THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*. V. COUPLING-REPULSION EFFECT OF SPONTANEOUS MUTANT POLYGENES CONTROLLING VIABILITY[1]

TERUMI MUKAI[2] AND TSUNEYUKI YAMAZAKI[3]

National Institute of Genetics, Mishima, Japan

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FROM recent studies of experimental population genetics, it has become apparent that a great amount of genetic variability is stored in heterozygous condition in natural populations of several species of Drosophila, and it is important to clarify whether this genetic variation has been chiefly maintained by the balance between mutation and selection pressures or by the superior fitness of heterozygotes relative to homozygotes.

MULLER (1950) argued that mutant genes with less extreme effects would tend to show more dominance than lethals and near lethals. GREENBERG and CROW (1960) directly analyzed WALLACE's apparently equilibrium experimental populations (1956) and a wild Madison, Wisconsin, population of *D. melanogaster*, and calculated the magnitudes of homozygous loads due to lethal genes (L) and detrimental genes (D), using their own data as well as those already published by other investigators. Based upon the D:L ratio, they suggested that "either (1) mutants with small effects occur with no greater frequency than lethals, or (2) they have more dominance than lethals and hence are eliminated relatively more rapidly as heterozygotes." The second suggestion corresponds to MULLER's idea (1950). MUKAI (1964) has reported an extremely high mutation rate per chromosome of polygenes controlling viability. Therefore, the first suggestion of GREENBERG and CROW (1960) can be eliminated. GREENBERG and CROW (1960) have suggested that, in comparing mild detrimentals with lethals, hs is more nearly constant than h, where h stands for the degree of dominance of mutant genes and s is the selection coefficient against mutant homozygotes.

On the other hand, WALLACE (1958) has tentatively concluded, on the basis of the increase in mean viability of heterozygotes with respect to radiation-induced mutations in an otherwise homozygous genetic background in *D. melanogaster*, that "on the average an individual member of the Drosophila population studied is heterozygous for genes at 50 percent or more of all loci," and has reported additional data which might support this conclusion (WALLACE 1963). In addition, correlation analysis with respect to viabilities of homozygotes and

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[2] Present address: Department of Genetics, North Carolina State University, Raleigh, North Carolina.

[3] Present address: Department of Zoology, University of Chicago, Chicago, Illinois.

corresponding heterozygotes in *D. melanogaster* (BAND and IVES 1963; WALLACE and DOBZHANSKY 1962), *D. pseudoobscura* (DOBZHANSKY and SPASSKY 1963), and *D. willistoni* (KRIMBAS 1959) have been interpreted by these investigators as showing a balanced load (segregation load of CROW 1958) in natural populations. However, KIMURA and CROW (1964) have questioned the universality of over-dominance for strongly selected genes in natural populations on the basis of the calculation of genetic load.

For a locus maintained by equilibrium between mutation and selection, the homozygous load becomes μ/h per locus where μ stands for mutation rate (MORTON, CROW, and MULLER 1956). If our estimate of total polygenic mutation rate, namely 0.1411 per second chromosome per generation in *D. melanogaster* (MUKAI 1964) is correct, h should be extremely large in comparison to that for lethal genes (0.01–0.015 CROW and TEMIN 1964), because the approximate range of the $D:L$ ratio was between 0.5 and 1.0 (GREENBERG and CROW 1960) and the lethal mutation rate was 0.0063 per second chromosome per generation. On the contrary, the experimental results of WALLACE (1958) and ourselves, (MUKAI, CHIGUSA, and YOSHIKAWA 1964; MUKAI, YOSHIKAWA, and SANO 1966) indicate that h is negative in a homozygous genetic background.

In the hope of reconciling this difference, we have conducted several experiments. The first (MUKAI, CHIGUSA, and YOSHIKAWA 1965) led to the hypothesis that there is an optimum level of heterozygosity and that overdominance prevails only below this level. Since this optimum is rather small, it is exceeded by most cross-fertilizing populations so that new mutants are generally not overdominant in their original populations.

In addition, we have evidence suggesting a second factor which restricts the expression of overdominance in natural populations, namely an apparent coupling-repulsion difference. This is the subject of the present paper. A preliminary report on this subject has been published in MUKAI and YAMAZAKI (1964).

MATERIALS AND METHODS

A single male $Pm/+_0$ from the cross $Cy/Pm \times +/+$ (W160S : an isogenic wild-type stock derived from DR. BURDICK's W160-po1) was sampled and multiplied by the cross Cy/Pm (♀ ♀) $\times$ $Pm/+_0$ (1 ♂) and 104 lines of $Cy/Pm \times Pm/+_i$ ($i = 1, 2, \ldots, 104$) were established. The genetic background of Cy/Pm is the same as that of W160S. In each line, the second chromosome has been maintained through a single male by the cross Cy/Pm (5 ♀ ♀) $\times$ $Pm/+_i$ (1 ♂) in a 3cm $\times$ 10 cm vial. Since Cy/Pm females were obtained from the original bottle population in every generation, the accumulation of mutations on the Cy chromosome is not serious. In fact, it has been proven that new mutations are slightly deleterious in heterozygous condition in inter-population crosses (MUKAI, CHIGUSA, and YOSHIKAWA 1965), so that new mutations would not be accumulated at a high frequency in the Cy/Pm stock. Details of the experimental materials and methods for the accumulation of mutant polygenes have been reported in a previous article of this series (MUKAI 1964). In Generations 32, 52, and 60 homozygous and heterozygous viabilities of these chromosome lines were tested.

Homozygous viability was tested by WALLACE's Cy method (1956). Five pair matings $Cy/+_i \times Cy/+_i$ were conducted with several replications in each line. In the offspring, the expected percentage of wild-type flies was 33.3% in the absence of mutation and 0% in the

presence of recessive lethal mutations. The percentages of wild-type flies were employed as viability indices throughout the experiments.

The viabilities of flies heterozygous for chromosomes from different lines were tested in Generations 32 and 52. Two mating schemes were used in order to secure random combinations of the chromosomes from different lines, namely, 1. $Cy/+_i$ (5♀♀) × $Cy+_{i+1}$ (5♂♂) crosses between lines having successive line numbers (1×2, 2×3, 3×4, . . .) and 2. $Cy/+_i$ (5♀♀) × $Cy/+_{i+2}$ (5♂♂) crosses between lines having alternate line numbers (1×3, 2×4, 3×5, 4×6, . . .). The former scheme is called, for the sake of convenience, Cross RS, and the latter, Cross RA. It has been proven that the viability of the *Cy* heterozygote is almost the same regardless of which second chromosome from a given population is used. Homozygously lethal and semi-lethal lines, supposedly due to major genic mutations, and their heterozygotes were disregarded in the present experiment.

Heterozygous viabilities of these chromosome lines with a chromosome supposed to be identical to the original chromosome (WO) were tested by the cross $+_0/+_0$ (7♀♀) × $Cy/+_i$ (5♂♂) in Generations 32 and 60, since the standards are always the same ($Cy/+_0$) in the estimation of the viability in the offspring. Line 92 was chosen for this purpose, because the results from the preliminary test just before the experiments showed that its viability in homozygous condition was normal.

In addition to the estimation of homozygote and heterozygote viabilities, recombination tests among mutant polygenes were conducted in Generation 56. The mating schemes are presented in Figure 1. A single pair mating was conducted between $Cy/+_i$ and $Cy/+_j$ of two different Lines *i* and *j*. From the progeny of this cross, phenotypically wild-type males and virgin females ($+_i/+_j$) were collected. In the first series (recombination series), three $+_i/+_j$ (5♀♀) × *Cy/Pm* (5♂♂) matings were performed. From the progeny of this cross approximately fifty $Cy/+_{ij}$ males were individually crossed to five *Cy/Pm* females ($+_{ij}$ indicates a second chromosome that originated from recombination between $+_i$ and $+_j$ chromosomes). After the above procedure, the homozygous viability of $+_{ij}$ chromosomes was estimated by WALLACE's *Cy* technique (1956). At the same time, the experiment of the second series (control series) was carried out as follows: Three *Cy/Pm* (5♀♀) × $+_i/+_j$ (5♂♂) matings were conducted. From the progeny of this cross, approximately fifty $Cy/+_{i\text{ or }j}$ males were picked up, and in the same way as that in the first series, the homozygous viability of $+_{i\text{ or }j}$ chromosome was estimated. Since there is no crossing over in Drosophila males, recombination between mutant polygenes, which occurred in different lines independently, takes place only in $+_i/+_j$ females of the recombination series. Thus, the effect of recombination can be tested by comparing the genetic variance among chromosome lines homozygous for the recombinant chromosomes with that for the parental chromosomes.

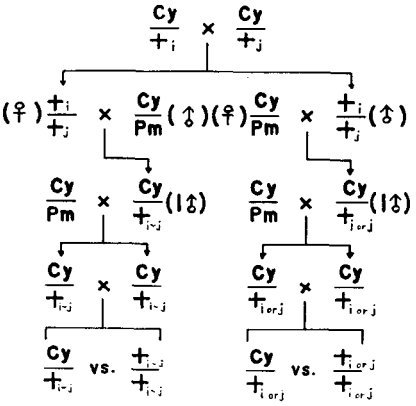

FIGURE 1.—Mating scheme for recombination test.

TABLE 1

Basic statistics and genetic parameters for homozygotes in Generations 32, 52, and 60

	Generations		
	32	52	60
Number of lines tested	80	75	77
Total number of flies counted	427,886	293,463	168,459
Harmonic mean of number of flies counted in single lines	5,322.21	3,856.78	2,100.02
Harmonic mean of number of flies counted in single observations	654.66	633.79	514.70
Number of observations per single line	8	6	4
Estimated error variance on individual observation basis	5.7845	8.8675	6.2432
Estimated genetic variance (homozygote basis)	5.6477	38.9486	66.2131
Average of control viability indices	32.84	33.12	32.42
Average viability of homozygote lines	28.04	21.29	16.48

The condition of the culture room, the formula for the culture medium, and the counting procedure were exactly the same as those described in the first article of the present series (MUKAI 1964).

EXPERIMENTAL RESULTS AND ANALYSIS

Homozygote viabilities: The outlines of the results for Generations 32, 52, and 60 are presented in Table 1. In Generation 32, 80 lines whose viability were higher than 20.00 were analyzed. Although there were four deleterious lines (viability indices were less than 20, but larger than 1), these were disregarded in this generation as well as in Generations 52 and 60. The control viability (v_0) for Generation 32 was estimated as follows: Three lines showing the highest viabilities in each of Generations 52 and 60 were selected, and their viability indices in Generation 32 were estimated. The weighted mean of these five lines (one line was highest in both Generations 52 and 60) was used as the v_0. The actual estimated v_0 was 32.84, a very reasonable value (see MUKAI 1964). The average of the observed viability indices was 28.04 which is significantly different from the v_0. The genetic variance (σ^2_G) among 80 lines was estimated to be $\hat{\sigma}^2_G = 5.6477$ by the analysis of variance.

The distribution pattern of viability indices is graphically shown in Figure 2, together with the 95% confidence interval (R) of the control distribution (a hypothetical distribution under the assumption that no lines had any mutations and that the same number of flies as in the actual observation was counted) which was estimated from Formula (1)

$$R = 1.96 \times \sqrt{\frac{\tilde{n}_I}{\tilde{n}_L}\hat{\sigma}^2_E} \qquad (1)$$

where n_I and n_L stand for the harmonic mean of the number of flies counted in single observations and in single lines, and $\hat{\sigma}^2_E$ is the estimated error variance on

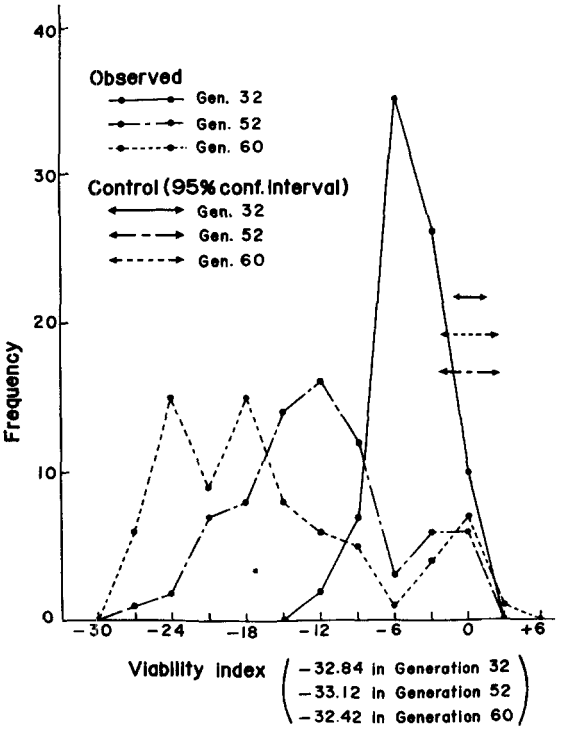

FIGURE 2.—Distribution patterns of homozygous viability indices in Generations 32, 52, and 60.

an individual observation basis. The 95% confidence interval thus obtained is ± 1.39.

After Generation 32, each line was divided into two groups (A and B) in order to avoid a decrease in the number of lethal-free lines. If a line in Group A was found to be carrying lethals, it was replaced by flies of the same line in Group B. Thus, 75 lines were tested in Generation 52. The control viability (v_0) for Generation 52 was estimated as above. The estimated v_0 was 33.12. The average of the observed viability indices was 21.29, and these two figures are, of course, significantly different. The distribution pattern of viability indices (deviation from the control viability) and the 95% confidence interval of the control distribution ($R = \pm 2.34$), which was estimated by the above method, are presented in Figure 2, together with those for Generations 32 and 60.

We were able to test 77 lines in Generation 60 by using several replacements. The control viability (v_0) was estimated by an indirect method: Three lines showing the highest viabilities were selected from the 77 lines, and the viability indices of the corresponding heterozygotes (WO) were estimated. The v_0 was estimated by multiplying this value by 2/3. The estimated v_0 became 32.42. The average of the observed viability indices in Generation 60 was 16.48. The distribution pattern of these viability indices and the 95% confidence interval of the control distribution ($R = \pm 2.42$) are presented in Figure 2. The genetic variance among the 77 lines was estimated to be $\hat{\sigma}_G^2 = 66.2131$.

From the experimental results, the following conclusions can be drawn: First, after Generation 32 the mean viability dropped rapidly and the genetic variance increased a great deal. The reduction in mean viability was 4.80 in Generation 32, while in Generation 60 it was 15.94. The genetic variance in Generation 60 was approximately 11.7 times as large as that in Generation 32. The relationships between these phenomena and the magnitude of genetic load in natural populations will be discussed in a subsequent article in this series. Second, the harmonic mean of the number of flies that emerged in single observations decreased with the increase in generation number. This indicates incomplete replacement by the *Cy* flies for the decreased number of wild-type flies and/or the decrease in egg laying capacity of *Cy*/+ females with the increase in generation number.

Viabilities of heterozygotes with the original chromosome: The outline of the results in Generation 32 is given in Table 2, together with those in Generations 52 and 60. The control viability (v_0') in Generation 32 shown in Table 2 was estimated as follows: Five lines showing the highest homozygous viability were selected from the 80 lines. These were crossed with Line 92 (assumed to be equivalent to the original chromosome because of its normal homozygous viability). The weighted mean of these heterozygotes was used as a control. The estimated v_0' became 49.91. The average of the observed viability indices for all 80 lines in heterozygotes with Line 92 was 51.51. These two figures are significantly different. The distribution pattern of the viability indices is presented in Figure 3 together with the 95 percent confidence interval of the control distribution ($R = \pm 1.66$). The genetic variance among the 80 lines was estimated to be $\hat{\sigma}^2_{G'} = 0.4081$.

The heterozygote viabilities (WO) of the 77 lines were examined in Generation 60. The outline of the results is presented in Table 2. The control viability was

TABLE 2

Basic statistics and genetic parameters for heterozygotes in Generations 32, 52 and 60

	Generation (and the type of heterozygotes)				
	32		52		60
	(WO)	(RS)	(RS)	(RA)	(WO)
Number of lines (or crosses) tested	80	77	69	68	77
Total number of flies counted	392,077	396,835	291,788	243,191	374,655
Harmonic mean of number of flies counted in single lines (or crosses)	4,864.82	5,113.96	4,166.18	3,500.61	4,841.69
Harmonic mean of number of flies counted in single observations	590.06	618.02	686.25	554.61	794.78
Average number of observations per single line (or cross)	7.99	7.99	6	6	6
Estimated error variance on individual observation basis ($\hat{\sigma}^2_{E'}$)	5.8858	5.8489	8.6418	10.2633	3.9597
Estimated genetic variance (heterozygote basis)	0.4081	8.2975	37.3218	29.7717	1.9718
Average of control viability indices	49.91	32.84	33.12		48.62
Average viability	51.51	29.56	24.78	25.88	52.28

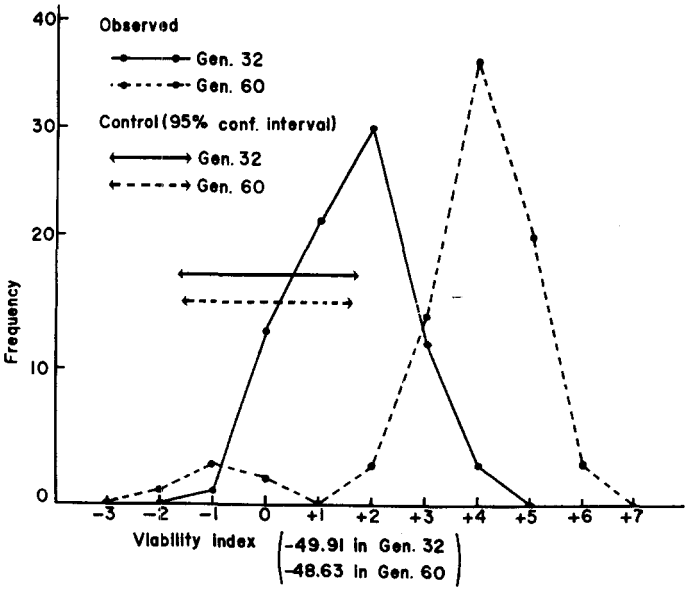

FIGURE 3.—Distribution patterns of viability indices of heterozygotes with the original chromosomes in Generations 32 and 60.

estimated by the same method as above, using the top three lines with respect to homozygous viability. The result thus obtained was $v_0' = 48.62$. The average of the observed viability indices was 52.28. The distribution pattern of the viability indices and the 95% confidence interval of their control distribution ($R = \pm 1.58$) are presented in Figure 3. The genetic variance among the 77 lines was estimated to be $\hat{\sigma}^2_{G'} = 1.9718$.

The following conclusion can be drawn from the above experimental results: The average viability of heterozygotes with the original chromosomes increases with the increase in generation number. Indeed, the above increments of viability indices were 1.60 in Generation 32 and 3.66 in Generation 60. The harmonic mean of the number of flies counted in single observations in Generation 60 is larger than that in Generation 32 (794.78 *vs.* 590.06). This result is in good accord with the hypothesis of increase in the average heterozygote viability. Thus, the experimental results of WALLACE (1958) and BURDICK and MUKAI (1958) with respect to radiation-induced mutations are supported.

Viabilities of random heterozygotes: In Generation 25, the viabilities of random heterozygotes (RS) were estimated at 21°C and 25°C on a small scale, and it was found that the average viability indices were 29.58 and 29.27 at 21°C and 25°C, respectively. The control viability was 32.99 (MUKAI 1964). Of course, the above two values are significantly lower than the control. The phenotypic correlation coefficients between the heterozygote viabilities and the sums of the corresponding homozygote viabilities were +0.51 at 21°C and +0.59 at 25°C. This result indicates that newly arising mutant polygenes are incompletely recessive and is inconsistent with the results of WALLACE (1958) and others. Therefore, experi-

ments on a large scale to confirm the above finding were conducted in Generations 32 and 52.

In Generation 32, only the RS experiment was conducted. The outline of the experimental result is given in Table 2. The average viability index was 29.56 which is significantly different from the control ($v_0 = 32.84$). The distribution pattern of the viability indices and the 95% confidence interval of the control distribution ($R = \pm 1.39$) are presented in Figure 4. It should be noted that the distribution pattern is bimodal. An interpretation of this phenomenon will be given later. The genetic variance among the 77 crosses was estimated to be $\hat{\sigma}^2_{G'} = 8.2975$. Thus, the result in Generation 25 was confirmed by the result in Generation 32.

In Generation 52, RS and RA experiments were conducted in order to obtain additional evidence on the above finding, and the summaries are presented in Table 2. The average viabilities are 24.26 and 25.38 in RS and RA, respectively. These two values are very close, and are significantly less than the control viability ($v_0 = 33.12$). The distribution pattern of the viability indices pooled over replicate observations in each of RS and RA is also graphically presented in Figure 4, together with that for Generation 32. The 95% confidence intervals of the control distributions are shown for respective distributions ($R = \pm 2.33$ in RS and ± 2.50 in RA). The genetic variances among the crosses were also estimated to be $\hat{\sigma}^2_{G'} = 37.3218$ and 29.7717 in RS and RA, respectively.

From all the above experimental results, the following general conclusions can be drawn: first, the average viability of random heterozygotes decreases in comparison with the individuals that are free of new mutations. This is in contrast with the result in the WO experiments described above. Second, the genetic

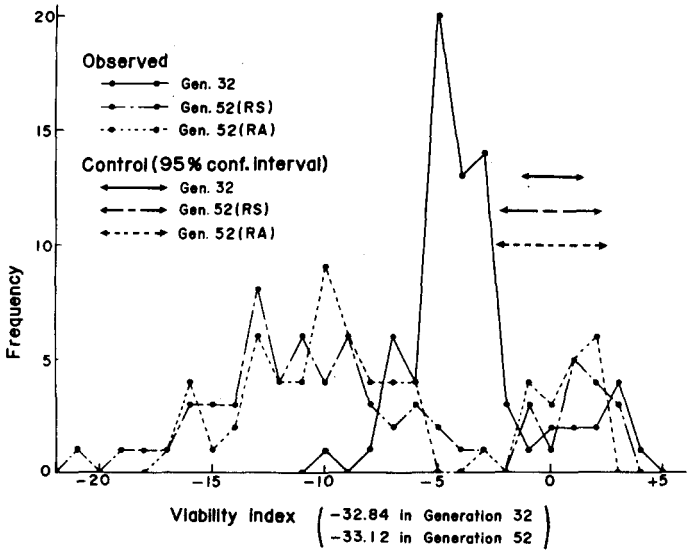

FIGURE 4.—Distribution patterns of viability indices of random heterozygotes in Generations 32 and 52.

variances are remarkably larger than in the WO experiments. Third, the distribution patterns are bimodal, which is also different from that obtained in the WO experiment (compare Figure 4 with Figure 3).

Correlation coefficient between homozygote and heterozygote viabilities and the degree of dominance of mutant polygenes: We might summarize the results of experiments WO, RS, and RA as follows: When new homozygously deleterious mutations accumulate in a homozygous background, they seem to show overdominance when they are in the same chromosome, but partial dominance when they are in homologous chromosomes. In order to confirm this conclusion, a further analysis was made.

(1) *Heterozygotes with the original chromosomes:* For the sake of simple presentation, homozygous lines were divided into five groups according to their viabilities. Thus, each group in Generation 32 consisted of 16 lines and in Generation 60 of 15 or 16 lines. The relationship between the average homozygote and the average heterozygote viability in each group was estimated and is graphically presented in Figure 5. From this figure, it is very clear that there is a negative correlation between the homozygote and the corresponding heterozygote viabilities. The correlation coefficients actually estimated on the line basis are −0.25

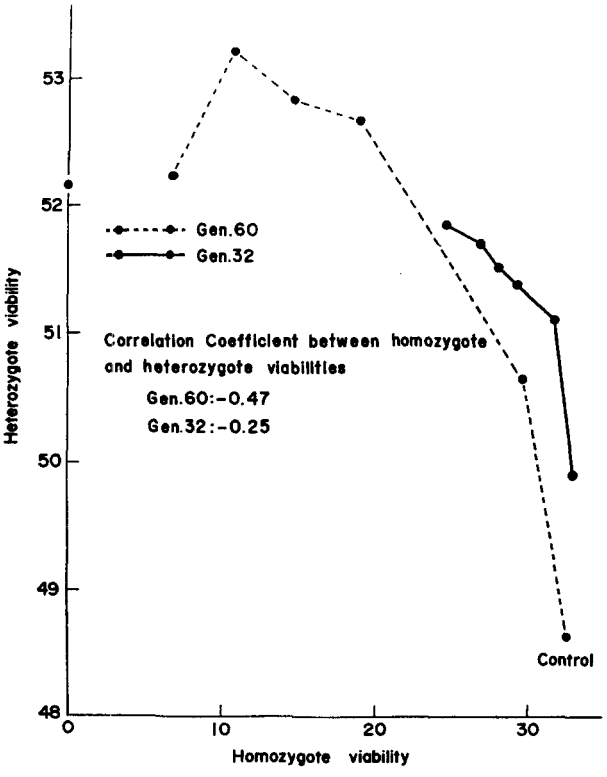

FIGURE 5.—Relationships between the viabilities of homozygotes and heterozygotes with the original chromosomes in Generations 32 and 60.

and −0.47 in Generations 32 and 60, respectively. These values are significantly different from zero. Thus, the above conclusion was confirmed—newly arising mutant polygenes show overdominance with respect to viability, an important component of fitness, in homozygous genetic background when the mutant polygenes are localized only in one chromosome. In addition, the following tendency is apparent: The viabilities of heterozygotes with the original chromosome increased with the number of mutant polygenes, but reached the optimum when approximately 11 mutant polygenes, on the average, have been accumulated. This figure was estimated from the polygenic mutation rate (0.1411/second chromosome/generation, MUKAI 1964) under the assumption of a Poisson distribution. Having reached the optimal level, the viabilities of heterozygotes might have decreased with the increase in the number of heterozygous loci. The same conclusion has been derived by MUKAI, CHIGUSA, and YOSHIKAWA (1965) from independent experimental results. It is necessary to confirm such a tendency after accumulating more mutant polygenes in the chromosomes. This might act as one of the restrictive factors against the manifestation of overdominance in natural populations, regardless of the existence of the optimum heterozygosity.

In general, it was impossible to estimate the average degree of overdominance because of nonlinearity between heterozygote viability and number of heterozygous loci. However, in Generation 32, an approximate linearity was found. Thus, the average degree of overdominance in the classical model (h) was estimated in a previous article of this series (MUKAI, CHIGUSA, and YOSHIKAWA 1965). The result was $h = -0.27$.

(2) *Random heterozygotes:* On our hypothesis there should be some random heterozygotes in which new mutant polygenes are localized only in single chromosomes, and they should show overdominance. In order to examine this hypothesis, a correlation table between viabilities of heterozygotes and the sums of those of the corresponding homozygotes was made, using the data in Generation 32. The result is presented in Figure 6.

A positive correlation can be seen on the whole in Figure 6 ($r = +0.71$), but a detailed inspection reveals that the correlation table can be separated into two parts at heterozygote viability index 32. In the upper group (Group 1), a significant negative correlation can be found ($r = -0.75$). It is reasonable to assume that only one chromosome of the members of this group carried newly arising mutations; therefore, they should correspond to the heterozygotes with the original chromosomes. Indeed, the viability indices of homozygotes for more viable chromosomes in heterozygous individuals of Group 1 (14 crosses) were larger than 30.90 which could be considered to be included in the category of new-mutation-free chromosomes. The number of new-mutation-free lines, which were the constituents of the above-mentioned 14 crosses were 8, i.e., Lines 15, 16, 37, 44, 58, 72, 91, and 92. These fourteen kinds of heterozygotes correspond to the small peak on the right side in Figure 4. Thus, the above hypothesis is supported.

In the lower group (Group 2), in which the viabilities of all the heterozygotes were less than 32.00, the viability indices of homozygotes for more viable chromo-

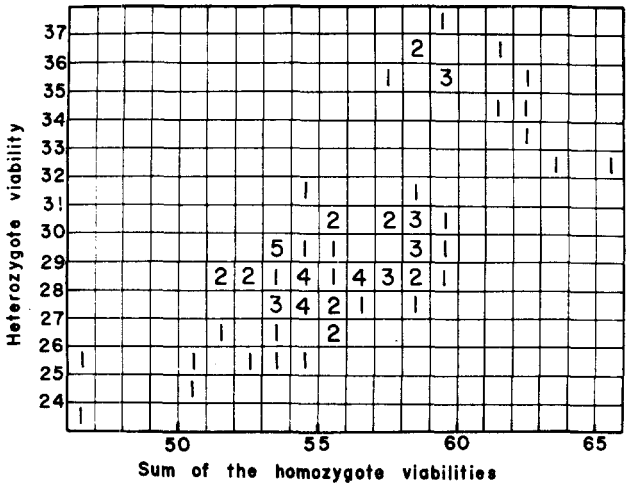

FIGURE 6.—Correlation between viabilities of random heterozygotes and sums of corresponding homozygote viabilities in Generation 32.

somes in heterozygous individuals were less than 32.54. There are five lines whose homozygous viabilities are larger than 30.00 but less than 32.54, i.e., 30.32 (Line 10), 30.11 (Line 27), 30.34 (Line 78), 32.53 (Line 89), and 31.62 (Line 104). It might be assumed that these five lines had newly arising mutations, but their effect might have been very small and some lines had almost normal viability indices owing to chance deviation or environmental effects. In fact, the viability indices of Lines 89 and 104 in Generation 25 were 31.15 and 30.31, respectively. The correlation coefficient between heterozygote viabilities and the sums of the corresponding homozygote viabilities, except for the above 14 heterozygotes, was calculated to be +0.67. These results are given in Table 3. Thus, the hypothesis was verified.

In Generation 52, the same tendency was found as in the random heterozygotes in Generation 32 both in RS and RA experiments. The correlation coefficients included all the random heterozygotes are +0.80 and +0.79 in the RS and RA experiments, respectively. However, all heterozygotic crosses can be separated into the two groups, i.e., Group 1 and Group 2 as in the case of Generation 32. The numbers of crosses in Group 1 are 13 in RS and 15 in RA experiments. The same eight lines as in Generation 32 were the components of the 13 or 15 heterozygotic crosses. The probability that no new mutation has taken place in these 8 chromosome lines in twenty generations is extremely low on the basis of the polygenic mutation rate reported previously (MUKAI 1964). Thus, it might be assumed that in some of these eight lines mutations took place, but their effects were so small that they were neutral with respect to the *trans*-effect of the mutant polygenes in random heterozygotes, and their heterozygotes with other new-mutation-carrying chromosomes manifested overdominance due to the mutations of their homologous chromosomes. Actually, the average homozygous viabilities of these 8 chromosome lines were 32.57 (32.84) and 32.35 (33.12) in Generations

TABLE 3

Correlation coefficients between homozygote and random heterozygote viabiliites

	Generation 25		32	52		60
	21°C(RS)	25°C(RS)	RS	RS	RA	
Random heterozygote						
As a whole	+0.51***	+0.59***	+0.71***	+0.80***	+0.79***	...
Group A	...	...	−0.75**	−0.68**	−0.32	...
Group B	...	...	+0.67***	+0.74***	+0.79***	...
Heterozygote with the original chromosome	...	...	−0.25*	...	...	−0.47***

RS stands for random heterozygotes between two lines of successive numbers.
RA stands for random heterozygotes between two lines of alternate numbers.
* Significant at the five percent level.
** Significant at the one percent level.
*** Highly significant.

32 and 52, respectively. The figures in parentheses indicate the control viability indices.

The phenotypic correlation coefficients between heterozygote viabilities and the sums of corresponding homozygote viabilities in Group 1 were −0.68 in RS and −0.32 in RA. The former is significantly different from zero at the 1% level. The correlation coefficients were also calculated for Group 2 to be +0.74 in RS and +0.79 in RA. These values are, of course, highly significant. All the estimated correlation coefficients are given in Table 3.

As in the case of WO heterozygotes, the sums of the viabilities of homozygotes corresponding to random heterozygotes (only Group 2) were divided into four groups in each of RS in Generation 32 and RS and RA in Generation 52, according to the order of their magnitudes. Actually, each group consisted of 15 or 16 members in Generation 32 and 13 or 14 members in Generation 52. The relationships between the averages of the sums of homozygote viabilities and those of corresponding heterozygote viabilities in respective groups are presented in Figure 7. It can be seen from Figure 7 that a clear linear relationship exists between the viabilities of homozygotes and heterozygotes at the average level. The regression function of the heterozygote viability (Y) on the sum of the corresponding homozygote viabilities (X) is as follows:

$$\hat{Y} = 0.3915\ X + 7.0007 \qquad (2)$$

Formula (2) and Figure 7 indicate that the degree of dominance of mutant polygenes might be independent of the viability of the genetic background, as long as the viability indices of these heterozygotes are larger than half of the wild-type homozygotes. Thus, the average degree of dominance of these mutant polygenes may be estimated in each cross combination of each generation. Indeed, the above regression coefficient (0.3915) is the estimate of $\bar{h}$ all over the generations.

The average dominance degree of mutant polygenes ($\bar{h}$) in the classical model

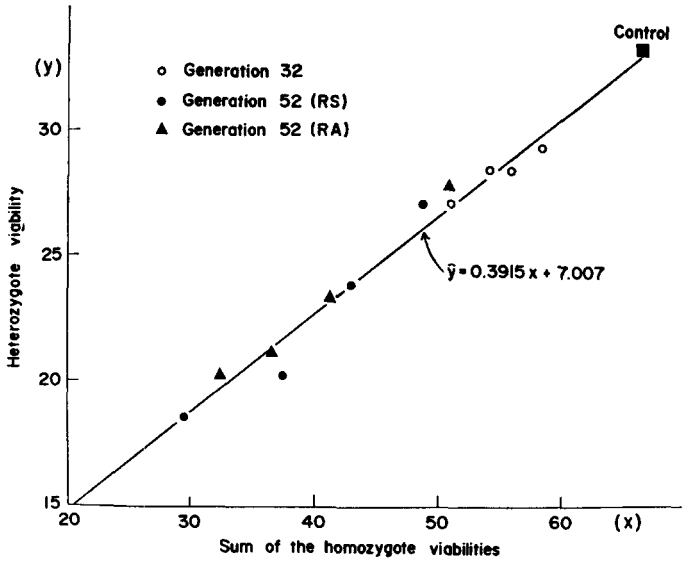

FIGURE 7.—Relationships between viabilities of homozygotes and random heterozygotes carrying chromosomes which both have new mutant polygenes.

was estimated. For the sake of reference, the genetic parameters defined previously (MUKAI 1964; MUKAI, CHIGUSA, and YOSHIKAWA 1964, 1965) are given here.

v_0 = viability index of the original homozygotes (approximately 33.3)

$\bar{v}$ = average viability of homozygote lines

$\bar{v}'_B$ = average viability of random heterozygotes carrying chromosomes which both have new mutant polygenes.

$\sigma^2_{G(B)}$ = genotypic variance among homozygote lines which are components of random heterozygotes of Group 2.

Cov ($v_i + v_j$ and v_{ij}) = covariance between the sum of homozygote viabilities of Line i and Line j and viability of their hybrid.
Line i and Line j should have newly arising mutant polygenes.

The average degree of dominance ($\bar{h}$) was estimated by the following two formulae, under the assumption of no correlation between the selection coefficient against mutant homozygotes and degree of dominance in the range of polygenes (MUKAI 1964). A linear relationship between homozygote and heterozygote viabilities in Figure 7 indicates the validity of this assumption.

$$\bar{h} = \frac{\text{Cov}\ (v_i + v_j \text{ and } v_{ij})}{2\,\hat{\sigma}^2_{G(B)}} \tag{3}$$

and

$$\bar{h} = \frac{v_0 - \bar{v}_B'}{2(v_0 - \bar{v})} \tag{4}$$

The necessary information for the estimation of $\bar{h}$ and the results are presented in Table 4. From this table, it can be concluded that the results estimated in

TABLE 4

The average degree of dominance ($\bar{h}$) of newly arising mutant polygenes and the basic statistics and the genetic parameters for its estimation (only random heterozygotes carrying chromosomes both of which have new mutant polygenes)

	Generation (cross) 32 (RS)	52 (RS)	52 (RA)
Number of crosses	63	56	53
Number of chromosome lines which are constituents of the above crosses	72	67	64
Covariance*	2.6604	25.0860	22.7371
Genetic variance among homozygote lines	3.6836	26.9569	27.7608
Average viability index of random heterozygotes	28.36	22.49	23.51
Average viability index of homozygotes	27.54	19.97	19.91
Average degree of dominance† Formula (3)	0.3611	0.4652	0.4095
Formula (4)	0.4226	0.4042	0.3561

* Covariance between the sums of homozygote viabilities and the corresponding heterozygote viabilities.

† See Formulae (3) and (4) in the text.

different generations, different cross combinations, and different methods are mutually consistent and the estimated $\bar{h}$ values are remarkably large (average $\bar{h} = 0.40$) in comparison with that of lethal genes ($\bar{h} = 0.01 \sim 0.015$, CROW and TEMIN 1964).

Recombination test: In order to examine whether or not these viability-decreasing mutations had occurred in different loci, recombination tests were conducted in Generation 56. In the first experiment, Lines 10 and 14 were employed. Their viability indices in Generation 52 were 26.7 (Line 10) and 29.9 (Line 14). A single pair mating was conducted between *Cy*/+ heterozygotes of Lines 10 and 14. For reference, the viability index of the heterozygotes was ca. 29.0. Following the procedure described in MATERIALS AND METHODS, fifty-two recombinant chromosomes and forty-eight parental chromosomes were recovered, and their homozygous viabilities were tested in three replicated cultures by the *Cy* method. The distribution patterns of viability indices for the recombinant group and the parental group are graphically presented in Figure 8 (A). The following facts can be deduced from this figure: First, the effect of recombination between polygenes can be clearly found from the increase of variance in the recombinant group in comparison with that in the parental group. The genetic variances were estimated by the analysis of variance to be $\hat{\sigma}^2_{G(R)} = 19.1465$ in the recombinant group and $\hat{\sigma}^2_{G(C)} = 7.1126$ in the control. The difference of these two values is highly significant. Second, supposedly normal chromosomes were recovered from the hybrid between two chromosomes carrying newly arising mutant polygenes, although their frequency is quite low. This indicates that at least in Lines 10 and 14 polygenic mutations occurred in different loci. Third, the homozygous viabilities of the parental chromosomes were approximately 21 and 26, the average

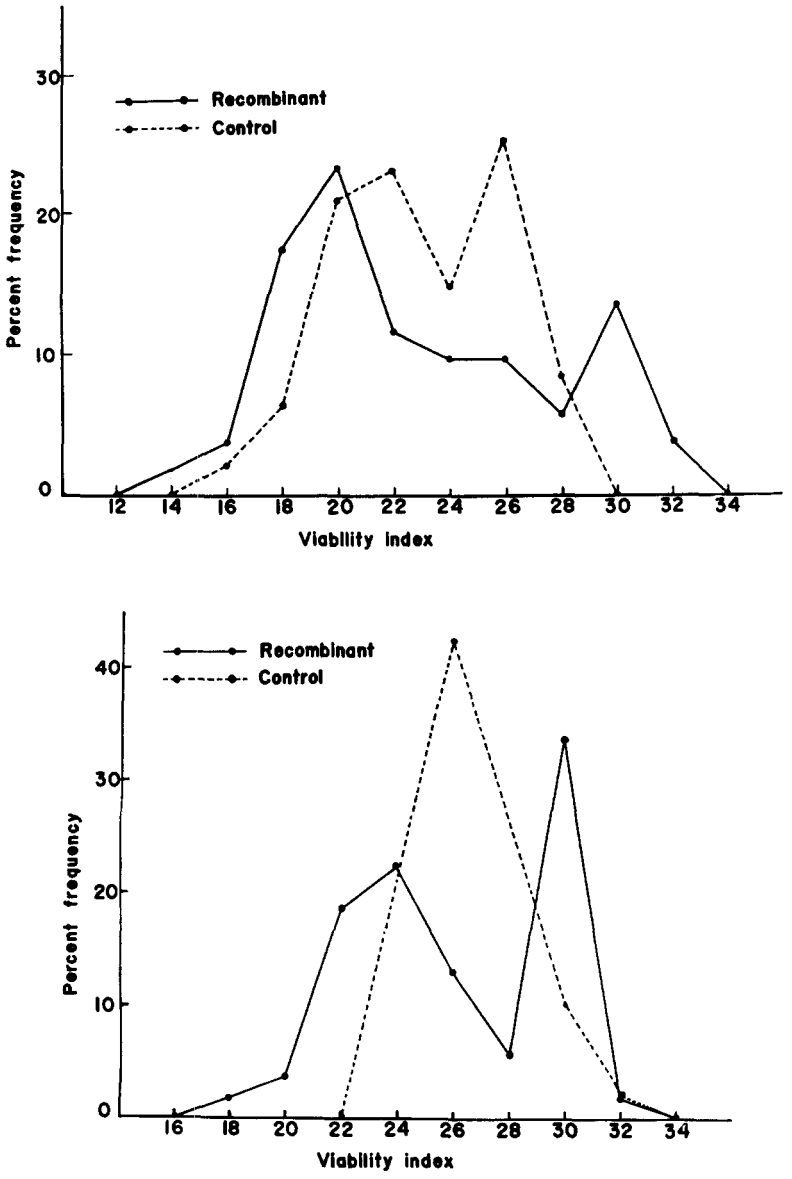

FIGURE 8.—Distribution patterns of viabilities of original and recombinant chromosomes. Above (A): Line 10 × Line 14; Below (B): Line 10 × Line 82.

viability of the parental group was 23.05 and that of the recombinant group was 22.92. No significant difference can be seen between the figures.

In the second experiment, Lines 10 and 82 were employed, and exactly the same experiment as the first one was conducted. The viability indices of these chromosome lines were, when homozygous, 26.7 (Line 10) and 26.4 (Line 82) in Generation 52. For reference, the viability index of their heterozygotes was

ca. 27.5. Fifty-four recombinant chromosomes and fifty parental chromosomes were recovered, and their homozygous viabilities were tested in three replicated cultures by the *Cy* method. The distribution patterns of viability indices are graphically presented in Figure 8 (B). From this figure, almost the same as the above conclusion could be drawn: The effect of recombination among mutant polygenes can be clearly seen. The estimated genetic variances are $\hat{\sigma}^2_{G(R)} = 11.5532$ in the recombination group and $\hat{\sigma}^2_{G(C)} = 1.3622$ in the control. The difference between these values is highly significant. The homozygous viabilities of the parental chromosomes were approximately 26 both in Lines 10 and 82, the average viability of the parental group was 26.78 and that of the recombinant group was 26.02. No significant difference could be observed between them. In the second experiment, it was impossible to recover normal chromosomes by recombination between chromosomes of Lines 10 and 82, although a significant increase of genetic variance was observed. This result might indicate that polygenic mutations took place in many loci.

In conclusion, it can be said that recombination was demonstrated between newly arising mutant polygenes controlling viability.

DISCUSSION

The present experimental results suggest some kind of position effect of spontaneous mutant polygenes controlling viability; namely, they show overdominance when located in the same chromosome, while, when located heterozygously in both homologous chromosomes, they are not only deleterious, but their degree of dominance is surprisingly high. An alternative hypothesis to the above will be discussed here.

Alternative hypothesis to the position effect hypothesis of newly arising mutant polygenes: The results of the present experiments might be explained without assuming a position effect, if we suppose that there were a few highly mutable loci where polygenic mutations were taking place. If so, random heterozygotes would not have been heterozygotes with respect to the newly arising mutations but would in some cases be homozygotes. This alternative hypothesis includes two cases. 1. when any chromosome line carrying polygenic mutations has at least one mutant polygene in the same locus (case 1), and 2. when polygenic mutations are distributed at random among a few mutable loci (case 2).

The most significant evidence against the alternative hypothesis is furnished by the results of recombination test between mutant polygenes. In the first experiment, six recombinant chromosomes out of fifty-two showed a viability index higher than 30 when homozygous (the maximum was 31.64), while none did in the control. The chromosome with the highest index had almost the same viability as the original viability. In the second experiment, the recovery of normal chromosomes from the heterozygotes could not be shown (see Figure 8), but the variance among recombinant chromosomes increased significantly. These experimental results argue against the alternative hypothesis (both cases 1 and 2). This conclusion is supported by the consideration that the mutation rate to recessive

lethal genes estimated by using the present experimental result was ca. 0.0063 per second chromosome per generation (MUKAI 1964), which is almost the standard recessive lethal mutation rate (see CROW and TEMIN 1964). If there had been such mutable loci, the recessive lethal mutation rate would have been higher than 0.0063, because lethal mutation rates in such loci would be expected to be correspondingly high (MUKAI 1964).

Furthermore, supporting evidences for position effect can be found in the present experimental results, when we assume that case 1 of the alternative hypothesis has been already rejected, i.e. that spontaneous polygenic mutations took place randomly at the several mutable loci. In Group 2 of the RS experiment in Generation 52, progenies of all the crosses should have been homozygous in at least one of such mutable loci under the assumption of no position effect mentioned above, because any heterozygote whose parental homozygotes carried mutant polygenes did not approximate normal viability. Such a probability is extremely low. Furthermore, even if the above phenomenon happened by chance, the probability that the same phenomenon would occur in Group 2 of the RA experiment would be also quite low, because we might expect in some frequency the appearance of complete heterozygotes for the mutant polygenes. Actually, we could not find any such exceptional hybrids in the RA experiment. Indeed, the distribution patterns of Group 2 in the RS and RA experiments almost completely overlapped. The χ^2 value with 4 degrees of freedom is 2.97 which corresponds to $0.5 < P < 0.6$. These results are inconsistent with an alternative hypothesis which assumes mutable loci and complete recessiveness of mutant polygenes.

Limitation of the manifestation of overdominance in natural populations: As far as our experimental results are concerned, we can accept a position effect (coupling-repulsion effect) of mutant polygenes. However, the present experiment was started from a single normal second chromosome from a natural population. Therefore, it is necessary to test the universality of this effect by using different experimental materials. Indeed, we have shown that viability polygenes, which were newly induced in a normal isogenic line, synthesized using 64 unrelated lines, did not manifest overdominance in the coupling phase (MUKAI, YOSHIKAWA, and SANO 1966).

Assuming the generality of the position effect tentatively, however, the following argument will be made about the limitation of the manifestation of overdominance in natural populations: A high proportion of the second chromosomes in natural or artificial equilibrium populations in *D. melanogaster* have at least one gene that would be deleterious if made homozygous (e.g., see GREENBERG and CROW 1960; OSHIMA 1963). Therefore, the frequency of individuals in which both homologous chromosomes carry deleterious genes is fairly high, and in these individuals overdominance would not be manifested. This might be the first restrictive factor against the manifestation of overdominance in natural populations.

As described in previous articles (MUKAI, CHIGUSA, and YOSHIKAWA 1965; MUKAI, YOSHIKAWA, and SANO 1966) and in the present report, even in heterozygotes between normal and deleterious chromosomes, overdominance in single

loci could not be manifested when heterozygosity in the other loci exceeds some magnitude (estimated number of heterozygous loci was 11 on the average). This might be the second restrictive factor against the manifestation of overdominance in natural populations.

WALLACE (1958) concluded the universality of overdominance on the basis of experimental results obtained under an exceptional condition, namely, a few mutant polygenes located only in one of the originally identical second chromosomes, and GREENBERG and CROW (1960) derived their conclusion from the direct analysis of natural populations where individuals satisfying WALLACE's condition are rare. BAND and IVES (1963) have supposed on the basis of negative corrrelation between the viabilities of heterozygotes and the averages of corresponding homozygote viabilities that the genetic load is primarily a balanced one in the South Amherst population of *D. melanogaster*. However, the corresponding homozygote viabilities for a given heterozygote are based on the average performance in homozygous condition of the two chromosomes making up the heterozygote. It could be that one of the two homozygotes whose average viability index is 6 or less (the index of normal individuals is ca. 33.3) is actually lethal, in which case the heterozygote carries a lethal chromosome. Ignoring this class of heterozygotes (although all the lethal heterozygotes are not excluded in this way), the correlation coefficients then become positive (ca. 0.13 at 25°C and ca. 0.10 at 18°C), but not significantly different from zero. Analyzed in this way the results might imply that the genetic load in the South Amherst population would not be, in the main, a balanced one. This result indicates that the degree of dominance of lethal genes is much less than that of polygenic mutations affecting viability, and it is consistent with the present experimental results. The phenomenon of the lack of a negative correlation in homozygote-heterozygote viabilities has recently been clearly demonstrated for *D. pseudoobscura* by WILLS (1966).

Homozygous loads and the genetic structure of natural populations: GREENBERG and CROW (1960) have developed a method for testing the existence of overdominance in natural populations, using homozygous loads. On the basis of the experimental results described in the present article, let us calculate homozygous loads assuming no overdominance. According to WRIGHT (1929), the frequency of mutant allele ($\hat{q}$) can be expressed as μ/hs in an equilibrium population assuming h is much larger than $\sqrt{\mu/s}$, where μ, s, and h have been defined above. Using this relationship, the homozygous load can be calculated by the following formula (MORTON, CROW, and MULLER 1956):

$$\Sigma \frac{\mu}{hs} s = \overline{\left(\frac{1}{h}\right)} \ \Sigma\mu \qquad (5)$$

In this derivation, it was assumed that $1/h$ and μ are uncorrelated. It should be noted here that h is almost uncorrelated with the homozygous viability of mutation-carrying individuals at least as far as only polygenes are concerned, as may be seen from the linearity between homozygote and heterozygote viabilities in

Figure 7. Thus, Formula (5) might be approximately valid even though there might be a small amount of epistatic interaction among mutant polygenes.

As reported in a previous article of this series (MUKAI 1964), the total polygenic and recessive lethal mutation rates per second chromosome in *D. melanogaster* were estimated to be 0.1411 and 0.0063 per generation, respectively. The average degree of dominance of mutant polygenes was also estimated to be ca. 0.4 as described above. YOSHIKAWA and MUKAI (in preparation) have estimated the average degree of dominance of 41 homozygously lethal genes in the second chromosomes derived from the original second chromosome in the present experiment. The estimated average h values were slightly different in accord with the genetic background, and ranged from 0.0176 to 0.0376 (without assuming any replacement (see MUKAI 1964)) in the genetic background of the same population. Thus, it might be reasonable to assume the $\bar{h}$ of lethal genes as being 0.02. It should be noted here that the 41 lethal genes employed in this experiment were accumulated under the minimum pressure of natural selection. There is one difficulty in the estimation of homozygous load by using the above estimates, namely, it is impossible to estimate $\overline{(1/h)}$ in Formula (5). However, as far as the coefficient of variation of h values is very small, $\overline{(1/h)}$ is nearly equal to $1/\bar{h}$. Although we do not know the coefficient of variation of h values, the homozygous loads due to detrimental genes (polygenes) and lethal genes in equilibrium populations were estimated under the assumption of $\overline{(1/h)} \approx 1/\bar{h}$. The results are: D (detrimental load) $= 0.3528$ and L (lethal load) $= 0.3150$; The D:L ratio becomes 1.12. On the other hand, TEMIN (1966) calculated the D and L values from the pooled data of the Madison, Wisconsin population and DR. WALLACE's cage population ($D = 0.147$ and $L = 0.262$) on the basis of the average viability of the populations. These values can be approximately converted to the values calculated based upon the optimum genotypes. The results turn out to be $D = 0.299$ and $L = 0.262$ (D:L ratio $= 1.14$), assuming that synergistic interaction (quadratic) exists among loci (in such a case the magnitude of the mutation load is approximately the total mutation rate of the haploid set of chromosomes (KIMURA and MARUYAMA 1966)). Based upon the assumptions given above in making the calculations, the two D:L ratios are found to be almost the same.

On the other hand, the homozygous loads of newly arising mutations were calculated by using the results in Generation 32 of the present experiment, assuming that different mutants act independently. In fact, until this generation, the average homozygote viabilities decreased almost linearly. In that generation, the control viability, the average viabilities of all homozygotes and non-lethal homozygotes were estimated to be 32.84, 22.82, and 27.44, respectively. By applying these estimates to the formulae derived by GREENBERG and CROW (1960), D and L values in Generation 32 were estimated to be: $D = 0.1797$ and $L = 0.1843$. The ratio of D to L becomes 0.9750. Thus, the D:L ratio of equilibrium populations to that of newly arising mutants becomes 1.15. This ratio is very close to 1 which can be expected from $hs =$ constant where h is positive

(GREENBERG and CROW 1960). Actually our data almost satisfy this relationship.

Under the assumption that different mutants act independently, the average number of mutant genes in the second chromosomes in equilibrium random mating populations can be calculated: The magnitudes of detrimental load and lethal load in equilibrium populations correspond to the detrimental load in Generation 62.8 and the lethal load in Generation 54.7 with respect to newly arisen mutations. The number of detrimental genes (polygenes) per second chromosome in that generation can be calculated to be 8.86 and the total number of mutant genes becomes 9.18 per second chromosomes on the average. Thus, the total number of heterozygous loci in autosomes is estimated to be 36.72, a very small number. Recently, LEWONTIN and HUBBY (1966) reported that there is a large amount of genetic variability with respect to isozymes in populations of *D. pseudoobscura*. Their tentative estimate of the amount of heterozygosity is approximately 8~15% per individual. Unfortunately it is impossible at present to state the relationship between our polygenic mutations and the isoalleles controlling isozyme variation which LEWONTIN and HUBBY studied. However, it may be said that recessive lethal genes, which might have been caused by the change of structural genes, are independent of the coupling-repulsion effect (YOSHIKAWA and MUKAI, in preparation). The isoalleles that control isozyme variations might be selectively neutral or nearly neutral in equilibrium populations as stated by KOJIMA and YARBROUGH (1967).

It should be pointed out here that the above calculation was made under several assumptions, some of which might not be valid, e.g., there might be a synergistic interaction among mutant polygenes in homozygous condition as reported by DOBZHANSKY, SPASSKY, and TIDWELL (1963) for *D. pseudoobscura*, by MALOGOLOWKIN-COHEN, LEVENE, DOBZHANSKY, and SIMONS (1964) for *D. willistoni*, and by MUKAI (1967) for *D. melanogaster*. (Even if synergistic interaction exists, its effect might not be so large as to change our conclusions.) Furthermore, so-called co-adapted gene complexes might have been developed in natural populations owing to the pressure of natural selection (e.g. WALLACE and VETUKHIV 1955). Thus, it might be dangerous to apply the present conclusion directly to natural populations, but the real situation might not be very far from the present situation in which the mathematical model was derived. In conclusion, it might, at least, be said that the manifestation of overdominance is restricted a great deal in natural populations and the experimental results of WALLACE (1958) and BURDICK and MUKAI (1958) showing overdominance of mutant polygenes can not be generally applied to natural populations. Furthermore, the present experimental results and the theoretical calculations derived from them might suggest that the genetic variation due to polygenic mutations of *D. melanogaster* in natural populations has been mainly maintained by the balance between mutation and selection pressures. However, it is necessary to study the evolutionary significance of overdominance as far as it is a product of natural selection (DOBZHANSKY 1950; MUKAI, YOSHIKAWA, and SANO 1966), even though the frequency of loci where overdominance is manifested might be small in equilibrium populations. This will be discussed in detail in another report of this series.

Finally, it should be pointed out that the coupling-repulsion effect of mutant polygenes in different loci can not be well understood at present on the basis of molecular genetics. It is an attractive further problem to clarify this phenomenon on a molecular basis.

The authors, while taking full responsibility for any errors which might be discovered in this paper, wish to express sincere appreciation to PROFESSOR JAMES F. CROW for his critical review of the manuscript and to DR. MOTOO KIMURA for his interest in this investigation and many constructive criticisms.

SUMMARY

Starting with a single second chromosome from a wild population of *Drosophila melanogaster*, 104 replicate lines were derived. In each of these lines spontaneous mutations were allowed to accumulate for many generations during which this chromosome was maintained in heterozygous condition with minimum selection. The viability of flies heterozygous and homozygous for new polygenic mutations was measured in Generations 32, 52, and 60. The average homozygous viability decreased greatly during this time. Random combinations of these chromosomes with a normal chromosome, presumed to be the same as the original chromosome from which the lines were derived, showed a slight increase in viability, negatively correlated with homozygous viability, unless the homozygous viability of the tested chromosome was very low. On the other hand, random combinations of chromosomes in general showed a viability positively correlated with the homozygous effect. By recombination tests, these mutant polygenes were proved to be nonallelic.—These results are interpreted as follows: Mutant viability genes show overdominance when they are on the same chromosome and the homologue is normal; this effect increases as the number of linked mutants increases until a maximum of about 11 mutants is reached, at which point the heterozygous viability begins to decrease. On the other hand, when the mutant genes are in both homologues they show a high degree of partial dominance. Thus there appears to be some kind of position effect, with mutant genes showing a different result in *cis* and *trans* positions. It is recognized that this is a surprising result, but the data are quite reproducible and this interpretation is tentatively put forward.—

It is suggested on the basis of these interpretations that overdominance is rarely manifested in natural populations because the number of mutant genes is likely to be above the optimum heterozygosity number and because many are in the *trans* position. The homozygous lethal and detrimental loads in equilibrium populations and those due to newly arising mutations were compared. The results are consistent with the absence of overdominance as an important factor for determining the frequency of viability genes in natural populations.

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