THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER.* XVII. A POPULATION CARRYING GENETIC VARIABILITY EXPLICABLE BY THE CLASSICAL HYPOTHESIS

SHINICHI KUSAKABE AND TERUMI MUKAI

Department of Biology, Kyushu University 33, Fukuoka 812, Japan

ABSTRACT

About 400 second chromosomes were extracted from the Aomori population, a northernmost population of *D. melanogaster* on Honshu in Japan, and the following experimental results were obtained. (1) The frequency of lethal chromosomes was 0.23. (2) The effective size of the population was estimated to be about 3000, from the allelism rate of lethal chromosomes and their frequency. (3) The detrimental and lethal loads for viability were 0.243 and 0.242, respectively, and the D/L ratio became 1.00. (4) The average degree of dominance for mildly deleterious genes was estimated to be 0.178 ± 0.056. (5) Additive (σ_A^2) and dominance (σ_D^2) variances of viability were estimated to be 0.00276 ± 0.00090 and 0.00011 ± 0.00014, respectively. (6) There was no significant difference in environmental variances between homozygotes and heterozygotes. Using these estimates, we discuss the maintenance mechanisms of genetic variability of viability in the population. The mutation-selection balance explained these experimental results.

THE results of recent analyses of natural populations of *Drosophila melanogaster* indicate that there is a north-to-south cline in magnitude of genetic variability of viability [the Raleigh, North Carolina, population in MUKAI *et al.* (1974), the Orlando-Lake Placid, Florida, population in MUKAI and NAGANO (1983) and the Ishigakijima, Japan, population in TACHIDA, *et al* (1983)], and that some kind of balancing selection is operating in the southern population, although a mutation-selection balance holds at the majority of loci (MUKAI and NAGANO 1983).

If this result is extended to a northern population, it may be expected that there is a population in which mutation-selection balance can explain the whole genetic variation with respect to viability in a population. Thus, we analyzed a northern population of this species in Japan, and the expectation was borne out.

MATERIALS AND METHODS

Extraction of second chromosomes: Two samples of a natural population of *D. melanogaster* were collected with a net at two different times in the fall of 1977 in a fruit-farming area in Hirosaki, Aomori prefecture (a northern population of this species). These samples are called AM-77 in this

paper. The first collection was made in vineyards. From this sample, 112 second chromosomes were extracted from males and 133 from females. The second collection was made mainly in apple orchards, and 137 second chromosomes were extracted from males and 50 from females. These two collections are hereafter called sample A and sample B, respectively. All of the extractions were made with the Cy/Pm $[In(2LR)SM1/In(2LR)bw^{V1}]$ stock, following the scheme described by WALLACE (1956). Each of these chromosomes was maintained at 18° as a line balanced with $In(2LR)SM1$, Cy chromosomes.

Cytological examination and enzyme assay: Cytological examination of salivary gland chromosomes was conducted for each chromosome line after a cross was made to the *cn bw* line with the standard chromosomes. The following four enzyme loci known to be located in the second chromosome were examined: $\alpha GPDH$ (2-17.8), MDH (2-35.3), ADH (2-50.1), $HEX\text{-}C$ (2-74.5) and AMY (2-77.3). The four dehydrogenases were assayed by starch gel electrophoresis (*cf.* SHAW and PRASAD 1970), whereas α-amylase was assayed by acrylamide gel electrophoresis (*cf.* PRAKASH, LEWONTIN and HUBBY 1969). For the analysis of linkage disequilibria the χ^2 method was employed (*cf.* MUKAI, WATANABE and YAMAGUCHI 1974).

Estimation of the rate of allelism among lethal chromosomes: For estimating the effective size of the population, the allelism tests among lethal chromosome lines were done for the two samples. Forty-six lines bearing recessive lethal genes were used from sample A, whereas 48 lines were used from sample B. Crosses were made between Cy/l_i and Cy/l_j where l indicates a lethal-bearing chromosome, and subscripts i and j stand for line numbers. If the lethals of the chromosomes of lines i and j are allelic, all of the progeny show the Cy phenotype; otherwise the Cy and wild-type flies segregate.

Estimation of relative viabilities: A total of 239 wild second chromosomes from sample A were used in the estimation of relative viabilities. Homozygote and heterozygote viabilities were estimated with the Cy method as in WALLACE (1956). The procedure for the estimation of viability was the same as that of MUKAI and YAMAGUCHI (1974). Crosses were made between five $Cy/+_i$ females and five $Cy/+_i$ males with four simultaneous replications in each chromosome line, where i indicates line number. Random heterozygote cultures were made simultaneously in the following fashion; $Cy/+_i \times Cy/+_{i+1}(i = 1 - n)$, where n is the number of lines in one set of the experiments, for random combination of different chromosome lines. The last line was crossed to the first line. In the offspring, the expected segregation ratio of the Cy and wild-type flies in both the homozygote and heterozygote crosses was 2:1. Relative viability was expressed as $2b/(a + 1)$, where b is the number of $+/+$ flies and a stands for the number of marker flies, and 1 in the denominator being HALDANE's (1956) correction for the bias in averaging ratios. Ten sets of crosses were made at ten different times, and 16–31 lines were employed in each set. The chromosome lines for each set were chosen at random from those originating from males and females. Homozygote and heterozygote viabilities were estimated at the same time within each set after standardizing relative to the average viability of the heterozygotes in each set. Altogether, 998,254 flies were counted in these viability crosses. Detrimental load to lethal load ratio (D/L ratio) was estimated using the method of GREENBERG and CROW (1960).

Estimation of the average degree of dominance and the environmental variance for viability polygenes: Ninety-seven second chromosome lines that originally showed homozygous viability indices larger than 0.6 of the average viability of random heterozygotes in the previous section were used in this experiment to estimate the average degree of dominance of viability polygenes. Homozygote and heterozygote viabilities were estimated in the same way as in the previous section. Two sets of crosses were made at two different times within a month. Forty-six and 51 chromosome lines were employed in these sets, respectively. The number of simultaneous replications per line was eight. There was no missing replicate. After the experiment, it was found that eight of 97 chromosome lines showed their homozygous viabilities to be less than 0.6, and one heterozygote showed a large reciprocal difference in its viability. Thus, the crosses including these lines were excluded in the analyses. As a result, 82 crosses were employed for the analysis. The total number of constituent chromosome lines was 87. Fifty-six of 82 were inversion-free heterozygotes. In total, 765,527 flies were counted in this experiment.

The average degree of dominance for viability polygenes was estimated by the regression method of MUKAI and YAMAGUCHI (1974). The expected regression of heterozygote viability (Y) on the sum of the two constituent homozygote viabilities (X) on chromosome basis ($\beta_{Y.X}$) estimates

the harmonic mean of the degree of dominance of newly arisen mutant viability polygenes ($\bar{h}_N$) and, at the same time, this gives the average degree of dominance of viability polygenes in an equilibrium population ($\bar{h}_E$) (MUKAI and YAMAGUCHI 1974; also see MORTON, CROW and MULLER 1956). On the other hand, the regression coefficient of X on Y ($\beta_{X,Y}$) approximately gives the inverse of the arithmetic mean of the average degree of dominance of newly arisen mutant viability polygenes (MUKAI and YAMAGUCHI 1974).

All of the heterozygotes whose constituent chromosomes have viability indices larger than 0.6 were chosen for the estimation of the average degree of dominance. For the calculation of the regression coefficients, the genetic variance of homozygotes, the genetic variance of heterozygotes and the genetic covariance between heterozygotes and the sum of the corresponding homozygote viabilities were estimated by the analysis of variance removing nongenetic variations. If the $In(2LR)SM1,Cy$ chromosomes show incomplete dominance over the wild chromosome, then this estimate of h should become a slight underestimate (MUKAI 1980).

The environmental variance was estimated according to the following procedure. With the Cy method, $Cy/+$ and $+/+$ flies segregate in the expected ratio of 2:1. The expected sampling variance (V_S) of viability indices is given by the following formula (MUKAI, CHIGUSA and KUSAKABE 1982; MUKAI and NAGANO 1983),

$$V_S = \frac{4n(m + n)}{m^3} \tag{1}$$

where m and n indicate the number of $Cy/+$ and $+/+$ flies emerged, respectively. Thus, environmental variance (V_{EN}) is given by subtracting the average of the expected sampling variances ($\bar{V}_S$) of viability indices for respective replicates from the observed error variance ($\hat{\sigma}_E^2$) of viability indices among replicates within lines or crosses.

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

Using this formula, we estimated V_{EN}.

Estimation of the genetic variance components with respect to viability: One hundred and forty second chromosome lines were randomly chosen from 245 extracted chromosome lines in sample A. Ten sets of partial diallel crosses with seven rows and seven columns were carried out. Five $Cy/+_i$ females from the ith line (row) were crossed to five $Cy/+_j$ males from the jth line (column) in two replicates ($i = 1 - 7$, $j = 8 - 14$). The reciprocal crosses were also made with two simultaneous replications. The experimental design is equivalent to design II of COMSTOCK and ROBINSON (1952) with reciprocal crosses. The additive and dominance variances were estimated from the components of genetic variance obtained in the analysis of variance following the procedure described by MUKAI *et al.* (1974). In this procedure, the incomplete dominance of the Cy chromosomes over wild-type chromosomes was taken into consideration. The viability of each set of the partial diallel crosses was standardized by the average viability of its set (average viability = 1.0000).

RESULTS AND ANALYSES

Genetic variation in the Aomori population: As usual, two types of cosmopolitan polymorphic inversions [$In(2L)t$ and $In(2R)NS$] were observed in 432 chromosome lines. Since there was no significant difference in the frequencies of chromosome types between the two samples [$\chi^2_{df=3} = 5.65$ $(0.25 > P > 0.10)$], the data for these two samples were pooled. For enzyme variation, about 400 chromosome lines were assayed. Among the five enzymes examined, the *MDH* locus was monomorphic, whereas two alleles were detected in other enzyme loci (*ADH*, $\alpha GPDH$, *HEX-C* and *AMY*). No significant linkage disequilibria were detected except for the case between $In(2L)t$ and *ADH* ($\chi^2_{df=1} = 64.90$) and between $In(2L)t$ and $\alpha GPDH$ ($\chi^2_{df=1} = 4.10$). The frequencies of inversions and the fast alleles of enzymes are summarized in Table 1.

TABLE 1

Frequencies of inversions and fast alleles at the five enzyme loci

	Frequency
Inversions	
In(2L)t	0.1412 ± 0.0168
In(2R)NS	0.0764 ± 0.0128
Isozymes	
ADH	0.542 ± 0.026
αGPDH	0.873 ± 0.017
AMY	0.920 ± 0.014
HEX-C	0.031 ± 0.009
MDH	0

ADH: alcohol dehydrogenase; *αGPDH*: α-glycerolphosphate dehydrogenase; *AMY*: α-amylase; *HEX-C*: hexokinase C; *MDH*: malate dehydrogenase.

TABLE 2

Frequencies and allelism rates of lethal chromosomes and the effective population sizes estimated by NEI's formula

	Sample A	Sample B	Pooled estimates
No. of chromosomes examined	239	187	
No. of lethal chromosomes	49	48	
Frequency of lethal chromosomes	0.205	0.257	0.227
No. of lethal chromosomes tested for lethal allelism	46	48	
No. of crosses performed	1035	1128	
No. of allelic crosses	20	23	
Allelism rate for lethals	0.0193	0.0293	0.0245
Effective population size			
($\mu = 10^{-5}$)	ca. 3700	ca. 2500	ca. 2900
($\mu = 2 \times 10^{-6}$)	ca. 3300	ca. 2300	ca. 2600

μ: spontaneous recessive lethal mutation rate per locus per generation.

Allelism rate of the lethals: The estimated allelism rates were 0.0193 and 0.0293 in samples A and B, respectively. Since there were no significant differences between the lethal frequencies [$\chi^2_{df=1} = 1.67$ (0.25 > P > 0.10)] and between the allelism rates [$\chi^2_{df=1} = 2.23$ (0.25 > P > 0.10) of these two samples, a pooled estimate of the allelism rate was calculated to be 0.0245. Based on NEI's (1968) formula, the effective size of the population (N_e) was calculated. In this calculation, the lethal mutation rate (U) per second chromosome per generation and the rate per locus (μ) were assumed to be 0.005 and 10^{-5} per generation, respectively (*cf.* CROW and TEMIN 1964). The estimate of the effective size of the population is shown in Table 2 along with the lethal frequencies and allelism rates. The results show that the effective size of the Aomori population is about 3000, which is not so much deviated from the typical value of the Japanese populations (YAMAGUCHI *et al.* 1980). The present

TABLE 3

Distributions of homozygote and heterozygote viabilities

| | Homozygotes | | |
	Total	Inversion-bearing	Heterozygotes
0.00–0.05	49	8	
0.05–0.15	7	1	
0.15–0.25	3	1	
0.25–0.35	3	1	
0.35–0.45	7	0	
0.45–0.55	7	3	
0.55–0.65	14	3	
0.65–0.75	20	2	
0.75–0.85	36	4	1
0.85–0.95	66	13	45
0.95–1.05	25	4	154
1.05–1.15	2	2	38
1.15–1.25	0	0	1
Total	239	42	239

estimate of the effective size of the population is an underestimate, since the mutation rate $\mu = 10^{-5}$ [*i.e.*, the number of loci (n) where lethal mutations take place is assumed to be 500 per second chromosome] is a maximum estimate. If the number of loci $n = 2000$, then N_e becomes ca. 2600.

Distribution of viabilities: Two hundred and thirty-nine of 242 chromosome lines in sample A were examined for their homozygote and heterozygote viabilities. The distributions of these viabilities are tabulated in Table 3 and graphically shown in Figure 1. There was no significant difference between the distribution patterns of the inversion-bearing lines and the inversion-free lines by the Kolmogorov Smirnov nonparametric test ($P > 0.50$). Furthermore, there were no significant differences in the frequencies of lethal chromosomes among three karyotypes ($\chi^2_{df=1} = 2.18$ for standard *vs.* *In(2L)t*; $\chi^2_{df=1} = 1.16$ for standard *vs.* *In(2R)NS*). Thus, there was no significant linkage disequilibrium between inversions and lethal genes.

Homozygous load: The average relative viability of all homozygotes (*B*) was 0.6156 ± 0.0065 and that of homozygote lines excluding lethal-bearing chromosome lines (*C*) was 0.7843 ± 0.0083. The average relative viability of random heterozygotes (*A*) was 1.000 ± 0.0034. From these values, the total homozygous load (*T*), detrimental load (*D*) and lethal load (*L*) can be estimated as follows (*cf.* GREENBERG and CROW 1960).

$$T = \ln A - \ln B = 0.485$$

$$D = \ln A - \ln C = 0.243$$

$$L = \ln C - \ln B = 0.242$$

The detrimental load estimated ($D = 0.243$) is much smaller than those in the Raleigh ($D = 0.333$, MUKAI and YAMAGUCHI 1974) and the Florida pop-
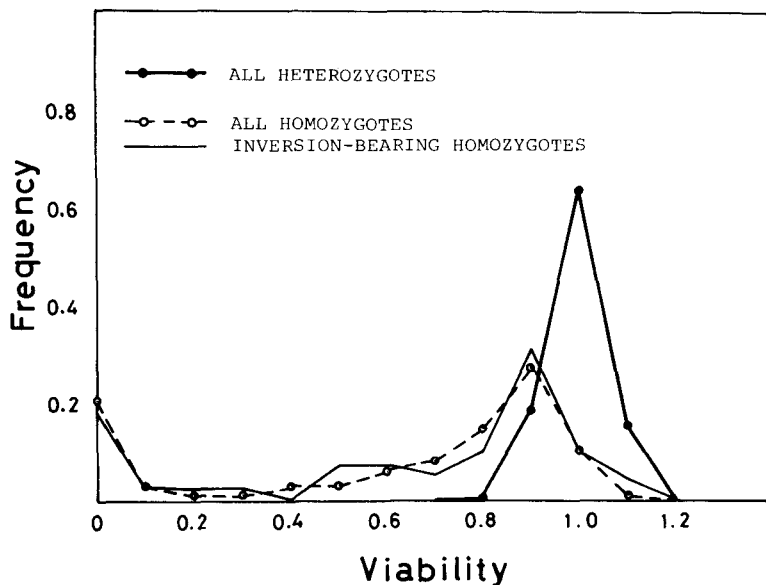
FIGURE 1.—Frequency distributions of homozygote and heterozygote viabilities for the second chromosomes. The average viability of the heterozygotes is adjusted to 1.00.

ulations ($D = 0.403$, MUKAI and NAGANO 1983). This shows that much more deleterious genes are concealed in the southern populations than in the Aomori population, a northernmost population of Japan. IVES (1945) reported a similar tendency with respect to the frequency of lethal chromosomes in the United States. The D/L ratio was 1.00. Although this ratio falls in a not unreasonable range listed in the review by SIMMONS and CROW (1977), it is slightly larger than the previous estimates [0.636 by TEMIN et al. (1969), 0.584 by KOSUDA (1971) and 0.667 by MUKAI and YAMAGUCHI (1974)].

Of 239 random heterozygotes, there are 146 lethal-free heterozygotes, 82 single-lethal heterozygotes and 11 double-lethal heterozygotes. The average viabilities of these classes are as follows: 0.9974 ± 0.0042 for lethal-free heterozygotes, 1.0033 ± 0.0058 for single-lethal heterozygotes and 1.0096 ± 0.0184 for double-lethal heterozygotes. No significant differences were detected as a whole, which was presumably the result of an insufficient number of chromosome lines employed in each experimental set that might have masked the heterozygous effects of lethal genes.

Average degree of dominance: The analyses were done for the three groups: all heterozygotes, inversion-bearing heterozygotes and inversion-free heterozygotes. The relationship between homozygote and heterozygote viabilities can be seen in Figure 2. All of the estimates from these analyses were shown in Table 4. Standard errors were calculated following the method used by TALLIS (1959). The estimates of the average degree of dominance for viability polygenes were 0.178 ± 0.056 for all heterozygotes, 0.301 ± 0.119 for inversion-bearing heterozygotes and 0.130 ± 0.060 for inversion-free heterozygotes. These values agree very well with the previous estimates, 0.210 ± 0.066 (MU-
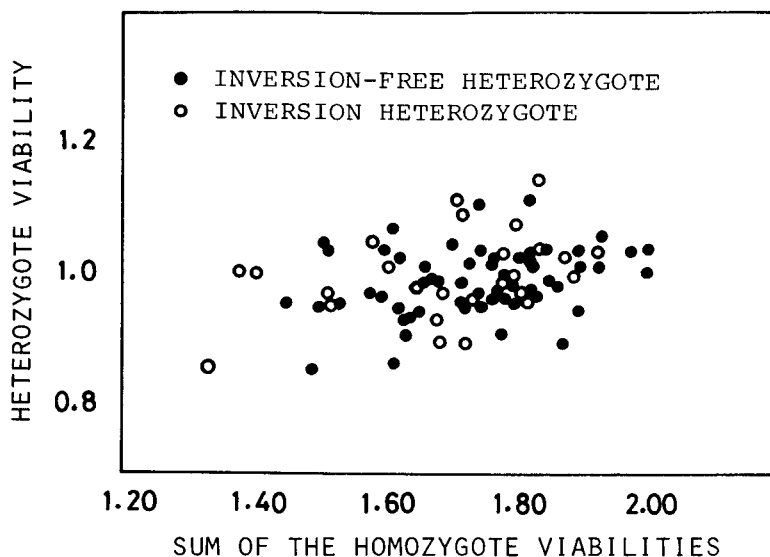
FIGURE 2.—The relationship between heterozygote viability and the sum of the corresponding homozygote viabilities with viability indices larger than 0.6.

TABLE 4

Estimation of the average degrees of dominance for viability polygenes

	All heterozygotes	Inversion heterozygotes	Inversion-free heterozygotes
No. of crosses	83	26	57
No. of simultaneous replications	8	8	8
Variance $(Y) \times 10^5$	165.94	302.71	108.68
Variance $(X) \times 10^5$	1391.77	1394.00	1394.29
Covariance $(X, Y) \times 10^5$	247.09	419.96	181.82
$\hat{h}_E$	0.178 ± 0.066	0.301 ± 0.119	0.130 ± 0.060
$\hat{h}_N$	0.672 ± 0.231	0.721 ± 0.303	0.598 ± 0.315

KAI *et al.* 1972) and 0.293 ± 0.071 (MUKAI and YAMAGUCHI 1974). These values also occur in the range 0.17–0.27 predicted from the harmonic mean of the values of newly arisen mutants, $\bar{h}_N$ (MUKAI 1969). Again, the inflation of the estimated value in inversion-bearing heterozygotes was detected as in MUKAI and YAMAGUCHI (1974), but its magnitude was much smaller than that. The average degree of dominance of newly arisen mutant viability polygenes $(\bar{h}_N)$ was estimated using the method described earlier. These estimates were 0.675 ± 0.231 for all heterozygotes, 0.721 ± 0.303 for inversion-bearing heterozygotes and 0.598 ± 0.315 for inversion-free heterozygotes. Thus, the estimates in the present investigation are close to those obtained by the direct estimate $(\bar{h}_N = 0.43 \pm 0.008$, MUKAI 1969; MUKAI and YAMAZAKI 1964, 1968). If there are partially recessive and overdominant polygenes, $1/\beta_{X,Y}$ can be expressed as follows.

$$1/\beta_{X,Y} = \frac{\mathrm{Var}(Y) + \mathrm{Var}(Y)'}{\mathrm{Cov}(X, Y) + \mathrm{Cov}(X, Y)'} \tag{3}$$

where $\mathrm{Var}(Y)'$, is the variance of heterozygotes, and $\mathrm{Cov}(X, Y)'$ is covariance between heterozygotes and the sums of the corresponding homozygotes for the overdominant loci or loci receiving some form of balancing selection. In an equilibrium population, $\mathrm{Cov}(X, Y) = 0$ at the overdominant loci, and $1/\beta_{X,Y}$ becomes as follows (MUKAI and YAMAGUCHI 1974; WATANABE, YAMAGUCHI and MUKAI 1976).

$$1/\beta_{X,Y} = \bar{h}_N\left(1 + \frac{\mathrm{Var}(Y)'}{\mathrm{Var}(Y)}\right) \tag{4}$$

Thus, a large value of $1/\beta_{X,Y}$ for an equilibrium population makes one suspect the presence of overdominance or some form of balancing selection (cf. MUKAI 1978). However, in the present population, those estimates were 0.58 to 0.72, which are close to the earlier predicted value and not significantly different from them. Therefore, this suggests that, even if some form of balancing selection is operating, the number of its loci is very limited, and mutation-selection balance may be seen at almost all loci.

Variance component analysis: Ten sums of squares for respective sources of variations in the analyses of variances were pooled over all of the ten sets of experiments, and the genetic variance components were estimated from the pooled data. There was no missing cell. The additive (σ_A^2) and dominance (σ_D^2) variances can be estimated from the following formulas considering the incomplete dominance of the Cy chromosomes with respect to viability, based on the two-way analysis of variance,

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

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

where $\hat{\sigma}_R^2$, $\hat{\sigma}_C^2$ and $\hat{\sigma}_{R\times C}^2$ stand for the estimated variance components for row, column and row $\times$ column interaction effects, respectively (MUKAI et al. 1974). The overall analysis of variance is shown in Table 5 together with expected mean squares. The components of genetic variance were calculated based on these values. Their standard errors were estimated on the basis of the following approximate relationships $V(MS) \cong \frac{2(MS)^2}{\mathrm{df}}$, where MS stands for mean square, $V(MS)$ is its sampling variance and df is its degree of freedom.

The estimated value of the additive genetic variance is 0.00276 ± 0.00091, which is significantly different from 0 ($0.001 < P < 0.01$), whereas that of the dominance variance is 0.000105 ± 0.000138. No appreciable difference was detected by including inversion-bearing chromosomes in comparison with the case of inversion-free chromosomes alone (Table 5). Two points should be noted here: (1) The estimated additive genetic variance in the Aomori population is the smallest of those ever estimated (MUKAI et al. 1974; MUKAI et al.

TABLE 5

Analysis of variance for ten sets of 7 × 7 partial diallel crosses for the second chromosomes of D. melanogaster *sampled from the Aomori population and estimated values for the additive and dominance variances with their standard errors*

Inversion	Source	d.f.	SS	Mean squares	EMS
	Rows	60	1.45568	0.02426	$\sigma_e^2 + 4\sigma_{R\times C}^2 + 28\sigma_C^2$
	Columns	60	1.34416	0.02240	$\sigma_e^2 + 4\sigma_{R\times C}^2 + 28\sigma_R^2$
Including[a]	Row × column	360	4.65727	0.01294	$\sigma_e^2 + 4\sigma_{R\times C}^2$
	Error	1470	18.39803	0.01294	σ_e^2
	Total	1950	25.85515		
	Rows	52	1.11208	0.02139	$\sigma_e^2 + 4\sigma_{R\times C}^2 + 28\sigma_C^2$
	Columns	48	1.19999	0.02500	$\sigma_e^2 + 4\sigma_{R\times C}^2 + 28\sigma_R^2$
Excluding[b]	Row × column	248	3.25392	0.01312	$\sigma_e^2 + 4\sigma_{R\times C}^2$
	Error	1074	13.81613	0.01286	σ_e^2
	Total	1422	19.38212		

[a] Additive variance = 0.00276 ± 0.00091; dominance variance = 0.000105 ± 0.00013.
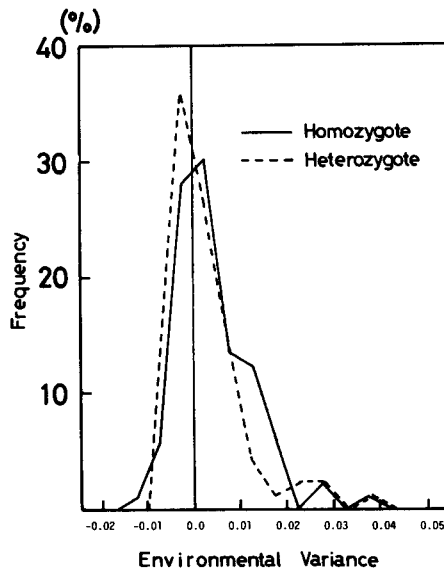[b] Additive variance = 0.00323 ± 0.00116; dominance variance = 0.00006 ± 0.00017.

FIGURE 3.—Distribution patterns of environmental variances of homozygotes and heterozygotes.

1982) and is very close to that expected from mutation-selection balance as will be described in DISCUSSION. (2) Nearly zero value of the dominance variance seems to be general among natural populations (*cf.* MUKAI *et al.* 1982).

Environmental variances: The estimation for environmental variances was done for all of the heterozygotes and homozygotes with viability indices larger than 0.6. The frequency distribution patterns of heterozygotes were almost the same as homozygotes (Figure 3). The average environmental variance for heterozygotes was 0.00218 ± 0.00081 and that of homozygotes was 0.00430

± 0.00085. There was no significant difference between them, although that of heterozygotes is smaller than that of homozygotes at face value. This is slightly different from the results on the environmental variances in the Raleigh (MUKAI, CHIGUSA and KUSAKABE 1982) and the Florida (MUKAI and NAGANO 1983) populations, where a clear significant difference was detected between homozygotes and heterozygotes. The situation is rather similar to that of the newly arisen mutations, which have not experienced natural selection at all (cf. MUKAI, CHIGUSA and KUSAKABE 1982).

<center>DISCUSSION</center>

Genetic variations maintained in the Aomori population and their approximate genetic equilibrium: The survey for the frequency of inversions, electrophoretic variation at the five enzyme loci, the frequency of recessive lethals and variability in viability shows that the amount of genetic variability carried by the Aomori population studied in the present investigation is not unusual for a natural population of *D. melanogaster* (cf. YAMAGUCHI et al. 1980).

The effective size of the Aomori population (ca. 2900) is not small as compared with those of other Japanese populations (ca. 2000–7000) estimated by the allelism rate of the lethal genes (MINAMORI et al. 1973; WATANABE, WATANABE and OSHIMA 1976; YAMAGUCHI et al. 1980). Over the wide range of the Japanese islands, large differences in the effective sizes of the populations cannot be seen. On the other hand, the effective sizes of populations of the United States (ca. 20,000 for the Raleigh, North Carolina, population and effectively infinite for the Orlando, Florida, population) are far greater than these. This may indicate the local isolation of the Japanese populations due to topographical complexity (YAMAGUCHI et al. 1980).

Comparison between the results of the present study and those obtained using the chromosome lines extracted from almost the same area in the previous year (the Hirosaki population, abbreviated as HS-76 hereafter) derived the following findings: (1) No significant difference in the array of inversions and alleles at the two polymorphic enzyme loci, *ADH* and *αGPDH*, between these two studies ($\chi^2_{df=12} = 19.2$; $P = 0.10$) was observed. Incidentally, there were no significant linkage disequilibria among isozyme genes excluding the cases involving polymorphic inversions. (2) There was no significant difference between the frequencies of lethal chromosomes ($\hat{Q} = 0.288$ for HS-76 and $\hat{Q} = 0.277$ for AM-77; $\chi^2_{df=1} = 2.58$; $0.25 > P > 0.10$). (3) The effective sizes of the populations estimated from the allelism rate of the lethal chromosomes were almost the same (ca. 2600 for HS-76 and ca. 2900 for AM-77). (4) The estimates of the detrimental to lethal load ratio (*D/L* ratio) were almost the same (0.964 for HS-76 and 1.00 for AM-77). These results suggest that the Aomori population is in approximate genetic equilibrium.

Maintenance mechanisms of genetic variability of viability polygenes in the northern marginal population: We could not detect significant deleterious effect of lethal genes in the heterozygous condition, although it is well established that the lethal genes are heterozygously deleterious to viability as well as to fitness as a whole (CROW and TEMIN 1964; MUKAI and YAMAGUCHI 1974; MUKAI and

NAGANO 1983). The reason is that the frequency of lethal chromosomes is too low unless we consider the deleterious effect on fitness in heterozygous condition. In fact, if WRIGHT's (1937) formula for the completely recessive lethal genes is used, the expected frequency of lethal chromosomes becomes 0.49 for the case of $\mu = 0.2 \times 10^{-5}$, $N_e = 3000$, and the number of loci = 2500; and 0.47 for the case of $\mu = 10^{-5}$, $N_e = 3000$, and the number of loci = 500. These values are much higher than the observed value ($\hat{Q} = 0.23$). The direct estimate of the degree of dominance of the mildly deleterious genes is much larger than zero. This verifies the deleterious effect of the mildly deleterious genes in heterozygous conditions. These results confirm those obtained in the United States (MUKAI and YAMAGUCHI 1974; MUKAI and NAGANO 1983).

The estimates of the homozygous load due to slightly deleterious genes and the average degree of dominance and the additive genetic variance enable us to evaluate the mutation-selection balance as a maintenance mechanism of genetic variability of viability polygenes. In the following discussion it is assumed that total fitness consists mainly of adult fertility and preadult viability. It should be stressed here that the subject of the present experiment is genetic variability of viability. Therefore, in addition to viability, only the pleiotropic effects of viability genes on other fitness components become the subject of the investigation in clarifying the maintenance mechanism of genetic variability of viability. The genetic parameters for a locus with two alleles in a large randomly mating population are expressed as follows (cf. MUKAI et al. 1974; MUKAI 1978):

	Genotype		
	AA	Aa	aa
Frequency	p^2	$2pq$	q^2
Relative viability in the laboratory	1	$1 - hs$	$1 - s$
Relative fertility in the laboratory	1	$1 - kt$	$1 - t$
Total fitness in the laboratory	1	$(1 - hs)(1 - kt) \cong 1$ $- (hs + kt)$	$(1 - s)(1 - t) \cong 1 -$ $(s + t)$
Total fitness in nature	1	$1 - chs$	$1 - c's$

We can regard A as a normal allele and a as a slightly deleterious mutant allele. hs is the deleterious effect of the mutant gene on viability, and kt is that on fertility in the heterozygous condition in the laboratory. The mean viability, $\bar{v}$, of a randomly mating population measured in the laboratory is $(1 - 2pqhs - q^2s)$, and that of a completely inbred population measured in the laboratory is $(1 - qs)$. Using this terminology, we can express the equilibrium gene frequency ($\hat{q}$) in a natural population as $\hat{q} \cong \dfrac{\mu}{chs}$, where $c = m\left(1 + \dfrac{kt}{hs}\right)$. m reflects the difference in fitness between laboratory and nature. Thus, it is possible to express $\hat{q}$ in terms of selection coefficient and the degree of dominance of viability measured in the laboratory.

It follows that inbreeding decline (i) from $F = 0$ to $F = 1$ (F is the inbreeding

coefficient) or homozygous load with respect to the average of the randomly mating population [$l_{(R)}$] measured in the laboratory can be expressed as follows;

$$l_{(R)} = i = \{(1 - qs) - (1 - 2pqhs - q^2s)\}/\bar{v}$$
$$= [pqs(1 - 2h)]/\bar{v} \tag{7}$$

In our experiment $\bar{v}$ is equated to be 1, and $\hat{q}$ must be extremely small in comparison with 1. Thus,

$$l_{(R)} \cong \hat{q}(1 - 2h)s$$
$$= \left(\frac{\mu}{c}\right)\frac{(1 - 2h)}{h} \tag{8}$$

In a similar way, additive genetic variance at the locus level (σ_a^2) measured in the laboratory can be expressed as

$$\sigma_a^2 = 2pq[h(p - q) + q]^2 s^2$$
$$\cong 2\hat{q}h^2 s^2$$
$$= 2\frac{\mu}{chs}h^2 s^2 \tag{9}$$
$$= 2\left(\frac{\mu}{c}\right)hs$$

The experiments on spontaneous mutations affecting viability (MUKAI 1964; MUKAI et al. 1972) showed that viability decreases with the increase in the number of mutations accumulated on a chromosome. The estimate of dominance variance (which contains epistatic components) is very small for all of the populations so far investigated (MUKAI et al. 1980). These two experimental results suggest that the epistatic interaction between loci affecting viability is quite small and support the additive effect on viability. Therefore, if additivity among loci is assumed, inbreeding decline on a chromosome basis (I) or homozygous load with respect to the mean of a randomly mating population on a chromosome basis [$L_{I(R)}$ or T] becomes

$$L_{I(R)} = \Sigma\frac{\mu}{c}\frac{(1 - 2h)}{h}$$

If we are concerned only with nonlethal deleterious genes, we may approximately assume that $\left(\frac{\mu}{c}\right)$ is not correlated with $\frac{1 - 2h}{h}$. Then $L_{I(R)}$ becomes $L_{Id(R)}$, and it is expressed as

$$L_{Id(R)} \cong \overline{\left(\frac{\mu}{c}\right)}\Sigma\left(\frac{1}{h} - 2\right) \tag{10}$$

In the same way, σ_a^2 becomes σ_A^2 on a chromosome basis neglecting possible correlation of c and hs and can be expressed as

$$\sigma_A^2 \cong 2 \overline{\left(\frac{\mu}{c}\right)} \Sigma hs \qquad (11)$$

If we calculate the ratio of σ_A^2 to $L_{Id(R)}$, it turns out to be

$$\sigma_A^2/L_{Id(R)} = \frac{2 \overline{\left(\frac{\mu}{c}\right)} \Sigma hs}{\left(\frac{\mu}{c}\right) \Sigma \left(\frac{1}{h} - 2\right)}$$

$$\cong \frac{2\overline{hs}}{\dfrac{1}{\tilde{h}} - 2} \qquad (12)$$

where $\tilde{h}$ is the harmonic mean of h and $\overline{hs}$ is the arithmetic mean of hs (MUKAI and NAGANO 1983; TACHIDA et al. 1983).

Formula (11) was approximately correct under the Classical Hypothesis. The estimates of $\overline{hs}$ and $\tilde{h}$ are available: $\overline{hs} \cong 0.015$ (MUKAI 1964; MUKAI and YAMAZAKI 1968), and $\tilde{h} \cong 0.25$. [In the present experiment, $\tilde{h}$ was estimated to be 0.18, but it is an underestimate (see MUKAI 1980)]. Thus, the predicted value of this ratio $2\overline{hs} \left/ \left(\dfrac{1}{\tilde{h}} - 2\right)\right.$ becomes 0.0084, whereas the estimated value $\sigma_a/L_{Id(R)}$ was $0.0027/0.243 = 0.01$. These values are close enough to support a mutation-selection balance. For the other population located in the south, we see a much larger value for this ratio (MUKAI and NAGANO 1983).

In addition, there is not as large a difference in environmental variance between homozygotes and heterozygotes as is found in the Raleigh and Orlando populations (MUKAI, CHIGUSA and KUSAKABE 1982; MUKAI and NAGANO 1983). In general, in the populations in which excesses of additive genetic variances were found relative to the value expected from the mutation-selection balance, significant decreases in environmental variances were detected in heterozygotes in comparison with homozygotes. This was used as part of the suggestive evidence for genotype-environment interaction or diversifying selection (MUKAI, CHIGUSA and KUSAKABE 1982; MUKAI and NAGANO 1983). Thus, no significant difference in environmental variance between homozygotes and heterozygotes indirectly supports this conclusion.

We should remark on chromosome-cytoplasmic interaction known as hybrid dysgenesis (KIDWELL, KIDWELL and SVED 1977). If this phenomenon was manifested in the extraction of the second chromosomes, the homozygous load due to detrimental genes estimated in the present study might be slightly overestimated. However, recent studies on the northern population (D. S. SUH and T. MUKAI, unpublished results) paying special attention to the cytotype and genotype characteristics and estimating the several parameters, show almost the same magnitude of homozygous load and additive genetic variance as those in the present study. Therefore, the effect of chromosome-cytoplasmic interaction could be negligibly small as far as the northern marginal populations are concerned.

Finally, the following two points should be noted: If we consider the clinal distribution of cosmopolitan inversions both in the United States (METTLER, VOELKER and MUKAI 1977) and in Japan (YAMAGUCHI et al. 1980), the maintenance of two types of inversions *In(2L)t* and *In(2R)NS* in the northern marginal population, although their frequencies are not high, suggests that some form of balancing selection (most probably diversifying selection) is operating at the inversion level. This may suggest that a slight level of diversifying selection is operating on viability polygenes. However, it is expected that the number of loci involved might be very small, since the genetic variance can be explained by mutation-selection balance. However, it is expected that the selection coefficients involved might be very small. In addition, if genetic variability of viability is maintained by a mutation-selection balance, it may indicate that the majority of protein polymorphisms are selectively neutral or nearly neutral for viability as claimed by MUKAI, TACHIDA and ICHINOSE (1980). This problem will be discussed in detail in the following paper of this series.

LITERATURE CITED

COMSTOCK, R. E. and H. F. ROBINSON, 1952 Estimation of average dominance of genes. pp. 494–516. In: *Heterosis*, Edited by J. W. GOWEN. Iowa State College Press, Ames, Iowa.

CROW, J. F. and R. G. TEMIN, 1964 Evidence for the partial dominance of recessive lethal genes in natural populations of *Drosophila*. Am. Nat. **98:** 21–33.

GREENBERG, R. and J. F. CROW, 1960 A comparison of the effect of lethal and detrimental chromosomes from *Drosophila* populations. Genetics **45:** 1154–1168.

HALDANE, J. B. S., 1937 The effect of variation on fitness. Am. Nat. **71:** 337–349.

HALDANE, J. B. S., 1956 Estimation of viabilities. J. Genet. **54:** 294–296.

IVES, P. T., 1945 The genetic structure of American populations of *Drosophila melanogaster*. Genetics **30:** 167–196.

KIDWELL, M. G., J. F. KIDWELL and J. A. SVED, 1977 Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility, and male recombination. Genetics **86:** 813–833.

KOSUDA, K., 1971 Synergistic interaction between second and third chromosomes on viability of *Drosophila melanogaster*. Jpn. J. Genet. **46:** 41–52.

METTLER, L. E., R. A. VOELKER and T. MUKAI, 1977 Inversion clines in natural populations of *Drosophila melanogaster*. Genetics **87:** 169–176.

MINAMORI, S., K. ITO, A. NAKAMURA, Y. ANDO and H. SHIOMI, 1973 Increasing trend in frequencies of lethal and semilethal chromosomes in a natural population of *Drosophila melanogaster*. Jpn. J. Genet. **48:** 41–51.

MORTON, N. E., J. F. CROW and H. J. MULLER, 1956 An estimate of the mutational damage in man from data consanguineous marriages. Proc. Natl. Acad. Sci. USA **42:** 855–863.

MUKAI, T., 1964 The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. Genetics **50:** 1–19.

MUKAI, T., 1969 The genetic structure of natural populations of *Drosophila melanogaster*. VIII. Natural selection on the degree of dominance of viability polygenes. Genetics **63:** 467–478.

MUKAI, T., 1978 *Population Genetics.* Kodansha Scientific, Tokyo.

MUKAI, T., 1980 The genetic structure of natural populations of *Drosophila melanogaster.* XIV. Effects of the incomplete dominance of the *In(2LR)SM1(Cy)* chromosome on the estimates of various genetic parameters. Genetics **94**: 169–184.

MUKAI, T., R. A. CARDELLINO, T. K. WATANABE and J. F. CROW, 1974 The genetic variance for viability and its components in a local population of *Drosophila melanogaster.* Genetics **78**: 1195–1208.

MUKAI, T., S. I. CHIGUSA and S. KUSAKABE, 1982 The genetic structure of natural populations of *Drosophila melanogaster.* XV. Nature of developmental homeostasis for viability. Genetics **101**: 279–300.

MUKAI, T., S. I. CHIGUSA, L. E. METTLER and J. F. CROW, 1972 Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster.* Genetics **72**: 335–355.

MUKAI, T. and S. NAGANO, 1983 The genetic structure of natural populations of *Drosophila melanogaster.* XVI. Excess of additive genetic variance of viability. Genetics **105**: 115–134.

MUKAI, T., H. TACHIDA and M. ICHINOSE, 1980 Selection for viability at loci controlling protein polymorphisms in *Drosophila melanogaster* is very weak at most. Proc. Natl. Acad. Sci. USA **77**: 4857–4860.

MUKAI, T., T. K. WATANABE and O. YAMAGUCHI, 1974 The genetic structure of natural populations of *Drosophila melanogaster.* XII. Linkage disequilibrium in a large local population. Genetics **77**: 771–793.

MUKAI, T. and O. YAMAGUCHI, 1974 The genetic structure of natural population of *Drosophila melanogaster.* XI. Genetic variability in a local population. Genetics **76**: 339–366.

MUKAI, T., O. YAMAGUCHI, S. KUSAKABE, H. TACHIDA, M. MATSUDA, M. ICHINOSE and H. YOSHIMARU, 1982 Lack of balancing selection for protein polymorphisms. pp. 75–114. In: *Molecular Evolution, Protein Polymorphism and the Neutral Theory,* Edited by M. KIMURA. Japan Scientific Societies Press, Tokyo.

MUKAI, T. and T. YAMAZAKI, 1964 Position effect of spontaneous mutant polygenes controlling viability in *Drosophila melanogaster.* Proc. Jpn. Acad. **40**: 840–845.

MUKAI, T. and T. YAMAZAKI, 1968 The genetic structure of natural populations of *Drosophila melanogaster.* V. Coupling-repulsion effect of spontaneous mutant polygenes controlling viability. Genetics **59**: 513–535.

NEI, M., 1968 The frequency distribution of lethal chromosomes in finite populations. Proc. Natl. Acad. Sci. USA **60**: 517–524.

PRAKASH, S., R. C. LEWONTIN and J. L. HUBBY, 1969 A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura.* Genetics **61**: 841–858.

SHAW, C. R. and R. PRASAD, 1970 Starch gel electrophoresis of enzymes: a compilation of recipes. Biochem. Genet. **4**: 297–320.

SIMMONS, M. J. and J. F. CROW, 1977 Mutation affecting fitness in *Drosophila* populations. Annu. Rev. Genet. **11**: 49–78.

TACHIDA, H., M. MATSUDA, S. KUSAKABE and T. MUKAI, 1983 Variance component analysis for viability in an isolated population of *Drosophila melanogaster.* Genet. Res. **42**: 207–217.

TALLIS, G. M., 1959 Sampling errors of genetic correlation coefficients calculated from analysis of variance and covariance. *Aust. J. Stat.* **1**: 35–43.

TEMIN, R. G., H. U. MEYER, P. S. DAWSON and J. F. CROW, 1969 The influence of epistasis on homozygous viability depression in *Drosophila melanogaster.* Genetics **61**: 497–519.

WALLACE, B., 1956 Studies on irradiated populations of *Drosophila melanogaster.* J. Genet. **54**: 280–293.

WATANABE, T. K., T. WATANABE and C. OSHIMA, 1976   Genetic changes in natural populations of *Drosophila melanogaster*. Evolution **30**: 109–118.

WATANABE, T. K., O. YAMAGUCHI and T. MUKAI, 1976   The genetic variability of third chromosomes in a local population of *Drosophila melanogaster*. Genetics **82**: 63–82.

WRIGHT, S., 1937   The distribution of gene frequencies under irreversible mutation. Proc. Natl. Acad. Sci. USA **24**: 253–259.

YAMAGUCHI, O., M. ICHINOSE, M. MATSUDA and T. MUKAI, 1980   Linkage disequilibrium in isolated populations of *Drosophila melanogaster*. Genetics **96**: 507–522.

Corresponding editor: M. T. CLEGG