THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*. XVI. EXCESS OF ADDITIVE GENETIC VARIANCE OF VIABILITY

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ABSTRACT

About 500 second and 500 third chromosomes were extracted, using the marked inversion technique, from the Orlando-Lake Placid, Florida, population. From the experiments using these chromosomes, the following findings were obtained: (1) The frequencies of lethal-carrying chromosomes were 0.37 in the second and 0.55 in the third chromosomes. (2) The size of the population was estimated to be effectively infinite, on the basis of the allelism rate of lethal-carrying chromosomes. (3) The detrimental and lethal loads for viability were, respectively, 0.40 and 0.45 for the second and 0.52 and 0.78 for the third chromosomes. Consequently, the detrimental to lethal load ratio is 0.90 for the second and 0.67 for the third chromosomes. (4) Lethal genes were shown to be deleterious when heterozygous. (5) The average degree of dominance for mildly deleterious genes (viability polygenes) was estimated to be nearly 0.5, although the confidence interval is large. (6) Additive (σ_A^2) and dominance (σ_D^2) variances of viability were estimated by using a partial diallel cross method. The results were $\hat{\sigma}_A^2 = 0.0227 \pm 0.0042$ and $\hat{\sigma}_D^2 = 0.00080 \pm 0.00062$ for the second chromosomes. (7) Environmental variances of viability were estimated. The result indicates that the heterozygotes are more homeostatic than the homozygotes. The most striking finding is that the additive variance is larger than expected on the classical hypothesis from the detrimental load. Several possible explanations for the discrepancy are offered. The most likely cause, we suggest, is genotype-environment interaction (diversifying selection) acting on viability polygenes. Overdominance is inconsistent with the low dominance variance, and frequency-dependent selection also appears unlikely as an explanation.

THE findings of recent experimental work appear to be unfavorable to the universality of overdominance (*e.g.*, MUKAI and YAMAGUCHI 1974; MUKAI, WATANABE and YAMAGUCHI 1974; MUKAI *et al.* 1974; MUKAI, TACHIDA and ICHINOSE 1980; MUKAI *et al.* 1982) and frequency-dependent selection (*e.g.*, DOLAN and ROBERTSON 1975; YOSHIMARU and MUKAI 1979) as mechanisms for the maintenance of genetic variability in random mating populations. The remaining candidate among balancing selection mechanisms is diversifying selection, or the maintenance of genetic variability due to genotype-environment interaction (LEVENE 1953), to which much attention has been paid by population geneticists.

Although in finite populations diversifying selection may either increase or

decrease genetic variability in comparison with neutral genes under some conditions (TAKAHATA 1981), it certainly increases genetic variability in comparison with mutation-selection balance. Previously, we analyzed genetic variability maintained in the Raleigh, North Carolina, population and found that the additive variance for viability is higher than expected on the basis of mutation-selection balance (MUKAI *et al.* 1974) and a very small dominance variance.

To obtain further information, we have analyzed the Orlando-Lake Placid, Florida, population: the homozygous genetic load, the components of genetic variance and the environmental variance for viability were estimated. The results agree with those of the North Carolina population and will be reported in this paper.

Before discussing the main subject, it should be stressed here that the subject of these experiments is *genetic variability of viability*. Therefore, in addition to viability, only the pleiotropic effects or possibly linkage effects of viability genes on other fitness components contribute to this variability. Genes affecting only fitness components other than viability are not studied in the present experiment. The object is to understand the maintenance of genetic variance of viability.

MATERIALS AND METHODS

Extraction of second and third chromosomes: Adult males were collected in Orlando and Lake Placid, Florida, in December 1972 by F. M. JOHNSON. From these flies about 500 second and 500 third chromosomes were extracted independently using the marked inversion method (*cf.* WALLACE 1956): *In(2LR) SM1/In(2LR)bw*^{V1} for the second and *In(3LR)TM3/Pr* for the third chromosomes were used. *In(2LR)SM1* and *In(3LR)TM3* are marked by the *Cy* (Curly) and *Sb* (Stubble) genes, respectively. After chromosome lines were established, they were maintained in the 19° stockroom, together with the *In(2LR)SM1* or *In(3LR)TM3* which help to maintain less viable or lethal chromosomes. Cytological examination of the salivary gland chromosomes was made for each chromosome line by R. A. VOELKER after making a cross to a standard wild type (W507). All experiments were completed approximately 1.5 years after collecting the flies. There was no evidence for a significant amount of accumulation of spontaneous mutations during this period since the genetic variance did not increase with later experiments.

Estimation of homozygote and heterozygote viabilities of the chromosome lines: Homozygote and heterozygote viabilities were estimated (WALLACE 1956). Crosses were made between $5Cy/+_i$ females and $5Cy/+_i$ males with two simultaneous replications in each chromosome line, where i indicates line number. *Cy* stands for *In(2LR)SM1*-carrying chromosome. In the offspring, $Cy/+_i$ and $+_i/+_i$ flies segregate in an expected ratio of 2:1. The viabilities of random heterozygotes were estimated in a similar manner, combining two successively numbered lines, *i.e.*, $5Cy/+_i$ (♀♀) × $5Cy/+_{i+1}$ (♂♂). The last line was crossed to the first. As in the case of homozygotes, two simultaneous replications were made. In both cases, 4 days after crosses were made, all ten flies in a vial were transferred to a second vial. Four days after the transfer was made, all flies were discarded. In both vials, the original and transferred, all emerged flies were counted at four different times until the 18th day after the cross or transfer was made. *Cy* flies and wild-type flies from the pair of vials were pooled and considered a single observation. The viability was expressed at first as the ratio of the number of wild-type flies to the number of *Cy* flies plus 1 (*cf.* HALDANE 1956). Crosses were made at eight different times (eight replications). About 60 lines were employed per replication. Homozygote and heterozygote viabilities were estimated at the same time within replications. Before the analyses were made, all viabilities were standardized to the average viability of the heterozygotes within replications.

The test for the third chromosomes was conducted in the same way as for the second chromosomes, but *In(3LR)TM3* marked by *Sb* was used instead of *In(2LR)SM1*.

Estimation of homozygous load: Using the formulas given by GREENBERG and CROW (1960), we estimated the total homozygous load (T), detrimental load (D) and lethal load (L) as follows:

$$\begin{aligned} T &= \ln A - \ln B \\ D &= \ln A - \ln C \\ L &= \ln C - \ln B \end{aligned} \tag{1}$$

where A is the average viability of random heterozygotes, B is the average viability of all homozygote lines and C represents the average viability of homozygote lines excluding lethal-carrying lines. In the present paper, any line having a standardized viability index less than 0.1 was classified as lethal. It should be noted that in the present method D and T estimate the homozygous loads with respect to the average viability of random heterozygotes, not to the optimum homozygote. Thus, their expected values can be expressed as follows:

$$\begin{aligned} T &= L_{II} + L_{Id} - L_R \\ D &= L_{Id} - L_R \\ L &= L_{II} \end{aligned} \tag{2}$$

where L_R, L_{Id} and L_{II} are random genetic load, detrimental load and lethal load, respectively, with respect to the optimum genotype in the population.

Estimation of average degree of dominance of viability polygenes: Since the average degrees of dominance of lethal genes and semilethal genes are different from the average degree of dominance of viability polygenes, heterozygotes whose constituent chromosomes showed viability indices larger than 0.6 (standardized viability) were employed for the estimation of the average degree of dominance of viability polygenes.

The average degree of dominance in an equilibrium population ($\bar{h}_E$) can be estimated approximately as follows. Under the classical hypothesis, namely, that genetic variability is maintained by the balance between mutation and directional selection pressures (*cf.* MUKAI and YAMAGUCHI 1974),

$$\hat{\bar{h}}_E \cong \beta_{Y \cdot X} = \frac{\mathrm{Cov}(X, Y)}{V(X)} \tag{3}$$

where $\beta_{Y \cdot X}$, $V(X)$ and $\mathrm{Cov}(X, Y)$ are the regression coefficient of the viability of a heterozygote (Y) on the sum of the homozygous viabilities of the two constituent chromosomes (X), the variance of X and covariance between X and Y, respectively. According to MORTON, CROW and MULLER (1956), $\bar{h}_E$ or $\beta_{Y \cdot X}$ is approximately equal to the harmonic mean of the degrees of dominance of newly arisen mutant viability polygenes ($\tilde{h}_N$). MUKAI and YAMAGUCHI (1974) showed that the inverse of the regression coefficient of the sum of the homozygous viabilities of two constituent chromosomes on heterozygote viability ($1/\beta_{X \cdot Y}$) in an equilibrium population estimates the average degree of dominance of newly arisen mutant viability polygenes; that is,

$$\hat{\tilde{h}}_N \cong 1/\beta_{X \cdot Y} = \frac{V(Y)}{\mathrm{Cov}(X, Y)} \tag{4}$$

where $V(Y)$ is the variance of Y.

Using formulas (3) and (4) we estimated average degrees of dominance.

Estimation of the components of genetic variance of viability: To estimate the components of genetic variance for viability, 7 × 7 partial diallel crosses [or North Carolina Design II of COMSTOCK and ROBINSON (1952), or Factorial experiment] were conducted in the same way described by MUKAI *et al.* (1974). For each set of partial diallel crosses, 14 chromosome lines were selected at random $Cy/+$ heterozygotes. Seven lines were used for the Row ($Cy/+_i$, i = 1–7), and the other seven were for the Column ($Cy/+_j$, j = 8–14). Within each cell, reciprocal crosses were made with two simultaneous replications. Twelve sets of experiments were carried out, employing randomly selected second chromosome lines, and 13 sets were done, excluding inversion-carrying second chromosomes. Relative viability was expressed as *ln*[number of wild-type flies/number of *Cy* flies], since this expression is convenient for comparing the estimate of genetic variance component

with that of homozygous load. Since there was no significant difference between reciprocal crosses, these were considered as replications. Thus, the statistical analysis was done for 7 × 7 partial diallel crosses with four simultaneous replications.

Analysis of variance was performed for each set of diallel crosses, and sums of squares were estimated. After pooling the sums of squares over all sets of experiments, an attempt was made to estimate the additive and dominance variances following the procedure used by MUKAI *et al.* (1974). The average of the components of Row (σ^2_R) and Column (σ^2_C) was used to estimate the component among chromosome lines (σ^2_g), and the component of interaction between Row and Column ($\sigma^2_{R\times C}$) was also estimated from the pooled data as described before. When one considers that the *Cy* chromosome does not suppress the effects of deleterious genes in the homologous chromosome, the additive (σ^2_A) and dominance variances (σ^2_D) were estimated by the following formulas (MUKAI *et al.* 1974):

$$\hat{\sigma}^2_A = 4(\hat{\sigma}^2_R + \hat{\sigma}^2_C - \tfrac{1}{2}\,\hat{\sigma}^2_{R\times C}) \tag{5}$$

$$\hat{\sigma}^2_D = \hat{\sigma}^2_{R\times C} \tag{6}$$

Estimation of environmental variance: Traditionally, the sampling variance of phenotypically *Cy* and wild-type flies in a vial using the *Cy* method has been assumed to be binomial (*cf.* DABZHANSKY and LEVENE 1955). However, C. C. COCKERHAM at North Carolina State University suggested to the senior author that this assumption is not correct, since the distribution of two terms from independent binomial distributions are not binomial. According to him, the numbers of *Cy* flies and wild-type flies are both distributed binomially. Following his suggestion, we calculated the approximate sampling variance of viability index, V_S, or the sampling variance of 2 × (the number of wild-type flies)/(the number of *Cy* flies) and the following formula was obtained:

$$V_S \cong \frac{4n}{M^2m^3}\,[Mm(M-2n) + Mn(M-m) + (M-2n)(M-m)] \tag{7}$$

where M is the number of eggs for *Cy* flies in a vial (in the present case, in a pair of vials whose numbers of flies were pooled), the number of eggs for wild-type flies being $M/2$; m and n are observed numbers of *Cy* and wild-type flies, respectively. The derivation is given in MUKAI, CHIGUSA and KUSAKABE (1982). In this derivation, the sampling variance of *Cy* eggs and wild-type eggs was disregarded, since many eggs are laid on culture media.

In the process of reviewing, F. TAJIMA calculated V_s considering the sampling variance of the numbers of *Cy* and wild-type eggs. It happened that the result was the same as formula (11) of MUKAI, CHIGUSA and KUSAKABE (1982), *i.e.*, the formula obtained under the assumption that the distribution of adult *Cy* and adult wild-type flies in a vial is binomial; that is,

$$V_s = \frac{4n(m+n)}{m^3} \tag{7'}$$

It has been shown that, when M approaches infinity, formula (7) approaches formula (7′) (MUKAI, CHIGUSA and KUSAKABE 1982). Although the effect of sampling variance is small (see Table 1 of MUKAI, CHIGUSA and KUSAKABE 1982), formula (7′) was included in the present analysis.

The environmental variance (V_{EN}) was calculated by the following formula:

$$\hat{V}_{EN} = \hat{\sigma}^2_E - \hat{\bar{V}}_S \tag{8}$$

where $\hat{\sigma}^2_E$ is the variance of the viabilities of simultaneously replicated cultures (error variance) and $\bar{V}_S$ is the average of the sampling variance among replicate cultures. For the heterozygotes and homozygotes whose viability indices are larger than 0.5 (standardized value), the environmental variances were calculated for both the second and third chromosomes. Formula (7′) was appropriately modified to take into account the standardization of viability indices.

RESULTS AND ANALYSES

Estimation of homozygous loads: Four hundred and seventy-five second and 450 third chromosomes were extracted, and the homozygous and heterozygous

viabilities of these chromosomes were estimated by the *Cy* method (WALLACE 1956). The heterozygotes were produced by crossing successively numbered lines and completing the circle by crossing the last to the first. The distribution patterns of homozygote and heterozygote viabilities are shown in Figure 1A for the second and Figure 1B for the third chromosomes.

The detrimental and lethal loads were calculated by using formula (1) and the results are as follows:

For the second chromosomes:

$$T = \ln(A/B) = \ln(1/0.4262) = 0.8529$$

$$D = \ln(A/C) = \ln(1/0.6684) = 0.4029$$

$$L = \ln(C/B) = \ln(0.6684/0.4262) = 0.4500$$

Thus, $D/L = 0.895$.

For the third chromosomes:

$$T = \ln(A/B) = \ln(1/0.2706) = 1.3073$$

$$D = \ln(A/C) = \ln(1/0.5931) = 0.5225$$

$$L = \ln(C/B) = \ln(0.5931/0.2706) = 0.7848$$

Thus, $D/L = 0.666$.

In general, the estimates of D and L in the present population are larger than those in the northern populations. This problem will be considered in DISCUSSION. The frequencies of lethal-carrying chromosomes are 0.368 (= 175/475) for the second and 0.549 (= 247/450) for the third chromosomes.

Estimation of the effective size of the population: The effective size of the population was estimated using NEI'S (1968) formula. In addition to the frequency of lethal-carrying chromosomes (Q), their allelism rate (I_C) was estimated to be

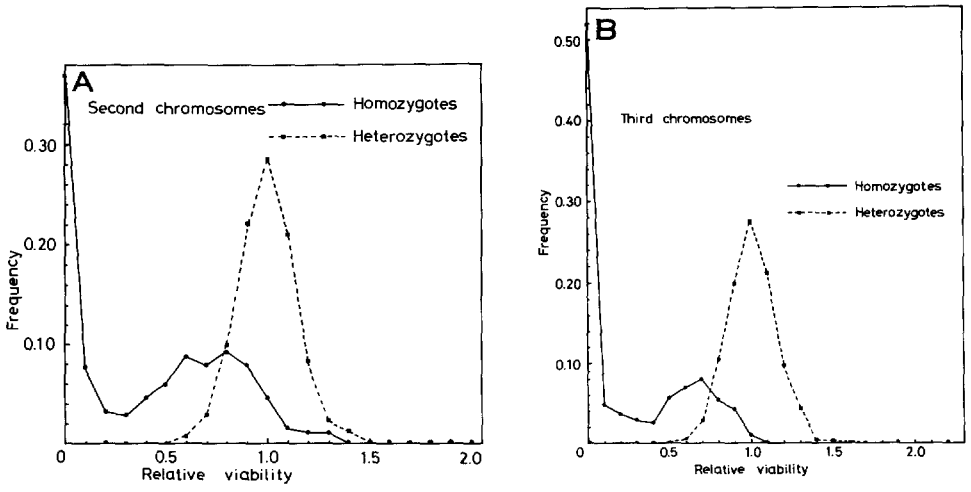

FIGURE 1.—The distribution of homozygote and heterozygote viabilities. A, second chromosomes; B, third chromosomes.

0.002603 (= 12/4610) for the second chromosomes, using 100 lethal-carrying chromosomes. The allelism rate of lethal genes (I_g) was estimated as follows:

$$\hat{I}_g = -ln(1 - I_C Q^2)/[ln(1 - Q)]^2 = 0.00167 \quad (9)$$

The effective size of the population can be estimated by formula (10).

$$N_e = (1 - \hat{I}_g)/[4(\hat{I}_g U - \mu)] \quad (10)$$

where U is the total lethal mutation rate per second chromosome per generation and μ is the lethal mutation rate per locus per generation. U has been estimated to be 0.0051 (see CROW and TEMIN 1964). Since the product of $\hat{U}$ and $\hat{I}_g$ is smaller than the estimate of μ (= 0.00001), the estimate of N_e becomes infinite. The value of μ may be one-fifth of the estimate. Then, $\hat{N}_e$ becomes about 40,000, which is still very large.

The same calculation could be made for the third chromosome, but this was not done since the allelism rate for this chrmosome was not available.

Estimation of the average degree of dominance for deleterious genes: (1) Lethal genes: Excluding abnormal crosses such as those showing extreme reciprocal differences and those having only a few individuals in the progeny, 463 and 435 random heterozygotes described before were used for the estimation of the average degree of dominance. The average viabilites of N/N', N/L and L/L' were estimated using the data of random heterozygotes, where N and L are the lethal-free and lethal-carrying chromosomes. Their numbers and the average viabilities are described in Table 1 both for the second and the third chromosomes, where the average viability of N/N' was assumed to 1.0000. N' and L' are different chromosomes of respective classes. Although the individual figures are not significantly different from 1, all of the face values of the four estimates are less than 1.0000. The estimates of the average degree of dominance on an individual lethal gene basis were made under the assumptions that (1) lethal genes are distributed on the chromosomes according to the Poisson distribution and (2) the heterozygous effects of nonhomologous lethal genes are additive. The results were 0.011 ± 0.0105 and 0.011 ± 0.0078 for the second and third chromosomes, respectively. The two estimates are almost the same but not significantly different from 0.

Using the numbers of progeny of the crosses to estimate the relative viability, *i.e.*, the offspring of the following crosses: $Cy/+_i \times Cy/+_j$, $Cy/+(l) \times Cy/+$ (including its reciprocal cross), $Cy/+(l) \times Cy/+(l')$ (including its reciprocal cross) or $Sb/+_i \times Sb/+_j$, $Sb/+(l) \times Sb/+$ (including its reciprocal cross), $Sb/+(l) \times Sb/+(l')$ etc., we estimated the average productivities of $Cy/+(l)$ or $Sb/+(l)$ relative to $Cy/+$ or $Sb/+$, where $+(l)$ is the chromosome carrying a lethal gene or genes. The average of the pooled relative productivity of males and females of lethal heterozygotes (Sb/l) was 1.0027 ± 0.0073 for the second chromosomes and 0.9866 ± 0.0086 for the third chromosomes, but they were not significantly different from the value of the wild type (= 1.0000).

On the basis of these two tests, it is speculated, as before, that lethal genes are deleterious, although not significantly so, in the heterozygous condition. This will be discussed in connection with heterozygous effects of mildly deleterious genes or viability polygenes in a later section.

TABLE 1

Average degree of dominance of lethal heterozygotes for viability

	Second chromosome		Third chromosome	
	n	Average relative viability	*n*	Average relative viability
N/N'	176	1	93	1
N/L	232	0.986 ± 0.017	209	0.978 ± 0.018
L/L'	55	0.976 ± 0.020	133	0.979 ± 0.020
$\bar{h}_E$		0.011 ± 0.0105		0.011 ± 0.0078

(2) Mildly deleterious genes: Among the random heterozygotes, 57 and 24 crosses were available for estimating the average degrees of dominance for the second and the third chromosomes under the criterion that the homozygous viabilities of their two constituent chromosomes are larger than 0.6 and less than 2.0. The value 0.6 was chosen to avoid using the chromosomes carrying major semilethal genes. Analyses of variance and covariance were performed to estimate the genetic variances and genetic covariances between the viabilities of random heterozygotes and the sums of the viabilities of respective homozygotes for their constituent chromosomes. On the basis of these estimated parameters, the average degrees of dominance of mildly deleterious genes in the approximately equilibrium population ($\bar{h}_E$) and for newly arisen mutations ($\bar{h}_N$) were estimated. Formulas (3) and (4) were employed for this purpose. The result for the third chromosomes does not give any significant information because of the small sample size ($n = 24$), so it was disregarded. The results for the second chromosomes are shown in Table 2 and the distribution pattern is shown in Figure 2. From the figure and table, it is concluded that the degree of dominance of mildly deleterious genes is large, *i.e.*, there is near-additivity. This finding is consistent with that from the Raleigh, North Carolina, population (MUKAI and YAMAGUCHI 1974; WATANABE, YAMAGUCHI and MUKAI 1976). The estimate of h_N is larger than 0.5. At face value, this finding suggests some form of balancing selection at a few loci (MUKAI and YAMAGUCHI 1974; WATANABE, YAMAGUCHI and MUKAI 1976), but the estimate is not significantly different from 0.5. Thus, the finding is not conclusive.

Components of genetic variance of viability: A total of twenty-five 7 × 7 partial diallel crosses were made, among which 12 sets included inversion-carrying chromosomes and 13 sets did not include them. In one set of the former group, a single chromosome line was missing. The analysis of variance was conducted for each set of the partial diallel crosses. There were some missing observations or plots, but exact analyses of variance were conducted by using the SCHAFFER-USANIS (1969) program for which we are grateful to H. E. SCHAFFER of North Carolina State University. Sums of squares of Row and Column of each set were pooled. After that, the results of all sets were pooled. The results are given in Table 3, where σ_g^2 is the average of the components of Row (σ_R^2) and Column (σ_C^2).

Using formulas (5) and (6), we estimated additive and dominance variances for each group. The results are also given in Table 4. The two estimates of

TABLE 2

Estimation of the average degree of dominance of mildly deleterious mutations (second chromosomes from the Orlando-Lake Placid, Florida, population)

Genetic variance of the heterozygote viabilities (y)	0.00841 ± 0.00359
Genetic variance of the sums of homozygotes for respective constituent chromosomes (x)	0.02636 ± 0.01181
Covariance between x and y	0.01259 ± 0.00493
Regression coefficient of y on x (average degree of dominance in equilibrium population $\bar{h}_E$)	0.48 ± 0.21
Inverse of the regression coefficient of x on y (average degree of dominance of newly arisen mutation $\bar{h}_N$)	0.67 ± 0.28

The number of heterozygotes = 57. The number of replications for the estimation of viability = 2.

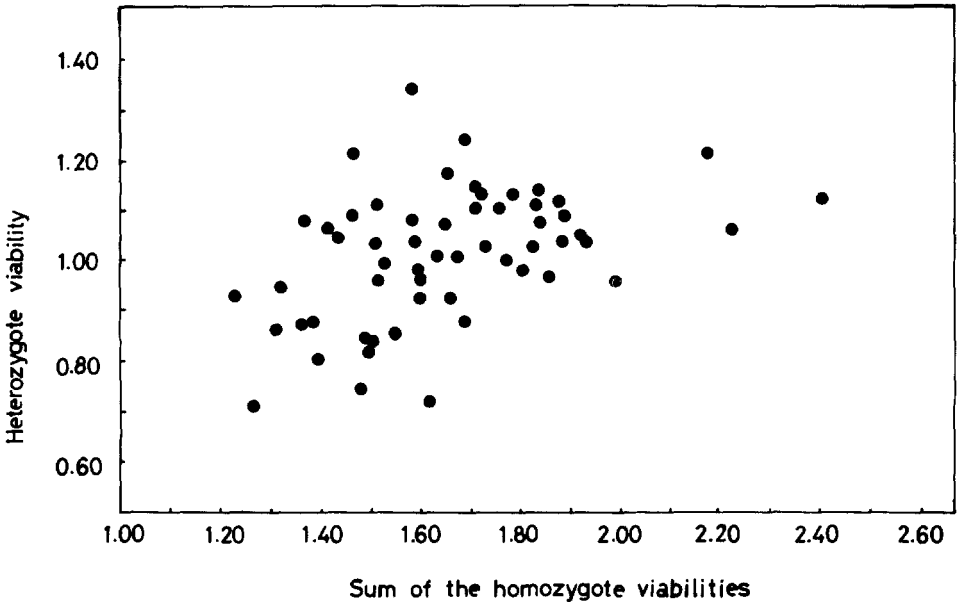

FIGURE 2.—The relationship between heterozygote viabilities and the sums of the viabilities of the homozygotes for their constituent chromosomes (second chromosomes). The regression coefficient is 0.48 ± 0.21.

additive variances are not significantly different from each other. The same is true of the dominance variances. It is the characteristic of the Florida population that the $\hat{\sigma}_A^2$ is extremely large as compared with the North Carolina population (0.0096), although the dominance variance in the former is smaller than that of the latter. This important finding will be used in DISCUSSION.

Environmental variance of homozygotes and heterozygotes: Environmental variances of viabilities of homozygotes and heterozygotes were estimated using formulas (7′) and (8). Because it was found that environmental variances of heterozygotes for polymorphic inversions are approximately equal to those of hetero-

TABLE 3

The pooled result of the analyses of variance for factorial experiments with respect to viability of the second chromosome lines

Source of variation	d.f.	Mean squares	Expected mean squares
A. Randomly sampled 167 second chromosome lines			
Rows and Columns	143	0.11576232	$\sigma_E^2 + 3.9647\sigma_{R\times C}^2 + 27.4321\sigma_g^2$
$R \times C$ interaction	424	0.03231488	$\sigma_E^2 + 3.9538\sigma_{R\times C}^2$
Error	1712	0.02914164	σ_E^2
Total	2279		
B. Excluding inversion-carrying second chromosome lines (no. of lines used = 182)			
Rows and Columns	156	0.09852960	$\sigma_E^2 + 3.8850\sigma_{R\times C}^2 + 26.8965\sigma_g^2$
$R \times C$ interaction	467	0.02895052	$\sigma_E^2 + 3.8397\sigma_{R\times C}^2$
Error	1814	0.02492594	σ_E^2
Total	2437		

σ_g^2 = genetic variance component of Row (or Column)

TABLE 4

Estimates of the components of genetic variance for viability

	Including inversions	Excluding inversions
$2\hat{\sigma}_g^2$	0.006083*** ± 0.001011	0.005170*** ± 0.000841
$\hat{\sigma}_{R\times C}^2$	0.000803 ± 0.000615	0.001048 ± 0.000538
$\hat{\sigma}_A^2$	0.022728*** ± 0.004228	0.018585*** ± 0.003535
$\hat{\sigma}_D^2$	0.000803 ± 0.000615	0.001048 ± 0.000538

σ_g^2 = genetic variance component of Row (or Column); $\sigma_{R\times C}^2$ = genetic variance component of interactions between Row and Column; σ_A^2 = additive genetic variance; σ_D^2 = dominance variance.
*** Highly significant.

zygotes for chromosomes with standard gene arrangement, the inversion-carrying chromosomes were not discriminated. In the case of homozygotes, only lines with viability indices larger than 0.5 were used in order to avoid the effect of severely deleterious major genic point mutations or semilethals. In heterozygotes, also, crosses with viability indices larger than 0.5 were used. Although some of the heterozygous crosses definitely include semilethals, it was disregarded. Lines and crosses with only one observation (one is missing) or with definitely accidental heterogeneous observations were excluded. The results for the second and the third chromosomes are shown in Tables 5 and 6 in connection with viability. Since no significant correlation was found between environmental variance and viability except for a small significant value of the heterozygotes for the third chromosomes ($\hat{r} = 0.110 \pm 0.048$, $P < 0.05$) (see Tables 5 and 6), the distributions of environmental variances are presented in Figure 3A (second chromosomes) and B (third chromosomes). From these tables and figures, it can be clearly seen that environmental variances of heterozygotes are significantly smaller than those of homozygotes as in the case of previous experimental results (DOBZHANSKY and LEVENE 1955; MUKAI, CHI-

TABLE 5

Environmental variance for second chromosomes extracted from a Florida population

Viability range	No. of lines	Mean viability	Environmental variance	Average no. of flies per observation
A. Homozygotes (no. of lines tested = 211)				
0.5–0.6	33	0.5442	0.0201734	280.1
0.6–0.7	46	0.6476	0.0200922	304.5
0.7–0.8	37	0.7551	0.0159770	315.3
0.8–0.9	37	0.8458	0.0099462	328.7
0.9–1.0	32	0.9445	0.0129744	310.9
1.0–1.1	13	1.0345	0.0209004	314.3
1.1–1.2	5	1.1615	0.0034810	331.9
1.2–1.3	4	1.2587	0.0056157	367.4
1.3–1.4	2	1.3204	0.0329335	323.2
1.5–1.6	1	1.5995	−0.0041017	373.0
1.9–2.0	1	1.9925	0.0005888	265.0
Mean (sum)	(211)	0.7949	0.0158 ± 0.0025	310.6
Correlation between environmental variance and viability = −0.079 ± 0.071				
B. Heterozygotes (no. of crosses = 446)				
0.5–0.6	3	0.5492	0.0005566	288.33
0.6–0.7	4	0.6703	−0.0033747	301.75
0.7–0.8	24	0.7584	0.0081168	315.69
0.8–0.9	82	0.8571	0.0052818	320.63
0.9–1.0	114	0.9518	0.0029134	327.32
1.0–1.1	116	1.0441	−0.0021025	326.66
1.1–1.2	67	1.1378	0.0034652	332.04
1.2–1.3	17	1.2276	0.0042587	356.06
1.3–1.4	12	1.3396	0.0499912	341.75
1.4–1.5	3	1.4499	−0.0070225	343.33
1.5–1.7	0	—	—	—
1.7–1.8	1	1.7042	−0.0330869	290.50
1.8–1.9	3	1.8322	0.1076980	316.67
Mean (sum)	(446)	1.0026	0.0042 ± 0.0014	326.95
Correlation between environmental variance and viability = 0.110 ± 0.048				

GUSA and KUSAKABE 1982); that is, developmental homeostasis for viability is manifested in heterozygotes more than in homozygotes. This finding will also be used for the discussion of the maintenance of genetic variability in DISCUSSION.

DISCUSSION

Deleterious effects of lethal genes: In the present experiment, it has been shown that "recessive" lethal genes were deleterious in the heterozygous condition, but the estimates of the average degree of dominance were not significantly different from 0. However, the detrimental heterozygous effect of lethal genes can clearly be concluded from the frequency of lethal-carrying chromosomes. The effective population size was estimated to be fininite when $\mu = 10^{-5}/$

TABLE 6

Environmental variance for third chromosomes extracted from a Florida population

Viability range	No. of lines	Mean viability	Environmental variance	Average no. of flies per observation
A. Homozygotes (no. of lines tested = 126)				
0.5–0.6	28	0.5507	0.0107884	269.20
0.6–0.7	37	0.6526	0.0216137	297.92
0.7–0.8	24	0.7437	0.0279049	271.25
0.8–0.9	24	0.8483	0.0269749	284.50
0.9–1.0	11	0.9330	0.0152947	305.18
1.0–1.1	2	1.0396	−0.0236107	235.75
Mean (sum)	(126)	0.7152	0.0202 ± 0.0040	283.55
Correlation between environmental variance and viability = 0.039 ± 0.094				
B. Heterozygotes (no. of crosses tested = 396)				
0.6–0.7	6	0.6819	0.0202994	306.17
0.7–0.8	23	0.7681	0.0115034	272.91
0.8–0.9	66	0.8570	0.0059806	289.90
0.9–1.0	98	0.9510	0.0057846	290.51
1.0–1.1	105	1.0465	0.0090510	302.06
1.1–1.2	66	1.1390	0.0074936	285.13
1.2–1.3	25	1.2405	0.0049501	265.76
1.3–1.4	5	1.3180	−0.0115534	290.30
1.4–1.5	1	1.4321	0.0026593	351.50
1.5–1.6	1	1.5822	−0.0324096	246.00
Mean (sum)	(396)	1.0030	0.0071 ± 0.0017	290.26
Correlation between environmental variance and viability = −0.002 ± 0.051				

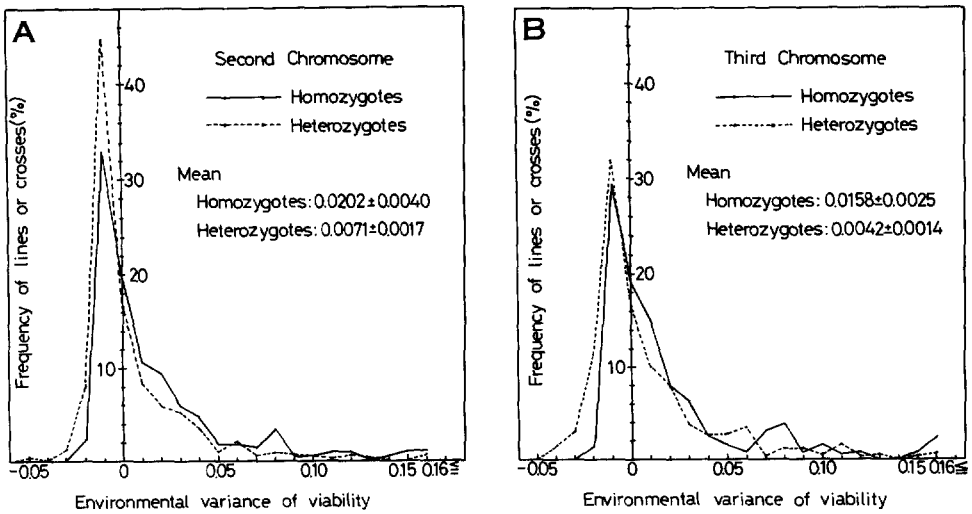

FIGURE 3.—The distributions of environmental variances for viabilities. A, second chromosomes; B, third chromosomes.

locus/generation and ca. 40,000 when $\mu = 2 \times 10^{-6}$, where μ is the lethal mutation rate. The latter is also a very large value and may be considered to be effectively infinite. Under the null hypothesis stating that heterozygous lethal genes are not deleterious to the carriers, the equilibrium frequency of lethal-carrying chromosomes can be predicted. The equilibrium frequency of a lethal gene is $\sqrt{\mu}$. If the number of lethal-producing loci is n, then the average number of lethal genes per chromosome is $n\sqrt{\mu}$. If one assumes that the lethal genes are distributed on the chromosomes according to a Poisson distribution, the expected frequency of lethal-carrying chromosomes (Q) can be estimated to be

$$E(Q) = 1 - e^{-n\sqrt{\mu}} \tag{11}$$

The μ and n have been reported to be 10^{-5}/locus/generation and about 500 (a minimum estimate) (IVES 1945). Thus, $E(Q) = 1 - 0.206 = 0.79$. If $n = 2500$, which corresponds to $\mu = 2 \times 10^{-6}$, then $E(Q) = 1 - 0.029 = 0.971$. The estimate of Q in the population is 0.368 ± 0.022, which is significantly smaller than 0.79. This finding rejects the null hypothesis and clearly supports the alternative hypothesis that the fitness of lethal heterozygotes is significantly smaller than that of lethal-free individuals (see CROW and TEMIN 1964). Incidentally, when the effective size of the population is 40,000 and the lethal genes are heterozygously neutral to their carriers, the expected frequency of lethal-carrying chromosomes becomes 0.87 on the basis of the formula given by WRIGHT (1937) and $\mu = 2 \times 10^{-6}$ and $n = 2500$.

Evidence of detrimentalness of the majority of mildly deleterious genes in heterozygous condition: The D:L ratio for the second chromosomes was estimated to be 0.90, which is larger than that in the Raleigh, North Carolina, population and other northern populations (GREENBERG and CROW 1960; MUKAI and YAMAGUCHI 1974; and others). It should be noted that the estimate of D is relative to the average heterozygote viability. Thus, under the classical model stating that the genetic variability is maintained by the balance between mutation and directional selection, L_{Id}, or the homozygous load due to detrimental genes relative to the optimum genotype becomes $D + 2\Sigma(\mu/c)$, when the equilibrium gene frequency ($\hat{q}$) is μ/chs. In this formula μ is the mutation rate, hs is the selection coefficient against heterozygotes with respect to viability alone and chs with respect to fitness as a whole. When $c = 2$ (MUKAI and YAMAGUCHI 1974), a single deleterious gene is harmful to its heterozygous carrier by the same amount to viability and fertility. Thus, L_{Id} becomes 0.543, when $\Sigma\mu = 0.14$ (MUKAI 1964) is assumed. Then, L_{Id}/L_{II} is 1.20. This value is close to that of newly arisen mutations (0.95 – 0.98, MUKAI and YAMAZAKI 1968; MUKAI *et al.* 1972). This fact implies that the majority of homozygously (nonlethal) deleterious genes are also harmful to their carriers in the heterozygous condition, since lethal genes are heterozygously deleterious to their carriers. In fact, if (nonlethal) deleterious genes were beneficial in the heterozygous condition, their frequencies would be increased and the detrimental load should become very large, and L_{Id}/I_{II} ratio should be very large in contrast to the

actual situation. This finding is consistent with the large average degree of dominance of mildly deleterious genes described before ($\hat{h}_E \cong 0.48 \pm 0.12$). Indeed, the average selection coefficient of viability of mildly deleterious genes is about 0.03 (MUKAI *et al.* 1972) and $\bar{h}_E$ is close to 0.50. Thus, $\overline{hs} \cong 0.015$, which is approximately equal to that of lethal genes. If so, mildly deleterious genes must be deleterious in fertility in the heterozygous condition too. Otherwise, the near equality in the L_{Id}/L_{Il} ratio between the population carrying newly arisen mutations alone and the natural population, especially northern populations (*cf.* MUKAI 1975), would not be observed.

However, a L_{Id}/L_{Il} ratio slighly larger than that of newly arisen mutations cannot be neglected. The value of 1.20 is definitely an underestimate if some form of balancing selection is operating on deleterious genes but not on lethal genes, since the random genetic load for estimating L_{Id} was estimated under the assumption of mutation-selection balance. Experimental data such as a large L_{Id}/L_{Il} value in comparison with that of newly arisen mutations, a large additive variance and a high degree of stability of heterozygote viability in contrast to that of homozygote viability may be difficult to explain under the assumption of mutation-selection balance. This will be discussed in the following section.

Mechanisms of the maintenance of excess genetic variability of viability in southern populations: It appears that there is a north-to-south cline of genetic variability both in homozygous load and in additive genetic variance, especially the latter. These results are tabulated in Table 7. The amount of genetic variability in the Aomori population can be explained by mutation-selection balance very well, but the southern population has an excess of genetic variability. This corresponds to the large value of L_{Id}/L_{Il} in the southern population in comparison with that for newly arisen mutations. It should be clarified what has produced this excess variability.

1. *High mutation rate in the southern population:* Under mutation-selection balance, the additive variance (σ_A^2) and homozygous load relative to the average viability of the population for nonlethal deleterious genes [$L_{Id\,(R)}$] can be expressed as follows:

$$\sigma_A^2 \cong 2\Sigma q_i h_i^2 s_i^2 = 2\Sigma \frac{\mu_i h_i s_i}{c_i} \tag{12}$$

$$L_{Id\,(R)} = \Sigma q_i s_i - 2\Sigma p_i q_i h_i s_i = \Sigma \frac{\mu_i}{c_i}\left(\frac{1}{h_i} - 2\right) \tag{13}$$

where c_i, μ_i, h_i and s_i were defined before. Disregarding a slight possible correlation between μ_i/c_i and $h_i s_i$ or $1/h_i$, the ratio of σ_A^2 to $L_{Ld\,(R)}$ cancels μ_i and c_i from the formula. Thus, the $\sigma_A^2/L_{Id\,(R)}$ ratio should be the same in different populations under mutation-selection balance. As shown in Table 7, there is a large variation of $\sigma_A^2/L_{Id\,(R)}$ ratio. Thus, high mutation rate alone cannot explain the excess of genetic variability.

2. *Negative correlation between viability and fertility:* It might be possible to explain the excess of additive variance by mutation-selection balance if there

TABLE 7

The relationship between homozygous load and additive genetic variance for viability in various populations

Population	Ishigakijima[a]	Florida	Raleigh[b]	Aomori[c]
Latitude	24.3°N	27.2°N	35.8°N	40.6°N
Additive genetic variance (σ_A^2)	0.0175 ± 0.0061	0.0227 ± 0.0042	0.0096 ± 0.0025	0.0027 ± 0.0010
Homozygous load relative to the population mean [$\hat{L}_{Id(R)}$]	0.243[d]	0.403	0.334	0.243[d]
$\frac{\sigma_A^2}{\hat{L}_{Id(R)}}$	0.075	0.056	0.029	0.011

[a] TACHIDA *et al.* (1983).
[b] MUKAI and YAMAGUCHI (1974), MUKAI *et al.* (1974).
[c] KUSAKABE and MUKAI (1982). The value 0.011 is close to the value expected on the basis of mutation-selection balance.
[d] Just a coincidence.

is a negative correlation between viability and fecundity at the genotypic level. However, as long as mutant genes are deleterious to fertility of the carriers in the heterozygous condition, it may be difficult to explain the present experimental results. The reason is that even if $c = 1$, which means no selection in fertility, the additive variance of the southern population is about five times larger than the expected value under mutation-selection balance.

There is a good suggestion for negative correlation between viability and fertility when one of them exceeds a certain value (*cf.* ROBERTSON 1955). This implies that there is an optimum viability that maximizes the fitness. If the optimum is the same as the maximum viability, then the classical load theory applies, and equations (12) and (13) predict a ratio of $\sigma_A^2/L_{Id(R)}$ of about 0.01. At the opposite extreme, if the optimum is at the mean and mutation is symmetrical, there is no increase in load with increased mutation rate, but mutation increases the genetic variance.

If the truth lies somewhere between these two extreme cases, but probably near to the first one (*cf.* HIRAIZUMI 1961), mutation enhances both the load and variance satisfying the condition $\sigma_A^2/L_{Id(R)} > 0.01$. One possibility, then, is that the relationship between viability and fitness changes with latitude, since mutation rate is higher in the south than in the north. This model may become realistic under the following condition: some fraction of deleterious viability polygenes becomes epistatically beneficial with respect to fertility in the homozygous and/or heterozygous condition. It is important to examine the reality of this model.

3. *Overdominance in viability:* Overdominance in viability with an appreciable amount of selection is clearly rejected by the extremely small amount of dominance variance. Indeed, MUKAI *et al.* (1974) have pointed out that, if all the genetic variance is due to overdominance in an equilibrium state, the genetic

variance consists entirely of dominance variance. The present experimental result is inconsistent with overdominance.

4. *Overdominance in fertility:* It is possible to assume that mutant genes are heterozygous deleterious to viability but overdominant in fertility. If the gene frequencies are determined mainly by fertility, the present experimental results may be explained. However, ROSE and CHARLESWORTH (1981) reported that the dominance variance of fertility is very small. Furthermore, MARINKOVIC (1967) has shown that the correlation coefficient in fecundity between homozygotes and heterozygotes is positive. These results are inconsistent with the hypothesis of overdominance in fertility.

5. *Frequency-dependent selection:* To explain protein polymorphisms, frequency-dependent selection was proposed (KOJIMA and YARBROUGH 1967 and others), but our large scale experiments (MUKAI *et al.* 1982; YOSHIMARU and MUKAI 1979) clearly denied its universality. Lethal and semilethal genes are not the subject of this type of selection because their gene frequencies are extremely low.

For mildly deleterious genes, few studies have been conducted. T. MUKAI, S. KUSAKABE and H. TACHIDA (unpublished results) indirectly examined whether this type of selection is working on mildly deleterious genes or viability polygenes, using the estimates of genetic variance components and the average degrees of dominance estimated by the *Cy* method, but they could not find any evidence for frequency-dependent selection. In our laboratory, this type of selection is being tested in the most direct way, but it appears that the results so far obtained do not support this type of selection.

6. *Diversifying selection:* The maintenance of the excess of genetic variance due to genotype-environment interaction or diversifying selection is an attractive hypothesis. The operation of diversifying selection in the Florida population (or southern population) could explain the excess additive variance, although, at the majority of loci, genetic variability is maintained by the balance between mutation and selection. The reasons are as follows: (1) The estimated additive variance is ten times larger than that expected on the basis of mutation-selection balance, but the dominance variance is negligibly small. (2) Environmental variance of viability of heterozygotes is significantly smaller than that of homozygotes. This phenomenon can be expected from the LEVENE (1953) model of diversifying selection, and similar results have been published by DOBZHANSKY and LEVENE (1955), WILLS (1975) and MUKAI, CHIGUSA and KUSAKABE (1982). (3) The average degree of dominance ($\bar{h}_E$) is larger in the southern population than that in the northern population: 0.178 ± 0.056 in the Aomori, 0.293 ± 0.071 in the Raleigh and 0.48 ± 0.21 in the Florida population. The $\bar{h}_N$ value estimated from the present equilibrium population (0.67 ± 0.28) is larger than the direct estimate (0.43 ± 0.008, MUKAI 1969). (4) Major candidates of balancing selection other than diversifying selection appear to be rejected.

There is considerable experimental evidence supporting diversifying selection. (1) Frequencies of polymorphic inversions [*In(2L)t, In(2R)2NS, In(3L)P, In(3R)P* and others] show north-to-south clines (METTLER, VOELKER and MUKAI

1977). These clines correspond well to the increase in additive variance in the southern populations. Furthermore, these polymorphic inversions tend to disappear in a constant laboratory environment. Disappearance or drastic decrease in polymorphic inversions in a constant laboratory condition was first reported by ANDERSON, DOBZHANSKY and PAVLOVSKY (1972) in *D. pseudoobscura*. (2) POWELL (1971), MCDONALD and AYALA (1974) and others found some experimental evidence for diversifying selection with *D. willistoni* and *D. pseudoobscura*. In their experiments, the average heterozygosities of isozyme genes decreased rather slowly under variable laboratory conditions in comparison with the case under a relatively constant laboratory condition. (However, we recently obtained experimental evidence indicating that the reaction to a varied environment is not due to polymorphic isozyme genes but due to the linked nonstructural genes.) (3) MUKAI, CHIGUSA and KUSAKABE (1982) showed that newly arisen mildly deleterious mutations were not heterozygously homeostatic with respect to viability in contrast to mildly deleterious genes sampled from a natural population. They explained this phenomenon as follows: Among newly arisen mutations only a few are homeostatic in heterozygotes. Natural selection acts positively on them, and the gene frequency of such heterozygously homeostatic genes has increased in equilibrium populations. Thus, we find heterozygote stability in the materials from natural population.

MUKAI, CHIGUSA and KUSAKABE (1982) suggested that the maximum number of loci involved in diversifying selection is not great (less than 200). However, it is speculated that the gene frequencies must be continuous from the value expected from mutation-selection balance to intermediate values expected from diversifying selection. Unfortunately, we do not have a theory concerning this subject. It is important to establish diversifying selection as an actual type of selection on the basis of the theory described and experimental data. It is also absolutely necessary to seek mechanisms other than diversifying selection to explain the excess additive genetic variance.

Does excess genetic variance originate from polymorphic isozyme loci? It is not possible to determine on the basis of the present experiment whether the excess additive variance is due to polymorphic isozyme genes. Our previous experimental result indicates that almost all of the mildly deleterious mutations are located at nonstructural sites (MUKAI and COCKERHAM 1977). If we compare the Florida and the Raleigh, North Carolina, populations with respect to the additive variance and the average of the isozyme gene frequencies, some additional information is obtained. The latter is approximately one-half the additive variance of the Florida population. The gene frequencies at several enzyme loci were reported for both of the populations (JOHNSON and SCHAFFER 1973). The frequencies of the most common alleles at seven loci are presented in Table 8. The averages of the allele frequencies are 0.840 for the North Carolina population and 0.856 for the Florida population. Thus, it can be expected that the average heterozygosities of protein loci of the two populations are not so different. Nevertheless, the additive variance of the Florida population is twice as large as that of the North Carolina population. A similar finding was obtained for Japanese populations. In the Ishigaki-jima population

TABLE 8

Isozyme gene frequencies in the North Carolina and Florida population of Drosophila melanogaster

Isozyme	Florida	North Carolina
Mdh	0.992	0.963
Adh	0.857	0.692
αGpdh	0.882	0.825
Est-C	0.854	0.916
Est-6	0.635	0.606
Acph	0.947	0.960
Odh	0.826	0.915
Average	0.856	0.840

Mdh = malate dehydrogenase; *Adh* = alcohol dehydrogenase; *αGpdh* = α glycerol-3-phosphate dehydrogenase; *Est-C* = esterase-C; *Est-6* = esterase-6; *Acph* = acid phosphatase; *Odh* = octanol dehydrogenase. Data from JOHNSON and SCHAFFER (1973); only the frequencies of the most common alleles are recorded.

(the southernmost population of Japan), the additive variance of viability was estimated to be 0.0175 ± 0.0061 and that of the Aomori population (a northernmost population of Japan) was 0.0027 ± 0.0010. This value is about ⅙ of that of the Ishigaki-jima population and is very close to the value expected on the basis of mutation-selection balance. The average heterozygosity of the former population with respect to ten randomly selected loci is 0.208 ± 0.052 and that of the latter population is 0.274 ± 0.045. These two estimates are not significantly different from each other (KUSAKABE and MUKAI 1982). Thus, it is most probable that the differences in additive variance between the two populations are due to mildly deleterious nonstructural genes or viability polygenes. If this finding is extended to all of the loci, most of the genetic variance in viability is likely to be due to nonstructural genes (T. MUKAI, K. HARADA and H. YOSHIMARU, unpublished results). If this conclusion is accepted, it is mildly deleterious genes or viability polygenes that are most important for the adaptive evolution of Drosophila since protein polymorphisms are selectively neutral or nearly neutral (KIMURA 1968; MUKAI, TACHIDA and ICHINOSE 1980). They may play a controlling role on structural genes (T. MUKAI, K. HARADA and H. YOSHIMARU, unpublished results).

Effects of hybrid dysgenesis: Finally, the following should be added. There is a possibility that excess genetic variance is due to hybrid dysgenesis induced on wild-type chromosomes (P type) in the cytoplasm of the *Cy/Pm* stock. However, it was found that the effects of newly induced mutations in and after the process of establishment of chromosome lines are negligible. This finding was obtained by comparing mean viabilities and mean squares in the analysis of variances in the different sets of experiments, respectively; that is, these statistics did not decrease or increase following the advance of experiments. One of the main reasons is that the chromosome lines after their establishment were maintained at 19°, at which the mutation rate was low and natural selection appears to have eliminated mutations easily from the lines. However, the effects of mutations induced in the second generation of the isolation of the

chromosomes were confounded with the estimated parameters, since only a single male (*Cy*/+ or *Pm*/+) was used in each line.

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