to use formula (17). It has been reported that $\mu \doteq 5 \times 10^{-5}$/cistron/generation (MUKAI *et al.* 1972), under the assumption that the number of cistrons is equal to the number of bands of salivary gland chromosomes (cf. JUDD, SHEN and KAUFMAN 1972). If $t_1 = t_2 = t$, then $\frac{t_1^2}{t_1+t_2}$ $(= t/2)$ 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. This simple calculation suggests that when the selection coefficient is large, we cannot expect many overdominant loci. For example, if $t = 0.1$, then the number of overdominant loci must be less than 5 per second chromosome. Conversely, when the selection coefficient is extremely small, we may expect many overdominant loci. A detailed study on this subject will be published elsewhere (MUKAI *et al.* 1974).

Variance ratio and the average degree of dominance: Ratios of the variances among the heterozygotes to the variances among the sums of the corresponding homozygotes were calculated and are shown in Table 4. The values range from 0.30 to 0.50. The theoretical expectation is $\bar{h}\,\tilde{h}$, as shown above [formula (14)],

or approximately $0.4 \times 0.2 = 0.08$, which is much smaller than the observed value. For newly arisen mutations, the expectation of variance ratio is $\bar{h}^2 + \sigma_h^2$ [formula (7)], or approximately 0.23. The observed value for the mutant viability polygenes accumulated for 32 generations is 0.27, excluding coupling heterozygotes (cf. MUKAI and YAMAZAKI 1968); this is very close to the expected value.

It is possible to estimate the average degree of dominance of newly arisen mutations using data from an equilibrium population [see formulae (9) and (12)]. The results are tabulated in Table 4. These estimates range from 0.42 to 2.30 and most of them are much larger than the directly observed value ($\bar{h}_N \doteq 0.43$, MUKAI 1969). These values were estimated by variance (Y)/covariance (X,Y) or variance $(Y)/[2 \times$ covariance $(X,Y)]$ depending on whether lethal-carrying chromosomes are included or not. Both variance (Y) and covariance (X,Y) can be separated into two parts: one due to partially dominant loci and the other due to overdominant loci. As described above for the case of overdominance, covariance (X,Y) can be zero $(c = 1)$, positive $(c > 1)$, or negative $(c < 1)$, depending on the value of c [cf. formula (3′)]. Thus, if $c = 1$ or $c < 1$, the overall regression of X on Y decreases since the covariance due to overdominant loci becomes zero or negative. Consequently, $\hat{\bar{h}}_N$ will be an overestimate. When $c > 1$, both variance and covariance due to overdominant loci become positive, but the ratio of the former to the latter is generally much larger than that for the partially dominant loci. Therefore, when overdominance is manifested at some, but not many other loci, the overall estimate of $V(Y)/\mathrm{Cov}(X,Y)$ becomes larger than the value due only to partially dominant loci. Thus, the above analyses suggest that overdominance, or overdominance and epistasis, or some forms of balancing selection are present. In such cases the overall value of $\mathrm{Cov}(X,Y)/V(X)$, an estimate of $\bar{h}_E$, becomes smaller than the value due only to partially dominant loci. However, the amount by which $\bar{h}_N$ is overestimated is much larger than the amount by which $\bar{h}_E$ is underestimated, since the values of $V(X)/V(Y)$ at overdominant loci are, in general, much smaller than those at partially dominant loci.

Comparison of viabilities between chromosomes extracted from males and females: Since there is no crossing over in males, there might be differences between mean viability and/or genotypic variances of chromosomes extracted from males and females in an equilibrium population, if there is epistasis. Thus, their mean viabilities and genotypic variances were respectively compared.

1) Homozygotes: There were 367 and 324 second chromosomes extracted from females and males, respectively. The difference in the distribution of viabilities was examined using the χ^2 contingency table method. The result is: $\chi^2_{\text{d.f.}=11} = 20.38$ $(P < 0.05)$, which indicates a significant difference between them. The overall mean values are 0.4271 for the chromosomes from female parents and 0.4422 for the chromosomes from male parents. Thus, it may be concluded that there is a small but significant epistasis among viability genes in homozygous condition.

2) Heterozygotes: Random heterozygotes in the present investigation can be classified into three categories. (1) Both homologous chromosomes originated

from females; (2) one homologous chromosome originated from a female and the other from a male; and (3) both homologous chromosomes originated from males. In the second category, there was one unusual cross that showed an extremely low viability. It was discontinuous from the remainder (viability index = 0.3599) and was excluded from the analysis. The distribution of the pooled result of categories 1 and 2 was compared with that of category 3 but no significant difference was detected. Analyses of variance were performed in order to examine the differences in mean viabilities and to estimate the genotypic variances in the above respective categories. The results are as follows:

Origin	N	Mean viability	Genotypic variance
Female and female	277	0.9954 ± 0.0059	0.005859 ± 0.000852
Female and male	176	1.0151 ± 0.0074	0.005689 ± 0.001062
Male and male	234	0.9969 ± 0.0058	0.003579 ± 0.000788

The results of the analyses show (1) that there are significant differences among mean viabilities of individuals belonging to categories 1, 2, and 3, i.e., the mean viability of category 2 being better than those of categories 1 and 2, and (2) that the genotypic variance in category 1 is significantly larger than in category 3 ($P < 0.05$); in category 2 it is larger than in category 3, but not significantly so. The genotypic variance pooled over categories 1 and 2 is significantly larger than that of category 3 ($P < 0.05$). Probably part of this inflation of the variance is due to the segregation of viability genes in the X chromosomes, but most of it may be due to slight but significant epistasis among viability genes.

Allelism rate of lethal chromosomes and effective population size

Among 153 randomly selected lethal chromosomes out of 275, 95 were from Site A and 58 were from Site B. Almost complete tests were made among lethals that came from the same sites, but all possible crosses between lethal lines that came from the different sites were not completed. The number of crosses made within Site A, within Site B, and between Sites A and B were 8,530, 3306, and 3,426, respectively; the rates of allelism were 0.00328 (28 lethal crosses), 0.00454 (15 lethal crosses), and 0.00525 (18 lethal crosses). An important finding is that for the distance as great as about 1.4 miles, the rate of allelism did not decrease. This differs from the results obtained by PAIK (1968) and WALLACE (1966). Indeed, at face value the allelism rate increased with distance but not significantly. This finding, together with the high frequency of lethal-carrying chromosomes (about 40%) and low allelism rates, indicates that the effective population size is very large. Since there is no significant difference among these three estimates of allelism rates ($\chi^2_{d.f.=2} = 2.70$, $0.30 > P > 0.20$), a pooled estimate of 0.0040 was obtained.

The allelism rate among lethal genes in a population of infinite size is 0.0025 (IVES 1945; WALLACE 1950; PROUT 1954). This means that the allelism rate of lethal-carrying chromosomes is 0.0038 based on the WRIGHT formula (cf. PROUT 1954). This value is very close to the above estimate. In fact, the allelism rate between lethal-carrying chromosomes sampled in 1968 and those sampled in 1969 was 0.0041 (16/3381).

TABLE 5

Estimates of the effective population size

Year	1968*	1969*	1970
Q	0.411	0.359	0.398
I_c	0.0067	0.0065	0.0040
I_g	0.0041	0.0043	0.0024
N_e: $\mu = 10^{-5}$	ca. 24,000	ca. 22,000	ca. 115,000
$\mu = 0.2 \times 10^{-5}$	ca. 14,000	ca. 13,000	ca. 25,000

Q = Frequency of lethal chromosomes; I_c = allelism rate of lethal chromosomes; I_g = allelism rate of lethal genes; N_e = effective population size; and μ = lethal mutation rate per locus.

* Partial data have been published in MUKAI *et al.* (1972) and YOSHIKAWA and MUKAI (1970).

The effective size of the population (N_e) can be estimated by the following formula (NEI 1968), where the degrees of dominance of lethal genes (H) and the mutation rates to the lethals (μ) per locus are assumed to be the same for all loci:

$$\hat{N}_e = (1 - \hat{I}_g)/[4(\hat{I}_g U\text{-}\mu)] \qquad (22)$$

where I_g stands for the allelism rate of *lethal genes* (and can be estimated by $-\log_e (1 - I_c Q^2)/[\log_e (1-Q)]^2$ where I_c stands for the allelism rate of lethal chromosomes); U is the total lethal mutation rate; and Q is the frequency of the lethal chromosomes. From the data, $\hat{Q} = 0.398$, $\hat{I}_c = 0.0040$, and $U = 0.005$ (cf. CROW and TEMIN v964), and μ is assumed to be 10^{-5} and 0.20×10^{-5}. The former estimate comes from the number of lethal-producing loci per second chromosome (n) = 500 (cf. IVES 1945; WALLACE 1950), and the latter is based on $n = 2400$ (total number of bands of salivary gland chromosomes $\times$ the proportion of the second chromosome to the entire genome: $6000 \times \frac{1}{2.5}$, from JUDD, SHEN and KAUFMAN 1972). The estimate of the effective size of the population is shown in Table 5 together with the results of 1968 and 1969. The result indicates that the effective size of the Raleigh population is extremely large (much greater than 10,000).

Although a reduction in size of the population occurs for several generations in winter, it does not decrease appreciably the effective size of the population. The population is apparently subdivided into many small groups which in spring will be mingled over a wide area. Indeed, we have obtained data suggesting that migration occurs over a wide area.

DISCUSSION

Approximate genetic equilibrium of the population: In the present investigation, it was assumed that the Raleigh population is approximately in genetic equilibrium. This assumption may be supported by the following findings: WALLACE (1956) maintained a large control population in the laboratory. The actual population number was about 10^4 and the effective size was estimated to be infinite (PROUT 1954). This population reached an equilibrium with respect to the

frequency of lethal-carrying chromosomes (Q) at $\hat{Q} \doteq 0.30$. The homozygous load of this population was estimated approximately 10 years after its initiation, and the detrimental load to lethal load ratio was estimated to be about 0.6 (GREENBERG and CROW 1960). In the Raleigh population, the effective size was estimated to be much greater than 10,000, the frequency of lethal-carrying chromosomes was 0.39, and the D:L ratio was 0.67: Thus, this population seems to be in approximate genetic equilibrium insofar as the WALLACE cage population was at equilibrium. Further evidences for the above speculation are as follows: Firstly, the lethal load of the Raleigh population is 0.50. Since the mutation rate of "recessive" lethals is reported to be 0.005 per second chromosome per generation on the average, the average selection coefficient of lethal heterozygotes (fitness as a whole) at equilibrium turns out to be about 0.01 (cf. NEI 1968), which is consistent with the estimate obtained in the present investigation as well as with other experimental results (cf. CROW and TEMIN 1964). Secondly, isozyme gene frequencies in the present population are approximately constant over seasons (JOHNSON, personal communication) and over years (MUKAI *et al.* 1971, unpublished). Thus, the most important assumption in the present investigation is shown to be approximately valid.

Mutation rates: As described above, we have indirectly estimated a recessive lethal mutation rate on the basis of homozygous genetic load and other estimated genetic parameters. The result is reasonably close to the direct estimate (0.005 per second chromosome per generation, CROW and TEMIN 1964; 0.006, MUKAI *et al.* 1972).

The mutation rate of polygenes affecting viability (mildly deleterious mutants) was estimated in the same manner and the result also agreed very well with the direct estimate. Thus, the estimates reported by MUKAI (1964) and MUKAI *et al.* (1972) can be generally accepted.

Degree of dominance: The average degree of dominance of lethal genes with respect to viability was estimated to be about 0.01. Also, from the rate of elimination of lethals from the population, the average degree of dominance with respect to fitness as a whole was estimated to be about 0.01, as described above. In addition, the allelism rate of lethal genes is very small. Thus, we have no evidence favoring the idea that an appreciable frequency of lethals are overdominant.

The D:L ratio of newly arisen mutants was 0.95 (MUKAI *et al.* 1972). On the other hand, the adjusted D:L ratio of mutant genes in the equilibrium population, comparable to the above, is 1.03. They are almost the same. This suggests that the elimination rates of lethals and deleterious genes are the same—that is to say, the average degree of dominance of mildly deleterious genes (viability polygenes) is higher than that of lethal genes. This was experimentally shown in the present experiment (0.01 *vs.* 0.20). In fact, the average degree of dominance of viability polygenes ($\tilde{h}$) in an equilibrium population was predicted to be $0.17 - 0.27$ on the basis of the characteristics of newly arisen mutation (MUKAI 1969). It should be noted here that there are probably a small number of overdominant loci. The magnitude of the homozygous load due to these overdominant loci was estimated to be about 0.10 (MUKAI, unpublished).

The degrees of dominance mentioned above are for viability alone. Following MUKAI *et al.* (1972), an attempt is made to estimate the average degree of dominance of total fitness in comparison with selection based on homozygous viability. The following parameters are defined:

D_m = mildly detrimental load or homozygous load due to viability polygenes (excluding the effect of major genic semi-lethals).

M = average reduction of homozygous viability due to polygenic mutations (or mildly detrimental mutations) per generation.

$\bar{h}_E$ = average degree of dominance of viability polygenes in an equilibrium population.

$\bar{s}$ = average selection coefficient of viability polygenes.

k_o = homozygous load due to overdominant viability polygenes.

$\tilde{h}'$ = harmonic mean of the degrees of dominance of newly arisen mutant viability polygenes (or arithmetic mean of the degrees of dominance of viability polygenes in an equilibrium population) with respect to the *total fitness* in comparison with the reduction of homozygous viability ($\tilde{h}'\bar{s}$ is the average amount of selection in heterozygotes with respect to the total fitness and $\bar{s}$ is the mean selection coefficient against mutant homozygotes in viability).

Then, $\tilde{h}' \doteq \dfrac{(1\text{-}2\bar{h}_E)M}{\bar{s}\,(D_m\text{-}k_o)}$ (MUKAI *et al.* 1972)

We already have the estimates: $M \doteq 0.004$ (MUKAI *et al.* 1972), $h_E \doteq 0.20$, $\bar{s} \leq 0.023$–0.027 (MUKAI 1964; MUKAI *et al.* 1972), k_o is unlikely to be greater than 0.10 (MUKAI, unpublished), and $D_m \doteq 0.30$. D_m was estimated as follows: The $\hat{D}$ in the present experiment includes the contribution of major-genic semi-lethals (D_{SL}). This can be estimated on the basis of their mutation rates and degrees of dominance, using the lethal load. The former two parameters were already used above. The D_{SL} thus obtained is 0.0313. Using this estimate, D_m can be estimated as $\hat{D} - \hat{D}_{SL} \doteq 0.30$. Then, h' can be calculated to be 0.44, which implies that the contribution of the fitness components other than viability is approximately the same as that of viability. This last finding supports the assumption in the estimation of polygenic mutation rate described above, i.e., $c \doteq 2$.

Mild epistasis: First, in the estimation of the average degree of dominance of lethal genes, it was detected that the $\bar{h}$ value in the inversion-heterozygotes is less than that in the inversion-free heterozygotes. Second, the ratio of genetic variance of viability of the random heterozygotes (free of major-genic lethals and semi-lethals) to that of the sums of two corresponding homozygotes is higher than the expected value on the basis of no epistasis. Third, the estimates of $\bar{h}_E$ values are larger in inversion heterozygotes than in inversion-free individuals. Fourth, there is a slight difference between the chromosomes extracted from males and females concerning the contribution to the homozygote and heterozygote viabilities. These four findings of the present investigation indicate *mild* epistasis. The result that linkage disequilibria were found between isozyme genes and inversions but not between isozyme genes themselves further supports the above suggestion (MUKAI, METTLER and CHIGUSA 1971).

Recently, MUKAI (unpublished) estimated the genetic variance components of relative viability of the chromosomes on which newly arisen mutations have been accumulated for more than 150 generations. Genetic backgrounds of the individuals examined were homozygous and homogeneous except for the loci where new mutations were accumulated. The result indicates that a great deal of additive variance was created, but not dominance variance. The estimator of the dominance variance in this experiment includes additive × dominance variance, dominance × dominance variance, and so on (excluding additive × additive variance, additive × additive × additive variance, and so on). Thus, it may be inferred that the epistatic variance contributed by loci with low gene frequencies is small in equilibrium populations. MUKAI *et al.* (1974) estimated dominance variance for viability in the present population. Its magnitude was about one-third of the additive variance. From these results, it may be speculated that the epistatic variance of viability in an equilibrium population is small.

This speculation will be verified in that the estimates of various genetic parameters in the present experiments agree very well with the results predicted, without assuming epistasis, from the result of newly arisen mutations that did not show appreciable amounts of epistasis.

Mechanisms of the maintenance of genetic variability: In the present studies, a relatively high frequency of inversion-carrying individuals were found, and two polymorphic inversions known to be cosmopolitan (cf. OSHIMA, WATANABE and WATANABE 1964) were also detected. The mechanism for the maintenance of these polymorphic inversions is not known. It may be speculated from Table 2 that slightly better viability of inversion heterozygotes is a basis for the maintenance of inversion polymorphisms, although the experimental result does not show a significant difference.

The fecundities of intra-populational heterozygotes of inversions extracted from the present population have not been examined, but we have no evidence that *Cy*/Inversion females produce more offspring than *Cy*/Non-inversion females (*Cy*/Inversion: 345.15 ± 3.09/pair of vials, N = 508; *Cy*/Non-inversion: 353.60 ± 1.36/pair of vials, $N = 2244$. These two figures are significantly different). However, this result could not be applied to the case of intra-populational crosses, since the *Cy* chromosome originated from an entirely different population and carries multiple inversions. Heterozygote superiority in fecundity in intra-populational crosses might be an important factor for the maintenance of polymorphic inversions. Meiotic drive or sperm competition of the inversion-carrying chromosomes and frequency-dependent selection (cf. TOBARI and KOJIMA 1967) should be tested. It has been reported that there is some interaction between these polymorphic inversions and some genes located outside the inversions: possibly permanent linkage disequilibria between *In(2L)t* and the *alcohol dehydrogenase* locus, between *In(2R)NS* and the *α Amylase* locus, and between *In(2R)NS* and the *alcohol dehydrogenase* locus (MUKAI *et al.* 1971). Further tests are necessary for clarifying the mechanisms for maintenance of polymorphic inversions. The rare inversions might have been induced by mutator genes such as *hi* and *mu-F* (CHIGUSA, METTLER and MUKAI 1969).

It is clear that lethal genes are heterozygously deleterious, at least in the present population. The evidences are as follows: (1) The allelism rate of lethal chromosomes was estimated to be 0.004, and the frequency of lethal-carrying chromosomes was about 0.4. Then the elimination rate of lethals due to homozygosity is: $0.004 \times (0.4)^2 = 0.00064$, which is much smaller than the average mutation rate per chromosome (0.005). Thus, elimination of the lethals must occur mainly in heterozygotes. This result is different from that of the population which WALLACE (1966) studied. (2) Direct estimate of the average degree of dominance of lethal genes was 0.018 in inversion-free heterozygotes and 0.0026 in inversion-carrying heterozygotes. These results indicate epistasis (cf. CRUMPUCKER 1968), but there is no evidence of overdominance both in viability and fecundity (but in the fecundity comparison, the numbers of offspring from $Cy/+$ and Cy/l were employed as described above). (3) From Table 5, it may be reasonable to assume that the effective size of the population is about 20,000, $\mu = 10^{-5}$/locus/generation, and the number of lethal-producing loci is 500 per second chromosome (cf. IVES 1945; WALLACE 1950). Using the formula given by WRIGHT (1937) for the frequency of completely recessive lethal genes:
$\hat{q} = \Gamma(2N\mu + 1/2)/[\sqrt{2N}\Gamma(2N\mu)]$ where N is the effective size of the population, the frequency of lethal-carrying chromosomes becomes 0.70. For the case of $\mu = 0.2 \times 10^{-5}$, $N = 10{,}000$, and the number of loci = 2400, the expected frequency of lethal-carrying chromosomes becomes 0.68. These values are much higher than the observed value ($\hat{Q} = 0.40$).

Although we do not have direct evidence for major genic semi-lethals, the situation is almost probably the same as for lethal genes.

The experimental results indicate that there are overdominant loci. The average degree of dominance of mildly deleterious genes (or viability polygenes) was estimated to be approximately 0.20 when formula (8) was employed. This estimate includes the contributions of overdominant loci. The estimated $\bar{h}_E$ value is very close to the predicted value (0.17–0.27) on the basis of $\bar{h}$ value of newly arisen mutations and the genetic variance of h value without assuming overdominance (MUKAI 1969). Thus, it is clear that the effect of overdominant loci on variance (X) is not large, as in the case where there are only a few overdominant loci with appreciable effects or a relatively large number of loci with very minor effects.

The D value due to mildly deleterious, non-overdominant loci ($D_m - k_o$) becomes about 0.20. If this value is applied to Figure 2 of MUKAI and MARUYAMA (1971), the $\bar{h}_E$ value obtained becomes 0.10–0.18. This value is only slightly smaller than the estimated value (0.20). This difference might be explained by assuming synergistic interaction among heterozygous loci is the same as found in the homozygous condition. Synergistic interaction in the heterozygous condition might not be appreciable. In general, the observed results agree well with the predicted result based upon simulation using the genetic parameters estimated in the experiment reported earlier (MUKAI and YAMAZAKI 1971 and earlier). Further studies are necessary to understand the coupling-repulsion

effect and to clarify whether or not the overdominance detected in the present experiment is due to the coupling effect (see MUKAI and YAMAZAKI 1968 and MUKAI and MARUYAMA 1971).

From the above experimental results and considerations, together with information from newly arisen mutations, it is clear that the genetic variability in most of the loci which carry homozygously deleterious genes has been maintained by the balance between mutation and selection pressures. On the other hand, it is now almost certain that most of the polymorphic isozyme genes are selectively neutral or nearly neutral, or at least selection is not perceptible per locus (cf. KIMURA and OHTA 1971; MUKAI 1968; MUKAI, METTLER and CHIGUSA 1971, MUKAI *et al.* 1974; YAMAZAKI 1971; YAMAZAKI and MARUYAMA 1972). OHTA and KIMURA (1971) have proposed a possible mechanism whereby isozyme polymorphisms can be maintained: according to this hypothesis, random genetic drift in finite population causes associative overdominance for neutral genes and this contributes to the retardation of the fixation of linked neutral genes. A few overdominant loci can be effective for creating associative overdominance. The present studies may give an experimental basis for the above hypothesis. Further studies are necessary for clarifying the mechanisms of the maintenance of isozyme or protein polymorphisms in natural populations.

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