VIABILITY MUTATIONS INDUCED BY ETHYL METHANESULFONATE IN *DROSOPHILA MELANOGASTER*[1]

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MUKAI and YAMAZAKI (1964, 1968) discovered a coupling-repulsion effect in *Drosophila melanogaster*. If mutant polygenes are located in only one of the homologous chromosomes, they show overdominance—this has been called the "coupling effect." On the other hand, if mutant polygenes are located in both homologous chromosomes, they are deleterious in the heterozygous condition in comparison to their wild-type alleles and their degree of dominance (h in the Wrightian fitness model) is large—this has been called the "repulsion effect." If this finding becomes generally accepted, the mechanism of maintenance of genetic variability with respect to fitness in populations will need to be reevaluated. In fact, MUKAI (1968a,b) has already proposed a model for the maintenance of genetic variability in populations on the basis of the coupling-repulsion effect. Since this effect is significant for understanding the genetic load problem and since it is a difficult phenomenon to understand on the basis of our present knowledge of molecular genetics, it is necessary to examine the generality of this phenomenon over different lines and different species of Drosophila. Any experimental techniques which would simplify or hasten such investigations would be highly useful.

LEWIS and BACHER (1968) published an effective feeding method for inducing mutations in *Drosophila melanogaster* using ethyl methanesulfonate (this was orally presented by DR. E. B. LEWIS at the Eighth Drosophila Research Conference held at the University of Chicago in May, 1966). If EMS induces many polygenic mutants affecting viability and if their characteristics are similar to those of spontaneous mutant polygenes, the generality of the coupling-repulsion effect could be examined very easily. Thus, an EMS experiment was conducted using the same experimental materials and methods as those of MUKAI and YAMAZAKI (1964, 1968). The results are reported in this article.

MATERIALS AND METHODS

Materials: The following two stocks were used in the present experiments: (1) W160S, an isogenic line. The origin of this stock was described in MUKAI (1964). Just before the present experiment, many isogenic lines were established from a derivative of W160S, and one such

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line, whose homozygous viability was normal (the viability being approximately the same as the average viability of random heterozygotes in the population from which this chromosome was derived), was employed in the present experiment. This line is abbreviated at $+_o/+_o$. (2) C160, + (from W160S); *In(2)SM1, al²Cy sp²/In(2)Pm, dp b Pm ds³³ᵏ*;+ (from W160S);+ (from W160S). This line is abbreviated as *Cy/Pm* (Curly/Plum).

Experimental procedure: The experiment was conducted at 25°C. Standard cornmeal-molasses medium in 2.4 cm × 9.4 cm vials was used throughout the experiment. Heavy yeast suspension was added to the vials before making crosses.

Two experiments were conducted; the first starting in February (Experiment 1) and the second in August (Experiment 2), 1968. In each experiment, about 30 W160S adult males (collected 8–48 hr after emergence) were fed on a 0.025 M solution of EMS in sterile 1% sucrose solution suspended in Kleenex tissue paper for 24 hr, following the method described by LEWIS and BACHER (1968). After that, all males were shaken into a fresh culture (half-pint milk bottle) containing the medium described above. Then, about 40 virgin *Cy/Pm* females were added to the bottle. Five days after the cross was made, all parental flies were transferred to a second bottle, and after five days they were discarded. F_1 $Cy/+'_i$ and $Pm/+'_i$ males ($+'_i$ stands for the EMS-treated second chromosome and *i* indicates line number) were collected. Although it is ideal to test the chromosomes following brood pattern, this could not be done since the EMS induced many dominant lethals, and there were not many $Cy/+'$ and $Pm/+'$ males.

(1) Test for homozygote viability: First, $Cy/+'_i$ or $Pm/+'_i$ males were individually mated to 20 *Cy/Pm* females. Second, approximately 30 $Cy/+'_i$ and $Pm/+'_i$ male offspring from a single male were mated to 30 *Cy/Pm* virgins in mass condition (in a half-pint milk bottle) in order to replicate $+'_i$ chromosomes. All parental flies were transferred to a second bottle. $Cy/+'_i$ virgin males and females were collected from the first and second half-pint milk bottles, and five-pair matings were conducted between them. Six contemporary observations were made for each line (6 vials were used). Four days after crosses were made, all flies were transferred to second vials, and five days after the transfer all flies were eliminated. In the next generation, phenotypically Curly and wild-type flies that emerged on or before the 18th day after crosses (or transfers) were made were counted. The counts of *Cy* flies from a pair of vials (the first and second vial) in each replicate of each line were pooled, and the sum was treated as a single value. The same sampling was done for wild-type flies. The homozygous viability of the second chromosome of each line ($+_i$) was expressed by the ratio of the number of wild-type flies to the number of *Cy* flies. Haldane's correction (HALDANE 1956) was not used since the denominator was large (always larger than 200). According to this expression, the expectation of normal viability is 0.50. Following GREENBERG and CROW (1960), a lethal line is defined as a line whose homozygous viability is less than one-tenth that of the control viability (the average viability of the homozygotes, or the heterozygotes between lines, without EMS-treatment. Cf. Control experiment). Genetic variance among nonlethal homozygotes was estimated by an analysis of variance.

(2) Test for the viabilities of coupling heterozygotes: In each line 5 $Cy/+'_i$ males were mated to 6 $+_o/+_o$ females with 6 contemporary replications. Exactly the same procedure was followed as for the case of the homozygotes, but the expectation of viability of normal flies is 1.00 for this mating scheme.

(3) Test for the viabilities of repulsion heterozygotes: Random heterozygotes were bred by making crosses between different line numbers. If both parental lines have mutant genes, the resulting heterozygote might be called a repulsion heterozygote. In the present paper, all random heterozygotes will be called repulsion heterozygotes for the sake of convenience, even if both of the parental chromosomes do not always carry mutant genes. The following mating scheme was used in order to secure random combinations of the chromosomes from different lines: $Cy/+_i$ (5 females) × $Cy/+_{i\pm1}$ (5 males) crosses between lines having successive line numbers (1×2, 2×3, $3 \times 4 \ldots$). The procedure was exactly the same as that for the homozygotes, except for the number of observations within crosses (6 in Experiment 1 and 8 in Experiment 2).

In all three above-mentioned cases (homozygotes, coupling heterozygotes, and repulsion heterozygotes), control experiments of approximately the same scale as the main experiments were conducted simultaneously, in exactly the same manner except for the feeding of EMS.

Estimation of the mutation rate to detrimental genes, the average of their individual effects, and their variance: According to BATEMAN (1959), the following formulae (1) can be obtained concerning the reduction of means and the increase of genetic variance caused by mutations under the assumptions that mutations are distributed on chromosomes according to a Poisson distribution and that effects at different loci are additive:

$$\bar{s}p = A \qquad (1)$$
$$(\bar{s}^2 + \sigma^2_s)p = B$$

where s is the effect of a single mutation with mean $\bar{s}$ and variance σ^2_s and p indicates the average number of mutations in the chromosome. A stands for the reduction of mean viability of homozygotes and B is the increase of genetic variance. A and B are estimated from the experimental data. From formulae (1), the following can be obtained (MUKAI 1964):

$$A^2/B \leqslant p \qquad (2)$$
$$\bar{s} \leqslant B/A \qquad (3)$$
$$0 \leqslant \sigma^2_s \leqslant B^2/(4A^2) \qquad (4)$$

Although the assumptions underlying the estimations are not always satisfied in the present experiment, limiting values of the genetic parameters $\bar{s}$, σ^2_s, and p were estimated using formulae (2), (3), and (4).

Estimation of detrimental load (D) *to lethal load* (L) *ratio*: GREENBERG and CROW (1960) proposed a method for estimating homozygous load which may be applied to the EMS-induced mutations. If

C = average homozygote viability for the control

E = average viability of all homozygotes for EMS-treated chromosomes

G = average viability of nonlethal homozgyotes for EMS-treated chromosomes

then, under the assumption of multiplicative gene action, it can be shown that the

$$\text{Total (homozygous) load } (T) = \ln C - \ln E$$
$$\text{Detrimental load } (D) = \ln C - \ln G \qquad (5)$$
$$\text{Lethal load } (L) = \ln G - \ln E$$

These formulae are employed for the analysis of the data of the present experiment. Attention was not given to fractional mutations.

RESULTS AND ANALYSES

Homozygotes: Experiment 1 consisted of two tests which were conducted at different times using different chromosomes which were treated with EMS at the same time. There were three tests in Experiment 2. Since environmental effects seemed to be different for the tests made at different times, every observation was standardized by the mean viability of the contemporary controls (control mean = 1.00). Analysis was conducted for each of the contemporary observations and their controls, and the results were later pooled. (The same thing was done for coupling and for repulsion heterozygotes.) The distribution of viability (line basis) is shown in Figure 1. Genetic variances among lines were estimated by analysis of variance. The minimum rate of detrimental mutations (both mild and severe), the maximum variance of the effects of single mutations, and the maximum value of the effects of single mutations were estimated by the formulae described above. Furthermore, the homozygous load was calculated and was partitioned into the Detrimental load (D) and Lethal load (L) and the D:L ratio was calculated. The summary of the analyses on the basis of about 0.4 million individuals is presented in Table 1.

From Table 1, Figure 1, and previously reported results, the following conclusions can be drawn: (1) EMS induced a considerably high frequency of muta-

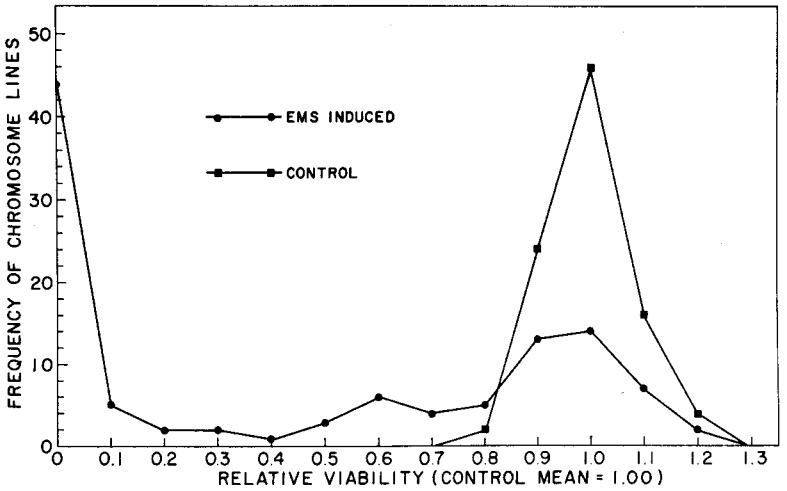

FIGURE 1.—Frequency distributions of the homozygous viabilities of second chromosomes carrying EMS-induced mutations and their controls.

tions. The frequency of lethal mutations is 0.55/second chromosome, on the average. This is the same as the total frequency of spontaneous lethal mutations for about 100 generations (cf. CROW and TEMIN 1964). (2) There is some variation between experiments with respect to the frequency of mutations, perhaps due to a difference in the amount of EMS taken by the parental flies. (3) The $D:L$ ratio for the mutations induced by EMS (an average of 0.45) is significantly lower than that of spontaneous mutations (about 0.98, reported by MUKAI and YAMAZAKI 1968). This indicates that the pattern of viability mutations induced by EMS is different from that of spontaneous mutations. (4) The maximum σ_s^2 and maximum $\bar{s}$ of EMS-induced nonlethal mutations are larger than those of spontaneous mutant polygenes, although these values might be overestimates due to possible nonrandom distribution of mutations on the chromosomes (the reason is that all the chromosomes carried by the sperm ejaculated by the 10th day after EMS was given were pooled). In fact in the present experiment $\sigma_s^2 \leqslant 0.035$ and $\bar{s} \leqslant 0.36$, while corresponding values for the spontaneous mutations were found to be $\sigma_s^2 \leqslant 0.00018$ and $\bar{s} \leqslant 0.027$ (MUKAI 1964). These results do not necessarily indicate that the σ_s^2 and $\bar{s}$ for EMS-induced mutations are larger than those of spontaneous mutant polygenes, but it is reasonable to suppose so according to the following reasoning.

First, from formulae (1), we can obtain the following relationship:

$$\sigma_s^2 = -(\bar{s} - \frac{B}{2A})^2 + \frac{B^2}{4A^2} \tag{6}$$

By substituting the actual observations for A and B in the present data, formula (6′) is obtained:

$$\sigma_s^2 = -(\bar{s} - 0.1875)^2 + 0.0352 \tag{6′}$$

This formula contains two variables, σ_s^2 and $\bar{s}$, for which a curve of possible values can be constructed to satisfy the expression. It is impossible to state which point

TABLE 1

Summary of the experimental results for homozygotes

	EMS-treated		Control	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Number of chromosome lines	52	56	38	54
	[108]		[92]	
Number of lethal lines	15	30	0	0
	[45]		[0]	
Overall mean viability	0.549	0.380	1.000	1.000
	(0.461)		(1.000)	
Mean viability of nonlethal lines	0.767	0.815	1.000	1.000
	(0.787 ± 0.037)		(1.000 ± 0.008)	
Genetic variance among nonlethal lines	0.0739 ± 0.0211	0.0826 ± 0.0260	0.0006 ± 0.0008	0.0049 ± 0.0018
	(0.0775 ± 0.0164)		(0.0032 ± 0.0012)	
Average number of flies counted per line	1674.8	1983.6	2045.6	2094.6
	(1841.1)		(2074.3)	
Detrimental load (D)	0.265	0.204		
	(0.24)			
Lethal load (L)	0.335	0.765		
	(0.53)			
$D : L$ ratio	0.79	0.27		
	(0.45)			

Detrimental mutations

Average number of detrimentals (p)	$0.59 \leq p$
(Average number of lethals	0.55)
Average effect of single mutations (s)	$s \leq 0.36$
Range of $\sigma^2{}_s$	$0 \leq \sigma^2{}_s \leq 0.035$

The figure in brackets indicates the sum of the results in Experiments 1 and 2; the same for Tables 2 and 4.

The figure in parentheses indicates the mean of the results in Experiments 1 and 2; the same for Tables 2 and 4.

on the curve most closely represents the true values involved. However, if we fix the variable σ_s^2 by assuming that the value for EMS mutants is the same as that for spontaneous mutants, which was found to be 0.00018 (MUKAI 1964), then there are two possible solutions for $\bar{s}$: 0.00050 (to which $p = 430.0$ corresponds) and 0.36 (to which $p = 0.59$ corresponds). The former $\bar{s}$ value is unrealistically

small, since the corresponding p value is far too great to account for the kind of distribution shown in Figure 1. On the other hand, the latter $\bar{s}$ value is unrealistically large, since a large proportion of the treated chromosomes had viabilities only slightly less than the control (Figure 1). Therefore, it can be concluded, under the assumption given, that the true value of $\bar{s}$ lies somewhere between the two solutions obtained. Now, any value of $\bar{s}$ between the two extremes 0.00050 and 0.36 would yield corresponding values of σ_s^2 greater than 0.00018 (σ_s^2 of spontaneous mutant polygenes). Thus, the σ_s^2 for EMS mutants is apparently greater than that for spontaneous mutants.

It may be further argued, based on formulae (1) and (6′), that the $\bar{s}$ value of EMS-induced mutations is larger than that for spontaneous mutant polygenes ($\bar{s} \leqslant 0.027$, MUKAI 1964). A value of $\bar{s}$ equal to 0.027 (to which $\sigma_s^2 = 0.0091$ corresponds, and this value is much larger than that for spontaneous mutant polygenes) would result in $p = 7.9$, a value again too large to account for the type of distribution actually found (Figure 1). In fact, if $p = 7.9$, the probability of the chromosomes carrying 0, 1, and 2 mutant genes is only 0.016 under the assumption of a Poisson distribution. This is clearly inconsistent with the result presented in Figure 1. Therefore, the true value of $\bar{s}$ for EMS-induced mutations is expected to be greater than 0.027. This would result in a larger estimate of σ_s^2 than that for spontaneous mutant polygenes. Accordingly, the real p value would become smaller than 7.9 [14.3 times larger than that of recessive lethal mutations].

From the above considerations, it might be said that the σ_s^2 value of EMS-induced detrimental mutants is larger than that of spontaneous mutant polygenes, and the $\bar{s}$ value is also larger than that of spontaneous mutant polygenes, and the ratio of EMS-induced detrimental mutation rate to lethal mutation rate is smaller than that of spontaneous mutations (less than 14.3 *vs.* more than 22.4 times).

TABLE 2

Summary of the experimental results for coupling heterozygotes

	EMS-treated		Control	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Number of chromosome lines tested	48(11*)	27(2*)	38	27
	[75(13*)]		[65]	
Mean viability	0.991	0.981	1.000	1.000
	(0.988 ± 0.007)		(1.000 ± 0.007)	
Genetic variance	0.00198 ± 0.00109	0.00085 ± 0.00093	0.00016 ± 0.00085	0.00024 ± 0.00091
	(0.00157 ± 0.00075)		(0.00020 ± 0.00063)	
Average number of flies counted per line	2079.8	2333.6	1902.0	2402.9
	(2001.2)		(2110.0)	

* The number of lethal lines employed

TABLE 3

The relationship between homozygote viabilities and the corresponding coupling heterozygote viabilities

Range of homozygote viability	Number of lines	Average homozygote viability	Average heterozygote viability	Average degree of chromosomal dominance ($\bar{H}$)
$v > 1.00$	16	1.068	0.976	...
$1.0 > v > 0.9$	16	0.943	1.025*	—0.22
$0.9 > v > 0.8$	9	0.851	0.983	0.12
$0.8 > v > 0.5$	12	0.617	0.980	0.05
$0.5 > v > 0.2$	5	0.330	0.975	0.04
$0.2 > v \geqslant 0$	17	0.051	0.975	0.03
$(v = 0.00)$†	(6)	(0)	(0.959)	0.04

v stands for homozygote viability.
† These are included in the range $0.2 > v \geqslant 0$.
* Significantly different from 0.976 ($P < 0.01$).

Coupling heterozygotes: As for the case of homozygotes, Experiment 1 was divided into two parts, conducted at separate times, while Experiment 2 was conducted at one time, excluding some lines carrying lethal mutations. The summary of the experimental results and their analysis is presented in Table 2. The mean viability of the EMS-treated group was less than that of the controls (difference = 0.0125 ± 0.0100), but not significantly so. The genetic variance due to EMS-induced mutations plus spontaneous mutations arising after establishing chromosome lines is significantly different from zero (0.00157 ± 0.00075, $P < 0.05$). These results are apparently contradictory to those of spontaneous mutations which showed overdominance in the coupling phase (MUKAI, CHIGUSA and

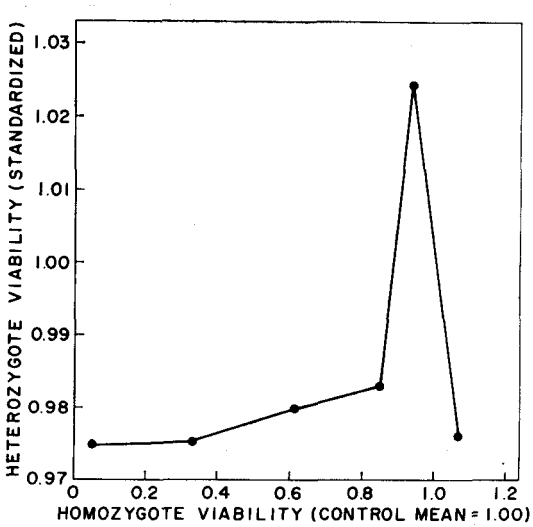

FIGURE 2.—The relationship between the viabilities of homozygotes and the viabilities of their corresponding heterozygotes (coupling).

YOSHIKAWA 1964, 1965; MUKAI and YAMAZAKI 1968). Therefore, further analysis was attempted.

Seventy-five lines were divided into 6 groups following the order in magnitude of the viabilities in the homozygous condition. The average viabilities of homozygotes and their corresponding heterozygotes are given in Table 3, and graphically presented in Figure 2. The average degree of chromosomal dominance ($\bar{H}$) was calculated for each group, using the following formula:

$$\bar{H} = \frac{1 - \bar{v}'}{1 - \bar{v}} \tag{7}$$

where $\bar{v}'$ stands for the average viability of heterozygotes and $\bar{v}$ for that of homozygotes. The results are tabulated in Table 3, and imply that (1) when the reduction of homozygote viability is small ($1 > v > 0.9$), overdominance is manifested, (2) when the v value is smaller than 0.9, the mutant genes are slightly deleterious in the heterozygous condition (in the coupling phase) and the values of $\overline{HS}$ within each of the groups seem approximately constant over groups where $\overline{S} = (1-\bar{v})$. This is in apparent contradiction to the fact that overdominance is manifested in the whole range of the homozygous viability for spontaneous polygenic mutations (MUKAI 1969a), which was constructed by gradual accumulation of mildly detrimental mutants (mutant polygenes).

In order to examine the overdominance which occurs when $1.0 > v > 0.9$, the relationship between the viabilities of homozygotes and their corresponding heterozygous viabilities is graphically shown in Figure 3 for $v > 0.9$. It can be seen from this figure that there is a negative correlation between homozygote and heterozygote viabilities ($r = -0.40$, $P < 0.05$), thus showing overdominance of mildly detrimental genes (polygenes). In fact, the difference in the average

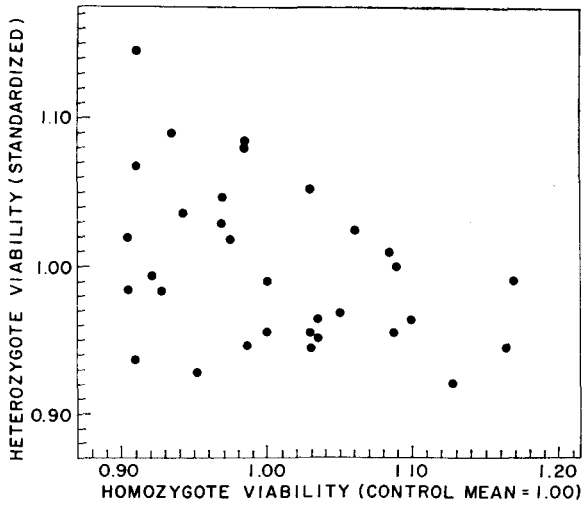

FIGURE 3.—Scatter diagram of homozygote and heterozygote viabilities in the coupling phase when the homozygous viabilities of EMS-treated chromosomes are larger than 0.9 of the control mean.

TABLE 4

Summary of the experimental results for repulsion heterozygotes

	EMS-treated		Control	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Number of crosses	35[4*]	27[2*]	35	27
	[62 (6*)]		[62]	
Mean viability	1.002	0.951	1.000	1.000
	(0.980 ± 0.009)		(1.000 ± 0.007)	
Genetic variance	0.00227	0.00188	0.00018	0.00224
	±0.00123	±0.00104	±0.00080	±0.00123
	(0.0021 ± 0.00083)		(0.00107 ± 0.00070)	
Average number of flies counted per line	2226.0	3469.7	2327.5	3275.9
	(2767.6)		(2740.5)	

* The number of lethal crosses employed.

heterozygote viabilities between the class of $v > 1.00$ and that of $1.0 > v > 0.9$ is -0.0486 ± 0.0175, which is significantly less than zero at the one percent level. The reason for the lack of overdominance when $v < 0.9$ will be discussed later.

Repulsion heterozygotes: Tests of the repulsion heterozygotes were conducted following the same procedure as for the coupling heterozygotes. The summary of the experimental results and their analysis is shown in Table 4. The mean viability of the EMS-treated group was significantly less than that of the controls (difference $= 0.0202 \pm 0.0115$, $P < 0.05$), and the genetic variance due to EMS-induced mutations plus spontaneous mutations occurring after establishing the chromosome lines is significantly larger than zero (0.00210 ± 0.00083, $P < 0.01$).

TABLE 5

The relationship between the viabilities of repulsion heterozygotes and the sums of the viabilities of corresponding homozygotes

Range of homozygote viabilities		Averages of homozygote viabilities		Number of crosses	Average viability of parents	Average viability of heterozygotes	The average degree of chromosomal dominance ($\bar{H}$)
H	L	H	L				
$H > 1.00$	$L > 0.98$	1.098	1.015	7	1.057	0.988	....
$H > 1.00$	$0.98 > L > 0.90$	1.043	0.935	4	0.989	1.076*	—3.542
$H > 1.00$	$0.90 > L > 0.40$	1.048	0.693	11	0.870	0.966	0.132
$H > 1.00$	$0.40 > L > 0.20$	1.109	0.313	5	0.711	0.934	0.115
$1.0 > H > 0.9$	$H > L > 0.9$	0.962	0.926	4	0.944	1.026	—0.234
$1.0 > H > 0.9$	$0.9 > L > 0.2$	0.941	0.707	13	0.824	0.978	0.063
$0.9 > H > 0.2$	$H > L > 0.2$	0.763	0.560	10	0.661	0.968	0.048
.....	$0.20 > L$	0.828	0.037	8	0.433	0.969	0.027
Average		0.956	0.626	(62)	0.791	0.980	....

* Significantly different from 0.988 ($P < 0.05$).
H stands for the viability of more viable chromosomes.
L stands for the viability of less viable chromosomes.

The following analysis was carried out to check whether or not overdominance is manifested in some of the repulsion heterozygotes, which can be expected since certain ones should give a "coupling" expression, if by chance one of the homologues is "mutant free." Seventy-five crosses were divided into 8 groups according to the magnitude of the homozygous viabilities of their constituent homologous chromosomes. The average viabilities of homozygotes and their corresponding heterozygotes are tabulated in Table 5. The average degree of chromosomal dominance was calculated using formula (8):

$$\bar{H} = \frac{1 - \bar{v}'}{2\ (1-\bar{v})} \tag{8}$$

where $\bar{v}$ indicates the mean of the homozygous viabilities of the constituent chromosomes of heterozygotes. The results are also presented in Table 5. Based on the results presented in this table, the following conclusions may be drawn: (1) If one of the homologous chromosomes is normal ($v > 1.0$) and the other chromosome is near-normal ($0.98 > v > 0.90$), overdominance is manifested. This result is consistent with that of coupling-heterozygote tests of EMS-induced mutations as well as spontaneous mutant polygenes (MUKAI, CHIGUSA and YOSHIKAWA 1964). (2) When both of the homologous chromosomes carried EMS-induced mutations whose homozygous effects were less than 0.10 (S), significant heterozygous effects were not detected; this was also true for spontaneous mutations (MUKAI and YAMAZAKI 1968; MUKAI 1969b). (3) When both homologous chromosomes carry detrimental mutants ($v < 0.9$), these mutants work deleteriously in the heterozygous condition and their average degrees of dominance ($\bar{h}$) are similar to those of semilethal and lethal genes (STERN *et al.* 1952; HIRAIZUMI and CROW 1960) but are much smaller than those of spontaneous mutant polygenes ($\bar{h} \approx 0.4$, MUKAI and YAMAZAKI 1968). [MUKAI (1969b) has shown that the expected value of H is $\bar{h}$ for newly arisen mutations if s and h are not correlated.] The values of $\overline{HS}$ within each of the viability classes seem approximately constant over classes, as for the case of coupling heterozygotes when $v < 0.8$.

DISCUSSION

Experimental method: Although EMS might induce mosaic (or fractional) mutations at the DNA level (ALTENBURG and BROWNING 1961), they would not cause a biased estimation in the present analysis for the following reason: The primordial cell of the gonad differentiates following a certain number of cell divisions after fertilization. After the first mitosis of the fertilized egg, any chromosome carrying a fractional mutation segregated as a whole mutant chromosome and a whole mutant-free chromosome. Thus, any primordial cell of the gonad must have a whole mutant chromosome or a whole mutant-free chromosome. It has also been shown that mutagenic effects of chemicals are sometimes delayed (AUERBACH 1946), but this too would not disturb the present analysis significantly.

The treated males were allowed to mate with females for 10 days in the present

experiment. The sperm which were ejaculated on the 10th day might have received EMS at the spermatogonial stage (AUERBACH 1954), and there might have been the possibility of clustering of mutations. However, since in the present experiment fresh virgins were not given successively, this probability is very low. In fact, no allelic cross was found in the allelism test among 15 lethals recovered in Experiment 1.

Comparison of EMS-induced mutations with spontaneous mutant polygenes: The nature of EMS-induced deleterious mutations is, as a whole, different from that of spontaneous mutant polygenes: (1) The Detrimental load to Lethal load ratio (D:L ratio) of EMS-induced mutations is less than that of spontaneous mutations as described above. This finding together with the other above results indicates that EMS induces more lethals than detrimentals in comparison with spontaneous mutations. (2) When the homozygous viability of one of the homologous chromosomes (in the coupling heterozygotes) or the homozygous viabilities of both the homologous chromosomes (in the repulsion heterozygotes) are less than 0.9, mutant detrimental genes work deleteriously in the heterozygous condition. The average degree of chromosomal dominance ($\overline{H}$) is smaller than that of spontaneous mutant polygenes in the repulsion phase, which is approximately 0.4, but much larger than that of spontaneous mutant polygenes in the coupling phase, which is negative. The probable reason for these differences will be discussed later. In addition, for both the coupling and the repulsion phases, $\overline{HS}$ is approximately constant. This relationship has already been discovered for repulsion heterozygotes of spontaneous mutations by GREENBERG and CROW (1960) and MUKAI and YAMAZAKI (1968). (3) When $0.98 > v > 0.90$, the nature of the EMS-induced mutant polygenes was found to be similar to that of spontaneous mutant polygenes: coupling heterozygotes manifested overdominance, and the heterozygous effects seen in the repulsion phase when the homozygous viabilities of both homologous chromosomes were between 0.9 and 1.0 were not significant. This result also shows a similarity between EMS-induced and spontaneous mutant polygenes (MUKAI and YAMAZAKI 1968; MUKAI 1969a).

All the above phenomena may be explained as follows: As noted above, there may be a large variation of the effects of single mutations induced by EMS. When the homozygous effects of EMS-induced mutations are small, their nature is very similar to that of spontaneous mutant polygenes. Overdominance is manifested in the coupling phase. If we accumulated these EMS-induced mutant polygenes in both homologues, the repulsion effect might be seen. From our previous reports (cf. Table 1, Figures 2 and 3 of MUKAI 1964, and Figure 2 of MUKAI and YAMAZAKI 1968), spontaneous viability mutations (excluding dominant lethals) may be classified into 3 categories: lethal, semilethal, and polygenic. Semilethals express homozygous viabilities near 0.5 of the normal while polygenic mutations show very small individual effects and have whole-chromosome mutation rates much higher than those of recessive lethals (e.g., Tables 1 and 8 in MUKAI 1964). The distribution of these three kinds of mutations appears discontinuous; it was possible to accumulate only polygenic mutant genes in single

chromosomes with spontaneous mutants. On this basis, the repulsion effect (MUKAI and YAMAZAKI 1968) and optimum heterozygosity (MUKAI 1969a) could be detected. In EMS-induced mutations, the distribution of homozygous effects of single mutations (s) may be somewhat continuous from $s = 0$ to $s = 1$, at least when flies were fed 0.025M solution of EMS for 24 hours. (The distribution has not been studied for the case where flies took a lower concentration of EMS). If the s values are large (probably larger than 0.1), the degrees of dominance of these mutant genes are similar to those of lethal and semilethal genes (h is positive and small). If EMS causes deletions at a high frequency (DR. E. B. LEWIS personally informed me that his data indicate that EMS induces not only large chromosomal aberrations but also deletions.) in addition to point mutations caused mainly by one of GC–AT transitions through the ethylation of guanine and probably by transversion (BAUTZ and FREESE 1960; FREESE 1961; KRIEG 1963), induction of mutations whose homozygous effects show a large variation can be expected.

Coupling-repulsion effect and the genetic load in random mating populations: The most significant finding in the present work is the discovery of overdominance with respect to viability (coupling effect) only when the selection coefficients of mutant genes are small ($s < 0.10$). This phenomenon has been discovered for spontaneous, as well as radiation-induced mutations (e.g., BURDICK and MUKAI 1958; MUKAI, CHIGUSA and YOSHIKAWA, 1964; MUKAI, YOSHIKAWA and SANO 1966; WALLACE 1958, 1963). Unfortunately, the repulsion effect could not be detected in the present data, but this does not necessarily indicate that it does not exist. Perhaps the experiment did not satisfy all the conditions which would produce a manifestation of the repulsion effect.

I do not want to claim overdominance of mildly deleterious genes or polygenes as a generality for coupling heterozygotes. I feel the necessary conditions for its manifestation is that the homozygous viability of the original chromosome should be normal and that the genes in the chromosome must be co-adapted (see WALLACE and VETUKHIV 1955). In fact, in a synthetic chromosome derived from 64 chromosomes which originated from different populations, overdominance was not manifested when mutations were induced by 500R X rays even though the chromosome showed a good homozygous viability (MUKAI, YOSHIKAWA and SANO 1966).

Recently several investigators have attempted to explain the coupling effect by proposing that segregation distortion in meiosis favors the chromosomes carrying mildly detrimental mutant genes (FALK 1967; MORTON, CHUNG and FRIEDMAN 1968; SAKAI and HIRAIZUMI 1969). However, this speculation does not seem applicable, at least for the results of spontaneous and X-ray-induced mutations obtained by MUKAI, CHIGUSA and YOSHIKAWA (1964, 1965), MUKAI and YAMAZAKI (1968), MUKAI, YOSHIKAWA and SANO (1966), and WALLACE (1963), since the same (or similar) mutant genes as those revealing overdominance showed deleterious effects in different genetic backgrounds (Figure 2 in MUKAI, CHIGUSA and YOSHIKAWA 1965; Table 2 in MUKAI, YOSHIKAWA and SANO 1966).

In equilibrium natural populations of *Drosophila melanogaster*, the frequency of individuals manifesting overdominance will be low, since the frequency of

chromosomes showing very high viability is low (accordingly, the frequency of coupling heterozygotes is low). However, overdominance together with optimum heterozygosity (MUKAI 1969a) in the coupling heterozygotes is important to the population even if its frequency is low, since overdominance and optimum heterozygosity in the coupling heterozygotes increase the mean fitness of the population without increasing genetic load. A detailed discussion on this problem has been published in MUKAI (1969c).

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SUMMARY

Homozygous and heterozygous effects on viability of EMS-induced second chromosome mutations were tested in an otherwise homozygous genetic background in experiments involving a grand total of approximately 1.02 million counted flies. The mutations were induced in normal chromosomes carried by isogenic males by feeding on Kleenex saturated with an 0.025M solution of EMS in sterile 1% sucrose solution for 24 hours. The results are as follows: (1) EMS induces mutations at an extremely high frequency (recessive lethals were induced on 42% of the EMS-treated chromosomes). (2) The total homozygous load (T) = 0.77 which consisted of a Lethal load (L) of 0.53 and a Detrimental load (D) of 0.24. The Detrimental load to Lethal load ratio (D:L ratio) was 0.45, which is significantly smaller than that of spontaneous mutations (0.98). (3) In the coupling phase, where induced mutant genes are located in only one of the homologous chromosomes, mutant genes are overdominant when the homozygous viability of the mutant-carrying chromosome (v) is larger than 0.9 that of the original chromosome, but they are deleterious when the v value is less than 0.9. (4) In the random heterozygote, mutant genes whose homozygous effects were small ($v > 0.9$) were overdominant when located by chance in only one of the homologous chromosomes (the other was normal), but they were deleterious in the heterozygous condition when $v < 0.9$, regardless of their relative positions in the chromosomes. When both homologous chromosomes carried minor mutations ($1 > v > 0.9$), nonsignificant heterozygous effects of these mutant genes were manifested. On the basis of these findings, a comparison of EMS-induced viability mutations with spontaneous mutations was made.

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