THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*. III. DOMINANCE EFFECT OF SPONTANEOUS MUTANT POLYGENES CONTROLLING VIABILITY IN HETEROZYGOUS GENETIC BACKGROUNDS¹

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OF expressed genetic loads in natural populations, mutation and segregation loads (Crow 1958, and others) have gained most attention, but their relative importance has not yet been estimated in any population. Since the difference in the causes of these two genetic loads is, in the main, due to whether or not overdominance with respect to fitness exists, numerous investigators have directed their attention to the degree of dominance of mutant genes controlling viability. Among them, MULLER (1950) argued that mutant genes with less extreme effects would tend to show more dominance than lethals and near-lethals, and GREENBERG and CROW (1960) have obtained experimental results supporting MULLER's idea.

On the other hand, DOBZHANSKY and SPASSKY (1963) tested, on a large scale, the viability of the second chromosomes of *D. pseudoobscura* collected from natural populations. Contrary to the results of GREENBERG and CROW (1960), they have arrived at a conclusion favoring the existence of overdominance on the basis of the correlation between the viability of homozygotes and that of the corresponding heterozygotes. The same conclusions have been drawn for *D. melanogaster* (BAND and IVES 1963; WALLACE and DOBZHANSKY 1962) and *D. willistoni* (KRIMBAS 1959).

In a previous article (MUKAI, CHIGUSA, and YOSHIKAWA 1964), we have reported that newly arising spontaneous mutant polygenes controlling viability are overdominant in homozygous genetic background in *D. melanogaster*. The same conclusion was drawn for radiation-induced mutant polygenes controlling viability (WALLACE 1958; BURDICK and MUKAI 1958; and others). However, the dominance effect of spontaneous mutants controlling viability in heterozygous genetic background has not yet been reported, although radiation-induced mutant polygenes have been reported to be detrimental in heterozygous condition (WALLACE 1963; MUKAI and YOSHIKAWA 1964).

The estimation of the degree of dominance in heterozygous genetic background should bring fundamental information about the genetic structure of natural

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populations. Therefore, using the experimental materials reported in the foregoing articles (MUKAI 1964; MUKAI, CHIGUSA, and YOSHIKAWA 1964), we have tested the dominance effect of spontaneous mutant polygenes in heterozygous genetic background. These mutant polygenes have been subjected to a minimum of natural selection before the testing.

MATERIALS AND METHODS

As previously reported (MUKAI 1964; MUKAI, CHIGUSA, and YOSHIKAWA 1964), spontaneous polygenic mutations affecting viability were accumulated under the minimum pressure of natural selection in 104 second chromosomes derived from a single normal chromosome. In Generation 32, homozygote and heterozygote viabilities of these chromosome lines were tested. The results of the viability test for homozygotes and heterozygotes with the original chromosome have already been published in a previous report (MUKAI, CHIGUSA, and YOSHIKAWA 1964).

The heterozygous effects of these mutant polygenes on viability were tested in two heterozygous genetic backgrounds in Generation 32, using two normal second chromosome lines; one (abbreviated as $+_w/+_w$) from the same population from which the original chromosome ($+_o$) had been extracted, and the other (abbreviated as $+_B/+_B$) from an unrelated population (Dr. A. E. BELL's Stock CP). The X and the third chromosomes of $+_w/+_w$ and $+_B/+_B$ have been substituted by those of W160S. Thus, the heterozygous genetic backgrounds are concerned with the second chromosome only.

The viabilities of intrapopulation hybrids ($+_w/+_i$ where i indicates line number) were estimated by counting the number of offspring in the cross $+_w/+_w$ (7 females $\times$ $Cy/+_i$ (5 males). Those of interpopulation hybrids ($+_B/+_i$) were estimated in the same manner. The relative viability was expressed by the percentage of phenotypically wild-type flies of the offspring in any culture. According to this method, the expectation of the viability index of normal flies is 50.00. Eighty quasi-normal lines, whose homozygous viability indices were larger than 20.00, were employed in the present experiment. As previously (MUKAI, CHIGUSA, and YOSHIKAWA 1964), the Curly method (WALLACE 1956) was employed in the estimation of homozygote viabilities. In this method, the expected control viability is 33.3. The methods for culturing and counting the present experiment were exactly the same as MUKAI (1964).

RESULTS AND ANALYSIS

Viabilities of homozygotes and heterozygotes with the original chromosome: Although the results were presented in MUKAI, CHIGUSA, and YOSHIKAWA (1964), the condensed data obtained on the basis of eight replicated observations per line are presented in Table 1 and are also described in this connection for the sake of comparison. In addition further information has been obtained. The distribution patterns of viability indices of homozygotes and heterozygotes on the line basis are presented in Figure 1A, together with the control for the heterozygotes. The method for the construction of the control distribution was described in detail by MUKAI, CHIGUSA, and YOSHIKAWA (1964). The viability indices of homozygotes in Figure 1A have been multiplied by the factor 3/2 to facilitate comparison, since the expected control viability of homozygotes is 33.3.

The average viability index of homozygotes ($\bar{v} = 28.04$) was significantly lower than that of the control ($v_o = 33.27$), and the genetic variance ($\hat{\sigma}_G^2 = 5.6477$) was also highly significant. The average viability index of heterozygotes ($\bar{v}'$) was 51.51 which is, of course, significantly different from v_o' (49.91). This result

TABLE 1

Basic statistics and genetic parameters obtained in the test of Generation 32

	Homozygote	Heterozygote		
		Homozygous genetic background	Intra-population	Inter-population
Total number of flies counted	427,886	392,077	371,296	446,233
Harmonic mean of the number of flies counted in single lines ($\bar{n}_L$ or $\bar{n}_{L'}$)	5,322.2	4,864.8	4,618.9	5,547.4
Harmonic mean of the number of flies counted in single observations ($\bar{n}_I$ or $\bar{n}_{I'}$)	654.7	590.1	546.0	677.9
Estimated error variance on the line basis ($\hat{\sigma}_L^2$ or $\hat{\sigma}_{L'}^2$)	0.7115	0.7139	0.8783	2.1066
Estimated error variance on the individual observation basis ($\hat{\sigma}_E^2$ or $\hat{\sigma}_{E'}^2$)	5.7845	5.8858	7.4302	17.2395
Estimated genetic variance ($\hat{\sigma}_G^2$ or $\hat{\sigma}_{G'}^2$)	5.6477	0.4081	0.2229	0.4771
Control viability index (v_o or v_o')	(33.27)	49.91	50.99	55.99
Mean viability index ($\bar{v}$ or $\bar{v}'$)	28.04***	51.51***	50.27*	54.87*

* Significantly different from v_o' at the 5% level.*** Significantly different from v_o or v_o' at a very low level.

Superscript ['] is concerned with heterozygotes.

FIGURE 1.—Distribution patterns of viabilities of heterozygotes with respect to newly arising mutant polygenes in Generation 32. (A) homozygous genetic background; (B) heterozygous genetic backgrounds—intra- and interpopulational.

clearly indicates the existence of overdominance, which can be intuitively seen in Figure 1A.

The average degree of overdominance was estimated as follows: The average number of mutant polygenes in the second chromosomes was calculated, on the basis of the previous estimation of polygenic mutation rate (MUKAI 1964), to be 4.5 in Generation 32 of the present experiment. Thus, the average overdominance effect of single mutations in homozygous genetic background becomes 0.00712 [i.e. = $(51.51 - 49.91)/(49.91 \times 4.5)$] of homozygote viability. That is to say, the degree of dominance ($\bar{h}$ value) in the classical model becomes -0.27 , where the average selection coefficient against mutant homozygotes ($\bar{s}$) = 0.0268 (= $0.8937/33.3$) (see Table 8 of MUKAI 1964).

Viabilities of heterozygotes with a normal chromosome extracted from the same population: In the test for heterozygote viabilities, as in the case of those of homozygotes, eight replicated observations were made in each line with the exception of only seven in one line. The outline of the observations is presented in Table 1. The result of the analysis of variance is given in Table 2. Although significant differences are not seen among the viabilities of 80 heterozygote lines, the probability value is very close to 5%. Thus, the genetic variance (σ_g^2) was estimated to be $\hat{\sigma}_g^2 = 0.0009$, and this value is about half the genetic variance of heterozygote lines in a homozygous genetic background ($\hat{\sigma}_g^2 = 0.4081$). The mean viability index was 50.27.

The distribution pattern of the viabilities of these intrapopulation hybrids is presented in Figure 1B, together with that of interpopulation hybrids. Furthermore, a control for the distribution of these heterozygote viabilities was constructed. Although the method for the construction is the same as that reported previously (MUKAI, CHIGUSA, and YOSHIKAWA 1964), it will be repeated here.

The error variance of heterozygotes on line basis ($\sigma_{L'}^2$) was estimated by Formula (1):

$$\hat{\sigma}_{L'}^2 = \hat{\sigma}_{E'}^2 \times \bar{n}_{L'}/\bar{n}_{L'} \quad (1)$$

where $\hat{\sigma}_{E'}^2$, $\bar{n}_{L'}$, and $\bar{n}_{L'}$ stand for the estimated error variance on the basis of individual observations, the harmonic mean of the number of flies counted in single observations, and that of single lines, respectively. Actual values are $\hat{\sigma}_{E'}^2 = 7.4302$, $\bar{n}_{L'} = 546.0$, and $\bar{n}_{L'} = 4618.9$. The $\hat{\sigma}_{L'}^2$ thus obtained is 0.8783.

The mean viability of the control (v'_0) was estimated by the following method: Five lines showing the highest viabilities in homozygous condition were chosen from the 80 lines, and the

TABLE 2

Analysis of variance for the viabilities of heterozygotes with a normal chromosome that originated from the same population (in Generation 32)

Source	Sum of squares	Degrees of freedom	Mean square	F	E.M.S.
Between lines	727.6330	79	9.2105	1.24	$\sigma_{E'}^2 + 7.99 \sigma_{G'}^2$
Within lines	4,153.4724	559	7.4302	...	$\sigma_{E'}^2$
Total	4,881.1054	638		...	

viability indices of the corresponding heterozygote lines were estimated. The weighted mean of these estimates was used as the ν'_o . This estimation was conducted on the assumption that these five selected lines have no mutation and the error of homozygote viability is uncorrelated with that of the corresponding heterozygote viability. Then the estimated ν_o on the basis of counting 22,731 flies becomes 50.99.

Assuming normality (mean ν'_o and variance $\sigma_{L'}^2$), the control for the distribution of heterozygotes was constructed and is presented in Figure 1B. It can be seen that newly arising mutant polygenes are heterozygously deleterious to their carriers. Indeed, the result of an approximate t-test indicates that the average viability of these heterozygotes ($\bar{\nu}' = 50.27$) is significantly lower than the control ($\nu'_o = 50.99$) at the 5% level (one-tailed test).

Viabilities of heterozygotes with a normal chromosome extracted from an unrelated population: Eight replicated observations of viability were made in each line with the exception of seven in one case. The outline of the observations is presented in Table 1. The analysis of variance was conducted (Table 3). Although significant differences are not seen among the viabilities of 80 heterozygote lines, the probability value is very close to 5%. Thus, the genetic variance ($\sigma_{G'}^2$) was estimated to be $\hat{\sigma}_{G'}^2 = 0.4771$. It should be stressed here that the error variance of the interpopulation hybrids ($\hat{\sigma}_{E'}^2 = 17.2395$) is significantly larger than that of the intrapopulation hybrids ($\hat{\sigma}_{E'}^2 = 7.4302$), although the number of flies counted is larger in the former. This difference might have been caused by eventual presence or absence of a co-adaptation system in the genetic background. The mean viability index was 54.87.

The distribution pattern of the viabilities of these interpopulation hybrids is given in Figure 1B, together with the control which was constructed in the same way as that for the intrapopulation hybrids. In that figure, the control mean (ν'_o) is 55.99. From the figure, we can intuitively recognize that newly arising mutant polygenes are heterozygously deleterious to their carriers in the genetic background of interpopulation hybrids as in the case of intrapopulation hybrids. An approximate t-test indicates that the average viability of the heterozygotes ($\bar{\nu}' = 54.87$) is lower than the control ($\bar{\nu}_o = 55.99$) at the 5% level (one-tailed test).

Correlation between homozygote and heterozygote viabilities: We can summarize our above findings in this way—newly arising mutant polygenes controlling viability have, when heterozygous, detrimental effects on viability in heterozygous genetic backgrounds both in intra- and in interpopulation hybrids.

TABLE 3

Analysis of variance for the viabilities of heterozygotes with a normal chromosome that originated from an entirely unrelated population (in Generation 32)

Source	Sum of squares	Degrees of freedom	Mean square	F	E.M.S.
Between lines	1,662.9190	79	21.0496	1.22	$\sigma_{E'}^2 + 7.99 \sigma_{G'}^2$
Within lines	9,636.8768	559	17.2395	...	$\sigma_{E'}^2$
Total	11,299.7958	638		...	

This is in contrast with the situation found in homozygous genetic background. In order to confirm the heterozygous detrimental effect, phenotypic and genotypic correlations between homozygote and heterozygote viabilities were estimated. The estimated phenotypic correlations are, on line basis, -0.2486 , $+0.2360$, and $+0.1147$ in homozygous and heterozygous intrapopulation and heterozygous interpopulational genetic backgrounds, respectively. The first two figures are significantly different from zero. The genetic correlation ($r_{GG'}$) can be calculated by the following formula:

$$r_{GG'} = \frac{Cov(\text{Homo and Hetero})}{\hat{\sigma}_G \hat{\sigma}_{G'}} \quad (2)$$

where $Cov(\text{Homo and Hetero})$ stands for the covariance between homozygote and heterozygote viabilities. The estimates of these covariances are -0.6803 (homozygous genetic background), $+0.6387$ (intrapopulation hybrids), and $+0.4429$ (interpopulation hybrids). The genotypic correlation coefficients calculated by using these values are -0.4482 , $+0.5621$, and $+0.2698$ in homozygous and heterozygous (intra- and interpopulation hybrids) genetic backgrounds, respectively. These values are significantly different from zero.

For the sake of simple presentation, the 80 lines were divided into five groups according to the order of the degree of homozygote viability. Thus, each group consisted of 16 lines. The relationship between the average of homozygote viabilities and that of heterozygote viabilities in each group was examined and is graphically presented in Figure 2. From this figure, the above conclusion can be intuitively drawn.

FIGURE 2.—Relationships of homozygote and heterozygote viabilities in Generation 32.

DISCUSSION

The results reported in the present article are not only compatible with those obtained for radiation-induced mutant polygenes (WALLACE 1963; MUKAI and YOSHIKAWA 1964) but are also more convincing in their clarity, and the following statements may be made: (1) Most of the spontaneous polygenic mutations are potentially heterotic (see Figure 1A); (2) If normal homozygotes gain a few mutant polygenes in one of the two identical second chromosomes, their viability can be as high as that of intrapopulation hybrids. Indeed, an intrapopulation hybrid in the present experiment showed the viability index of 50.99 (v'_0) before the accumulation of mutant polygenes has taken place, while the viabilities of the parental lines were approximately 50.00. It has been estimated previously (MUKAI, CHIGUSA, and YOSHIKAWA 1964) that approximately 4.5 new mutant polygenes have been accumulating through Generations 0 to 32, and the average viability of heterozygotes in homozygous genetic background increased from 49.91 to 51.51. In Generation 60, it increased further to 52.50 from 48.62 (of the control), owing to about 8.5 ($= 0.1411 \times 60$) heterozygous loci (MUKAI and YAMAZAKI 1964), on the average. These average heterozygote viabilities are larger than that of an intrapopulation hybrid; (3) The viability of an intrapopulation hybrid, which was less than the average viability of individuals heterozygous at only a few loci, decreased with an increase in the number of mutant polygenes.

On the basis of the increase in mean viability of heterozygotes with respect to radiation-induced mutations, WALLACE (1958) tentatively concluded 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 he obtained additional data which might support this conclusion (WALLACE 1963). However, KIMURA and CROW (1964) have cast doubt on WALLACE's statement on theoretical grounds, because the magnitude of segregation load becomes quite large under his condition. From the experimental results of MUKAI, CHIGUSA, and YOSHIKAWA (1964) and WALLACE (1958), and the theoretical calculation of KIMURA and CROW (1964), it is reasonable to assume epistatic interaction among loci.

Based upon our findings, we tentatively propose the hypothesis of "optimal heterozygosity" [once mentioned but rejected by WALLACE himself (WALLACE 1959)], i.e., a hypothesis according to which the existence of overdominance is admitted if the number of heterozygous loci is very small. But the viabilities of heterozygotes decrease with the increase in the number of heterozygous loci after viability has reached the optimal level. Under this rather new theory, the classical hypothesis could be applicable to most of the loci, as far as the optimum heterozygosity had been reached by only a few loci.

WALLACE (1959) rejected this hypothesis, since interpopulation hybrids are almost always more viable than are the members of a single population. In fact, in the present experiment, the viability of the original interpopulation hybrid was 55.99 and that of the intrapopulation hybrid was 50.99. However, if the

viabilities of individuals increased with the increase in the number of heterozygous loci, those of intrapopulation hybrids should increase when still more mutant polygenes are added because their proportion of heterozygous loci should be much less than 50% (KIMURA and CROW 1964). Contrary to the expectation, our experimental result shows a viability decrease when mutant polygenes are accumulated. It is our opinion that the luxuriant heterosis manifested by interpopulation F_1 hybrids might have been due mainly to specific interaction between two homologous chromosomes (not merely homologous genes) originated from different populations, and, therefore, cannot be discussed on the same basis as the intrapopulation hybrids. This speculation is supported by the breakdown of the heterosis of interpopulation F_1 hybrids in subsequent generations (WALLACE 1955; WALLACE and VETUKHIV 1955).

Finally, it should be pointed out that the present discussion was based on the characteristics of newly arising polygenic mutations which were placed in the originally identical normal chromosomes, whose homologous chromosomes showed normal viability when homozygous. This condition is not common in natural populations. That is, polygenic mutations arise in lethal, semilethal, subvital, or normal chromosomes, and their homologues are not always normal chromosomes. Since a position effect of newly arising polygenic mutations was discovered by MUKAI and YAMAZAKI (1964), the above discussion cannot be generally applied. A more detailed discussion will be given in the next article of this series.

We wish to express our appreciation to Dr. M. KIMURA for his interest in our investigation and many constructive criticisms.

SUMMARY

Spontaneous mutant polygenes controlling viability were accumulated in second chromosomes derived from a single chromosome, and their dominance effects were tested in two heterozygous genetic backgrounds (intra- and interpopulation). The results have shown that the newly arising mutant polygenes were heterozygously detrimental in heterozygous genetic backgrounds, in contrast with the result obtained for a homozygous genetic background. On the basis of these experimental results, the limitation of the manifestation of overdominance in equilibrium natural populations was discussed.

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