FREQUENCY DISTRIBUTION OF LETHAL CHROMOSOMES IN SMALL POPULATIONS OF *DROSOPHILA MELANOGASTER*

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STUDYING the frequencies and allelic rates of lethal chromosomes in natural populations of *Drosophila pseudoobscura*, DOBZHANSKY and WRIGHT (1941) and WRIGHT, DOBZHANSKY and HOVANITZ (1942) showed that the frequency of lethals is much less than would be expected of completely recessive lethals under random mating and this lower frequency is due to either partial inbreeding within populations or selection against lethal heterozygotes. The same conclusion has been reached by CROW and TEMIN (1964) in a survey of Drosophila data obtained by various authors. In these studies the effective sizes of populations and the migration rates between populations were unknown parameters, so that they could not separate the effects of inbreeding from the effects of selection against heterozygotes. Thus, if these parameters are artificially determined in experimental populations, selection against lethal heterozygotes, which is one of the most controversial problems in population genetics at present, would be studied more accurately, as pointed out by NEI (1968).

In recent years it has been shown with several organisms that the effective breeding size of natural populations is probably fairly small, though there is generally more or less migration between populations (e.g., KERSTER 1964; TINKLE 1965; NEI and IMAIZUMI 1966a; MERRELL 1968). In *Drosophila melanogaster*, WALLACE (1966) obtained a high rate of allelism of lethal genes when flies were collected from small areas. This strongly suggests that the effective size in this species is also quite small. Thus, it is desirable to know the population dynamics of lethal genes in small populations.

The theoretical distribution of lethal gene frequency in finite equilibrium populations was first obtained by WRIGHT (1937). Recently, extending WRIGHT's theory, NEI (1968) studied the frequency distribution of *lethal chromosomes* rather than *lethal genes*. This study is important, because in most cases the frequencies of individual lethal genes cannot be observed but the frequency of lethal-bearing chromosomes is determined. So far, however, no experimental studies have been conducted on the frequency distribution of lethal genes or lethal-bearing chromosomes.

The present study is intended to determine experimentally the frequency distribution of lethal second chromosomes in small populations of *Drosophila melanogaster* and to investigate the type and degree of selection against lethal heterozygotes. The relation between the mean gene frequency of lethals and population size will also be examined.

MATERIALS AND METHODS

Experimental populations: In the present study three different kinds of experimental populations (cage populations, small populations and extremely small populations) were used. These populations were made in the following ways from the descendants of flies captured in a natural population of *D. melanogaster*. In July 1965 about 300 males and 300 females were collected at Kofu-Katsunuma, Japan, and introduced into a population cage (30 × 40 × 13 cm) with 15 food cups. The progenies in the next generation were separated into 4 stock cages. After random mating for 2 generations more than 100 male flies were randomly sampled from these stock cages and used to examine the initial frequency of lethal second chromosomes in the experimental populations. At the same time, all the food cups of these 4 cages were removed and replaced by new cups. To initiate experimental cage populations No. 1 and No. 2, six cups with eggs deposited for 48 hours were withdrawn from each of the 4 stock cages and inserted into two new cages, 12 cups being used for cage No. 1 and the other 12 cups for cage No. 2. In these cage populations two cups were exchanged with new ones every other day as a rule. On the other hand, 52 small populations, No. 1 to No. 52, were initiated by using flies taken at random from the remaining cups of the stock cages. Each of these small populations was maintained by sampling 25 virgin females and 25 males in a culture vial (3 × 10 cm) every generation, though in some generations females were not necessarily virgin. One of the small populations (No. 12) was lost by a technical mistake at the 8th generation. In April, 1966, 50 extremely small populations were set up by using flies originating from the two experimental cage populations. Each of these 50 populations was kept by 5 females and 5 males in a small culture vial (2.3 × 8 cm) in every generation. Three out of 50 extremely small populations were lost in the 14th, 44th and 54th generation owing to inbreeding depression. All the experimental populations were kept in a constant temperature (25°C) room throughout the experiment. The humidity was not controlled.

Test of lethal second chromosomes: Using the *Cy/Pm* technique, the frequencies of lethal second chromosomes in the three groups of the experimental populations were periodically examined in order to see how the frequency of lethal chromosomes changes temporally. The genetic background of this *Cy/Pm* strain was not the same as that of the tested populations. In the examination of lethal chromosome frequency, more than 100 adult males per population were used in the cage populations, while in the small and the extremely small populations, a few males per vial, totaling 100 to 200 males for each of the two types of populations were sampled. In all cases, sampled males were individually crossed to *Cy/Pm* females and a single *Cy/+* male progeny from each cross was backcrossed to *Cy/Pm* females. In the next generation, a *Cy/+* × *Cy/+* mating was made for each backcross progeny. When *no* +/+ flies were observed in the following generation, the tested chromosome was defined as carrying one or more recessive lethal gene.

Frequency distribution of lethal chromosomes in small populations: The frequency distribution of lethal chromosomes was studied with the small population–i.e., the populations with 50 individuals. To apply NEI's theory, it is essential to use those populations which have reached the equilibrium state. In practice, however, it is very difficult to know whether the populations have reached equilibrium or not. Thus, the first examination was undertaken during a period from the 29th to the 43rd generations. To estimate the lethal chromosome frequency in each of the small populations, about 110 male flies were randomly sampled from the progenies of individual populations. The total number of chromosomes tested in this examination was 4,114. Because of this large number of chromosomes tested, more than ten generations were required for completing the examination.

As will be seen later, the result of the first examination indicated that the populations had possibly not yet reached equilibrium. Therefore, the frequency distribution was again examined during the 62nd to the 72nd generations in the same manner as before, although in this case about 130 male flies were sampled from each of the populations. The total number of chromosomes tested in the second examination was 5,483.

Allelism test of lethal chromosomes: The allelism frequency of lethal chromosomes plays

an important role in studying the heterozygous effect of lethal genes or the effective size of populations. Thus, in the cage populations as well as in the small populations, this parameter was estimated by the standard all-possible-cross method. In cage No. 1, 25 lethal chromosomes sampled at the 46th generation were used for this test, while in cage No. 2, 22 lethal chromosomes sampled at the 44th generation were used. In small populations, the lethal chromosomes sampled in the second test of chromosome frequency distribution were kept as *Cy*-balanced lethal stocks at low temperature (15°C) until the time for allelism tests. The number of lethal chromosomes tested in each population varied from 2 to 45 and the total number of crosses made reached 5,381. The allelism frequency between lethals of independent origin was also determined by making crosses between the lethal chromosomes taken at random from different small populations. In this case only those chromosomes that were nonallelic in the within-population test were used. Furthermore, not all possible combination crosses were made; namely, 764 out of 1,867 possible crosses were performed, using 64 lethal chromosomes.

EXPERIMENTAL RESULTS

Temporal changes in the frequencies of lethal second chromosomes: The temporal changes in the frequencies of lethal second chromosomes in the three different populations, i.e., cage populations, small populations with 50 individuals, and extremely small populations with 10 individuals, are shown in Figures 1 and 2. In this test of temporal changes a total of 4,078 chromosomes was examined. The initial frequency in these test populations was estimated from that in the stock cage populations, as mentioned earlier; it was 0.109. In the cage populations generations were completely overlapping, so that a period of 15 days was assumed to be equivalent to one generation. Figure 1 shows that the lethal chromosome frequency in the cage populations fluctuates considerably generation by generation, but there is no tendency to increase or decrease, on the whole. The weighted mean frequency over all generations is 0.148 for cage No. 1 and 0.175 for cage No. 2. There is no significant difference in the mean frequency between the two cage populations; the grand mean is 0.160.

The frequency of lethal chromosomes fluctuates also in the small populations (Figure 2). The high frequencies in the early generations are perhaps caused by genetic sampling error at the time of population initiation. In the small populations, however, there is a tendency for the lethal frequency to decrease gradually. The regression coefficient of frequency on generation is -0.0010, which is significantly different from zero ($t = 3.77$, $df = 12$, $P<0.01$). Since the population size was drastically reduced compared with that of wild populations, some amount of reduction in the lethal frequency is expected (cf. NEI 1969). The weighted mean frequency over all generations is 0.133, which is significantly smaller than the mean frequency in the cage populations ($\chi^2 = 4.94$, $df = 1$, $P<0.05$).

In the extremely small populations, data on lethal frequency are not so extensive, but there is again the tendency for the lethal frequency to decrease with generation time. In this case, however, the regression coefficient of frequency on generation is not statistically significant. The mean frequency is 0.110, which is slightly smaller than that in the small populations.

Frequency distribution of lethal chromosomes in the small populations: The distribution of lethal chromosome frequency in the first examination is shown in

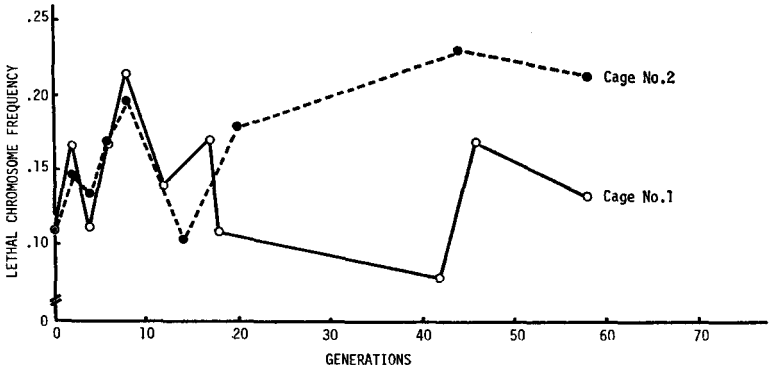

FIGURE 1.—Changes in the frequency of lethal second chromosomes in the cage populations.

Figure 3. In this figure the horizontal axis represents the *transformed* lethal chromosome frequency, i.e., $Q_1 = -\log_e (1 - Q)$, where Q denotes the lethal chromosome frequency. This transformation gives an estimate of the sum of lethal gene frequencies at individual loci on the assumption that lethal genes are randomly distributed on the chromosome. It is seen that Q_1 is widely distributed from 0 to 0.4. The distribution pattern is well in accordance with the theoretical expectation, as will be examined later. The mean and variance of Q_1 are 0.104 and 0.00851. The mean frequency of lethal chromosomes, before transformation, $\bar{Q}$, is 0.096, which is not significantly different from the value obtained in the test of temporal changes in lethal frequency–i.e., 0.133, but different from the grand mean in the cage populations–i.e., 0.160 ($\chi^2 = 50.62$, df = 1, $P<0.001$).

The frequency distribution of lethal chromosomes in the second test, which was conducted between the 62nd and 72nd generations, is presented in Figure 4.

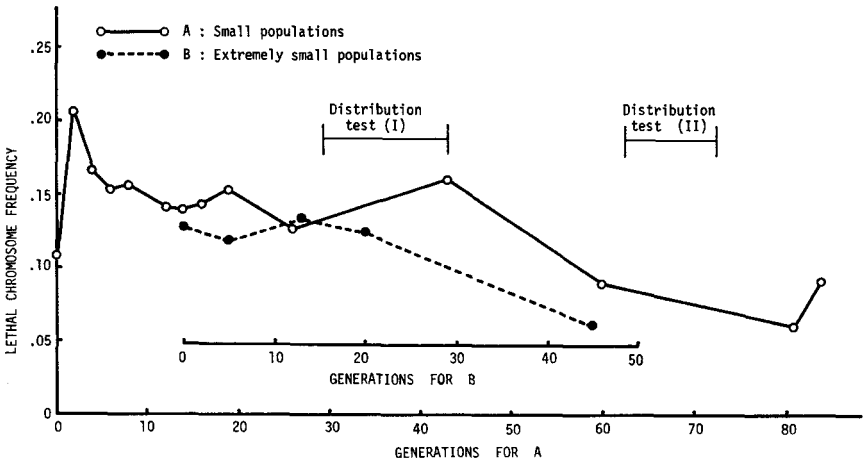

FIGURE 2.—Changes in the frequency of lethal second chromosomes in the small and in the extremely small populations.

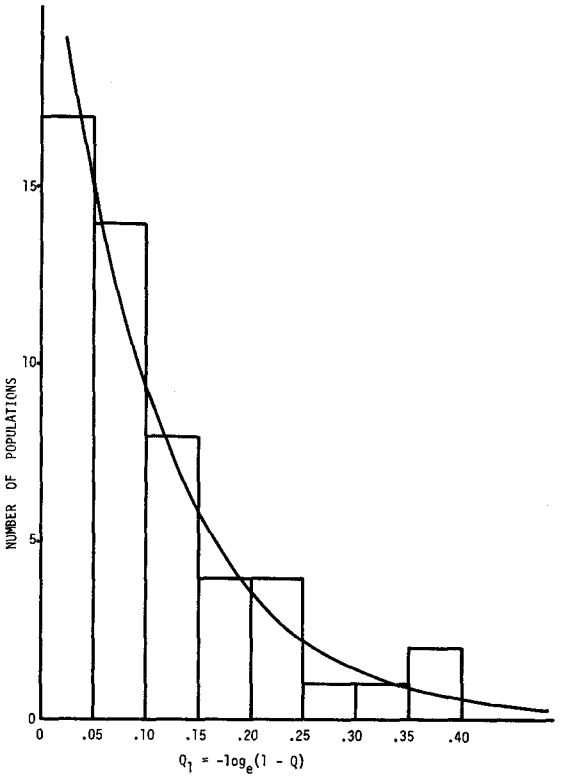

FIGURE 3.—Observed and expected frequency distribution of lethal second chromosomes in the small populations, first test. The theoretical curve is given by $51 \times 9.5 \times e^{-9.5Q_1} \times 0.05 = 24.23 \times e^{-9.5Q_1}$.

It is seen that the shape of this frequency distribution is very similar to that obtained in the first test. However, a close examination reveals that the numbers of populations in the intermediate classes (.05–.25) are reduced, while those in the lower (0–.05) and higher (beyond .25) classes are increased compared with those in the first test. The mean and variance of Q_1 are 0.115 and 0.01504, respectively. Thus, the variance is almost twice as large as that of the first distribution, but the mean remains almost the same. The value of $\bar{Q}$ is 0.103, which is also almost the same as the previous value ($\chi^2 = 1.25$, df = 1, $0.20<P<0.30$). It is, however, significantly different from the grand mean in the cage populations. The fact that the variance of Q_1 is larger in the second test than in the first test suggests that the distribution of Q_1 had not reached the equilibrium state when the first test was performed.

Allelism tests with lethal chromosomes: In Table 1 the result of allelism tests with lethal chromosomes from the cage populations is presented. The estimate of allelic rate of lethal chromosomes, I_c, is 7.0% for cage No. 1 and 4.7% for cage No. 2. The difference between the two cage populations is perhaps due to genetic random drift and sampling error at the time of test. The average rate of allelism

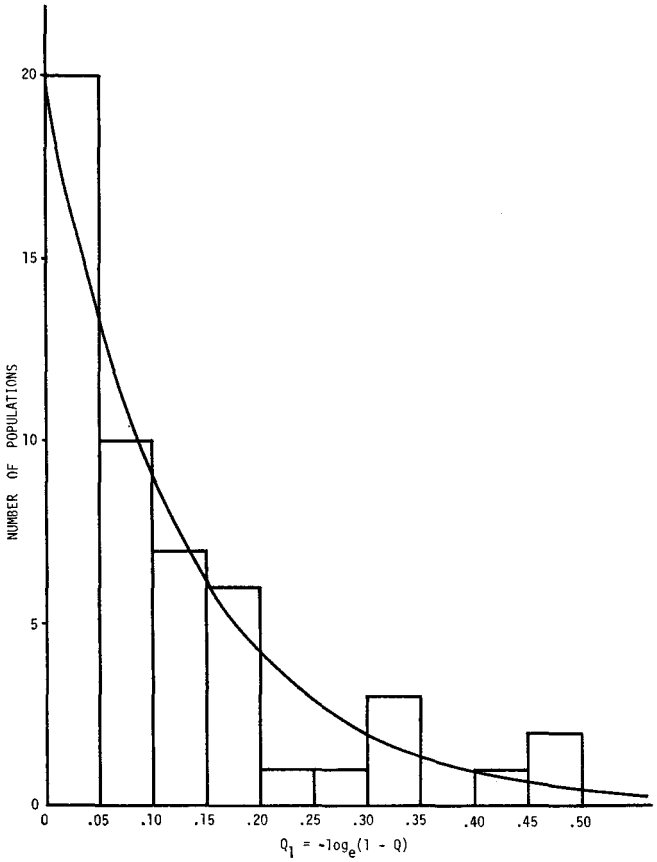

FIGURE 4.—Observed and expected frequency distribution of lethal second chromosomes in the small populations, second test. The theoretical curve is given by $51 \times 7.7 \times e^{-7.7Q_1} \times 0.05 = 19.64 \times e^{-7.7Q_1}$.

is estimated to be 6.0%. The allelic rate of *lethal genes* (I_g) instead of lethal chromosomes can be estimated by $-\log_e(1 - I_cQ^2)/Q_1^2$ (cf. NEI 1968). In the present case the average value of I_g becomes 5.1%.

In the small populations, the estimate of allelic rate of lethal chromosomes within population is variable from 0 to 100% with a mean of 46.6%. This allelic rate is extremely high compared with those reported by other workers in both

TABLE 1

Frequencies of allelism of lethal second chromosomes in the cage populations

Population	Generation	Number of lethal chromosomes	Number of crosses	Frequency of appearance 1	2	3	4	5	6	7	Allelic rate
No. 1	46	25	300	2	5	1	5	0	0	1	0.070
No. 2	44	22	231	4	9	0	0	0	0	0	0.047

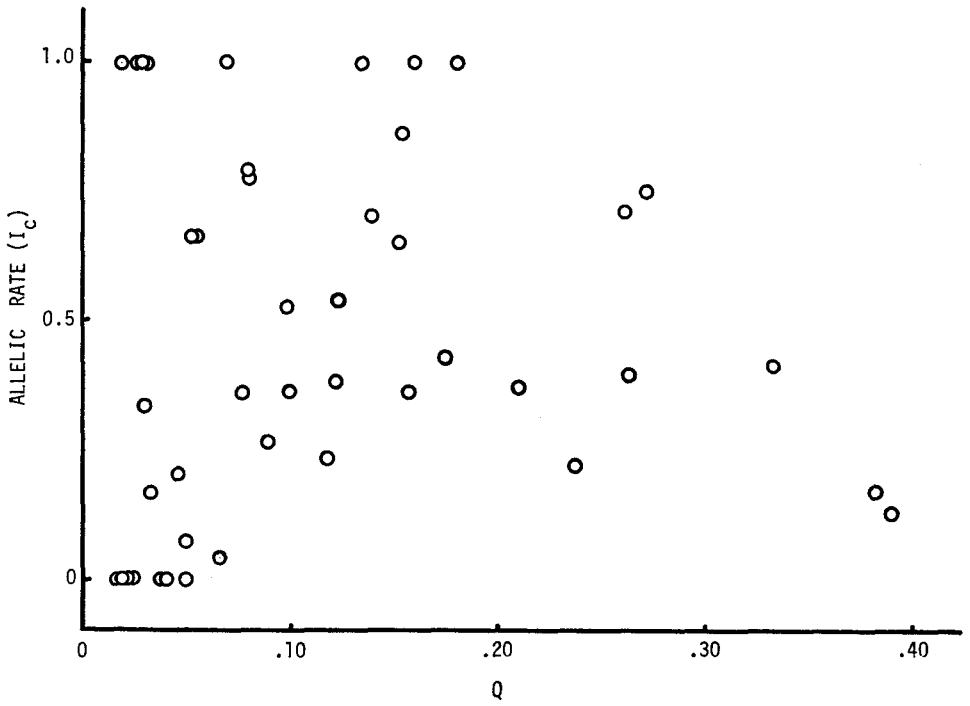

FIGURE 5.—Relation between the frequency of allelism and lethal chromosome frequency in the small populations.

natural and laboratory populations. This result is not, however, unexpected, since NEI (1968) has shown theoretically that the allelic rate becomes very high in small populations. The average value of I_g is 41.3%. No correlation is found between the frequency and the allelic rate of lethal chromosomes (Figure 5). The allelic rate of lethal chromosomes between populations was 0.4% (3/764), while the allelic rate of lethal genes was 0.35%. The former value is slightly larger than the allelic rate of lethal chromosomes obtained by WALLACE (1950).

STATISTICAL ANALYSES

Heterozygous effect of lethal genes in the cage populations: The mean lethal gene frequency strongly depends on the effective population size as well as on the heterozygous effect of the genes (WRIGHT 1937; NEI 1969). Thus, if one wants to estimate the heterozygous effect of lethal genes, it is essential to know the effective population size. The actual size of the cage population can be estimated by counting all the adult flies living in a population cage at a certain time. The data obtained in a preliminary experiment indicate that the actual population size is about 7,000. On the other hand, the effective size of the population, N_e, can be estimated by NEI's (1968) formula, $(1 - I_g)/4(I_gU - u)$, where u and U are the rates of lethal mutations per generation, per locus and per

chromosome, respectively. It seems that $u = 10^{-5}$ and $U = 0.005$ are well established (cf. CROW and TEMIN 1964). Thus, using these values and $I_g = 0.051$, we obtain $N_e = 968$. This value is considerably smaller than the actual number of adult flies in the cage populations. The probable cause for this difference will be discussed later. At the moment, we assume that the effective size is about 1,000 ~ 2,000.

WRIGHT (1937) has shown that if lethal genes are completely recessive, the mean gene frequency, $\bar{q}$, in finite populations is approximately given by

$$\bar{q} = \Gamma(2N_eu + \tfrac{1}{2})/\sqrt{2N_e}\,\Gamma(2N_eu)$$

where $\Gamma(\cdot)$ denotes the gamma function. $\bar{Q}_1$ $(= n\bar{q})$ is, therefore, obtained by the above formula, if n is known and all lethal genes are completely recessive. The value of n is approximately 500 $(= U/u)$. Using this value and $N_e = 1{,}000 \sim 2{,}000$, we obtain $\bar{Q}_1 = 0.386 \sim 0.532$. This is two to three times larger than the observed value, 0.174. This suggests that lethal genes are partially recessive and reduce the fitness of lethal heterozygotes to some extent. NEI (1968) has shown that if the amount of reduction in relative fitness of lethal heterozygotes, h, is much larger than $\sqrt{u}$, h is estimated by U/Q_1 approximately. If we apply this method, h is estimated to be 0.029. Thus, we are led to the conclusion that lethal genes reduce the heterozygote's fitness by about 3 percent on the average. The possibility that lethal genes are overdominant is, of course, ignored in the present case, because overdominant lethals give a value of Q_1 much larger than that for completely recessive lethal genes (cf. NEI 1969).

Heterozygous effect of lethal genes in small populations: NEI (1968, 1969) showed that, if $h \gg \sqrt{u}$ and $4N_eh \gg 1$, the distribution of Q_1 is approximately given by the following gamma distribution.

$$\phi(Q_1) = [(4N_eh)^{4N_eU}/\Gamma(4N_eU)]\, e^{-4N_ehQ_1}\, Q_1^{4N_eU-1}$$

The mean, $\bar{Q}_1$, and variance, V_{Q_1}, of Q_1 are:

$$\bar{Q}_1 = U/h \qquad V_{Q_1} = \bar{Q}_1/4N_eh\,.$$

The foregoing analysis of the data for the cage populations has indicated that lethal genes are partially recessive with respect to fitness. Thus, let us now analyze the data for the small populations by using the above theory.

As mentioned earlier, the observed mean and variance of the frequency distribution of Q_1 (in the first test) are 0.104 and 0.00851, respectively. The observed variance, however, includes the sampling variance (V_s) in addition to the variance due to random genetic drift (V_{Q_1}), since the number of chromosomes examined in a population was sometimes smaller than 2N (i.e., 100). Dr. M. NEI has suggested that if the number of chromosomes examined, n_o, is smaller than 2N, V_s is computed by $(2N - n_o)Q/(2N - 1)(1 - Q)n_o$, and if n_o is equal to or larger than 2N, $V_s = 0$. The mean of the sampling variances thus obtained is 0.00030. Therefore, we have $V_{Q_1} = 0.00821$. It is difficult to know the real effective size in this case, but it must be close to N (i.e., 50). Thus, equating N_e to N, h and U are estimated to be 0.064 and 0.0066, respectively. The estimate of U is close to the direct estimate of mutation rate—i.e., 0.005 (cf. CROW and TEMIN 1964)—but the estimate of h is twice as large as the value obtained in the cage populations.

In the second test, $\overline{Q}_1 = 0.115$ and $V_{Q_1} = 0.01503$ were obtained. Applying the same method as the above, the estimates of h and U are 0.038 and 0.0044, respectively. The value of h is now a half of that in the first test and nearly equal to the estimate for the cage populations, while the estimate of U is again close to 0.005. The large value of h obtained in the first test perhaps reflects that the populations had not reached equilibrium when the first test was performed, since h is expected to be overestimated before equilibrium is reached. How the populations reached equilibrium will be discussed later.

As already mentioned, it has been established that the value of U for the second chromosome is approximately 0.005. If we take this value as granted, 4NU becomes unity. In this case, therefore, the distribution of Q_1 is given by $4Nhe^{-4NhQ_1}$. Thus, h can be obtained by fitting this equation to the observed distribution. The least-squares estimate of h obtained in this way is 0.047 for the first distribution and 0.039 for the second test. The latter is very close to the value obtained by the previous method.

If we use the estimates of h obtained by the latter method, 4Nh becomes 9.5 for the first test and 7.7 for the second test. These numbers are not necessarily much larger than unity. Since $4Nh \gg 1$ has been assumed in NEI's theory, there is the possibility that the equation $4Nhe^{-4NhQ_1}$ does not fit the data well. In practice, however, the fit has been proved to be quite satisfactory, as will be seen from Figures 2 and 3. The deviations of the observed values from the expected are not statistically significant ($\chi^2 = 1.19$, $df = 4$, $P > 0.8$ for the first test, and $\chi^2 = 1.43$, $df = 4$, $P > 0.8$ for the second test). In this case the expected frequencies for the classes $0 \sim 0.05$, $0.05 \sim 0.10$, . . . etc. were obtained by $51 \int_0^{0.05} \phi(Q_1) dQ_1$, $51 \int_{0.05}^{0.10} \phi(Q_1) dQ_1$, . . . etc., respectively. We can then conclude that the transformed frequency of lethal chromosomes practically follows the gamma distribution in small populations, and that lethal genes are, on the average, slightly deleterious in the heterozygous condition. Although h is possibly slightly overestimated even in the second test because of the relatively small value of 4Nh, it is not much larger than the estimate obtained in the cage populations.

DISCUSSION

In small populations and in extremely small populations, there was a tendency for the frequency of lethal chromosomes to decline generation by generation. This decline is considered to be due to reduction in population size. When population size is very small, the lethal gene frequencies at individual loci are occasionally increased by random drift, and selection in the homozygous state becomes appreciable, even if there is selection against the lethal heterozygotes. NEI (1968, 1969) has shown that if $h \gg \sqrt{u}$ and $4Nh \gg 1$ the equilibrium gene frequency is approximately given by u/h, but otherwise it will be smaller than u/h. In the present case h is much larger than $\sqrt{u}$ if u is the order of 10^{-5}, but 4Nh is not necessarily much larger than unity in the small and extremely small populations. Therefore, the equilibrium frequency of lethal genes or lethal chromosomes can

be smaller in these populations than in the cage populations (cf. Figure 4 of NEI 1969). Nevertheless, we must keep in mind that the frequency of lethal chromosomes often shows a long-term fluctuation due to random drift, as seen in the data obtained by WALLACE (1956). Note also that the mean frequencies of lethal chromosomes in the distribution test (0.096 and 0.103) are very close to the initial frequency (0.109). Therefore, in order to obtain a decisive conclusion, a much larger period of study is required on the temporal changes of lethal chromosome frequencies.

In the present study, the effective size of the cage populations was estimated to be about 1,000, while the actual number of adult flies was about 7,000. Although there is the possibility that the effective size is an underestimate because of the relatively high rate of allelism of lethal genes in our cage population compared with those obtained by SETO (1961) and PROUT (1954) (the allelic rate obtained by TOBARI (1966) is close to our estimate), it is unlikely that the effective size is much larger than 1,000. This discrepancy between the actual and effective size may be accounted for by the probable non-Poissonian distribution of progeny number (CROW and MORTON 1955) and by the fact that generations were completely overlapping in the cage populations. If generations are overlapping, the effective size could be much smaller than the actual population size (NEI and IMAIZUMI 1966b).

As mentioned earlier, it is important to use equilibrium populations when applying NEI's theory of the frequency distribution of lethal chromosomes. In the present case, the distribution was examined during the 29th to 43rd generations in the first test and during the 62nd to 72nd generations in the second test. Since we now have an estimate of h, we have a rough idea about how the populations reach equilibrium. NEI (1969) showed that, if $h \gg \sqrt{u}$, $4Nh \gg 1$, and the initial frequency of Q_1, $Q_{1(0)}$, is equal to the equilibrium frequency, $\tilde{Q}_1$, the mean, $\overline{Q}_{1(t)}$, and variance, $V_{Q_{1(t)}}$, of Q_1 at the tth generation are approximately given by

$$\overline{Q}_{1(t)} = \overline{Q}_{1(0)}$$

$$V_{Q_{1(t)}} = \tilde{V}_{Q_1} - (\tilde{V}_{Q_1} - V_{Q_{1(0)}}) e^{-[2h+1/(2N)]t}$$

where $V_{Q_{1(0)}}$ and $\tilde{V}_{Q_1}$ are the initial and equilibrium values of the variance of Q_1, respectively. In the present case, 4Nh is not necessarily much larger than unity, but the above formulae may be used to know the approximate values of $\overline{Q}_{1(t)}$ and $V_{Q_{1(t)}}$. It is noted that the means of Q_1 in the first and second tests are very close to the initial value, as predicted by the above formula for $\overline{Q}_{1(t)}$. On the other hand, the variance of Q_1 is expected to increase generation by generation in the present case, since the population size has been reduced in the small populations. Namely, $\tilde{V}_{Q_1} - V_{Q_{1(t)}}$ is expected to be initially positive and to decrease exponentially as generations proceed and $V_{Q_{1(t)}}$ finally becomes equal to $\tilde{V}_{Q_1}$. How the difference $\tilde{V}_{Q_1} - V_{Q_{1(t)}}$ decreases can be evaluated by the above formula for $V_{Q_{1(t)}}$, putting $N = 50$. The values of $e^{-[2h+1/(2N)]t}$ for $h = 0.03$ and $h = 0.02$ and various values of t are as follows:

Generation (t)	30	40	50	60	70
h = 0.03	0.12	0.06	0.03	0.015	0.007
h = 0.02	0.22	0.16	0.08	0.049	0.030

The above values suggest that if $V_{q_1(0)}$ was quite small, the small populations had not necessarily reached the equilibrium state in the first test, but they were almost in equilibrium for the second test.

In the present study h was estimated to be about 0.03. This is slightly larger than the estimate (.015) obtained by CROW and TEMIN (1964) in a survey of Drosophila data obtained by various authors. Their estimate, however, could be an underestimate, since they used the sum of the frequencies of completely lethal and semilethal chromosomes, though their estimate of mutation rate apparently referred to completely lethal genes only (cf. NEI 1968). If we assume that one third of lethal chromosomes is semilethal and estimate h by the formula U/Q_1 using the data given in Table 3 of CROW and TEMIN (1964), h becomes 0.02 to 0.03. These are in agreement with our estimate.

WATANABE (1969) and KOSUDA (personal communication) studied the frequency of lethal second chromosomes in the same Kofu-Katsunuma population from which our flies were derived. The allelic rate of lethal chromosomes was estimated to be about 2% by WATANABE (1969) and 1.5% by KOSUDA. These values are much smaller than those for our cage populations. Thus, the effective size of the Kofu-Katsunuma population is considered to be larger than that of the cage populations. However, the estimates of the frequency of lethal chromosomes by WATANABE (1969) and KOSUDA are 0.155 and 0.15, respectively, which are very close to the frequency (0.16) in our cage populations. This apparent constancy of lethal chromosome frequency irrespectively of allelism frequency supports our conclusion that lethal genes are, on the average, slightly deleterious in the heterozygous condition. Note that this principle does not apply if 4Nh is not much larger than unity.

It is of great interest that the mean rate of allelism of lethal chromosomes in the small populations, 0.466, is tremendously higher than that in the cage populations, 0.060, and those reported by others for both natural and laboratory populations. This indicates that the allelic rate markedly increases as the effective population size, N_e, decreases, as predicted by NEI (1968).

There are many experimental studies on the effect of random genetic drift on gene frequency changes in Drosophila (e.g., KERR and WRIGHT 1954a,b; WRIGHT and KERR 1954; BURI 1956; NOZAWA 1963). Most of these studies were concerned with the frequency of a single mutant gene, and results obtained were mostly in agreement with the theoretical expectations based on WRIGHT's (1931, 1937) and KIMURA's (1955) studies. The present study indicates that the frequency of *lethal chromosomes* rather than *lethal genes* is also affected by random drift in both small and cage populations, as expected from the studies by NEI (1968, 1969). In small populations the frequency of lethal chromosomes has quite a large variance. Thus, it would often be erroneous to draw any conclusions from a single sample for lethal chromosome frequency or allelic rate.

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SUMMARY

The temporal changes in frequencies of lethal second chromosomes were examined in three different kinds of experimental populations of *D. melanogaster* (two cage populations, 51 small populations with 50 individuals, and 50 extremely small populations with 10 individuals) for about 80 generations. In all populations the frequency of lethal chromosomes showed a quite large random fluctuation. In the cage populations there was no directional change in the frequency of lethal chromosomes, but in the small and the extremely small populations it showed a slight tendency to decrease as generations proceeded. The frequency was significantly smaller in the small populations than in the cage populations. The frequency of allelism of lethal chromosomes was extremely high in the small populations, the average rate being 46.6%, while it was about six percent in the cage populations. The frequency distribution of lethal chromosomes was examined in the small populations during the 29th to 43rd generations and during the 62nd to 72nd generations. This distribution was in good agreement with the theoretical expectation, i.e., NEI's gamma distribution. Statistical analyses of the relation between the lethal chromosome frequency and population size and of the frequency distribution of lethal chromosomes in the small populations both indicated that lethal genes reduce the heterozygotes' fitness by about 3 percent, on the average.

LITERATURE CITED

BURI, P., 1956 Gene frequency in small populations of mutant Drosophila. Evolution **10**: 367–402.

CROW, J. F. and N. E. MORTON, 1955 Measurement of gene frequency drift in small populations. Evolution **9**: 202–214.

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

DOBZHANSKY, TH. and S. WRIGHT, 1941 Genetics of natural populations. V. Relations between mutation rate and accumulation of lethals in populations of *Drosophila pseudoobscura*. Genetics **26**: 23–52.

KERR, W. E. and S. WRIGHT, 1954a Experimental studies on the distribution of gene frequencies in very small populations of *Drosophila melanogaster*. I. Forked. Evolution **8**: 172–177.
——, 1954b Experimental studies on the distribution of gene frequencies in very small populations of *Drosophila melanogaster*. III. Aristapedia and spineless. Evolution **8**: 293–302.

KERSTER, H. W., 1964 Neighborhood size in the rusty lizard, *Sceloporus olivaceus*. Evolution **18**: 445–457.

KIMURA, M., 1955 Stochastic processes and distribution of gene frequencies under natural selection. Cold Spring Harbor Symp. Quant. Biol. **20**: 33–53.

MERRELL, D. J., 1968 A comparison of the estimated size and the "effective size" of breeding populations of the leopard frog, *Rana pipiens*. Evolution **22**: 274–283.

NEI, M., 1968 The frequency distribution of lethal chromosomes in finite populations. Proc. Natl. Acad. Sci. U.S. **60**: 517–524. ——, 1969 Heterozygous effects and frequency changes of lethal genes in populations. Genetics **63**: 669–680.

NEI, M. and Y. IMAIZUMI, 1966a Genetic structure of human populations. II. Differentiations of blood group gene frequencies among isolated populations. Heredity **21**: 183–190. ——, 1966b Genetic structure of human populations. III. Differentiation of ABO blood group gene frequencies in small areas of Japan. Heredity **21**: 461–472.

NOZAWA, K., 1963 Competition between a brown gene and its wild-type allele in *Drosophila melanogaster*. II. Estimations of relative viability of a brown homozygote and an analysis of changes in gene frequency. Japan J. Genet. **38**: 6–20.

PROUT, T., 1954 Genetic drift in irradiated experimental populations of *Drosophila melanogaster*. Genetics **39**: 529–546.

SETO, F., 1961 A developmental study of recessive lethals from wild populations of *Drosophila melanogaster*. Am. Naturalist **95**: 365–373.

TINKLE, D. W., 1965 Population structure and effective size of a lizard population. Evolution **19**: 569–573.

TOBARI, I., 1966 Effects of temperature on the viability of heterozygotes of lethal chromosomes in *Drosophila melanogaster*. Genetics **53**: 249–259.

WALLACE, B., 1950 Allelism of second chromosome lethals in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **36**: 654–657. ——, 1956 Studies on irradiated populations of *Drosophila melanogaster*. J. Genet. **54**: 280–293. ——, 1966 Distance and the allelism of lethals in a tropical population of *Drosophila melanogaster*. Am. Naturalist **100**: 565–578.

WATANABE, T. K., 1969 Frequency of deleterious chromosomes and allelism between lethal genes in Japanese natural populations of *Drosophila melanogaster*. Japan. J. Genetics **44**: 171–187.

WRIGHT, S., 1931 Evolution in Mendelian populations. Genetics **16**: 97–159. ——, 1937 The distribution of gene frequencies in populations. Proc. Natl. Acad. Sci. U.S. **23**: 307–320.

WRIGHT, S., TH. DOBZHANSKY and W. HOVANITZ, 1942 Genetics of natural populations. VIII. The allelism of lethals in the third chromosome of *Drosophila pseudoobscura*. Genetics **27**: 363–394.

WRIGHT, S. and W. E. KERR, 1954 Experimental studies of gene frequencies in very small populations of *Drosophila melanogaster*. II. Bar. Evolution **8**: 225–240.