The Genetic Structure of Natural Populations of *Drosophila melanogaster*. XX. Comparison of Genotype-Environment Interaction in Viability Between a Northern and a Southern Population

Toshiyuki Takano, Shinichi Kusakabe and Terumi Mukai

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

Manuscript received June 20, 1986
Revised copy accepted June 24, 1987

ABSTRACT

In order to examine the operation of diversifying selection as the maintenance mechanism of excessive additive genetic variance for viability in southern populations in comparison with northern populations of *Drosophila melanogaster*, two sets of experiments were conducted using second chromosomes extracted from the Ogasawara population (a southern population in Japan) and from the Aomori population (a northern population in Japan). Chromosomal homozygote and heterozygote viabilities were estimated in eight kinds of artificially produced breeding environments. The main findings in the present investigation are as follows: (1) Significant genotype-environment interaction was observed using chromosomes extracted from the Ogasawara population. Indeed, the estimate of the genotype-environment interaction variance for heterozygotes was significantly larger than that of the genotypic variance. On the other hand, when chromosomes sampled from the Aomori population were examined, that interaction variance was significant only for homozygotes and its value was no more than one quarter of that for the chromosomes from the Ogasawara population. (2) The average genetic correlation between any two viabilities of the same lines estimated in the eight kinds of breeding environments for the chromosomes sampled from the Ogasawara population was smaller than that for the chromosomes from the Aomori population both in homozygotes and in heterozygotes, especially in the latter. (3) The stability of heterozygotes over homozygotes against fluctuations of environmental conditions was seen in the chromosomes from the Ogasawara population, but not from the Aomori population. (4) From the excessive genotype-environment interaction variance compared with the genotypic variance in heterozygotes, it was suggested for the chromosomes from the Ogasawara population that the reversal of viability order between homozygotes took place in some environments at the locus level. On the basis of these findings, it is strongly suggested that diversifying selection is operating in a southern population of *D. melanogaster* on some of the viability polygenes which are probably located outside the structural loci, and the excessive additive genetic variance of viability in southern populations is maintained by this type of selection.

SINCE MUKAI *et al.* (1974) have estimated genetic variance and its components for viability in a natural population of *Drosophila melanogaster*, it has been found that there is more additive genetic variance in southern populations than expected on the basis of mutation-selection balance in both the United States and Japan (MUKAI and NAGANO 1983; TACHIDA *et al.* 1983). On the other hand, the genetic variability of a northern population in Japan can be explained by mutation-selection balance (KUSAKABE and MUKAI 1984a). MUKAI (1977), MUKAI, CHIGUSA and KUSAKABE (1982) and MUKAI and NAGANO (1983) have suggested that diversifying selection is responsible at a relatively small number of loci for the excessive additive genetic variance in southern populations, although mutation-selection balance holds at the majority of loci.

Two experimental results suggest the operation of diversifying selection in Drosophila populations. One is concerned with the stability of heterozygotes over homozygotes in viability (DOBZHANSKY and WALLACE 1953; DOBZHANSKY and LEVENE 1955; WILLS 1975; MUKAI, CHIGUSA and KUSAKABE 1982; MUKAI and NAGANO 1983). By using chromosomes from natural populations, the experimental results show that microenvironmental variance is smaller in heterozygotes than in homozygotes. In addition, this developmental homeostasis in heterozygotes has been suggested to be a product of natural selection and to be directly related to the amount of the genetic variability in natural populations. The reason is as follows: the difference in microenvironmental variance between heterozygotes and homozygotes could not be seen when a chromosome population with variation due only to new mutations and a northern population in Japan were tested (MUKAI, CHIGUSA and KUSAKABE 1982; KUSAKABE and MUKAI 1984a). The second result is the existence of genotype-environment inter-

action, which is essential to diversifying selection (DOBZHANSKY and LEVENE 1955; TACHIDA and MUKAI 1985). In particular, TACHIDA and MUKAI (1985) detected a significant genotype-environment interaction, using a population of *D. melanogaster* from southern Japan which has excessive additive genetic variance for viability.

In the present work, we carried out experiments using a southern Japanese population [Chichijima (Bonin) Island, Ogasawara] and a northern Japanese population (Aomori) of *D. melanogaster* in which viabilities were measured in various growing environments. The hypothesis that diversifying selection is operating in a southern population for the maintenance of genetic variability of viability was examined more directly by comparing genotype-environment interactions in a southern and in a northern population. Under this hypothesis, genotype-environment interaction is expected to be larger in a southern population than in a northern population, and this expectation was borne out.

MATERIALS AND METHODS

Stocks: The following stocks were used in the present experiments:

AO-711: This is the iso-female line collected at a locality of Hirosaki City, Aomori Prefecture in September 1981 and maintained at 18° in the Laboratory of Population Genetics, Kyushu University. It has the standard karyotype.

cn bw: A standard karyotype line whose second chromosomes carry two recessive eye color mutants, *cn* (cinnabar, 2-57.5) and *bw* (brown, 2-104.5).

C-160: *Cy/Pm* (*Cy*: Curly wings, included in *In(2LR)SM1* and *Pm*: plum eyes, carried by *In(2LR)bw*^{V1}) (MUKAI 1964).

C-160(G37): This is a stock which was developed by single-pair mating of C-160 stock for 37 generations.

Establishment of lines: Both experiments were carried out in the same manner except for the chromosomes used. All of the extractions of second chromosomes were made following the procedure described by WALLACE (1956) using the second chromosome balancer, *In(2LR)SM1* (abbreviated *Cy*). Details of the establishment of lines in each experiment are as follows:

Experiment 1. Adult male and female flies were collected in Chichijima (Bonin) Island, Ogasawara, in December 1982. Male flies were individually mated with five C-160(G37) females and a single F_1 *Cy*/+ male progeny was crossed with five C-160(G37) females. In order to make genetic background (*X*, third and fourth chromosomes and cytoplasm) uniform, such backcrosses of *Cy*/+ males to C-160(G37) were repeated for five or six generations. Finally, *Cy*/+ female and male progenies were collected and crosses among them were made to establish the chromosome line, and + chromosomes were maintained together with the *Cy* chromosomes. Iso-female lines were also established and one male from each iso-female line was chosen at random, and extraction was made in the same way as for male flies. About three hundred second chromosomes were extracted from each of the male and female groups, and these lines were named OG.

Experiment 2. The second chromosomes used in this experiment were extracted from female flies collected in September 1983 in two wineries near Hirosaki City, Aomori Prefecture. Before the extraction of the chromosomes, *Cy/Pm* stock (C-160(AO)) carrying the Aomori background was developed, using the iso-female line AO-711. The extraction was made in the same way as in experiment 1 but C-160(AO) was employed instead of C-160(G37). After repeated backcrosses to C-160(AO) for five generations, about 350 second chromosome lines carrying the genetic background of C-160(AO) were established and they are called AO in this paper.

All lines used in the above two experiments were maintained at 18° by mass mating. The experimental crosses were made at 25°. Before the experiments, two tests were made to choose appropriate chromosomes: estimation of homozygous viabilities and cytological surveys for each chromosome line.

Homozygote viabilities of OG and AO lines were estimated by the *Cy* method (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*/+ females and five *Cy*/+ males in each chromosome line. In the offspring, the expected segregation ratio of the *Cy* and wild-type flies is 2:1. Relative viability was expressed as $2b/(a + 1)$, where b is the number of +/+ flies and a stands for the number of the phenotypically *Cy* flies, and 1 in the denominator is HALDANE's (1956) correction for the bias in averaging ratios. 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 karyotype.

For experiments 1 and 2, 100 and 94 second chromosomes with the standard karyotype and viability indices of 0.5 or higher were randomly chosen from the OG and AO lines, respectively, to exclude genes with drastic effects and inversions from the analysis.

Experiment 1: Using second chromosomes of the Ogasawara population, homozygote and heterozygote viabilities were estimated in several different breeding environments with the *Cy* method as described above. Eight kinds of environments described below were employed as the breeding environments for the offspring. Two kinds of yeast, brewer's and baker's, and four kinds of culture medium, corn, banana, rice bran and tomato, were combined to constitute $2 \times 4 = 8$ environments. Details of the culture media are summarized in Table 1.

Homozygote crosses were made between five *Cy*/+$_i$ females and five *Cy*/+$_i$ males with two simultaneous replications in each chromosome line, where i indicates line number. The viabilities of random heterozygotes were estimated simultaneously with two replications, combining two lines at random in each set of experiments, *i.e.*, five *Cy*/+$_i$ females × five *Cy*/+$_j$ males. Four days after crosses were made, all ten parental flies were transferred to a second vial and after four more days they were discarded. The offspring were counted until the 18th day after the crosses (or transfers) were made. The counts from the original and transferred vials were pooled. In the offspring, *Cy* and wild-type flies in both homozygote and heterozygote crosses segregate at an expected ratio of 2:1. Viabilities were expressed as the logarithm of the ratio of twice the number of wild-type flies to the number of *Cy* flies without the HALDANE's (1956) correction, assuming the multiplicative contribution of each locus and environmental factor.

In each set of the experiments, six to nine chromosomes were tested simultaneously in the eight environments. In total 13 sets of the experiments were carried out.

Experiment 2: The AO lines were tested in the same way as in experiment 1. Twelve sets of the experiments were

TABLE 1

Formulas of the four culture media used in the experiment[a]

	Water (ml)	Base[b] (g)	Agar (g)	Sugar (g)	Molasses (ml)	Ebios[c] (g)	Propionic acid (ml)
Corn	1130	123*	6.7	15.7	31.7	22.3	4.5
Rice bran	1130	112**	6.7	40.0	56.0	30.0	4.5
Tomato	1130	470***	6.7	21.0		30.0	5.9
Banana	1130	616****	10.0	19.2		30.0	6.2

[a] These formulas each yield about 120 vials of culture media.

[b] As the base constituents, * corn meal, ** rice bran, *** tomato paste and **** banana were used.

[c] Dead brewer's yeast.

carried out and the total number of constituent chromosome lines was 94.

Analytical methods: Homozygote and heterozygote viabilities were analyzed separately. In the first place, two-way analyses of variance were conducted for each set under a random model. The environments represented are not a random sample of typical wild environments present in the north or south of Japan. However, the range of wild environments could be perhaps much more varied than this set of controlled media, thus the size of the genotype-environment interaction under natural conditions may be underestimated. The purpose in this paper is to compare genotype-environment interactions in a southern and a northern population. Hence, the results in the present experimental conditions based on the random model is conservative. Each sum of squares and degrees of freedom for respective sources of variations in the analyses of variance were pooled over all sets of experiments and variance components, that is, environmental variance, genotypic variance, genotype-environment interaction variance and error variance were estimated. Standard errors were calculated on the basis of the relationship $V(MS) \cong 2(MS)^2/\text{d.f.}$, where MS stands for a mean square, $V(MS)$ is its sampling variance and d.f. is its degree of freedom.

Genotypic variance (σ_G^2) and genotype-environment interaction variance ($\sigma_{G\times E}^2$) can be interpreted as follows: $\sigma_{G_iG_i}$ ($\sigma_{G_i}^2$) and $\sigma_{G_iG_j}$ can be defined as the variance for random entries in the ith environment and the covariance of entries between ith and jth environments. Then, the total variance (σ_{GT}^2) and the genotypic variance are given by:

$$\begin{aligned}\sigma_{GT}^2 &= E[\sigma_{G_iG_i}] \\ &= E[\sigma_{G_i}^2] \\ \sigma_G^2 &= E[\sigma_{G_iG_j}],\end{aligned}$$

where $E[\cdot]$ stands for expectation. The difference

$$\sigma_{GT}^2 - \sigma_G^2 = \sigma_{G\times E}^2.$$

This difference can be due to differences among the σ_{G_i}'s or lack of a perfect correlation between the σ_{G_i}'s and σ_{G_j}'s or both.

In this context, the average genetic correlation coefficient ($\bar{r}_g$) between any two viabilities tested in the eight breeding environments can be estimated as follows:

$$\hat{\bar{r}}_g = \frac{\widehat{\overline{\sigma_{G_iG_j}}}}{\widehat{\overline{\sigma_{G_i}\sigma_{G_j}}}}$$

where the bars stand for means.

Environmental variance due to random fluctuation of environments between replicated cultures, *i.e.*, microenvironmental variance, was calculated using the method of MUKAI, CHIGUSA and KUSAKABE (1982). The expected sampling variance (V_S) of viability indices for the multiplicative model is given by:

$$V_s = \frac{m+n}{m\times n}$$

where m and n indicate the number of the adult *Cy* flies and adult wild-type flies, respectively. Thus, microenvironmental variance was estimated by subtracting the average expected sampling variance of viability indices from the observed error variance. In contrast to this variance, the environmental variance estimated from the variance component analysis was called macroenvironmental variance.

THEORETICAL CONSIDERATION

To see the manifestation of diversifying selection in more concrete terms, assume the following simple model with one locus, two alleles and two environments:

Genotype	AA	Aa	aa
Frequency	p^2	$2pq$	q^2
Viability in environment 1	1	$1-hs$	$1-s$
Viability in environment 2	$1-s$	$1-hs$	1

where: $0 < s < 1$ and $0 < h < 1/2$, and p and q are the frequency of allele A and a, respectively. What can be seen from this model is as follows: (1) genotype-environment interaction exists mainly because of the lack of perfect correlation between genotypic values in environment 1 and 2. Based on the above model, genotype-environment interaction variance ($\sigma_{g\times e}^2$) and genotypic variance (σ_g^2) for these two environments are given by:

$$\sigma_{g\times e}^2 = \frac{1}{2}pqs^2 \tag{1}$$

$$\sigma_g^2 = \frac{1}{2}pq(p^2+q^2)(2h-1)^2s^2. \tag{2}$$

For $p = 0.9$, $q = 0.1$, $s = 0.1$ and $h = 0.4$, then

$$\begin{aligned}\sigma_{g\times e}^2 &= 0.000450 \\ \sigma_g^2 &= 0.000015\end{aligned}$$

and

$$\frac{\sigma_{g\times e}^2}{\sigma_g^2} = 30.$$

About 97% of the total variance is due to the genotype-environment interaction variance. (2) The stability of heterozygotes over homozygotes against fluctuations of environmental conditions. (3) The reversal of viability order between homozygotes at the locus level.

It should be noted here that the experimental estimates for genotypic variance and genotype-environment interaction variance are representing all of the loci affecting viability in the second chromosome. In the present experiment, it is assumed that there is no epistatic interaction and no linkage disequilibrium

between any two loci. This assumption has perhaps been confirmed by the experimental data (MUKAI *et al.* 1974; MUKAI and NAGANO 1983; TACHIDA *et al.* 1983; KUSAKABE and MUKAI 1984a; H. HONDA and T. MUKAI, unpublished data). Then the estimates of genotypic variance (σ_G^2) and genotype-environment interaction variance ($\sigma_{G\times E}^2$) can be expressed as the sum over all loci affecting viability in the second chromosome. Let us assume that the genotype-environment interaction variance for the whole chromosome is M times as large as the genotypic variance. Since any genotype-environment interaction variance and genotypic variance for a single locus cannot be negative, the genotype-environment interaction variance should be M times as large as the genotypic variance at least at one locus. To be more exact, the maximum ratio of $\sigma_{g\times e}^2$ to σ_g^2 at the locus level should be equal to or greater than that ratio at the chromosome level. In order to examine the reversal of viability order between homozygotes at the locus level, the following consideration was made by using this M value.

Before the analysis, it will be necessary to take account of the effect of incomplete dominance of the *Cy* chromosome. Then, the following three models will be analyzed separately.

Cases where *Cy* chromosome is complete dominance: The relative viability array for a locus with two alleles is defined as in Figure 1, where s_i, h_i and u_i are selection coefficient, degree of dominance and the common environmental effect in the ith environment. Using the random model, the genotypic variance (σ_g^2) and genotype-environment interaction variance ($\sigma_{g\times e}^2$) for heterozygotes are given by:

$$\sigma_g^2 = pq[2(p^2 + q^2)\bar{h}^2 - 4q^2\bar{h} + q(1 + q)]\bar{s}^2 \quad (3)$$

$$\sigma_{g\times e}^2 = pq[2(p^2 + q^2)\,\bar{h}^2 - 4q^2\bar{h} + q(1 + q) + 2(p^2 + q^2)\sigma_h^2]\sigma_s^2 + 2pq(p^2 + q^2)\sigma_h^2\bar{s}^2 \quad (4)$$

where $\bar{s}(\bar{h})$ and σ_s^2 (σ_h^2) are the arithmetic mean of $s_i(h_i)$ and the variance of $s_i(h_i)$, respectively, and it was assumed that h_i is not correlated with s_i. If the genotype-environment interaction variance is M times as large as the genotypic variance, the condition for this phenomenon is:

$$\frac{\sigma_s^2}{\bar{s}^2} = M - \frac{2(M + 1)(p^2 + q^2)\sigma_h^2}{2(p^2 + q^2)\bar{h}^2 - 4q^2\bar{h} + q(1 + q) + 2(p^2 + q^2)\sigma_h^2}. \quad (5)$$

The formulas for homozygotes corresponding to formulas (3) and (4) can be expressed as follows (using the same notations as above):

$$\sigma_g^2 = pq\bar{s}^2 \quad (6)$$

$$\sigma_{g\times e}^2 = pq\sigma_s^2. \quad (7)$$

	Genotype			Mean
	AA	*Aa*	*aa*	
Frequency	p^2	$2pq$	q^2	--
Environment 1	u_1	$u_1(1-h_1s_1)$	$u_1(1-s_1)$	$u_1(1-2pqh_1s_1-q^2s_1)$
Environment 2	u_2	$u_2(1-h_2s_2)$	$u_2(1-s_2)$	$u_2(1-2pqh_2s_2-q^2s_2)$
.	.	.	.	.
Environment k	u_k	$u_k(1-h_ks_k)$	$u_k(1-s_k)$	$u_k(1-2pqh_ks_k-q^2s_k)$

FIGURE 1.—Relative viability array for a locus with two alleles. s_i, h_i and u_i are selection coefficient, degree of dominance and the common environmental effect in the ith environment, respectively.

If

$$\frac{\sigma_{g\times e}^2}{\sigma_g^2} = M, \quad (8)$$

then

$$\frac{\sigma_s^2}{\bar{s}^2} = M. \quad (9)$$

Cases where *Cy* chromosome carries wild-type alleles (*A*): The relative viability array for *AA*, *Aa* and *aa* can be expressed as 1, $(1 - h_is_i)/(1 - h_is_i/2)$ and $(1 - s_i)/(1 - h_is_i)$, respectively. Assuming that s_i, and h_is_i are much smaller than one, the relative viability of *AA*, *Aa* and *aa* can be expressed approximately as follows:

$$\left.\begin{array}{l} AA : 1 \\ Aa : 1 - hs/2 \\ aa : 1 + hs - s \end{array}\right\} \quad (10)$$

where subscripts are omitted. Then,

$$\sigma_g^2 = \frac{pq}{2}[(8q^2 + 1)\bar{h}^2 - (8q^2 + 4q)\bar{h} + 2q^2 + 2q]\bar{s}^2 \quad (11)$$

$$\sigma_{g\times e}^2 = \frac{pq}{2}[(8q^2 + 1)\bar{h}^2 - (8q^2 + 4q)\bar{h} + 2q^2 + 2q + (8q^2 + 1)\sigma_h^2]\sigma_s^2 + \frac{pq}{2}(8q^2 + 1)\sigma_h^2\bar{s}^2. \quad (12)$$

The condition for formula (8) is:

$$\frac{\sigma_s^2}{\bar{s}^2} = M - \frac{(M + 1)(8q^2 + 1)\sigma_h^2}{(8q^2 + 1)\bar{h}^2 - (8q^2 + 4q)\bar{h} + 2q^2 + 2q + (8q^2 + 1)\sigma_h^2}. \quad (13)$$

As in the case where *Cy* is complete dominance the formulas for homozygotes corresponding to formulas (11) and (12) can be given as follows:

$$\sigma_g^2 = pq(1 - \bar{h})^2\bar{s}^2 \quad (14)$$

$$\sigma_{g\times e}^2 = pq[(1 - \bar{h})^2 + \sigma_h^2]\sigma_s^2 + pq\sigma_h^2\bar{s}^2. \quad (15)$$

For $\bar{h} = 1.0$, $\sigma_g^2 = 0$. For $\bar{h} \neq 1.0$, the condition for formula (8) is:

$$\frac{\sigma_s^2}{\bar{s}^2} = M - \frac{(M+1)\sigma_h^2}{(1-\bar{h})^2 + \sigma_h^2}. \tag{16}$$

Cases where *Cy* chromosome carries mutant alleles (*a*): The relative viability array for *AA*, *Aa* and *aa* can be expressed as $1/(1-hs)$, $(1-hs)/\{1-1/2(hs+s)\}$ and 1. As in the case where *Cy* carries wild-type alleles, they can be expressed approximately as follows:

$$\left.\begin{array}{l} AA : 1 \\ Aa : 1 - 1/2(3hs - s) \\ aa : 1 - hs. \end{array}\right\} \tag{17}$$

Then,

$$\sigma_g^2 = \frac{pq}{2}[(8q^2 - 16q + 9)\bar{h}^2 - (8q^2 - 12q + 6)\bar{h} + (2q^2 - 2q + 1)]\bar{s}^2 \tag{18}$$

$$\sigma_{g\times e}^2 = \frac{pq}{2}[(8q^2 - 16q + 9)\bar{h}^2 - (8q^2 - 12q + 6)\bar{h} + (2q^2 - 2q + 1) + (8q^2 - 16q + 6)\sigma_h^2]\sigma_s^2 + \frac{pq}{2}(8q^2 - 16q + 9)\sigma_h^2\bar{s}^2. \tag{19}$$

The condition of formula (8) is

$$\frac{\sigma_s^2}{\bar{s}^2} = M - \frac{(M+1)(8q^2 - 16q + 9)\sigma_h^2}{(8q^2 - 16q + 9)\bar{h}^2 - (8q^2 - 12q + 6)\bar{h} + 2q^2 - 2q + 1 + (8q^2 - 16q + 9)\sigma_h^2}. \tag{20}$$

For homozygotes,

$$\sigma_g^2 = pq\bar{h}^2\bar{s}^2 \tag{21}$$

$$\sigma_{g\times e}^2 = pq(\bar{h}^2 + \sigma_h^2)\sigma_s^2 + pq\sigma_h^2\bar{s}^2. \tag{22}$$

For $\bar{h} = 0$, $\sigma_g^2 = 0$. For $\bar{h} \neq 0$, the condition of formula (8) is:

$$\frac{\sigma_s^2}{\bar{s}^2} = M - \frac{(M+1)\sigma_h^2}{\bar{h}^2 + \sigma_h^2}. \tag{23}$$

As mentioned above, the maximum ratio of $\sigma_{g\times e}^2$ to σ_g^2 at the locus level is equal to or greater than the ratio of the estimates ($\sigma_{G\times E}^2$ to σ_G^2) at the chromosome level. Therefore, formulas (5), (9), (13), (16), (20) and (23), respectively, turn out to be as follows:

$$\frac{\sigma_s^2}{\bar{s}^2} \geqq M - \frac{2(M+1)(p^2+q^2)\sigma_h^2}{2(p^2+q^2)\bar{h}^2 - 4q^2\bar{h} + q(1+q) + 2(p^2+q^2)\sigma_h^2} \tag{5'}$$

$$\frac{\sigma_s^2}{\bar{s}^2} \geqq M \tag{9'}$$

$$\frac{\sigma_s^2}{\bar{s}^2} \geqq M - \frac{(M+1)(8q^2+1)\sigma_h^2}{(8q^2+1)\bar{h}^2 - (8q^2+4q)\bar{h} + 2q^2 + 2q + (8q^2+1)\sigma_h^2} \tag{13'}$$

$$\frac{\sigma_s^2}{\bar{s}^2} \geqq M - \frac{(M+1)\sigma_h^2}{(1-\bar{h})^2 + \sigma_h^2} \tag{16'}$$

$$\frac{\sigma_s^2}{\bar{s}^2} \geqq M - \frac{(M+1)(8q^2 - 16q + 9)\sigma_h^2}{(8q^2 - 16q + 9)\bar{h}^2 - (8q^2 - 12q + 6)\bar{h} + 2q^2 - 2q + 1 + (8q^2 - 16q + 9)\sigma_h^2} \tag{20'}$$

$$\frac{\sigma_s^2}{\bar{s}^2} \geqq M - \frac{(M+1)\sigma_h^2}{\bar{h}^2 + \sigma_h^2}. \tag{23'}$$

RESULTS

Experiment 1: Viability estimation was performed for second chromosomes extracted from the Ogasawara population of *D. melanogaster*. One hundred homozygous lines and the same number of heterozygous crosses were employed in the analysis.

The pooled results of two-way analyses of variance conducted for homozygote and heterozygote viabilities are shown in Table 2. All variance components were significantly larger than zero. Variance components were estimated from these analyses and are shown in Table 3. Among these components, macroenvironmental variance and genotype-environment interaction variance are due to variability of viability in different environments. These results indicate that viabilities of homozygotes and heterozygotes varied when the breeding environments were changed and the genotype-environment interaction existed, which, in fact, significantly exceeded the component of variance for main effects of environments. Furthermore, estimates for homozygotes with respect to both the variance components were significantly larger than those for heterozygotes. This implies that heterozygotes are more stable against fluctuations of environmental conditions than homozygotes. In addition, it should be stressed here that in heterozygotes the estimate of genotype-environment interaction variance is significantly larger than that of genotypic variance, whereas in homozygotes the latter is significantly larger than the former. This excessive interaction variance in heterozygotes will be discussed in detail below.

Microenvironmental variance of viabilities for homozygotes and heterozygotes were estimated using the formulas described in the MATERIALS AND METHODS. Lines and crosses with only one observation (one is missing) or with definitely accidental heterogeneous observations were excluded from the analysis. Fur-

TABLE 2

Pooled results of the two-way analyses of variance for viability of the Ogasawara population

Source	d.f.	Sum of squares	Mean square	F	EMS
A. Homozygotes					
Genotype (G)	87	63.1258	0.725584	9.73**	$\sigma_e^2 + 2\sigma_{G\times E}^2 + 16.000\sigma_G^2$
Environment (E)	91	18.1942	0.199936	2.68**	$\sigma_e^2 + 2\sigma_{G\times E}^2 + 15.385\sigma_E^2$
$G \times E$	609	45.4262	0.074591	2.35**	$\sigma_e^2 + 2\sigma_{G\times E}^2$
Error	796	25.3069	0.031793		σ_e^2
Total	1583	152.0531			
B. Heterozygotes					
Genotype (G)	87	5.8171	0.066863	2.40**	$\sigma_e^2 + 2\sigma_{G\times E}^2 + 16.000\sigma_G^2$
Environment (E)	91	5.4913	0.060344	2.17**	$\sigma_e^2 + 2\sigma_{G\times E}^2 + 15.385\ \sigma_E^2$
$G \times E$	609	16.9429	0.027820	1.67**	$\sigma_e^2 + 2\sigma_{G\times E}^2$
Error	798	13.2730	0.016632		σ_e^2
Total	1585	41.5243			

** Significant at the 1% level.

TABLE 3

Estimates and standard deviations of variance components for viability of the Ogasawara population

Variance component	Homozygotes	Heterozygotes
Genotype (G)	0.040687 ± 0.006881**	0.002440 ± 0.000641**
Macroenvironment (E)	0.008147 ± 0.001947**	0.002114 ± 0.000591**
$G \times E$ interaction	0.021399 ± 0.002281**	0.005594 ± 0.000899**
Error	0.031793 ± 0.001594	0.016633 ± 0.000833

** Significant at the 1% level.

thermore, if there was any cross whose viability index without logarithmic transformation was less than 0.5, it was not used in the present analysis. Regarding observations in different environments for the same cross as being independent, 723 homozygote and 795 heterozygote crosses were employed in the analysis. The estimated microenvironmental variances for homozygotes and heterozygotes were 0.00715 ± 0.00142 and 0.00316 ± 0.00085, respectively, and the difference between them was significant ($P < 0.025$). As in the case of macroenvironmental variance and genotype-environment interaction variance, it can be seen from the microenvironmental variance that heterozygotes are more homeostatic than homozygotes.

The average genetic correlation coefficients between viabilities estimated in the eight kinds of breeding environments were calculated and they are shown in Table 6 together with the results of experiment 2. As pointed out by COCKERHAM (1963), genotype-environment interaction variance could arise for two reasons, one being that genetic variances in the different environments are different and the other being a lack of perfect correlation of the genetic effects in the different environments. It is the latter that is important to maintain polymorphisms (TACHIDA and MUKAI 1985). Therefore, this correlation coefficient can be a measure for the test of the operation of diversifying selection, *i.e.*, this coefficient is expected to be reduced in the case of the operation of this type of selection. The estimates for both homozygotes and heterozygotes of the present population were lower than those for the Aomori population, especially in heterozygotes, as will be described below.

Experiment 2: Using the second chromosomes extracted from the Aomori population, the viabilities of 89 homozygous lines and 88 heterozygous crosses in total were estimated in each of the eight kinds of breeding environments. Analyses were conducted in entirely the same way as in experiment 1.

The pooled results of two-way analyses of variance and the estimated variance components are shown in Tables 4 and 5, respectively. The genotype-environment interaction variance was significant only in homozygotes. On the contrary, the macroenvironmental variance was significantly different from zero only in heterozygotes. These findings are in striking contrast to that for the Ogasawara population.

The microenvironmental variances for homozygotes and heterozygotes respectively were estimated to be 0.00464 ± 0.00118 and 0.00300 ± 0.00089, using 666 homozygote and 652 heterozygote crosses. The difference between them was not statistically significant.

The above results suggest that the viabilities of homozygotes and heterozygotes fluctuated when the breeding environments were changed. However, the existence of genotype-environment interaction was

TABLE 4

Pooled results of the two-way analyses of variance for viability of the Aomori population

Source	d.f.	Sum of squares	Mean square	F	EMS
A. Homozygotes					
Genotype (G)	77	16.9940	0.220701	7.56**	$\sigma_e^2 + 2\sigma_{G\times E}^2 + 16.000\sigma_G^2$
Environment (E)	84	2.6264	0.031267	1.07	$\sigma_e^2 + 2\sigma_{G\times E}^2 + 14.833\sigma_E^2$
$G \times E$	539	15.7295	0.029183	1.49**	$\sigma_e^2 + 2\sigma_{G\times E}^2$
Error	674	13.2151	0.019607		σ_e^2
Total	1374	48.5650			
B. Heterozygotes					
Genotype (G)	76	2.0940	0.027552	1.72**	$\sigma_e^2 + 2\sigma_{G\times E}^2 + 16.000\sigma_G^2$
Environment (E)	84	2.0158	0.023997	1.50**	$\sigma_e^2 + 2\sigma_{G\times E}^2 + 14.667\sigma_E^2$
$G \times E$	532	8.5192	0.016014	1.06	$\sigma_e^2 + 2\sigma_{G\times E}^2$
Error	652	9.8478	0.015104		σ_e^2
Total	1344	22.4768			

** Significant at the 1% level.

TABLE 5

Estimates and standard deviations of variance components for viability of the Aomori population

Variance component	Homozygotes	Heterozygotes
Genotype (G)	0.011970 ± 0.002226**	0.000721 ± 0.000286**
Macroenvironment (E)	0.000141 ± 0.000347	0.000544 ± 0.000261**
$G \times E$ interaction	0.004788 ± 0.001037**	0.000455 ± 0.000645
Error	0.019607 ± 0.001068	0.015104 ± 0.000837

** Significant at the 1% level.

TABLE 6

Average genetic correlation coefficients between any two viabilities of the same lines estimated in the eight kinds of breeding environments

Population	Homozygotes	Heterozygotes
Ogasawara	0.688	0.351
Aomori	0.750	1.180

not detected in heterozygotes. Furthermore, consistent evidence of developmental homeostasis in heterozygotes was not found in contrast to the results obtained for the Ogasawara population. Hence, it appears that the fluctuation of viabilities found in the present experiment has little relation to diversifying selection.

The estimated average genetic correlations between viabilities tested in the eight environments were shown in Table 6. For homozygotes and heterozygotes, they were 0.750 and 1.180, respectively. Compared with the results of the Ogasawara population, their absolute values are larger than those in the southern population both in homozygotes and in heterozygotes. Indeed, the estimate of the correlation coefficient for heterozygotes in this population is quite close to 1.

Comparison of the results between the Ogasawara population and the Aomori population: The main results of the present work are shown graphically in Figure 2. Significant genotype-environment interaction was observed using the Ogasawara population. Indeed, the estimate of the genotype-environment interaction variance for heterozygotes was significantly larger than that of the genotypic variance. On the other hand, when the chromosomes sampled from the Aomori population were examined, that interaction variance was significant only for homozygotes and its value was no more than one quarter of that for the chromosomes from the Ogasawara population. Moreover, the average genetic correlation coefficient between any two viabilities of the same lines estimated in the eight kinds of breeding environments was smaller for the chromosomes sampled from the Ogasawara population than that for the chromosomes from the Aomori population both in homozygotes and in heterozygotes, especially in the latter. This implies that the Ogasawara population has an excess of genotype-environment interaction variance, due to the lack of perfect correlation of the genetic effects in the different environments. The stability of heterozygotes over homozygotes against fluctuations of environmental conditions was seen in the chromosomes from the Ogasawara population, but not from the Aomori population. This finding was also confirmed by the results of the microenvironmental variance.

The next step is to examine whether or not the reversal of viability order between homozygotes at the locus level takes place in different environments. As

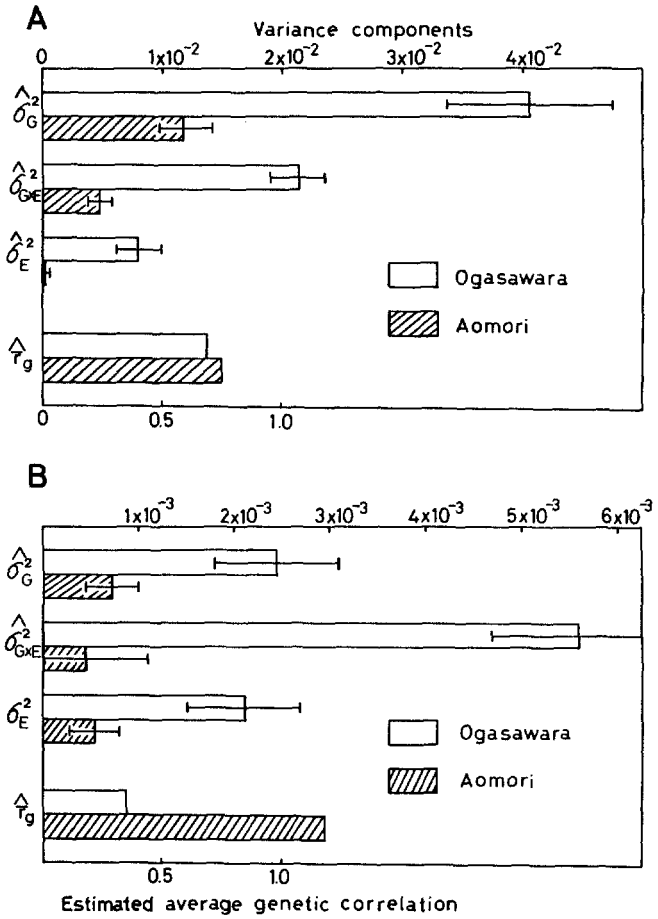

FIGURE 2.—Estimates and standard deviations of variance components for viability and average genetic correlation coefficients between two viabilities of the same lines estimated in the eight kinds of breeding environments ($\hat{r}_g$)·σ^2_G, σ^2_E and $\sigma^2_{G\times E}$ are genotypic, macroenvironmental and genotype-environment interaction variances, respectively. A, Homozygotes; B, heterozygotes.

TABLE 7

Minimum estimates of square of coefficient of variation of selection coefficient ($\sigma_s^2/\bar{s}^2$) for various gene frequencies of mutant allele (q), means ($\bar{h}$) and variances (σ_h^2) of degrees of dominance

		Gene frequency					
		0.4	0.2	0.1	0.01	0.001	0.0001
(1) $\bar{h}=0.3$	$\sigma_h^2=0.0001$	2.29	2.29	2.29	2.29	2.29	2.29
		2.29	2.29	2.29	2.29	2.29	2.29
		2.28	2.26	2.21	2.06	2.03	2.02
	$\sigma_h^2=0.001$	2.29	2.28	2.27	2.26	2.26	2.26
		2.28	2.28	2.27	2.26	2.26	2.26
		2.19	1.96	1.62	0.87	0.75	0.73
	$\sigma_h^2=0.01$	2.22	2.16	2.09	1.98	1.97	1.96
		2.14	2.14	2.10	1.99	1.97	1.96
		1.51	0.56	−0.08	−0.62	−0.67	−0.67
(2) $\bar{h}=0.4$	$\sigma_h^2=0.0001$	2.29	2.29	2.29	2.29	2.29	2.29
		2.29	2.29	2.29	2.29	2.29	2.29
		2.28	2.27	2.26	2.23	2.22	2.22
	$\sigma_h^2=0.001$	2.29	2.28	2.28	2.27	2.27	2.27
		2.27	2.28	2.28	2.27	2.27	2.27
		2.21	2.09	1.95	1.72	1.69	1.69
	$\sigma_h^2=0.01$	2.22	2.18	2.15	2.10	2.10	2.10
		2.08	2.12	2.12	2.10	2.10	2.10
		1.61	0.97	0.53	0.07	0.02	0.01
(3) $\bar{h}=0.5$	$\sigma_h^2=0.0001$	2.29	2.29	2.29	2.29	2.29	2.29
		2.29	2.29	2.29	2.29	2.29	2.29
		2.29	2.29	2.28	2.28	2.28	2.28
	$\sigma_h^2=0.001$	2.29	2.28	2.28	2.28	2.28	2.28
		2.26	2.28	2.28	2.28	2.28	2.28
		2.24	2.21	2.20	2.18	2.18	2.18
	$\sigma_h^2=0.01$	2.23	2.21	2.19	2.17	2.17	2.17
		2.02	2.13	2.16	2.17	2.17	2.17
		1.85	1.65	1.53	1.43	1.42	1.42

The estimates can be applied only to one locus with the largest ratio of genotype-environment interaction variance to genotypic variance ($\sigma^2_{g\times e}/\sigma^2_g$). The calculation is made based on $\sigma^2_{G\times E}/\sigma^2_G = 2.293$. The upper row assumes the complete dominance of the *Cy* chromosome. The middle and lower rows assume that the *Cy* chromosome carries a wild-type allele (*A*) and a mutant allele (*a*), respectively.

described above, the estimate of genotype-environment interaction variance was significantly larger than that of the genotypic variance. Therefore, it is appropriate here to investigate the condition for this phenomenon. The ratio of the genotype-environment interaction variance to the genotypic variance was estimated to be 2.293. Using this value and Formulas (5), (13) and (20), the squares of the coefficients of variation of the selection coefficient ($\sigma_s^2/\bar{s}^2$) are shown for various q, h and σ_h^2 values in Table 7. The range of the average degree of dominance for mildly deleterious genes was estimated to be 0.2–0.3, for a southern Japanese population (Ishigakijima) and two northern Japanese populations (Aomori and Akayu) (TACHIDA *et al.* 1983; KUSAKABE and MUKAI 1984a; SUH and MUKAI 1987). As in the case of the populations in the United States (MUKAI and YAMAGUCHI 1974; MUKAI and NAGANO 1983), the additive genetic variance for the southern population was estimated to be larger than that for the northern populations (about seven times). However, there is no difference for detrimental load between the southern and the northern populations. This implies that a relatively small number of loci are responsible for an excessive additive genetic variance for viability, and the degrees of dominance for such genes are very close to 0.5. (MUKAI, CHIGUSA and KUSAKABE 1982; MUKAI and NAGANO 1983). From Table 7, the square of the coefficient of variation for selection coefficient can be estimated to be 1.5–2.3. This finding strongly suggests the reversal of viability order between homozygotes in different environments at the locus level. The exception can be seen when $\sigma_h^2 = 0.01$ and the *Cy* chromosome carries a mutant allele (*a*), especially in the case of a low frequency of the mutant allele. The *Cy* chromosome has standard viability in comparison with the chromosomes from a natural population. Hence, only a small number of loci can carry mutant

alleles. As described above, a relatively small number of loci are responsible for an excess of additive genetic variance for viability in southern populations. Then it can be neglected that the *Cy* chromosome carries a mutant allele for a locus responsible for the excessive additive genetic variance. Thus, the above suggestion can be seriously violated.

Finally, inconsistency between the results in heterozygotes and that in homozygotes should be discussed. An excess of genotype-environment interaction variance in comparison with genotypic variance was not observed in homozygotes. Indeed, this ratio was estimated to be 0.526 and this value is about one quarter of that in heterozygotes. This difference between heterozygotes and homozygotes may be explained as follows: only the second chromosomes with viability indices of 0.5 or higher were chosen in the present work. However, semilethal genes with small h values (*cf.* HIRAIZUMI and CROW 1960) may have been accidentally involved in the present materials. In this situation, such genes can contribute greatly to genotypic variance in homozygotes, but not in heterozygotes. If such genes are stable against fluctuation of environmental conditions, the present result in homozygotes can not be contradictory to that in heterozygotes.

All of the above findings, together with the previous results in our laboratory, may be explained most straightforwardly as follows: a mutation-selection balance holds at the majority of the polygenic loci affecting viability in *D. melanogaster* and genotype-environment interaction can not be detected so much at such loci. However, genotype-environment interaction at some fraction of the polygenic loci is much larger and the gene frequencies at such loci become more intermediate in the southern population than in the northern population. Thus, it is most probable that diversifying selection is operating at such loci in the southern population. Indeed, the variety of the fauna and the flora is generally much greater in the southern environments than in the northern environments. This suggests that niche variability in the southern populations is much larger than in the northern populations.

In conclusion, the present results strongly suggest the operation of diversifying selection for viability in the Ogasawara population.

DISCUSSION

On the basis of the findings in the present work, the operation of diversifying selection for viability was suggested in the southern population of *D. melanogaster*. Consequently, additional genetic variability of viability may be maintained in the southern population in comparison with the northern population. It should be noted again that there is a north-to-south cline of additive genetic variance of viability and the amount of the genetic variability in the northern population can be explained by mutation-selection balance (MUKAI 1985; MUKAI *et al.* 1974; MUKAI and NAGANO 1983; TACHIDA *et al.* 1983; KUSAKABE and MUKAI 1984a). MUKAI and NAGANO (1983) have suggested that diversifying selection appears to be most responsible for the excess of additive genetic variance of viability in the southern populations. Further analyses for this north-to-south cline of genetic variance of viability have been made based on a finite population model (KUSAKABE and MUKAI 1984b), and they have reached the conclusion that the changes in mutation rate and population size can not explain the observed results. The present results are concordant with the above suggestion. Indeed, it has been shown that the frequencies of heterozygously homeostatic alleles at a small fraction of loci increase through natural selection (MUKAI, CHIGUSA and KUSAKABE 1982).

On the other hand, the evidence against the operation of diversifying selection on the protein polymorphic loci have been reported as follows (review in MUKAI 1977; MUKAI *et al.* 1982): (1) From the data in a carefully designed experiment (YAMAZAKI *et al.* 1983), it has been shown that the previously published supporting evidence for this type of selection in the cage experiments (POWELL 1971; MCDONALD and AYALA 1974; MINAWA and BIRLEY 1978) was caused by linkage disequilibria between the genes in question and fitness-affecting factors. (2) Most protein polymorphisms are selectively neutral or nearly neutral (*cf.* KIMURA 1968, 1979; KIMURA and OHTA 1971). In fact, the maximum confidence interval of the average selection coefficient of allozyme genes is estimated to be of the order of 10^{-4} which includes 0 (MUKAI, TACHIDA and ICHINOSE 1980). (3) In contrast to the north-to-south cline of additive genetic variance, the average heterozygosities at the loci coding for proteins are very similar over a wide range of geographical locations (MUKAI 1977; MUKAI and NAGANO 1983; KUSAKABE and MUKAI 1984b). (4) On the basis of mutation studies, it has been speculated that most viability and the other fitness polygenes are located in controlling regions outside the structual genes (MUKAI and COCKERHAM 1977). In addition, it is suggested that many mutations controlling the activity of alcohol dehydrogenase occurred in regions different from the alcohol dehydrogenase locus itself, mainly in the noncoding DNA (MUKAI, HARADA and YOSHIMARU 1984).

On the basis of the above findings, it can be deduced that the diversifying selection on viability suggested from the present experiment does not operate on structual loci, but on factors outside the structual loci.

In conclusion, the present results strongly suggest that in a southern population of *D. melanogaster*, diversifying selection is operating for viability polygenes

which are most probably located outside the structual loci.

We would like to thank Asahi Breweries Limited for providing the brewer's yeast. We are also grateful to C. C. COCKERHAM and T. TACHIDA for their constructive criticism and suggestions on this paper. This is Paper No. 34 from the Laboratory of Population Genetics, Department of Biology, Kyushu University, Japan. This investigation was supported mainly by research grants from the Ministry of Education, Science and Culture of Japan and also in part by a National Institutes of Health research grant GM32518 from the National Institute of General Medical Science of the U.S.A. to B. S. WEIR of North Carolina State University, to whom we are grateful.

LITERATURE CITED

COCKERHAM, C. C., 1963 Estimation of genetic variances. pp. 53–94. In: *Statistical Genetics and Plant Breeding*, Edited by W. D. HANSON and H. F. ROBINSON. National Research Council, Publication 982, Washington, D.C.

DOBZHANSKY, TH. and H. LEVENE, 1955 Genetics of natural populations. XXIV. Developmental homeostasis in natural populations of *Drosophila pseudoobscura*. Genetics **40:** 797–808.

DOBZHANSKY, TH. and B. WALLACE, 1953 The genetics of homeostasis in Drosophila. Proc. Natl. Acad. Sci. USA **39:** 162–171.

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

HIRAIZUMI, Y. and J. F. CROW, 1960 Heterozygous effects of viability, fertility, rate of development and longevity of Drosophila chromosomes that are lethal when homozygous. Genetics **45:** 1071–1083.

KIMURA, M., 1968 Evolutionary rate at the molecular level. Nature **217:** 624–626.

KIMURA, M., 1979 The neutral theory of moleuclar evolution. Sci. Am. **241:** 94–104.

KIMURA, M. and T. OHTA, 1971 *Theoretical Aspects of Population Genetics*. Princeton University Press, Princeton, N.J.

KUSAKABE, S. and T. MUKAI, 1984a The genetic structure of natural populations of *Drosophila melanogaster*. XVII. A population carrying genetic variability explicable by the classical hypothesis. Genetics **108:** 393–408.

KUSAKABE, S. and T. MUKAI, 1984b The genetic structure of natural populations of *Drosophila melanogaster*. XVIII. Clinal and uniform genetic variation over populations. Genetics **108:** 617–632.

MCDONALD, J. F. and F. J. AYALA, 1974 Genetic response to environmental heterogeneity. Nature **250:** 572–574.

MINAWA, A. and A. J. BIRLEY, 1978 The genetical response to natural selection by varied environments. I. Short-term observations. Heredity **40:** 39–50.

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., 1977 Lack of experimental evidence supporting selection for the maintenance of isozyme polymorphisms in *Drosophila melanogaster*. pp. 103–126. In: *Proceedings of the Second Taniguchi International Symposium on Biophysics: Molecular Evolution and Polymorphism*, Edited by M. KIMURA. National Institute of Genetics, Mishima.

MUKAI, T., 1985 Experimental verification of the neutral theory. pp. 125–145. In: *Population Genetics and Molecular Evolution*, Edited by T. OHTA and K. AOKI. Japan Scientific Societies Press, Tokyo.

MUKAI, T. and C. C. COCKERHAM, 1977 Spontaneous mutation rates at enzyme loci in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **74:** 2514–2517.

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

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., K. HARADA and H. YOSHIMARU, 1984 Spontaneous mutations modifying the activity of alcohol dehydrogenase (ADH) in *Drosophila melanogaster*. Genetics **106:** 73–84.

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., 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., O. YAMAGUCHI, S. KUSAKABE, H. TACHIDA, M. MATSUDA, M. ICHINOSE and H. YOSHIMARU, 1982 Lack of balancing selection for protein polymorphisms. pp. 81–120. In: *Molecular Evolution, Protein Polymorphism and the Neutral Theory*, Edited by M. KIMURA. Japan Scientific Societies Press, Tokyo/Spring-Verlag, Berlin.

POWELL, J. R., 1971 Genetic polymorphisms in varied environments. Science **174:** 1035–1036.

SUH, D. S. and T. MUKAI, 1987 Genetic variability due to mutation-selection balance in a northern population of *Drosophila melanogaster* in Japan. Jpn. J. Genet. **62:** 95–107.

TACHIDA, H. and T. MUKAI, 1985 The genetic structure of natural populations of *Drosophila melanogaster*. XIX. Genotype-environment interaction in viability. Genetics **111:** 43–55.

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.

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

WILLS, C., 1975 Marginal overdominance in Drosophila. Genetics **81:** 177–189.

YAMAZAKI, T., S. KUSAKABE, H. TACHIDA, M. ICHINOSE, H. YOSHIMARU, Y. MATSUO and T. MUKAI, 1983 Reexamination of diversifying selection of polymorphic allozyme genes by using population cages in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **80:** 5789–5792.

Communicating editor: B. S. WEIR