THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER. II. OVERDOMINANCE OF SPONTANEOUS MUTANT POLYGENES CONTROLLING VIABILITY IN HOMOZYGOUS GENETIC BACKGROUND[1]

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EXPERIMENTAL evidences indicating overdominance, in homozygous genetic background, of radiation-induced mutant polygenes controlling viability have been accumulated (WALLACE 1958, 1963, BURDICK and MUKAI 1958, MUKAI 1961, and MUKAI and YOSHIKAWA 1964). However, almost none of the examples of this kind have been presented with respect to spontaneous mutant polygenes. When the overdominance exists in natural populations, it greatly contributes to the maintenance of genetic variability and therefore is of evolutionary significance. Thus, using the experimental materials reported in the foregoing article (MUKAI 1964), we have tested the existence of overdominance in relation to spontaneous mutant polygenes which have not been subjected to natural selection, and the result is presented in this report.

MATERIALS AND METHODS

Details of the experimental materials and methods for the accumulation of mutant polygenes, and the test for viability have been reported in the first paper of this series (MUKAI 1964). A single male $Pm/+$ from the cross Cy/Pm (the genetic background is substituted by that of W160S) $\times$ $+/+$ was sampled (W160S: an isogenic stock derived from BURDICK's W160). This selected $Pm/+$ male was crossed to Cy/Pm (females) to replicate the $+$ chromosome from which 104 lines of $Cy/Pm \times Pm/+_i$ were established ($i = 1, 2, \ldots, 104$). In each line, the second chromosome has been maintained through a single male by the cross Cy/Pm (5 females) $\times$ $Pm/+_i$ (1 male). In Generation 32, homozygote viabilities and heterozygote viabilities of these chromosome lines were tested.

In the estimation of homozygote viability, the Curly-method (WALLACE 1956) was employed. Following this method, the cross $Cy/+_i$ (5 females) $\times$ $Cy/+_i$ (5 males), was conducted. In the next generation, phenotypically Cy- and wild-type flies segregate in a culture. The percentage of the wild-type flies was employed as viability index. Thus, the expectation of the viability index for the wild-type flies is 33.3 (Cy-homozygotes are lethal). The viabilities of heterozygotes were tested in heterozygous condition with a chromosome supposed to be identical to the original chromosome, since it did not undergo any mutation. Line 92 ($+_o/+_o$) was chosen for this purpose on the basis of the result from the preliminary test in which its viability in homozygous condition was estimated to be normal (viability index = 32.08). The heterozygote viabilities were estimated by counting the number of offspring in the cross $+_o/+_o$ (7 females) $\times$ $Cy/+_i$

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(5 males). The relative viability was expressed as the percentage of phenotypically wild-type flies in any culture. According to this method, the expectation of the viability index of normal flies is 50.00. Eighty quasinormal lines, whose homozygous viabilities exhibited a greater than 20.00 viability index, were employed in the present experiment.

RESULTS AND ANALYSIS

Homozygote viability: An analysis of variance was performed for the estimated viabilities of the 80 lines with eight replicate observations in each line. The result is shown in Table 1. It is evident from Table 1 that there is a significant diversity among these lines with respect to viability. The genetic variance caused by mutation was estimated to be $\hat{\sigma}^2_G = 5.6477$. The mean viability index was 28.04. These figures are consistent with those reported previously (MUKAI 1964).

The distribution pattern of viabilities is presented in Figure 1 on the line basis (viability index pooled over replicate observations in each line). In this figure, each homozygote viability index was multiplied by a factor of 3/2 for the sake of comparison with those of heterozygotes.

Heterozygote viability: In the test for heterozygote viability, eight replicate observations were made in each line with the exception of one case where the number of observations was seven. The result of the analysis of variance is given in Table 2. Although significant differences are seen among the viabilities of 80

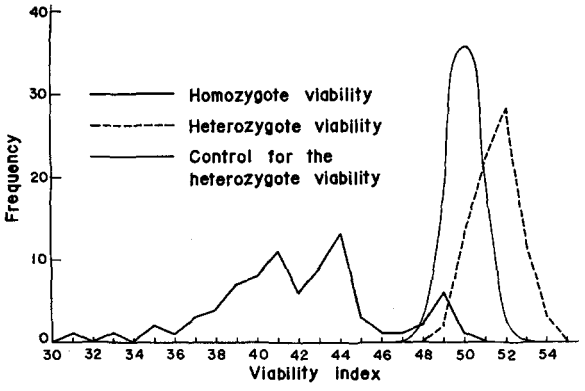

FIGURE 1.—Distribution pattern of homozygote and heterozygote viabilities in Generation 32. Homozygote viabilities have been multiplied by the factor 3/2 to facilitate comparisons.

TABLE 1

Analysis of variance for the homozygote viabilities of quasinormal lines in Generation 32

Source	Sum of squares	Degrees of freedom	Mean square	F	Expected mean square
Between lines	4026.30	79	50.9658	8.81***	$\sigma^2_E + 8.0\,\sigma^2_G$
Error	3239.31	560	5.7845	...	σ^2_E
Total	7265.61	639	.....	...	...

*** Highly significant.

TABLE 2

Analysis of variance for the viabilities of heterozygotes with the original chromosome in Generation 32

Source	Sum of squares	Degrees of freedom	Mean square	F	Expected mean square
Between lines	722.4081	79	9.1444	1.55**	$\sigma^2_{E'} + 7.99\,\sigma^2_{G'}$
Error	3290.1684	559	5.8858	...	$\sigma^2_{E'}$
Total	4012.5765	638	.....	...	...

** Significant at the 1 percent level.

heterozygote lines, the estimated genetic variance ($\hat{\sigma}^2_{G'} = 0.4081$) is, as expected, markedly smaller than that of homozygotes. The mean viability index was 51.51.

The distribution pattern of heterozygote viabilities is presented in Figure 1 together with that of the homozygotes. Furthermore, a control for the distribution of heterozygote viabilities was constructed as follows: The error variance of heterozygotes on the pooled basis ($\sigma^2_{L'}$) was estimated by Formula (1)

$$\hat{\sigma}^2_{L'} = \hat{\sigma}^2_{E'} \times \frac{\bar{n}_{I'}}{\bar{n}_{L'}} \tag{1}$$

where $\hat{\sigma}^2_{E'}$,$\bar{n}_{I'}$ and $\bar{n}_{L'}$ stand for the estimated error variance on the basis of individual observations, harmonic mean of number of flies counted in single observations, and that in single lines, respectively. Actual values are $\hat{\sigma}^2_{E'} = 5.8858$, $\bar{n}_{I'} = 590.1$ and $\bar{n}_{L'} = 4864.8$. The $\hat{\sigma}^2_{L'}$ thus obtained is 0.7139.

The mean viability of the control (v_o) was estimated by the following method: The five lines showing the highest viabilities in homozygous condition were chosen from the 80 lines, and the viability indices of the corresponding heterozygotes of these five selected lines were estimated. The weighted mean of these estimates was used as the v_o. This estimation was conducted assuming that these selected five lines do not have any mutation and the error of homozygote viability is uncorrelated with that of corresponding heterozygote viability. The estimated v_o on the basis of counting 24,555 flies becomes 49.91. This value corresponds to 33.27 of the *Cy*-method and is a very reasonable estimate (see MUKAI 1964).

Assuming normality (mean v_o and variance $\hat{\sigma}^2_{L'}$), the control for the distribution of heterozygotes was constructed and presented in Figure 1. From this figure, the existence of overdominance can be easily seen.

Correlation between homozygote and heterozygote viabilities: Phenotypic correlation between homozygote and heterozygote viabilities was calculated on a line basis. The result is -0.2486. This estimate is significantly different from zero at the 5 percent level. Since the expectation of correlation between the errors of homozygote and heterozygote viability is zero, the genotypic correlation between the homozygote and heterozygote viabilities ($r_{GG'}$) can be calculated by the following formula

TABLE 3

Relationship between homozygote and heterozygote viabilities in Generation 32

	Rank of homozygote viability						Total number of of flies counted
	0*	1	2	3	4	5	
Homozygotes†	....	31.66	29.18	27.98	26.82	24.61	427,886
Heterozygotes‡	49.91	51.11	51.38	51.51	51.70	51.85	392,077

* Control viability.
† Expected viability of normal flies = 33.3.
‡ Expected viability of normal flies = 50.0.

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

where Cov (Homo and Hetero) indicates the covariance between the homozygote and heterozygote viabilities. The result obtained on the basis of $\hat{\mathrm{Cov}}$ (Homo and Hetero) $= -0.6803$, $\hat{\sigma}_G = 2.3764$, and $\hat{\sigma}_{G'} = 0.6388$ is -0.4482.

In the interest of simplified presentation, the 80 lines were divided into five groups in the order of degree of homozygote viability. Thus, each group consisted of 16 lines. The relationship between the average of homozygote viabilities and that of heterozygote viabilities is presented in Table 3.

The negative correlation between homozygote and heterozygote viabilities, which can be intuitively seen in Table 3, clearly indicates that newly arising mutant polygenes show overdominance with respect to viability, an important component of fitness, in homozygous genetic background at least when the mutant polygenes are localized only in one chromosome.

DISCUSSION

In the present experiment, it was clearly shown that newly arising mutant polygenes were overdominant. This result is compatible with that of radiation-induced mutant polygenes (WALLACE 1958, 1963; BURDICK and MUKAI 1958; MUKAI 1961; and MUKAI and YOSHIKAWA 1964). However, in all the experiments including the present one, the existence of overdominance was shown in otherwise homozygous genetic background, and only a few loci were heterozygous for mutant polygenes. Indeed, the average number of mutant polygenes in the second chromosomes is 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, i.e. the proportion of the heterozygous loci was extremely low. Therefore, it is questionable that overdominance can always be seen when the proportion of heterozygous loci is increased, although a nearly linear increase of the heterozygote viability is found with the decrease of homozygote viability as observed in Table 3. Hence, further tests are necessary after accumulating more mutant polygenes.

SUMMARY

After accumulation of spontaneous mutant polygenes controlling viability in the second chromosome, the dominance of these polygenes was tested in homozygous genetic background by counting 819,963 flies. The result indicates that most of the mutant polygenes show overdomiance, at least when they are localized in one chromosome.

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