HETEROZYGOUS EFFECTS ON VIABILITY OF SPONTANEOUS LETHAL GENES IN *DROSOPHILA MELANOGASTER*¹⁾

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Received August 17, 1970

In recent years, considerable attention has been paid to the mechanism involved in the maintenance of genetic variability in random mating populations, and it has been suggested theoretically (e.g., Lewontin 1964) and experimentally (cf. Dobzhansky 1950; Wallace 1956; Lewontin and White 1960 and others) that gene interaction or chromosome interaction might be significant in the maintenance of genetic variability in populations. Especially gene interaction should be reconsidered now that a large number of polymorphisms of isozyme genes have been discovered (e.g., Lewontin and Hubby 1966).

In the studies of lethal genes, Wallace (1962) found a very interesting phenomenon; he established experimental populations with fourteen quasi-normal second chromosomes of *D. melanogaster*, and then continuously irradiated the entire populations with chronic γ -rays; along with the irradiation he sampled second chromosomes periodically and tested the average effects on viability of individuals heterozygous for lethal and semi-lethal chromosomes. In early generations, these chromosomes decreased heterozygote viability, but in later generations it was enhanced. An experimental result similar to this was obtained by Dobzhansky and Spassky (1968), using 45 lethal carrying and 50 quasi-normal second chromosomes of *D. pseudoobscura* which were extracted from natural populations. That is, the average viability of lethal heterozygotes measured on a chromosome basis was better than that of lethal-free heterozygotes in their own genetic background, but the result was reversed in foreign genetic backgrounds. On the basis of these experimental results, Wallace (1962) and Dobzhansky and Spassky (1968) stressed the importance of genetic backgrounds and criticized the work of Hiraizumi and Crow (1960) who studied the heterozygous effects of lethal-carrying chromosomes in a foreign genetic background. Stern, Carson, Kinst, Novitski, and Uphoff (1952) also examined the heterozygous effects of lethals in a foreign genetic background.

Another interesting phenomenon was discovered by Oshima (1963) using second chromosomes extracted from natural populations of *D. melanogaster*. The average viability of heterozygotes in which both the homologous chromosomes carried lethal

¹⁾ Contribution number 596 from the National Institute of Genetics, and Paper number 3264 of the Journal Series of the North Carolina State University Agricultural Experiment Station. This work was supported by Public Health Service Research Grants RH-00034, GM-11546, and FR-00011.

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genes (double-lethal heterozygotes) was found to be better than that of lethal-free individuals (control), although the average viability of heterozygotes carrying single lethal chromosomes (single-lethal heterozygotes) was less than the control.

We have accumulated spontaneous mutations in the second chromosomes of *D. melanogaster* that originated from a single second chromosome extracted from an Erie, Pennsylvania population (see Mukai 1964) and in the second chromosomes that originated from a Madison, Wisconsin population (Mukai and Crow, unpublished). Furthermore, a number of lethal-carrying and lethal-free chromosomes were extracted from a Raleigh, North Carolina population. Using these chromosomes, we have carried out two experiments: In the first experiment, we attempted to estimate what percent of newly arisen *lethal genes* show overdominance in viability and to examine how the degrees of dominance of newly arisen lethal genes are modified by their genetic backgrounds in connection with the experimental result obtained by Dobzhansky and Spassky (1968). In the second experiment, the Oshima effect was re-examined using the chromosomes extracted from the Raleigh, North Carolina population and the chromosomes carrying lethal genes which arose in the Madison genetic background.

MATERIALS AND METHODS

Experiment 1: As a by-product of accumulation of spontaneous mutant polygenes controlling viability (Mukai 1964), we obtained many lethal-carrying second chromosomes which were employed in the first experiment. Although details of the accumulation of mutations have been reported in Mukai (1964), they will be briefly mentioned here. A single $Pm/+_o$ male was sampled from the cross $Cy/Pm \times +_o/+_o$. The Cy/Pm females are from the C160 stock, in which the genetic background is that of W160. The $+_o/+_o$ males are from W160S, a normal isogenic line derived from Dr. Burdick's W160-pol. This selected $Pm/+_o$ male was crossed to Cy/Pm females to replicate the $+_o$ chromosome, and 104 lines of $Cy/Pm \times Pm/+_i$ (1 male) were subsequently established ($i=1, 2, \dots, 104$). In each line, the second chromosome has been maintained through a single male by the cross Cy/Pm (5 females) $\times$ $Pm/+_i$ (1 male). In generation 33, each line was duplicated to A and B, and the two replications were then maintained independently. By generation 60, 41 independent lethal-carrying second chromosomes had been obtained. Allelism tests were conducted among lethal chromosomes of independent origin, and two allelic groups consisting of two lethal chromosomes each were detected. All these lethal lines were maintained by the method described above. In generation 60, homozygous viabilities of group A lines were tested, and 41 lethal-free chromosome lines were randomly chosen to serve as controls for the lethal lines. The average genetic backgrounds of the lethal-carrying lines are thus expected to be the same as that of the control lines. The experimental lines were redesignated L-1, L-2, $\dots$, L-40, L-41; N-1, N-2, $\dots$, N-40, and N-41 for the lethal-carrying lines and the control lines, respectively, following the order of their original line numbers.

Three isogenic normal lines were chosen for the test of viabilities of lethal heterozygotes: line 92 (abbreviated as $+_o/+_o$), assumed to be identical to the original chromosome because the viability index indicated that this line had not accumulated any new

mutations; W170S (abbreviated as $+_W/+_W$), an isogenic normal line extracted from the same population from which the original W160 was extracted; and W202 (abbreviated as $+_B/+_B$), an isogenic normal line extracted from Dr. Hiraizumi's wild-type stock (Tokyo). The X and the third chromosomes of W170S and W202 had been substituted by those of W160S.

The viabilities of heterozygotes for single-lethal chromosomes were tested in three genetic backgrounds, i.e., homozygous ($+_o/l_i$), and heterozygous-intrapopulational ($+_W/l_i$) and interpopulational ($+_B/l_i$). The mating scheme was $+/+$ (5 females) $\times$ Cy/l_i (5 males). Three replicate crosses were made for each background with each lethal line in $3\text{ cm} \times 10\text{ cm}$ vials. Four days after the cross was made, all flies were transferred to a second set of vials and five days later the parents were discarded. If any flies were dead at the time of the transfer, flies of the same age were substituted. In the next generation, phenotypically wild-type and Curly flies that emerged before any F_2 flies could appear were counted. The counts of Cy flies from a pair of vials in each replicate of each cross were pooled; likewise the counts of the $+$ flies were also pooled. The ratio of the number of wild-type flies to the number of Cy flies plus one (Haldane 1965) was employed as viability index. In the present analysis, it was assumed that replacement did not take place by an increased number of Cy flies compensating for the decreased number of wild-type flies resulting from viability reduction caused by heterozygosity of lethal genes. Thus, we used the ratio of the two types of flies as viability index (cf. Mukai 1964). In the present case, there is some possibility that a single chromosome has two lethal genes. The possibility for such a case will be discussed later.

The controls, consisting of the forty-one lethal-free lines mentioned above were assayed using the same procedure and at the same time as the lethal lines.

Experiment 2: Accumulation of spontaneous mutant polygenes controlling viability was conducted again, using three second chromosomes extracted from a Madison, Wisconsin population (Mukai and Crow, unpublished). The method of accumulating mutations is exactly the same as above. As a by-product of this accumulation experiment, we obtained many lethal-carrying second chromosomes which were employed in the second experiment. By generation 100, 22 lethal-carrying chromosomes were obtained from 50 lines that originated from a single chromosome (CH), and 18 lethal-carrying chromosomes were obtained from 50 lines that originated from a second single chromosome (PQ). They were employed as experimental materials. Following the original line numbers, new numbers were given to the lethal-carrying lines as LM-1, LM-2, ..., LM-40. The same numbers of lethal-free chromosomes were sampled at random from lines that originated from the CH and the PQ chromosomes, respectively. They are redesignated as FM-1, FM-2, ..., FM-40.

The viabilities of heterozygotes of single lethal chromosomes were tested by the following mating scheme: Cy/l_i (5 females) $\times$ $Pm/+_i$ (5 males) ($i=1, 2, \dots, 40$) where $+_i$ indicates a second chromosome of FM- i , l_i stands for a second chromosome of LM- i , and Pm stands for the inversion chromosome carrying Pm gene. The experimental schedule after crosses were made was exactly the same as in Experiment 1. The viabilities of heterozygotes of two lethal-carrying chromosomes were tested by the following mating scheme: Cy/l_i (5 females) $\times$ Pm/l_{i+1} (5 males) but within the CH and

the PQ group, respectively, considering the effect of genetic background. As the controls, $Cy/+_i$ (5 females) $\times$ $Pm/+_{i+1}$ (5 males) avoiding the crosses between lines of the CH group and those of the PQ group.

In all crosses, 8 contemporary replications were made. Crosses were made using 2.4 cm $\times$ 9.4 cm vials. In the following generation, phenotypically Cy Pm , Cy , Pm , and wild-type flies segregate at a ratio of 1:1:1:1 in expectation. Because of the large variance of the relative viabilities of Cy Pm flies, the viabilities were expressed by three times the ratio of phenotypically wild-type flies to the total of Cy Pm , Cy , and Pm flies plus one.

We have extracted 145 second chromosomes from a Raleigh, North Carolina population in 1969. Among them 52 were lethal-carrying chromosomes. We used 49 chromosomes out of 52. New numbers were given to these lines as DL-1, DL-2, ..., DL-49. As the control, 49 lethal-free chromosomes were chosen at random and new numbers were given to these lines as DF-1, DF-2, ..., DF-49. The genetic backgrounds of these lines were mostly substituted by those of the Raleigh population, but not completely so. For the estimation of viabilities, the following crosses were made: $Cy/+_i$ (5 females) $\times$ Cy/l_i (5 males) for single-lethal heterozygotes; Cy/l_i (5 females) $\times$ Cy/l_{i+1} (5 males) for double-lethal heterozygotes; and $Cy/+_i$ (5 females) $\times$ $Cy/+_{i+1}$ (5 males) for the control. Notations were the same as above. The experimental procedure was exactly the same as above, except for the number of contemporary replications. In the present experiment, the number was four per cross. In the offspring, phenotypically Cy and wild-type flies segregate at a ratio of 2:1 in expectation. The viabilities were expressed as twice the ratio of the number of phenotypically wild-type flies to the number of phenotypically Cy flies plus one.

RESULTS AND ANALYSES

Experiment 1: Distribution patterns of the viabilities of lethal heterozygotes and their controls are presented in Figure 1. From this figure, it can be seen that lethal genes are, generally, detrimental to their heterozygous carriers in all of the genetic backgrounds utilized. Only in one cross, the lethal heterozygote of L-10, did the viability exceed the best control viability in the homozygous genetic background (1.3449 vs. 1.3216), but in the other genetic backgrounds, the heterozygote viabilities of this lethal line did not show good viabilities: 0.9940 in the intrapopulational genetic background and 1.1159 in the interpopulational genetic background. Thus, it was speculated that the extremely good viability shown in the homozygous genetic background by L-10 heterozygotes might be due to the extreme overdominance of mutant viability polygenes, which were carried by the L-10 chromosome, and to environmental effect. Mukai, Chigusa, and Yoshikawa (1965) showed using the same experimental material that mutant viability polygenes were overdominant when they were located only in one of the homologous chromosomes (in the coupling phase) and the degree of heterozygosity in the genetic background is low. Indeed, the $+$ chromosome was considered to have no *new* mutant genes. Thus, the condition of polygenes in coupling phase probably explains the high viability.

An analysis of variance was performed assuming that lethality (lethal and normal),

Fig. 1. The distribution of the viabilities of lethal heterozygotes and their controls in various genetic backgrounds.

Table 1. Analysis of variance with respect to the viabilities of lethal heterozygotes and their controls in various genetic backgrounds

Source of variation	Degrees of freedom	Mean square	F
Lethality	1	0.2287	7.63**
Genetic background	2	0.6732	37.35***
Lethality $\times$ genetic background	2	0.0093	0.52
Lines within lethality	80	0.0299	2.32**
Genetic background $\times$ lines within lethality	160	0.0180	1.40*
Error	492	0.0129	
Total	737		

* significant at the 5 percent level.

** significant at the 1 percent level.

*** highly significant.

genetic backgrounds (homozygous, intrapopulational, and interpopulational) are fixed and lines are random. The results are presented in Table 1. It is clear that, on the average, lethal genes significantly decrease the viabilities of their heterozygous carriers in all the genetic backgrounds utilized.

The result of the analysis of variance indicates that there are viability differences

among lines within lethality on the average. However, it is necessary to test whether or not lethal *genes* have different heterozygous effects from each other. Thus, separate variance analyses were conducted in the lethal-carrying group and the control group. The results are shown in Tables 2 and 3. There are significant differences in viabilities among lines in the lethal-carrying group but not in the control group. Indeed, the genetic variance between lethal lines minus the genetic variance between control lines (=the genetic variance in the heterozygous effects of lethal genes among chromosomes) is estimated to be 0.00338 ± 0.00120 , which is significantly different from zero (on a single lethal gene basis $\hat{\sigma}_h^2 = 0.0027$). Thus, it is concluded that there is a variance in the heterozygous effects of *newly arisen lethal genes*.

Table 2. Analysis of variance with respect to the viabilities of lethal heterozygotes in various genetic backgrounds

Source of variation	Degrees of freedom	Mean square	F
Line	40	0.0441	4.08***
Genetic background	2	0.4247	31.48***
Genetic background $\times$ line	80	0.0135	1.25
Error	246	0.0108	
Total	368		

*** highly significant

Table 3. Analysis of variance with respect to the viabilities of the controls for lethal heterozygotes

Source of variation	Degrees of freedom	Mean square	F
Line	40	0.0177	1.19
Genetic background	2	0.2915	13.48***
Genetic background $\times$ line	80	0.0216	1.46*
Error	246	0.0148	
Total	368		

* significant at the 5 percent level.

*** highly significant.

It was found that there was a significant difference among genetic backgrounds. It should be noted here that the average viability of the controls in the homozygous background was the best of the three. This is rather abnormal but was caused by the overdominance of mutant viability polygenes. They were located in only one of the homologous chromosomes as described above, and perhaps showed extreme overdominance (cf. Mukai, Chigusa, and Yoshikawa 1965).

In Table 1, the interaction between genetic background and lines within lethality is significant. These interaction variance components ($\sigma_{GB \times LINE}^2$) were calculated in the control group and lethal group separately. The results are: $\hat{\sigma}_{GB \times LINE}^2 = 0.00089 \pm 0.00078$

for the lethal group and $\hat{\sigma}_{GB \times LINE}^2 = 0.00227 \pm 0.00122$ for the control group (the figure 0.00227 is significantly different from zero). Their difference, which is considered to be due to lethal genes, is 0.0013 ± 0.0015 . This value is not significantly different from zero. Thus, the significance of the interaction between genetic background and lines within lethality in Table 1 might be due to the mutant polygenes in the genetic background, and not due to lethal genes in question. For this reason, it may be concluded that the newly arisen lethal genes were almost always deleterious in heterozygous condition.

The average degree of dominance of lethal genes ($\bar{h}$) was estimated. The average degree of dominance of lethal-carrying chromosomes was estimated at first using the following formula: $\bar{H} = 1 - \bar{v}'/\bar{v}$ where $\bar{v}'$ is the average viability of lethal heterozygotes and $\bar{v}$ stands for the average viability of the control group. Estimation was carried out in each genetic background. $\bar{h}$ was estimated as $\bar{h} = \bar{H}/\bar{n}$ where $\bar{n}$ is the average number of lethal genes in lethal carrying chromosomes. As reported previously (Mukai 1964), the mutation rate to lethals in the present experimental materials has been estimated to be 0.0063 per second chromosome per generation. On the basis of this figure and the number of generations during which lethal genes had been accumulated in the second chromosomes, the average number of lethal genes in the lethal-carrying chromosomes was calculated to be 1.20 under the assumption of a Poisson distribution. The estimated $\bar{h}$ values are presented in Table 4. All the $\bar{h}$ values are significantly

Table 4. The average degree of dominance of lethal genes in various genetic backgrounds

Genetic background	Average viability index		Average degree of dominance of lethal genes ($\bar{h}$)
	Control	Lethal	
Homozygous	1.1717	1.1455	
[+ _o /+ _i vs. + _o /l _i]	± 0.0116	± 0.0109	0.019* ± 0.011
Intrapopulational	1.0803	1.0310	
[+ _w /+ _i vs. + _w /l _i]	± 0.0121	± 0.0126	0.038** ± 0.013
Interpopulational	1.1062	1.0761	
[+ _B /+ _i vs. + _B /l _i]	± 0.0113	± 0.0096	0.023* ± 0.011
Grand mean of $\bar{h}$ valuee			0.0265** ± 0.0069

* significant at the 5 percent level.

** significant at the 1 percent level.

different from zero, which means that the newly arisen lethal genes are heterozygously detrimental to their carriers in all the genetic backgrounds utilized on the average. The data in Table 4 also suggest that there may be a negative correlation between the $\bar{h}$ values and the relative viabilities of corresponding wild-type individuals. Of course, the viabilities of the standard *Cy* flies (*Cy*/+_o, *Cy*+_w, and *Cy*/+_B) are not the same, but the data of Wallace and Dobzhansky (1962) and Wallace (1962) suggest that their differences may be small.

Experiment 2: Analyses of variances were carried out on the basis of individual ob-

servations for both newly arisen lethals and lethals that originated from the natural population, but significant results were not obtained. Thus, all the flies within crosses were pooled (8 observations in newly arisen mutations and 4 observations in natural lethals per cross) and the viability indices were calculated.

In the experiment of newly arisen mutations, the average numbers of flies counted per cross were 2640.8 in the control group, 2710.0 in the single-lethal heterozygote group, and 2632.6 in the double-lethal group (total number of flies counted was 316,706). LM-34 and LM-35 were allelic. Thus, the numbers of crosses were 40 in the control and single-lethal group, respectively, and 39 in the double-lethal group. The average viability indices were given in Table 5 together with their standard errors. These

Table 5. The average heterozygous viabilities and degrees of dominance of newly arisen¹⁾ and natural lethals²⁾

Heterozygote	Newly arising lethal			Natural lethal		
	Control	Single	Double	Control	Single	Double
Average viability index	0.9544 ±0.0183	0.9489 ±0.0277	0.9369 ±0.0194	0.9458 ±0.0125	0.9390 ±0.0147	0.9325 ±0.0153
Standardized viability	1	0.9942	0.9817	1	0.9929	0.9859
Average number of flies counted per cross	2640.8	2710.0	2632.6	1324.0	1327.4	1388.7
Regression coefficient	-0.0092±0.0019 with d.f.=1			-0.0071***±0.00005 with d.f.=1		
Average degree of dominance	0.0076±0.0016			0.0057***±0.00004		

*** highly significant

1) 40 newly arisen lethals were employed

2) 49 natural lethals were employed.

values were standardized by the average value of the control, and then a linear regression was applied. If X is the number of lethal chromosomes and $\hat{Y}$ is the predicted viability, then we can obtain the following regression equation:

$$\hat{Y} = -0.0092X + 1.0009 \quad (1)$$

The standard error of the regression coefficient (s_b) is 0.0019 with one degree of freedom. The regression coefficient is not significantly different from zero but very close to it ($0.05 < P < 0.10$).

The mutations were accumulated in the chromosomes to generation 100. The estimated mutation rate of recessive lethals was 0.0051 per second chromosome per generation. This is almost exactly the same as the standard mutation rate (cf. Crow and Temin 1964). Based upon this mutation rate the average number of lethal genes per lethal-carrying chromosome was calculated to be 1.28 under the assumption of a Poisson distribution. Thus, the average degree of dominance was estimated to be 0.0076 ± 0.0016 .

Fig. 2. The relationship between the average viabilities of lethal heterozygotes and the numbers of lethal-carrying chromosomes in them.

These results are tabulated in Table 5 and also shown in Figure 2.

In the experiment on the natural lethals, the average numbers of flies counted per cross were 1324.0 in the control group, 1327.4 in the single-lethal heterozygote group, and 1388.7 in the double-lethal group (total number of flies counted was 196,576). DL-27 and DL-28 were allelic. Thus, the numbers of crosses were 49 in the control and single-lethal group, respectively, and 48 in the double-lethal group. The average viability indices were given in Table 5 together with their standard errors. These values were standardized by the average value of the control, and then, as before, a linear regression was applied. Using the same notations, the following regression equation was obtained:

$$\hat{Y} = -0.0071X + 1.0000 \quad (2)$$

The standard error of the regression coefficient (s_b) is 0.000049 with one degree of freedom. The regression coefficient is highly significant.

In the natural population from which the lethal-carrying and their control chromosomes were extracted, the frequency of lethal chromosomes was 0.36. Using this figure, the average number of lethal genes per lethal-carrying chromosome was calculated to be 1.24 under the assumption of a Poisson distribution. Thus, the average degree of dominance was estimated to be 0.0057 ± 0.00004 . These results are presented in Table 5 and also shown in Figure 2.

In the above analysis, we found that the viabilities of lethal heterozygotes decrease, on the average, approximately in proportion to the number of the lethal genes which they carry in both newly arisen and natural lethals. Even a tendency of synergistic interaction among lethal genes can be seen in the former group as shown by Kitagawa (1967). The result of natural lethals is different from the result reported by Oshima (1963). The probable cause of this difference will be discussed below.

DISCUSSION

Detrimental effects of newly arisen lethal genes in heterozygous condition: In the experiments for newly arisen lethal genes, a total of 81 lethal-carrying chromosomes and the same number of lethal-free chromosomes sampled at random were used to estimate the average degree of dominance of lethal genes. The genetic backgrounds were the same on the average, since randomly arising non-lethal mutations were accumulated under minimum pressure of natural selection in both lethal-carrying and control chromosomes. Thus, we could examine the average heterozygous effect of *lethal genes (not lethal chromosomes)* in a nearly homozygous genetic background (heterozygosity occurred in loci where non-lethal mutations took place). However, it would be impossible to carry out experiments of this type, using lethal-carrying chromosomes extracted from natural or artificial populations, if linkage disequilibrium generally occurs between lethal genes and their closely adjacent genes. Under this condition, we could not conduct the control experiment to estimate heterozygous effects of lethal genes.

The results of the experiments for newly arisen lethal genes can be summarized as follows: (1) Perhaps all of the newly arisen lethal genes studied were detrimental in heterozygous condition in all genetic backgrounds employed (the effects of individual lethal genes were not estimated in the second experiment). This is different from the case of lethals from equilibrium populations in *D. pseudoobscura* shown by Dobzhansky and Spassky (1968). (2) The average degrees of dominance of lethal genes were negatively correlated with the average viabilities of their genetic backgrounds. (3) Lethal genes that showed more deleterious effects in one genetic background seemed to be more detrimental in any other genetic background. According to Ives (1945) and Wallace (1950), the number of loci that can mutate to lethal genes is 400-500 in the second chromosome of *D. melanogaster*. Thus, it is dangerous to conclude from the present study that all newly arisen lethal genes are detrimental in heterozygous condition to their carriers since we tested only a small proportion of loci.

Lethal genes in random mating populations: Nei (1968) has shown that the following formula is a better one for the estimation of the average degree of dominance of lethal genes in equilibrium population when $4N\bar{h}$ is much larger than 1:

$$\hat{\bar{h}} = U / [-1n(1-Q)] \quad (3)$$

where U is the total lethal mutation rate per chromosome, Q is the frequency of lethal-carrying chromosomes and N is the effective size of the population. Using $U=0.00503$ (Crow and Temin 1964) and $Q=0.36$ for the Raleigh, N. C. population, $\hat{\bar{h}}$ can be estimated to be 0.011. This value is very close to the previous estimate (0.0057), if we consider the range of $\bar{h}$ values which have been estimated by many investigators. It should be noted here that the estimate of $\bar{h}$, 0.011, is for the fitness as a whole, but the estimate in the present experiment is concerned with only viability.

Wright (1937) obtained the following formula for the equilibrium frequency of a completely recessive lethal gene ($\hat{q}$):

$$\hat{q} = \Gamma\left(2N\mu + \frac{1}{2}\right) / [\sqrt{2N}\Gamma(2N\mu)] \quad (4)$$

where μ is the lethal mutation rate per locus. The effective size of the Raleigh population was estimated to be about 22,000, applying the allelism rate of lethal chromosomes and the equilibrium frequency of lethal chromosomes to Nei's (1968) formula (Mukai, unpublished). Estimated $\hat{q}$ was 0.00238. Assuming that the number of loci where lethal mutations take place is 400 per second chromosome (Wallace 1950), the frequency of lethal-carrying chromosomes is estimated to be 0.61. A direct estimate of Q was 0.36 in 1969. This is significantly less than the estimated value assuming complete recessivity of lethal genes.

Allelism tests were carried out between the lethal-carrying chromosomes extracted in 1968 and 1969. The results are 0.0041 (16/3381) (Mukai, unpublished). This figure is almost equal to the allelism rate of lethal-carrying chromosome in an infinite population with $Q=0.36$ (0.0038). This indicates that perhaps almost all the lethal genes would be eliminated within one year after their appearance in the Raleigh populations, as the theory predicts (cf. Fisher 1958).

All the above results show the deleterious effects on fitness of lethal genes in heterozygous condition. In an infinite population and if h is much larger than $\sqrt{\mu/s}$, the arithmetic mean of h values in equilibrium population is the harmonic mean of the h values of newly arising lethal genes, if there is no gene interaction (Hiraizumi and Crow 1960; Morton, Crow, and Muller, 1956). Thus, the change of $\bar{h}$ values in the Raleigh, North Carolina population might be explained by simple natural selection. In fact, we have estimated that the genetic variance of h values of lethals on a single chromosome is 0.00338 ± 0.00120 .

In the present experiment, we could find neither gene interaction such as the case observed by Oshima (1963) nor the superiority of lethal heterozygotes in their native genetic background such as the case observed by Dobzhansky and Spassky (1968). The phenomena observed by Oshima (1963) and Dobzhansky and Spassky (1968) cannot be explained by a simple linkage disequilibrium created by random genetic drift shown by Ohta and Kimura (1969) mathematically. Co-adapted gene complexes were perhaps formed around the lethal genes in their cases. Indeed, Oshima (1963) has shown that three and two lethal genes of the twenty-four isolated in 1960 were retained at least for one year and two years, respectively in the same natural populations. In the Raleigh population, such a phenomenon could not be seen.

The difference of our results from those of Oshima (1963) and Dobzhansky and Spassky (1968) might have been caused partly by the difference in the effective sizes between the populations. The effective size of the population which Oshima (1963) studied can be estimated as about 1,800 on the basis of the parameters reported by Watanabe (1969), i.e., allelism rate=0.0348 and the frequency of lethal-carrying chromosomes=0.149. Dobzhansky and Wright (1941, 1943) and Wright, Dobzhansky, and Hovanitz (1942) estimated the effective size of *D. pseudoobscura* in the Mount San Jacinto populations to be between 500 and 1000. They are much smaller than the Raleigh, N. C. population (22,000). An intermediate effective size of the populations such as the Japanese populations of *D. melanogaster* and the *D. pseudoobscura* populations which Dobzhansky and others studied might be necessary for the formation of coadapted gene complexes around lethal genes in the chromosomes. This speculation may be consistent

with the theory of evolution of species founded by Wright (Wright 1949; Kimura 1960) who has stated on the basis of his mathematical theory of population genetics that the most favorable condition for the evolution of species is that the species exists as many subpopulations which are incompletely isolated from one another.

We are grateful to Dr. M. Nei for general discussions we have had with him on the present topic.

SUMMARY

Heterozygous effects of lethal genes on viability in *Drosophila melanogaster* were examined using 81 second chromosomes carrying newly arisen lethal genes and 49 second chromosomes carrying lethal genes extracted from a Raleigh, N. C. population. The same number of lethal-free control chromosomes were employed. The following findings were obtained: (1) No evidence of superiority of lethal heterozygotes over the control was obtained in either almost completely homozygous or in intra- and interpopulational heterozygous genetic backgrounds. (2) The average degrees of dominance of lethal genes ($\bar{h}$) are probably negatively correlated with the viabilities of their genetic backgrounds. The $\bar{h}$ values were 0.0076–0.038 for newly arisen lethals and 0.0057 for lethals from the natural populations. (3) The newly arisen lethals that showed more deleterious effects in one genetic background seemed to be also more detrimental in all the other genetic backgrounds employed. (4) Significant synergistic interaction could not be observed when single individuals had two lethal genes. For the natural lethals, a clear linear relationship was observed between the average viabilities of heterozygotes and the numbers of lethal genes per individual, while in newly arisen lethals, a tendency of synergistic interaction was observed but not significantly so.

On the basis of the above findings and of the experimental results of Dobzhansky and Spassky (1968) and Oshima (1963), the behavior of lethal genes in natural populations was discussed.

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