ACCUMULATION OF DELETERIOUS GENES IN A CAGE POPULATION OF DROSOPHILA MELANOGASTER

WON HO LEE AND TAKAO K. WATANABE

National Institute of Genetics, Mishima, 411 Japan

Manuscript received January 31, 1977
Revised copy received March 21, 1977

ABSTRACT

Lethal and sterility mutations were accumulated in a cage population which was initiated with lethal- and sterility-free second chromosomes of D. melanogaster. It took about 2,000 days for the frequencies of these genes to reach equilibrium levels, i.e., 18% lethal and 9% male-sterile chromosomes. Two other cage populations which were initiated with random chromosomes sampled from natural populations and kept for more than eleven years in the laboratory showed 19–20% lethal content. The elimination rates of lethals by homozygosis in these populations were smaller than the mutation rate. By using Nei's formulae, the deleterious effect of a lethal gene in heterozygous condition \((h)\) was estimated to be 0.035. The effective population number in the cage populations was estimated to be 1,000–2,900, while the actual population number was 3,500–7,800.

MURATA and TOBARI (1973) studied the frequency changes of lethal chromosomes in experimental cage populations that were derived from an irradiated population of D. melanogaster. After termination of irradiation, the lethal frequency decreased to the original nonirradiated levels more rapidly than expected for completely recessive lethals. The rapid decrease of the radiation-induced lethal genes was ascribed to the partial dominance of the genes. The degree of deleterious effects of the lethal genes in heterozygous condition \((h)\) was calculated by using Nei's (1969) formulae. It was large in the early generations, but decreased thereafter.

The present experiment was intended to accumulate spontaneous mutations in a mutation-free cage population until equilibrium was attained. The frequencies of lethal and sterile chromosomes were monitored during the approach to equilibrium. In addition, two other cage populations that were started with material derived from two different natural populations were studied.

MATERIALS AND METHODS

Cage populations: A cage population (designated LF) was begun in January, 1968, with 400 males and 400 females whose second chromosomes were completely normal in viability and carried no sterility genes. These flies were derived from 20 chromosome lines isolated from natural populations of Katsumuma, Japan, in October, 1967. Two other cage populations were started from about 1,000 flies captured at Suyama and Katsumuma in 1962 and 1963, designated

1 Contribution No. 1,110 from the National Institute of Genetics.
as Su and Ka respectively. Each population was maintained in a plastic cage (30 × 40 × 13 cm) with 15 food cups, and kept in a constant temperature (25°) room under a fluorescent lamp. Three food cups (4 × 6 cm) with 50 ml of cornmeal, agar, molasses and yeast medium were provided twice a week.

Test of lethal and sterile chromosomes: Using the Cy/Pm technique, the frequencies of lethal and sterile second chromosomes in the cage populations were periodically examined. Approximately 200 male flies were randomly sampled from the cage, and individually crossed to Cy/Pm virgin females. Then a single Cy/+ male taken from each cross was backcrossed to Cy/Pm females. In the next generation, five pairs of Cy/+ flies were crossed within each backcross progeny. When non-Cy flies (+/+?) did not appear in the following generation, the tested chromosome was regarded as lethal. To test for male sterility among nonlethal chromosome lines, five non-Cy males (+/+?) from each line were mated with five Oregon-R virgin females. When no larvae could be found, the tested chromosome was regarded as male sterile. In a similar fashion, the test for female sterility was performed by crossing +/+ females with Oregon-R males.

Allelism test of lethal and sterile chromosomes: The allelism frequencies of lethal and sterile chromosomes were examined by the all-possible-cross method. Using the Cy-balanced lethal (l/Cy) or sterile (st/Cy) chromosomes sampled in each of the tested generations, all possible combinations of l?l, or st/st were made. If, by the above criteria, a combination was determined to be lethal or sterile, then its constituent chromosomes were regarded as carrying allelic mutations (l = l or st = st).

RESULTS AND DISCUSSION

Accumulation of lethals and steriles in the cage population: The frequency changes of lethal and sterile second chromosomes and their allelisms in the LF population are shown in Table 1 and graphically presented in Figure 1. Initially, lethal and sterile chromosomes were absent from the population, but because of spontaneous mutation, they accumulated during the course of the experiment. It seems to have taken about 2,000 days for the frequencies of lethals and steriles to reach equilibrium levels. At 2,023 days, the frequencies of lethal, male-sterile and female-sterile chromosomes were 18%, 11% and 3%, respectively. Although they showed some reduction at 2,347 days (probably due to random drift), they recovered at 2,621 days.

| TABLE 1 |

| Frequencies of lethals and steriles, and their allelisms in LF cage population |

<table>
<thead>
<tr>
<th>Days</th>
<th>Lethal test</th>
<th>Sterility test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chr.</td>
<td>% Le.</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>193</td>
<td>3.63</td>
</tr>
<tr>
<td>162</td>
<td>169</td>
<td>7.10</td>
</tr>
<tr>
<td>430</td>
<td>224</td>
<td>10.71</td>
</tr>
<tr>
<td>720</td>
<td>217</td>
<td>15.21</td>
</tr>
<tr>
<td>1,374</td>
<td>210</td>
<td>16.67</td>
</tr>
<tr>
<td>2,023</td>
<td>207</td>
<td>17.87</td>
</tr>
<tr>
<td>2,347</td>
<td>230</td>
<td>11.30</td>
</tr>
<tr>
<td>2,621</td>
<td>231</td>
<td>18.18</td>
</tr>
</tbody>
</table>
Though the age structure of the population was complex, a period of 15 days was regarded as one generation (Crow and Chung 1967). Therefore about 135 generations were required for the population to reach equilibrium.

In the course of accumulation of mutations, the frequency of lethals was always higher than that of male steriles, and the frequency of male steriles was always higher than that of female steriles. The relative frequencies of lethals and steriles in natural populations were studied by Watanabe, Watanabe and Oshima (1976), and the present results agree with theirs.

Allelism frequencies of lethals and steriles: From Table 1 and Figure 1, it is obvious that the allelism frequency of lethal chromosomes increased as the lethal mutations accumulated. It reached 10% at 720 days, but thereafter decreased to 3% at 2,023 days. At 2,347 days it increased slightly, apparently in conjunction with the sudden drop in the frequency of lethal chromosomes. This was probably due to random genetic drift. The allelism frequency at the final sampling (2,621 days) decreased again to 2.3%.

The allelism frequency of male-sterile chromosomes was extremely high compared with that of lethal chromosomes. At one time, it declined (42% at 430 days to 22% at 1,347 days), but by 2,621 days it had increased to 65%. The high frequency of the allelism at 2,621 days was mainly due to a specific sterility gene which was found in 12 second chromosomes among 16 chromosomes carrying male-sterility genes. In the natural population of Katsunuma, the allelism fre-
frequency of male-sterile chromosomes was variable from year to year (0.6%–15.1%, Oshima and Watanabe 1973), while that of lethal chromosomes was relatively constant (about 3.5%, Watanabe, Watanabe and Oshima 1976). The fluctuation of the allelism frequency can be ascribed in some degree to sampling error due to the low frequency of male-sterile chromosomes in the population. It seems that the number of loci associated with male-sterility is considerably smaller than that for lethal genes.

The frequency of the female-sterile chromosomes is much lower than that of male-sterile chromosomes. The allelism rate also seems to be very low, though our estimate is not very reliable because of the small number of crosses examined. Accurate mutation rates for male-sterility and female-sterility genes are still not known. A lower frequency of female-sterile chromosomes than of male-sterile chromosomes was also observed in natural populations (Oshima and Watanabe 1973). Of course, the possibility that the mutation rate for male-sterility genes is higher than that for female-sterility genes cannot be ruled out.

Equilibrium cage population: Two cage populations (Su and Ka) which have been kept in the same environment as the LF population for over 11 years were analyzed, and the results are shown in Table 2. The initial frequency of lethals was 13% for Su and 17% for Ka, and their allelism frequency was about 3% for both populations. After more than 260 generations, the frequency of lethal chromosomes (Q) increased to 19–20%, whereas the allelism frequency (Ic) remained practically unchanged (3–5%). When these were compared with those of the LF population at the 175th generation, no significant difference was found in Q and Ic. Thus, these populations seem to have reached the equilibrium state under the cage condition.

In equilibrium populations, the effects of mutation and selection should be balanced. The lethal mutation rate (U) for the second chromosome is believed to be about 0.5% per chromosome per generation (Crow and Temin 1964). In our experiments, we found 14 lethals among 96 chromosomes examined after 20 generations of maintenance in heterozygous condition with Cy balancer chromosomes. This gives an estimate of U = 0.73%.

The elimination rate of lethal mutations by homozygosis can be obtained by IcQ² and shown in Table 2 for three cage populations. It varies from 0.08% to 0.19% in the equilibrium cage populations. On the other hand, the IcQ² in natural populations was 0.05% for Suyama (Su) and 0.08% for Katsunuma (Ka).

### Table 2

<table>
<thead>
<tr>
<th>Population</th>
<th>Initial population</th>
<th>Present population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q</td>
<td>Ic</td>
</tr>
<tr>
<td>LF</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Su</td>
<td>0.1314</td>
<td>0.0303</td>
</tr>
<tr>
<td>Ka</td>
<td>0.1706</td>
<td>0.0292</td>
</tr>
</tbody>
</table>

* t: Generations passed in the cage population.
Comparing the mutation rates and the elimination rates by homozygosis, it is obvious that the former is always much larger than the latter in both natural and cage populations. Therefore, a large part of selection against lethal genes must occur in heterozygous condition.

**Degree of heterozygous disadvantage of lethal genes:** Direct estimates of the deleteriousness of lethal heterozygotes have been reported by many investigators. The degree of disadvantage of the lethals in the heterozygotes \( h \) was estimated to be 4% by Stern et al. (1952), 2.6% by Hiraizumi and Crow (1960), and 1.2% by Mukai and Yamaguchi (1974). These estimates were obtained from the results of "viability" experiments. Although viability is an important component of fitness, other characters influence it. Recently, Watanabe and Ohnishi (1975) and Watanabe, Yamaguchi and Mukai (1976) showed that lethal heterozygotes have lower "productivity" and "fertility," compared with non-lethal heterozygotes.

An estimate of the \( h \) value obtained from the population dynamics of lethal genes refers to the total fitness, not just viability. Using Nei's (1969) formula, Tobari and Murata (1970) and Murata and Tobari (1973) estimated the \( h \) value from the frequency changes of lethal genes in irradiated populations after termination of irradiation. It was as large at 7.3% in the early generations, but gradually decreased to become about 2% in the later generations. The large value of \( h \) in the early generations was explained as due to some chromosomal aberrations induced by X rays or to the synergistic interaction between numerous lethal genes.

From the frequency change of lethals in the LF population, we can estimate the \( h \) value by using Nei's (1969) formula. We assume that the equilibrium frequency of lethal chromosomes \( Q(t) \) is 0.1818, the equilibrium frequency of lethal chromosomes at 2,621 days. The \( h \) value is then estimated by

\[
\hat{h} = \Sigma w_i y_i t / \Sigma w_i t^w \,
\]

The \( \hat{h} \) is a weighted regression coefficient; \( w_i \) is the reciprocal of the large sample variance of \( y_i \); \( w_i = n [\widetilde{Q}_i - Q(t)]^2 \) \([1 - Q(t)] / Q(t)\), where \( y_i = -\ln (1 - Q_i(t) / \widetilde{Q}_i) \), and \( \widetilde{Q}_i = -\ln (1 - \widetilde{Q}) = 0.2006 \), where \( t \) is generation and \( n \) is the number of chromosomes examined in each generation. \( Q(t) \) and \( Q_i(t) \) are the frequencies of lethal chromosomes and lethal genes at the \( t \)-th generation, respectively \([Q_i(t) = -\ln (1 - Q(t))].\)

The results obtained are as follows: If we use the data from generations up to 48 and 135, \( \hat{h} \) becomes 0.035 and 0.033, respectively. If we know the \( h \) value, the expected frequency of lethal genes \( \tilde{Q}_i(t) \) and that of lethal chromosomes \( \tilde{Q}(t) \) in each generation can be obtained by the following formulae,

\[
\tilde{Q}_i(t) = \widetilde{Q}_i (1 - e^{\hat{h} t}), \tilde{Q}(t) = 1 - 1/e^{\hat{h} t} \,.
\]

Using these formulae with \( \hat{h} = 0.035 \), we fitted the theoretical curve to actual data.
The results obtained are shown in Figure 2. It is clear that the theoretical curve agrees well with experimental observations. In Figure 2, the results from population B in Murata and Tobari's (1973) experiments are also shown. Up to the 50th generation or so, the observed frequencies of lethals agree with the expected frequencies with $h = 0.035$, but after that they tend to be higher than the expected frequencies. If we consider all generations, $h = 0.02$ gives a better fit. The $h$ decreases in the course of time due to the elimination of the most detrimental mutations in early generations. This change in the value of $h$ is not seen in our data because we observed mutation accumulation, not elimination. Presumably lethals occurred at a constant rate, and with fairly constant heterozygous effects.

Another estimate of $h$ may be obtained by

$$h = \frac{\hat{U}}{\overline{Q}_1}$$

(NEI 1968). Our estimate of $\hat{U}$ was 0.0073 and the $\overline{Q}_1$ was 0.2006 for LF, 0.2159 for Su and 0.2254 for Ka. Therefore, $\hat{h}$ becomes 0.036 for LF, 0.034 for Su, and 0.032 for Ka. These estimates are very close to the previous estimates from the accumulation curve of lethals for the LF population.

**Effective and actual population sizes:** In equilibrium populations with no migration like our cage populations, both the actual and effective population sizes
TABLE 3

<table>
<thead>
<tr>
<th>Population</th>
<th>$t$</th>
<th>$Ne$</th>
<th>$Na \pm$ s. e.</th>
<th>$Ne/Na$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>135</td>
<td>1,900</td>
<td>6,300 ± 700</td>
<td>0.30</td>
</tr>
<tr>
<td>LF</td>
<td>155</td>
<td>1,000</td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>LF</td>
<td>175</td>
<td>2,900</td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>Su</td>
<td>310</td>
<td>1,200</td>
<td>7,800 ± 3,100</td>
<td>0.15</td>
</tr>
<tr>
<td>Ka</td>
<td>260</td>
<td>1,200</td>
<td>3,500 ± 1,200</td>
<td>0.69</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1,900</td>
<td>5,900</td>
<td>0.32</td>
</tr>
</tbody>
</table>

The effective population number ($Ne$) can be obtained. The effective population size ($Ne$) may be estimated by Nei’s (1969) formula,

$$Ne = (1 - Ig)/(4 (Ig U - u)),$$

where $Ig = -\ln (1 - Ic Q^2)/(\ln(1 - Q))^2$, $Ic$ stands for the allelic rate of lethal chromosomes and $Q$ for the frequency of lethal chromosomes, whereas $U$ and $u$ are the mutation rates per chromosome and per lethal locus, respectively. In our computation $U = 0.005$ and $u = 10^{-5}$ were used.

The actual population number ($Na$) was obtained by counting the adult flies three times at intervals of one month for each cage population immediately after the final sampling for the genetic analysis. The results are shown in Table 3. $Ne$ was estimated three times for the LF population, since this population was near equilibrium after the 135th generation. The $Ne$ varied from 1,000 to 2,900, while the $Na$ ranged from 3,500 to 7,800. Therefore, the ratio, $Ne/Na$, was between 0.15 and 0.69, the average being 0.32. Thus, about 30% of the censused adult population contributes to the next generation.

The authors are very much indebted to Dr. Masatoshi Nei for advice concerning the statistical analysis of the data, and also to Mr. Masaoki Kawanishi for technical assistance.

LITERATURE CITED


Corresponding editor: J. F. Kidwell.