THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER. I. SPONTANEOUS MUTATION RATE OF POLYGENES CONTROLLING VIABILITY[1]

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MUTATIONS are the raw materials from which evolutionary changes are compounded. Although, up to the present time, a great number of researches have been conducted by numerous investigators with respect to the mutations of so-called major genes, studies on polygenic mutations, especially on their rate, are not abundant.

EAST pointed out as early as 1935, on the basis of his long-continued genetical investigations in plants, that polygene mutations are predominant among the raw materials of evolution, their spontaneous occurrence rates being of large magnitude in comparison with those of major genes; he also believed that the mutant polygenes are not always recessive.

Using doubled monoploids, SPRAGUE, RUSSELL, and PENNY (1960), estimated the rate of mutations controlling some agronomic quantitative characters to be 4.5 mutations per attribute per 100 gametes on the average. This finding indicates that an appreciably large number of spontaneous quantitative mutations must occur per individual in comparison with qualitative mutations.

At present, we have no reliable data on the spontaneous mutation rate of polygenes controlling viability, one of the most important components of fitness. However, some data are available for radiation-induced mutation rates of polygenes controlling viability. In the reports of the present series, the author would like to call all such mutations that slightly alter the viabilities of their homozygous carriers mutations of polygenes controlling viability, following DARLINGTON and MATHER (1949), for the reason that the individual effects of those genes are similar, supplementary to one another, and much smaller than the environmental effects. Under this definition, most of the major genes, whose main effects are qualitative (e.g. eye color genes), could work as polygenes controlling viability as far as their pleiotropic effects are concerned with viability.

On the basis of the proportion of deleterious mutations which cause probably more than a 10 percent reduction of normal viability, to recessive lethal mutations, found among radiation-induced mutations in *D. melanogaster* (MULLER 1934, etc.), MULLER (1950) predicted that the mutation rate of polygenes, if expressed by the present terminology, may be of perceptibly large magnitude in

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comparison with that of all the more markedly detrimental mutations taken together, and this was substantiated by BURDICK and MUKAI (1958), BATEMAN (1959), and MUKAI (1961).

From these experimental evidences, it seems natural to predict that the spontaneous mutation rates of polygenes of this type should be substantially higher than those of major genes, although DURRANT and MATHER (1954), CLAYTON and ROBERTSON (1955), and PAXMAN (1957) have obtained data indicating that the spontaneous mutation rates of polygenes controlling the bristle characters in *D. melanogaster* are rather low.

Considering that the genetical properties of an organism are the products of its evolutionary history, if the hypothesis is valid that polygenic mutation rates are essentially higher than those of major genes, the former genes should play a different role from the latter in evolution. From an accurate estimate of the rate of mutations of this kind a considerable amount of information could be drawn with respect to the genetic structure of natural populations. Accordingly, a series of experiments was started in May, 1961; the results through Generation 25 are reported here.

MATERIALS AND METHODS

Materials: The following two stocks were used in the present experiments: (1) W160S, an isogenic wild-type stock maintained by sib-mating for about five generations after the fourth chromosome of the isogenic line W160-*pol* (MUKAI 1961) was substituted by a wild-type chromosome. (2) C160, + (from W160S); *Ins(2)SM1, al²Cy sp²/In(2)Pm, dp b Pm ds³³ᵏ*; + (from W160S); + (from W160S), abbreviated as *Cy/Pm* (Curly/Plum).

The experimental materials were maintained in a culture room at about 24°C. The estimations of relative viabilities were conducted at 25°C except for the experiment in Generation 15 and one set of experiments in Generation 25, which were conducted at 21°C. In the maintenance of experimental lines as well as in the estimation of relative viability, 3cm × 10cm vials were employed. The medium used throughout the experiments consisted of water 1200ml, dry yeast 50g, agar 14g, molasses 100ml, cornmeal 50g, and propionic acid 5ml.

Experimental procedure: The methods for accumulating spontaneous mutations in the present experiments are essentially the same as those in the experiments of DOBZHANSKY, SPASSKY and SPASSKY (1952) and PAXMAN (1957). A single *Pm/+* male from a cross C160 × W160S was sampled and multiplied by the cross *Cy/Pm* (♀ ♀) × *Pm/+* (1 ♂), and 104 lines of *Cy/Pm* × *Pm/+$_i$* (i=1, 2, . . . , 104) were established. In each line, the second chromosome has been maintained through a single male by the cross *Cy/Pm* (5 ♀ ♀) × *Pm/+$_i$* (1 ♂) for the purpose of accumulating spontaneous mutations affecting viability. In every generation, *Cy/Pm* females were sampled from the original stock C-160. This procedure is schematically represented in Figure 1. According to this method, every recessive mutation has an almost equal probability to be established in the line, because it is introduced in heterozygous condition after selection in only one generation.

The test for the mutation rate is efficient if it is conducted in homozygous condition after the mutations are accumulated in the chromosome. In Generations 10, 15, 20, and 25, about 20 *Pm/+$_i$* males were sampled and crossed in the mass to *Cy/Pm* females in each line. In the first generation, *Cy/+$_i$* virgin females and males were collected and five pair-matings were conducted between them. Four days after the crosses were made, all flies in the vials were transferred to second vials and five days after the transfer all flies were eliminated from the vials. In the second generation, phenotypically Curly and wild-type flies that emerged before any F_2 fly could appear were counted. Consequently, the effects of spontaneous mutations which occurred

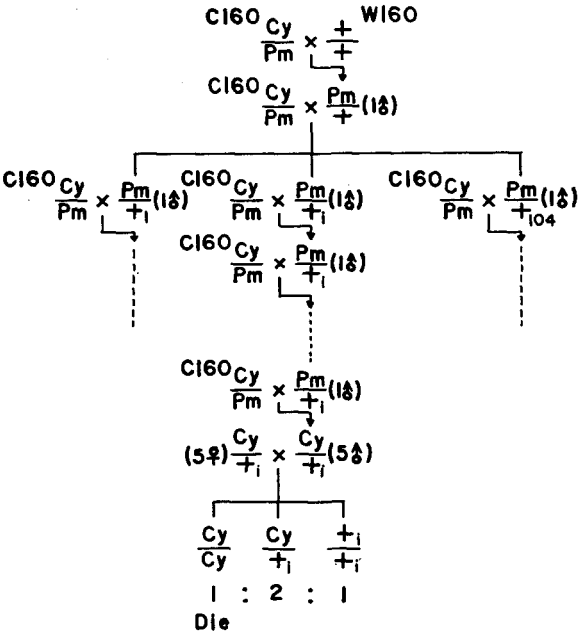

FIGURE 1.—Mating scheme for accumulation of mutations.

during the testing of two generations were confounded with those of environmental errors. The *Cy*-method of WALLACE (1956) was employed for the estimation of homozygotic viability of these second chromosomes. The counts of *Cy* flies from a pair of vials in each replicate of each line were pooled, and the sum was treated as a single value. The same sampling was done for wild-type flies. The numbers of replicates per line were not constant owing to the shortage of the parental *Cy* flies.

Suppose that there are r_i observations in Line i ($i = 1, 2, \ldots, i, \ldots, k$). In the jth observation (pooled data of two vials) of Line i, we count phenotypically Curly and wild-type flies whose numbers are represented by $(Cy/+_i)_j$ and $(+_i/+_i)_j$, respectively. The viability index of wild-type flies in the jth observation of Line i (v_{ij}) is expressed by Formula (1)

$$v_{ij} = 100 \times \frac{(+_i/+_i)_j}{n_{ij}} \tag{1}$$

where $n_{ij} = (Cy/+_i)_j + (+_i/+_i)_j$.

According to the *Cy*-method, the expected ratios of wild-type flies to the totals are 33.3 percent and 0 percent in the cases of absence of mutation and of recessive lethal mutations, respectively, and the percentages of wild-type flies were employed as viability indices.

In order to test the differentiation among quasi-normal lines, which are defined in this report as lines having viability index larger than 20, the method of analysis of variance is employed with respect to v_{ij}. The definitions of the parameters which can be estimated by the analysis of variance are as follows: σ^2_E = Error variance for line viability (variance among replicates in lines pooled over different lines). σ^2_G = Genetic variance between lines (variance among homozygotes, excluding lethal and deleterious lines).

Estimation of the average number of mutations in the second chromosome: According to BATEMAN (1959), the following Formula (2) can be obtained concerning the reduction of means and the increase of genetic variance caused by mutations, on the assumption that spontaneous mutations are distributed on the chromosome according to a Poisson distribution.

$$\left.\begin{aligned} \bar{a}\,p &= v_0 - \bar{v} \\ (\bar{a}^2 + \sigma^2{}_a)p &= \hat{\sigma}^2{}_G \end{aligned}\right\} \tag{2}$$

where a is the effect of a single mutation whose mean and variance are $\bar{a}$ and $\sigma^2{}_a$, respectively. The p indicates the average number of mutations in the second chromosome, and $\bar{v}$ stands for the mean of the viability indices of quasi-normal lines, and v_0 for its control. For the sake of simplicity, $v_0 - \bar{v}$ is equated to A and $\hat{\sigma}^2{}_G$ to B. Consequently, A and B are the estimated values. From Formula (2), the maximum $\sigma^2{}_a$ can be estimated. The $\sigma^2{}_a$ should be 0 or positive. Thus, with the condition that p takes a real value, the range of $\sigma^2{}_a$ becomes:

$$0 \leq \sigma^2{}_a \leq \frac{B^2}{4A^2} \tag{3}$$

The lower limit of p and the upper limit of $\bar{a}$ can be calculated from Formulae (2) and (3), and the results are:

$$\bar{a} \leq \frac{B}{A} \tag{4}$$

$$\frac{A^2}{B} \leq p \tag{5}$$

By the aid of Formula (5), the mutation rates of polygenes controlling viability may be estimated in terms of per chromosome per generation, and easily converted to an individual basis by multiplying by a factor proportional to the length of the chromosomes.

EXPERIMENTAL RESULTS AND ANALYSIS

Mutation rates to recessive lethal genes: It is impossible to ascertain the exact number of polygenic loci controlling viability. Thus, in this report, comparison of the polygenic mutation rate with that of major genes was performed on the basis of the second chromosome. Hence, the mutation rate to recessive lethal genes was estimated as a base for comparison with that of polygenes.

The number of lethal lines detected in each of the tested generations, i.e., Generations 10, 15, 20, and 25, is presented in Table 1. Since the results of Generation 25 are most reliable owing to the large number of chromosome generations, the rate was estimated on its basis.

When the test for Generation 25 was begun, we had 101 lines, i.e., three lines out of 104 had been lost by accident in the course of the experiment, and 15 out of 101 lines were recessive lethal lines. Under the assumption that recessive lethal genes are distributed on the chromosome according to the Poisson distribution, the mutation rate was estimated to be 0.0063 per second chromosome per generation. This value is entirely consistent with that of WALLACE (1956). From his data, the recessive lethal mutation rate is estimated to be 0.006 per second chromosome per generation. Thus, it can be concluded that W160S line does not have any mutators, and the mutation rate 0.0063 can be employed as a base for comparison of the mutation rate of polygenes controlling viability.

The following findings were obtained with respect to the origin of recessive lethals. Eight out of 101 lines had been classified as deleterious lines, at least once in the four tests including that of Generation 25. The present author calls mutations deleterious if they reduce homozygotic viability of the carriers to a viability

TABLE 1

Basic statistics and genetic parameters obtained in Generations 10, 15, 20, and 25

	Generation (and temperature)				
				25	
	10 (25°C)	15 (21°C)	20 (25°C)	(21°C)	(25°C)
Number of lethal lines	2	5	9	15	15
Number of deleterious lines	3	2	4	2	2
Number of quasi-normal lines	98	97	89	84	84
Number of missing lines	1*	0	2	3	3
Total number of flies counted in quasi-normal lines	140,328	240,473	383,543	453,999	472,720
Harmonic mean of number of flies counted in single lines ($\tilde{n}_L$)	1,225.26	2,356.21	3,864.79	5,261.22	5,584.25
Harmonic mean of number of flies counted in single observations ($\tilde{n}_I$)	270.86	262.39	509.37	594.55	602.94
Estimated error variance on the individual observation basis (σ^2_E)	10.987	16.898	8.019	5.639	6.645
Estimated error variance on the line basis (σ^2_L)	2.429	1.881	1.509	0.638	0.718
Estimated genetic variance (homozygote basis) (σ^2_G)	0.698	2.092	1.848	3.513	3.810
Average of the control viability indices (v_0)	32.94	29.69	32.23	33.06	32.92
Average of the viability indices of quasi-normal lines ($\tilde{v}$)	31.60**	27.89**	30.93**	28.37**	28.35**

* This line was not lost, but there was failure in expanding it to provide data for the particular test.
** Significantly different from v_0 at a very low level.

index less than 20 but more than 1. Five of the eight lines mutated to recessive lethals, one recovered to quasi-normal condition, one was still deleterious, and the last one was just detected as deleterious in Generation 25. Thus, five out of the 15 recessive lethals classified in Generation 25 were detected as deleterious at least once in the previous tests.

In Generation 10, two recessive lethal lines and three deleterious lines were detected. Thus, it is supposed that the mutation rate from a normal allele to a recessive lethal is not relatively higher than that from a normal to a deleterious allele. When these two findings are taken into consideration, the following conclusion is drawn, although the evidence is not very strong: The great majority of recessive lethal genes might arise after the change of wild-type genes to deleterious genes, and the mutation rate from deleterious to lethal is of surprisingly large magnitude.

Details of the work with special emphasis on the significance of this phenome-

non for the understanding of the genetic structure of natural populations will be published in another report of this series.

Estimation of the control viability: No control in a strict sense is available for this kind of experiment. However, the average viability of the control distribution (v_0) is the key to the estimation of the polygenic mutation rate in question. Thus, a detailed procedure of the estimation of the control viability index should be given here.

The control viability index is estimated by the following procedure: Five lines showing the best viabilities are sampled in each of Generations 15, 20, and 25. We assume that these selected lines do not contain any mutations in the second chromosome. In the estimation of control viability index in Generation 10, phenotypically Curly flies and wild-type flies of Generation 10 in the lines which are selected on the basis of data in Generations 15, 20, or 25 are pooled, respectively, and the percentages of wild-type flies are estimated on the basis of the pooled numbers as the control viability index in Generation 10. Those of Generations 15 and 20 are estimated by the same method as that in Generation 10.

It should be stressed here that an error of a line chosen above in a certain generation was uncorrelated with that of the same line in advanced generations, because they were tested in different environments and the genetic backgrounds of the experimental lines, which were originally the same as that of C160, were continuously substituted by that of C160. Thus, the present method provided us with unbiased estimates so long as the five lines showing the best viabilities did not contain any viability mutations. Should viability mutations have occurred, the estimates obtained are underestimates rather than overestimates.

The five lines showing the best viabilities in each of Generations 15, 20, and 25 are as follows: Generation 15: Lines 77, 15, 91, 72, and 21; Generation 20: Lines 104, 98, 99, 40, and 32; Generation 25: Lines 15, 91, 72, 37, and 92. The order in Generation 25 was decided on the basis of the sum of viability indices in the 21°C and 25°C experiments, because of no temperature effect and no interaction between temperatures and lines, as will be described later. Lines 15, 72, and 91 were

TABLE 2

Estimation of the average viability index of control distribution in Generation 10

Line No.	Number of observations	Number of (*Cy*)	Number of (+)	Total number	Viability index
15	4	1,083	530	1,613	32.86
21	3	848	371	1,219	30.43
32	6	954	475	1,429	33.24
37	3	665	308	973	31.65
40	5	1,468	688	2,156	31.91
72	4	757	432	1,189	36.33
77	6	1,406	699	2,105	33.21
91	5	899	471	1,370	34.38
92	4	1,016	549	1,565	35.08
98	5	1,205	606	1,811	33.46
99	5	1,191	527	1,718	30.68
104	4	877	419	1,296	32.33
Total	54	12,369	6,075	18,444	395.56
Average viability index			32.94 (weighted)		32.96

TABLE 3

Estimation of the average viability index of control distribution in Generation 15

Line No.	Number of observations	Number of (*Cy*)	Number of (+)	Total number	Viability index
15	7	1,301	643	1,944	33.08
32	7	1,163	454	3,001	28.08
37	10	2,080	921	2,089	30.69
40	9	1,490	599	2,246	28.67
72	8	1,530	716	1,617	31.88
91	10	2,021	987	3,008	32.81
92	8	1,651	713	2,364	30.16
98	9	2,306	816	3,122	26.14
99	10	2,268	935	3,203	29.19
104	9	2,275	852	3,127	27.25
Total	87	18,085	7,636	25,721	297.95
Average viability index			29.69 (weighted)		29.80

chosen in two generations. Thus, the control viability index (v_0) in Generation 10 was estimated by the average of Lines 15, 21, 32, 37, 40, 72, 77, 91, 92, 98, 99, and 104. The observed numbers of flies and viability indices in those lines are shown in Table 2. The result thus obtained was 32.94 (weighted mean) and 32.96 (arithmetic mean). These two estimates were almost equal, and the mean weighted by the number of observed flies, 32.94, was employed as the v_0 in this generation, because those chosen lines were assumed to have no mutations and to be identical as far as the second chromosomes are concerned.

The selected lines in Generation 15 on the basis of the order of degree in Generations 20 and 25, and the numbers of flies counted in those lines of Generation 15, are listed in Table 3. The v_0 became 29.69.

The selected lines and the numbers of flies counted in those lines in Generation 20 are presented in Table 4. The result shown in Table 4 indicates that the control viability in Generation 20 was estimated to be 32.23.

The above method cannot be applied for the estimation of the control viability in Generation 25. Fortunately, we have estimated homozygotic viabilities at 21°C and 25°C for the same lines, and the calculated phenotypic correlation coefficient

TABLE 4

Estimation of the average viability index of control distribution in Generation 20

Line No.	Number of observations	Number of (*Cy*)	Number of (+)	Total number	Viability index
15	8	4,464	2,197	6,661	32.98
37	8	3,560	1,608	5,168	31.11
72	7	2,412	1,115	3,527	31.61
91	7	1,807	906	2,713	33.39
92	4	1,504	713	2,217	32.16
Total	34	13,747	6,539	20,286	161.25
Average viability index			32.23 (weighted)		32.25

($r = 0.86$) indicates that the great majority of lines have at least one viability mutation. As will be seen below, interaction between lines and temperatures is negligible. Thus, the correlation between the two estimates of the same lines were employed for the estimation of the control viability. The correlation coefficient between the two estimates for each of nine lines, whose viability indices were larger than 30.00 both at 21°C and 25°C, was 0.87, but if the top line (Line 15) (which was assumed to have had supervital mutation as will be described later), and the last two lines in the order of degree of the nine lines, were eliminated, the correlation coefficient in the remaining six lines became 0.0228. Thus, these six lines (16, 37, 58, 72, 91, and 92) were chosen as control lines. The observed numbers of flies and the viability indices in these six lines are shown in Table 5. From this table, v_0 in Generation 25 was estimated to be 32.99. It should be stressed here that this estimated v_0 value is highly reliable because it was estimated on the basis of counting 77,007 flies.

Thus, the estimated v_0's are 32.94, 29.69, 32.23, and 32.99 in Generations 10, 15, 20, and 25. They seem to be mutually consistent except for Generation 15. A conceivable reason for this phenomenon will be discussed later.

Generation 10: The outline of the observations in Generation 10 is presented in Table 1 with the data for Generations 15, 20, and 25. The distribution pattern of viability indices on line basis ($100 \sum_j (+_i/+_i)_j / \sum_j n_{ij}$) is presented in Figure 2 with those of Generations 20 and 25 (estimation at 25°C). An analysis of variance was performed with respect to the estimated viabilities of 98 quasi-normal lines, with two to seven replicated observations for each line. The result is given in Table 6. Although no significant differentiation among these lines with respect to viability was found, the probability value was very close to 0.05. Genetic variance among quasi-normal lines in homozygous condition estimated from Table 6 becomes $\hat{\sigma}^2_G = 0.6980$.

The average viability index ($\bar{v} = 31.60$) was compared with the control ($v_0 = 32.94$) by an approximate t-test. The result indicates that these two values are

TABLE 5

Estimation of the average viability index of control distribution in Generation 25

Line No.	21°C				25°C			
	(*Cy*)	(+)	Total number	Viability index	(*Cy*)	(+)	Total number	Viability index
16	4,490	2,143	6,633	32.31	4,420	2,121	6,541	32.43
37	4,297	2,064	6,361	32.45	4,336	2,175	6,511	33.41
58	4,068	1,996	6,064	32.92	3,830	1,867	5,697	32.77
72	4,289	2,198	6,487	33.88	4,235	2,016	6,251	32.25
91	4,289	2,232	6,521	34.23	4,361	2,193	6,554	33.46
92	4,380	2,113	6,493	32.54	4,608	2,286	6,894	33.16
Total	25,813	12,746	38,559	198.33	25,790	12,658	38,448	197.48
Average viability index	33.06 (weighted)			33.06	32.92 (weighted)			32.91

Pooled estimates: Weighted mean = 32.99; arithmetic mean = 32.99.
The number of observations in each line was nine in each experiment.

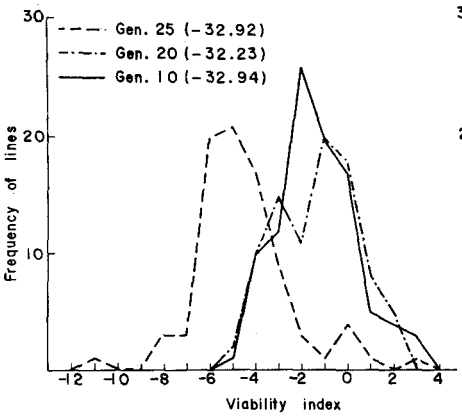

FIGURE 2.—Distribution patterns of viability indices in Generations 10, 20, and 25 (25°C).

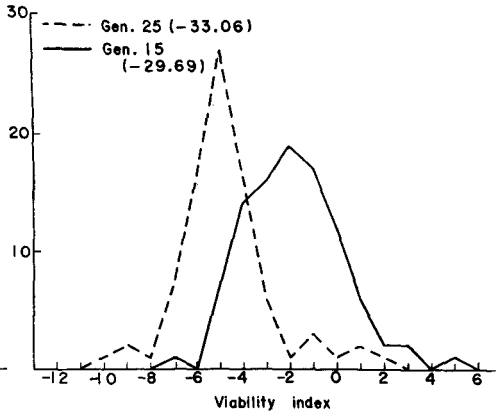

FIGURE 3.—Distribution patterns of viability indices in Generations 15 and 25 (21°C).

significantly different at the one percent level. Thus, it can be concluded that many mutations having small effects on viability had taken place in these quasi-normal lines.

Generation 15: After the crosses of $Cy/+_i \times Cy/+_i$ for the estimation of viability indices, we came to realize that we have been working at about 21°C, owing to a change in the temperature of the culture room. Thus, greater care was taken in classifying the *Cy* flies. The result obtained in this generation was not always consistent with those from Generations 10, 20, and 25, especially in the mean viability of quasi-normal lines, as described above.

TABLE 6

Analysis of variance with respect to viabilities of quasi-normal lines

Source	Sum of squares	Degrees of freedom	Mean square	F	Expected mean square
(a) Generation 10					
Between lines	1,336.61	97	13.78	1.25	$\sigma_E^2 + 4.0\,\sigma_G^2$
Error	3,592.87	327	10.99		σ_E^2
Total	4,929.48	424			
(b) Generation 15					
Between lines	3,308.41	96	34.47	2.04**	$\sigma_E^2 + 8.4\,\sigma_G^2$
Error	12,385.93	733	16.90		σ_E^2
Total	15,694.34	829			
(c) Generation 20					
Between lines	1,884.21	88	21.41	2.67**	$\sigma_E^2 + 7.2\,\sigma_G^2$
Error	4,586.81	572	8.02		σ_E^2
Total	6,471.02	660			

** Significant at a very low level.

Condensed data for this generation are given in Table 1. The distribution pattern of viability indices on a line basis is presented in Figure 3, together with that of Generation 25 (estimation at 21°C). Analysis of variance was conducted with respect to the estimated viabilities of 97 quasi-normal lines with six to ten replicated observations in each line; the result is given in Table 6. It is clear from Table 6 that there is a significant differentiation among these lines with respect to viability. The genetic variance caused by mutations was estimated to be $\hat{\sigma}^2_G = 2.0918$. As described above, the control viability (v_0) was estimated to be 29.69. This value is significantly smaller than those for Generations 10, 20, and 25 given in Table 1. It was thought at the beginning that low temperature might have caused the decrease of v_0, but the experimental results in Generation 25 indicate that this speculation was not sound. Some bacteria were prevalent in the culture media in early generations, especially in the test cultures in Generation 15. They might have caused the decrease of v_0 in question, in cooperation with the low temperature. Indeed, the average number of flies in one observation was extremely low in this generation (289.7) as compared with Generations 20 and 25 (560.7 and 622.0), and the error variance was large.

The difference between the average viability and the control ($\bar{v} - v_0$) was tested by an approximate t-test and the result indicates that the difference is significantly different from zero, and it is consistent with that of Generation 10.

Generation 20: Similarly as in Generations 10 and 15, the condensed data are presented in Table 1, and the analysis of variance was carried out with respect to the viabilities of 89 homozygously quasi-normal lines with four to eight replicated observations for each line; the result obtained is shown in Table 6. Significant differentiation in viabilities can be detected among these lines. Genetic variance on homozygote basis was estimated to be $\hat{\sigma}^2_G = 1.8481$. The distribution pattern of viabilities of quasi-normal lines is presented in Figure 2. In this figure, it is impossible to detect intuitively the difference between the distribution pattern of Generation 10 and that of Generation 20. This is mainly caused by the larger error variance in Generation 10 than that in Generation 20, because we counted a smaller number of flies in the former than in the latter.

The difference between the average viability ($\bar{v} = 30.93$) and the control ($v_0 = 32.23$) was tested by the same method employed in early generations; it is significantly different from zero.

Generation 25: As mentioned above, it was thought that moderately low temperature might have caused the decrease of average viability and increased the genetic variance in Generation 15. Thus, the experiments were conducted both at 21°C and 25°C for this generation. The outlines of the observations in the 21°C and 25°C experiments are presented in Table 1. Analysis of variance was performed with respect to the estimated viabilities of 84 quasi-normal lines with four to nine replicated observations for each line of each experiment. The results are given in Table 7. Significant differentiation was detected among lines as in the previous generations. Since there was no effect due to temperature and the interaction between temperatures and lines was not significant, genetic variance was estimated on the pooled basis of the two temperature conditions. In the

TABLE 7

Analysis of variance with respect to viabilities of quasi-normal lines in Generation 25

Source	Sum of squares	Degrees of freedom	Mean square	F	Expected† mean square
Between lines	5,908.72	83	71.19	11.58**	$\sigma^2_E + 17.9\,\sigma^2_G$
Between temperatures	0.02	1	0.02		$\sigma^2_E + 8.8\,\sigma^2_{L\times T} + 738.4\,\Sigma\tau^2_i$
Interaction between temperatures and lines	460.19	83	5.54		$\sigma^2_E + 8.8\,\sigma^2_{L\times T}$
Error	8,124.65	1,321	6.15		σ^2_E
Total	14,493.58	1,488			

** Significant at a very low level.
† Approximation but very close to the real values.
τ_i indicates effect of the temperature and $\Sigma\tau_i = 0$.
$\sigma^2_{L\times T}$ stands for the variance of the effects of interaction between lines and temperatures.

calculation of the expected mean square in Table 7, the harmonic mean of subclass sample size was substituted for the formulae for equal subclass sample size. Hence, the formulae presented in Table 7 are approximate but very close to the exact formulae, because the variance of subclass sample size is extremely small, i.e., in most of the lines, the number of observations was nine. The distribution patterns of homozygote viabilities in the 21°C and 25°C experiments are presented in Figures 3 and 2, respectively. From these two figures, we can intuitively recognize that polygenic viability mutations accumulated in the lines with succeeding generations.

Contrary to the expectation, detection of a difference in the effect of temperature on the relative viability of wild-type and *Cy* flies was impossible. The difference between the average viability ($\bar{v} = 28.36$) and the control ($v_0 = 32.99$) was tested by an approximate t-test, which indicates that these two values are significantly different from each other, and this is consistent with the results in the previous generations.

The correlation coefficient between the estimates of the same line at the two temperatures was calculated; the correlation table is given in Figure 4. The phenotypic correlation coefficient (r) became +0.86. In addition, the correlation coefficient between the two estimates for each line whose viability index was greater than 30.00 in the 25°C experiment was also calculated to be $r = +0.91$. Such large values seem to have been obtained for the following reasons: First, almost all of the lines had at least one homozygously deleterious viability mutation. As an exceptional case, Line 15 might have had at least one supervital mutation in homozygous condition. The viability index of this line was 35.32 ± 0.74 at 21°C and 35.88 ± 0.28 at 25°C. Secondly, since a large number of flies were counted (more than 10,000 flies at any single point in the correlation table), the high correlation coefficient was estimated accurately.

Rate of increase of variance and rate of decrease of mean of viability indices of quasi-normal homozygotes: If the effects of polygenic viability mutations are

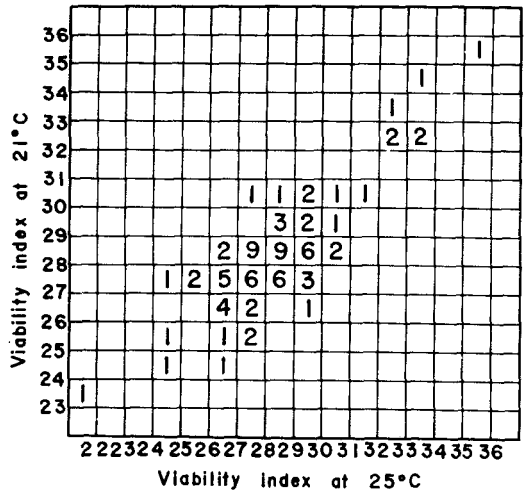

FIGURE 4.—Correlation table between the estimates of viabilities at 21°C and at 25°C in Generation 25.

additive or multiplicative, although in the latter case the effect of each mutation should be small as compared with the viability of standard normal homozygotes, the genetic variance among the lines increases approximately linearly in the course of generations and the mean of quasi-normal homozygote viabilities decreases also linearly. The linearity of variances and means might be examined graphically. Indeed, the number of degrees of freedom is too small to carry out an appropriate statistical test for linearity.

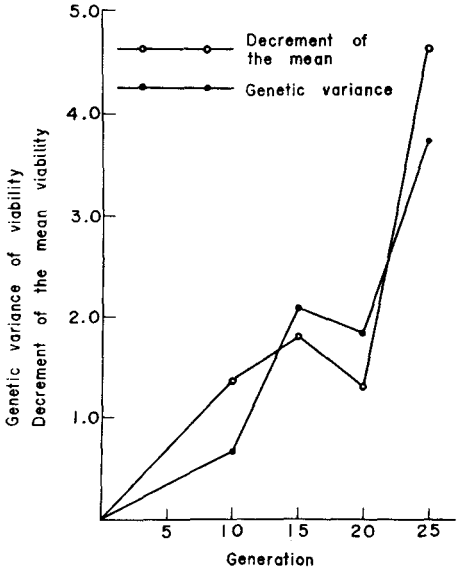

FIGURE 5.—Relationships of generation number to the genetic variance of viability and the decrement of mean viability.

The relationships of generation number to reductions of means, $v_0 - \bar{v}$ (estimated from the results presented in Table 1), and to genetic variances are represented graphically in Figure 5. From Figure 5, it might be said that the variance of viability increases and the mean decreases approximately linearly, although the decrement of mean viability in Generation 20 was unusual. This has probably been caused by underestimation of the control viability ($v_0 = 32.23$). Hence, it was concluded that: (1) The average number of mutations per individual chromosome increases linearly with generations; and (2) effects of interaction of new mutant genes with each other, and with already existing genes in homozygous condition, are not of appreciably large magnitude in the homozygous genetic background, at least when the number of mutations per individual is small. For reference, it should be mentioned that the result of the analysis, which will be described below, indictates that at least two polygenic mutations were, on the average, included in single second chromosomes in Generation 25.

Under the assumption of linear relationships, regression coefficients of the decrement of the mean on generation (b_M) and that of the variance on generation (b_V) were calculated without weighting. The results are: $b_M = 0.1261 \pm 0.0247$, and $b_V = 0.1127 \pm 0.0189$. These values are significantly different from zero at the 1 percent level. The estimates of mean viability and the genetic variance might have to be weighted by the inverse of the variance, when the regression coefficients are calculated. However, estimated variances of the genetic variance are not reliable, since fourth degree statistics are involved in those calculations. Thus, weighting was not carried out.

Estimation of the rate of polygenic mutations controlling viability: The lower limit of the rate of polygenic mutations controlling viability was estimated by the aid of Formula (5) and the regression coefficients of the decrease of the mean of viability and the increase of the variance of viability.

A and B in Formula (5) were substituted by $b_M = 0.1261$ and $b_V = 0.1127$, respectively. At the same time, the range of σ_a^2 and the upper limit of $\bar{a}$ were estimated by using Formulae (3) and (4). These results are given in Table 8, together with the mutation rate of recessive lethals estimated above. It should be noted here that the present estimate of polygenic mutation rate is not the lower limit in statistical sense. It could become smaller than the present estimate if A and B have variances and σ_a^2 is nearly zero. However, the assumption concerning σ_a^2 is not real. Hence, it is actually impossible to calculate the statistical lower limit of polygenic mutation rate estimated by the present method.

TABLE 8

The characteristics of polygenic mutations affecting viability

Item	Estimate
Number of mutations/generation (p)*	$0.1411 \leqslant p$
Average effect of single mutations ($\bar{a}$)	$\bar{a} \leqslant 0.8937$
Range of σ_a^2	$0 \leqslant \sigma_a^2 \leqslant 0.1997$

* Second chromosome basis.

From Table 8, the following conclusions were obtained: First, the rate of polygenic mutations affecting viability is of extremely large magnitude (ca. 0.15 per second chromosome per generation), when it is compared with that of recessive lethal genes (ca. 0.0063 per second chromosome per generation), and approximately 20 times as high as that of recessive lethal mutations estimated simultaneously. Secondly, the magnitudes of single mutations are extremely small. That is to say, polygenic mutations, whose individual effects are very small, are occurring at a high rate, and are supplying natural populations with genetic variation. Further experimental evidences supporting the present conclusion are given in the Discussion.

DISCUSSION

Experimental technique: In the present experiment, we did not use the *Cy-Pm* method (WALLACE 1956), because classification would be too tedious and the error variances estimated by this method would be larger than those by the *Cy*-method (SPASSKY, SPASSKY, PAVLOVSKY, KRIMBAS, KRIMBAS and DOBZHANSKY 1960). Although the viability of $Cy/+_i$ as the standard in the *Cy*-technique might vary to some extent from line to line owing to the independent occurrence of mutations, the amount of variation is negligible compared with that in the homozygote $+_i/+_i$. Indeed, WALLACE and DOBZHANSKY (1962), and WALLACE (1962) have shown by their abundant data almost complete dominance of *Cy* chromosomes over various kinds of chromosomes that originated from artificial populations. With the *Cy* method, if complete replacement takes place in a vial by an increased number of *Cy* flies compensating for the decreased number of wild-type flies resulting from viability reduction caused by mutations, the percentage of the number of wild-type flies, as viability index, is better than the ratio of wild-type flies to *Cy* flies in the same vial, because the number of polygenic mutations is, on the average, proportional to the amount of viability-index reduction. When the replacement does not take place, the latter expression is more appropriate than the former on the same criterion. Although correlation between the number of observed individuals in a vial and the viability index expressed as the percentage of wild-type flies was about 0.3, which indicates that there was not complete replacement by the *Cy* flies for the decreased number of wild-type flies due to mutations, a compromise was made after the following considerations: When the percentage of wild-type flies is employed as viability index, its distribution is theoretically binomial, where the viability index (v) and the variance are positively correlated, if v is less than 50 percent. Usually, in such a case, a transformation of the data is performed. However, a binomial distribution will approach the normal when the number of individuals counted in one observation becomes large. In the present experiment, we have counted a number of flies in one observation, and we can expect an approximately normal distribution. In addition, correlation between the v and its variance might not exist on the ground of a biological characteristic. Error variance has two components: sampling variance, $[v(1-v)]/n$, and environmental variance. The former is positively correlated with v, when v is smaller than 50 percent. However, if the latter is negatively

correlated with v, we might expect approximately no correlation between viability index and error variance and might be able to accept the homogeneity of error variance. In reality, this expectation was almost borne out in the present experiment (in Generations 10, 15, 20, and 25, $r = +0.282$, -0.083, $+0.161$, and -0.073, respectively); the figures are not significantly different from zero except for Generation 10, where $P < 0.01$. Thus, the analysis was conducted on the percentage basis without any transformation.

High mutation rate of polygenes: From the present experiment, the mutation rate of polygenes controlling viability has been estimated to be, as expected, of extremely large magnitude, i.e., it is approximately 20 times as high as that of recessive lethal genes on the chromosome basis, and the average contribution of mutant polygenes has been estimated to be very small.

The former finding is supported by the following experimental results. First, the genotypic correlation between the viabilities estimated at 21°C and those estimated at 25°C in the same lines was estimated to be 1.0 in Generation 25. This high value can be understood only by assuming that most of the lines had differentiated from the original owing to polygenic mutations that occurred independently, because in the starting generation, all the lines had exactly the same genotype with respect to the second chromosome.

Second, as described above, the line showing the second greatest viability and five lines following in the order of degree in Generation 25 were assumed to have had no mutation because the phenotypic correlation between viabilities at 21°C and 25°C was 0.0228, i.e., six lines out of 84 had no viability mutations at all. The mutation rate per second chromosome per generation (p) can be estimated to be 0.1056 under the assumption of a Poisson distribution. This estimate is very close to 0.1411 and supports the result of the high mutation rate of polygenes controlling viability. The latter figure is much more reliable than the former, because the former was estimated on the basis of only one bit of information, i.e., six lines out of 84 probably did not have any viability mutation.

Third, in parallel with matings for the Generation-25 test, crosses were made to obtain flies heterozygous for chromosomes from different lines. The crossing scheme used was to secure random combinations of second chromosomes from different lines with homozygous genetic backgrounds. Viabilities of these heterozygotes were determined, and a correlation analysis was made between heterozygote viability and mean viability of the two corresponding homozygotes (MUKAI, CHIGUSA, and YOSHIKAWA, in preparation). The estimate of genotypic correlation obtained was 0.75. This result not only indicates incomplete dominance of original polygenes over mutant polygenes in *trans*-phase in the homozygous genetic background, but also supports the view of the high mutation rate of polygenes. In a *cis*-phase test, the genotypic correlation between heterozygote viability and homozygote viability became negative. Therefore, the positive genotypic correlation obtained above does not necessarily imply absence of overdominance with respect to viability (MUKAI, CHIGUSA, and YOSHIKAWA, in preparation).

Evidence supporting the high mutation rate of polygenes can be obtained from an experiment of DURRANT and MATHER (1954). They conducted the test for heritable variation in viability of second chromosomes sampled from an inbred Oregon line of *D. melanogaster* maintained

for more than 300 generations of continuous brother-sister mating, and found a sizable amount of heritable variation which might have been caused by the balance between mutation and selection pressures during the inbreeding. This finding supports the occurrence of polygenic viability mutations at high rate.

As a conflicting evidence, PAXMAN's experimental result (1957) might be mentioned. He carried out an experiment which was essentially the same as the present author's, but he used only seven lines. The result of the analysis with respect to viability indicated neither a change of overall means nor any difference between lines even in the test of Generation 41. This finding is completely inconsistent with the present experimental result. The contradiction might have been caused by the small number of flies counted in each line and especially by the small number of lines employed in PAXMAN's experiment.

As to the radiation-induced mutation rate, several investigators have conducted experiments. Although he did not intend to estimate the mutation rate, WALLACE (1957, 1958, 1959) irradiated sperm of *D. melanogaster* with 500r of X rays, and tested heterozygous effects of the induced mutations on viability. He detected that the viability of the heterozygotes with respect to the induced mutations increased, on the average, by approximately 1.5 percent of the control. MULLER and FALK (1961) have criticized WALLACE's data on the basis that the average heterotic effect of the induced mutations in WALLACE's experiment should have been of the order of 9 percent, if the experimental results of MULLER and MEYER (1959) were accepted with respect to the rate of mutations, whose viability effects are as little as 5 percent reduction in homozygous condition. However, the following inference might be more reasonable than the above critique: Mutations which reduce the viability of the homozygous carriers by less than 5 percent have occurred much more frequently than more deleterious mutations, and have contributed a great deal to the improvement of viability of their heterozygous carriers. Thus, the result of WALLACE's large scale experiment directly indicates a high rate of radiation-induced mutations controlling viability.

MUKAI (1961) estimated the number of viability mutations in the second and third chromosome of *D. melanogaster* to be about 0.37 on the average per one set of those chromosomes, using the results of BURDICK, MUKAI, and KRAWINKEL's experiment (1959) in which they tested the heterozygous effect of mutations induced with 100r of X rays at the spermatid stage. This result indicates that the radiation-induced mutation rate of polygenes controlling viability is substantially larger on a chromosome basis than that of major genes, and that the magnitude of the so-called "doubling dose" of polygenes is of the same order as that of major genes, when their experimental result, although they irradiated spermatids, is compared with the present estimated rate of spontaneous polygenic viability mutations.

All the above experimental evidence except that of PAXMAN (1957) indicates that both spontaneous and radiation-induced mutation rates of polygenes controlling viability are extremely high, and supports the conclusion of the present experiment.

As an alternative hypothesis, it might be assumed that there are several mutable loci where most of the mutations detected in the present experiment took place. However, this hypothesis might be rejected, although the evidence is not strong, on the basis of the consideration that the mutation rate to recessive lethal genes estimated in the present experiment was ca. 0.0063 per second chromosome per generation, as described above, and this rate is the standard recessive lethal mutation rate (WALLACE 1956). If there had been such mutable loci, the recessive lethal mutation rate would have been higher than the present estimate, because lethal mutation rates in such loci would be generally high.

Mutation rate of polygenes and the genetic structure of natural populations: There are two principal hypotheses concerning the genetic structure of natural populations. According to the terminology of DOBZHANSKY (1955), they are the classical hypothesis, founded on mutant genes being detrimental both in homo-

zygous and heterozygous condition, and the balance hypothesis, in which the existence of overdominance is accepted.

Recently, the concept of genetic load was defined by CROW (1958), and was introduced in discussions of the genetic structure of natural populations. If the classical hypothesis is valid, the genetic load is in the greater fraction of loci mutational, and its magnitude is approximately 2μ per locus (μ = rate of mutation from wild-type gene to mutant allele) in autosomes at equilibrium in a random mating population (MULLER 1950), disregarding completely-recessive genes with respect to fitness. For the sex chromosome, the magnitude is 1.5μ ($\frac{(3\text{-}h)\mu}{1+h}$ in males and $\frac{4h\mu}{1+h}$ in females) where a very small h is the degree of dominance of a mutant gene over its wild-type allele (KIMURA 1961) ($\bar{h} = 0.04$, STERN, CARSON, KINST, NOVITSKI and UPHOFF 1952).

We have estimated a high mutation rate of viability polygenes in the second chromosome. This result may be extended to all the other chromosomes, because we have no reason to single out the second chromosome since all genes in an individual are anyhow related to the expression of viability. Thus, we can estimate the total polygenic mutation rate of one gamete on the basis of the proportionality between the frequency of mutations and the length of the chromosomes. It becomes approximately 0.35 per X-chromosome-bearing gamete, a surprisingly large magnitude.

Hence, it can be said that natural populations are supplied with a great amount of genetic variation by spontaneous polygenic mutations. If the classical hypothesis is valid, the high rate of polygenic mutation results in a large mutational load. Detailed discussion about this problem will be given in another report of this series, after obtaining additional information.

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SUMMARY

Spontaneous polygenic mutations affecting viability were accumulated under minimum selection pressure in 104 second chromosomes that originated from a single chromosome of an isogenic strain. A *Cy-Pm* inversion technique was used.

The magnitudes of the decrements of mean viabilities and the increments of genetic variances were estimated in homozygous condition for Generations 10, 15, 20, and 25 by counting approximately 1.7 million flies in total. The increase in genetic variance and the decrease in the mean, thus obtained, are 0.1127 and 0.1261 per generation, respectively, on the scale of the *Cy* method (WALLACE 1956).

The lower limit of mutation rate was estimated to be 0.1411 per chromosome per generation by use of the above estimates. This is approximately 20 times as high as the recessive lethal mutation rate (ca. 0.0063 in the present experiment).

The range of the variance of the effect of single mutations on viability was estimated to be 0 to 0.1996, and the upper limit of the average effect of single mutations to be 0.8937.

From these findings, it was concluded that natural populations have been supplied with a large amount of genetic variation by spontaneous polygenic viability mutations whose individual effects are very small.

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