A CASE OF HIGH RATE OF SPONTANEOUS MUTATION AFFECTING VIABILITY IN DROSOPHILA MELANOGASTER

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ABSTRACT

A spontaneous lethal mutation rate approximately twenty to thirty times greater than normal has been discovered in second and third chromosomes derived from an irradiated isogenic line and paired with marked inversion chromosomes. Mutations resulting in reductions of viability of varying magnitude short of complete lethality apparently also occur at a very high rate in the third but not in the second chromosome. The pattern of accumulation of lethal mutations over several generations and viability frequency distributions within generations have been studied in a number of independent experiments. High mutation rate occurs in heterozygous isogenic-derived second and third chromosomes, either together or apart, irrespective of the genetic constitution of nonhomologous chromosomes. High mutation rates were not observed using the same methods with chromosomes of an inbred line from a different source. The possible mechanisms responsible for these results are discussed.

HIGH rates of spontaneous mutation have been observed in a wide range of species from prokaryotes to higher plants and animals. They have been variously ascribed to mutator genes (e.g. TREFFERS, SPINELLI and BELSER 1954, in E. coli; IVES 1950, and GREEN 1970, in Drosophila melanogaster), controlling elements (e.g. MCCLINTOCK 1951, in corn and GREEN 1967, in D. melanogaster), and an effect of structural heterozygosity (THOMPSON 1960, in D. melanogaster). In most cases the mode of action of these mutating mechanisms is not clearly understood. Here we describe experiments in which spontaneous mutations affecting viability were accumulated at an extremely high rate. The changes in the pattern of viability frequency distributions with time are described, and the adequacy of various theories to explain the high spontaneous mutation in this case is examined.

DISCOVERY OF HIGH SPONTANEOUS MUTATION RATE

The line to be described was one of several isogenic sublines produced in an earlier experiment to investigate the effect of irradiation on a metric trait, wing length (KIDWELL et al 1971). An isogenic line (all homologous loci identical by

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descent) was produced by the marked-inversion-outcross technique as described by Kidwell (1963). A six-day-old virgin female from this line was irradiated with 500r and mated to a male of the marked-inversion stock to establish a sub-line which was isogenic for the irradiated chromosomes. Wing length of this sub-line was significantly shorter than that of the original line. After completion of this experiment the isogenic subline was maintained by single-pair full-sib mating for several years prior to the start of the present investigation.

Accumulation of lethal mutations in sheltered chromosomes: The possibility that nonfunctional genes may be accumulated in sheltered chromosomes has important implications for the way by which the Y and B chromosomes may have evolved in higher organisms (Muller 1914; Nei 1970). In theoretical studies Nei (loc. cit.) has shown that the probability of accumulation of nonfunctional mutations on sheltered chromosomes is highly dependent on the effective population size, the heterozygous effect of nonfunctional mutations and the recombination value. The assumption is generally made that lethal genes are accumulated on sheltered chromosomes according to the Poisson process in probability theory. Experiment 1 of this study was designed to investigate the validity of this assumption by studying the pattern of accumulation of lethal mutations on chromosomes which were artificially sheltered from selection by inversion chromosomes.

The method for accumulating mutations was similar to that of Dobzhansky and Wright (1941), Durant and Mather (1954) and Mukai (1964). Two sets of 120 lines each were established from the irradiated isogenic line described above. They were continued by a mating scheme which artificially sheltered the second and third chromosomes from recombination and selection (Figure 1). In one set the males were fed ethyl methanesulfonate (EMS) each generation according to the method of Lewis and Bacher (1968) to induce a high lethal mutation rate. The second set of males was untreated and used to provide an estimate of the spontaneous mutation rate.

The frequencies of lethal second and third chromosomes were examined simultaneously for the first five generations in both sets of lines. In this as in subsequent experiments, the generation number identifying a lethal test (or viability test in later experiments) always refers to the number of generations in which the tested chromosomes have been sheltered from selection and recombination and in which viability mutations have been allowed to accumulate. In these tests only (see Figure 1) a line was designated as lethal if no wild-type flies emerged and as non-lethal if at least one wild-type individual was observed. An average of about 30 flies were counted in those lines designated as lethal. The low number of progeny examined per line is partly a consequence of the use of inversion markers which are homozygous lethal. Even when neither the second nor the third unmarked chromosomes carry lethals, it is expected that 7/16 of the possible gametic combinations will die (see Figure 1). This fraction rises to 5/8 for a recessive lethal second or third unmarked chromosome and to 3/4 for both second and third chromosomes carrying lethals. This difficulty was overcome to some extent in later generations by testing more than one pair of flies per line.
Figure 1.—Mating scheme for perpetuating sheltered second and third chromosomes and testing for accumulated lethals in Experiment 1.

The no-EMS lines were also tested at generations 12 and 20. In the latter tests all progeny were counted and classified and an average of 70 progeny were counted per line. Thus in addition to the lethal frequency the viability of non-lethal lines was estimated.

The results are summarized in Table 1 and illustrated in Figure 2. In the EMS set the frequency of third chromosomes with at least one lethal after one generation of accumulation (40.5%) was twice as high as that of the second chromosome (20.2%). The reliability of these results may be questioned due to the low

<table>
<thead>
<tr>
<th>Generation</th>
<th>II No-EMS</th>
<th>III</th>
<th>II EMS</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.8 (103)*</td>
<td>5.5 (102)</td>
<td>20.2 (89)</td>
<td>40.5 (84)</td>
</tr>
<tr>
<td>2</td>
<td>16.8 (107)</td>
<td>25.3 (99)</td>
<td>40.8 (71)</td>
<td>57.9 (76)</td>
</tr>
<tr>
<td>3</td>
<td>28.3 (106)</td>
<td>57.0 (100)</td>
<td>62.9 (89)</td>
<td>75.3 (85)</td>
</tr>
<tr>
<td>4</td>
<td>38.7 (111)</td>
<td>66.0 (103)</td>
<td>81.1 (90)</td>
<td>92.0 (87)</td>
</tr>
<tr>
<td>5</td>
<td>53.2 (100)</td>
<td>77.7 (94)</td>
<td>93.4 (91)</td>
<td>98.9 (87)</td>
</tr>
<tr>
<td>12</td>
<td>81.1 (95)</td>
<td>89.7 (97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>89.5 (95)</td>
<td>97.2 (109)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses denote number of chromosomes tested.
Figure 2.—Percentage of second and third chromosomes with at least one lethal mutation in EMS and no-EMS lines in Experiment 1. Dashed lines indicate the theoretical exponential curves with \( U \) estimated by the method of least squares. The dotted line indicates the theoretical exponential curve with \( U = 0.005 \).

Figure 3.—Frequency distribution of viabilities for no-EMS lines after twelve generations of accumulation of mutations in Experiment 1.
HIGH SPONTANEOUS MUTATION RATE

number of flies examined per line. After five generations, however, the difference had narrowed considerably and over 90% of both chromosomes were lethal.

In the no-EMS set the rate of accumulation of lethals on both second and third chromosomes was unexpectedly high. As seen in Figure 3, at generation 12, in addition to over 80% of lines carrying complete lethals, a high proportion of ‘non-lethals,’ as defined in the strict sense, had a very low viability.

Accumulation of lethal mutations on a sheltered chromosome is expected to follow a Poisson distribution if the mutation rate is the same for all loci and there is no interaction among loci with respect to mutability and natural selection. Hence the expected frequency of chromosomes with at least one lethal mutation, $Q$, is

$$ Q = 1 - e^{-Ut} $$

where $U$ is the rate of accumulation of lethal mutations per chromosome per generation and $t$ is time in generations. The least-squares estimator of $U$ is

$$ \hat{U} = \sum \frac{W_t Y_t t}{\Sigma W_t t^2} $$

where:

- $Q_t$ = the percent observed lethal chromosomes in the $t^{th}$ generation
- $N_t$ = the number of chromosomes tested in the $t^{th}$ generation
- $W_t = N_t (1 - Q_t)/Q_t$
- $Y_t = -\log_e (1 - Q_t)$

The goodness of fit of the exponential curve for $Q$ can be tested by $\chi^2$ with degrees of freedom equal to the number of generations examined minus two. The four estimates of $U$ are:

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>No-EMS</th>
<th>EMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>0.119</td>
<td>0.330</td>
</tr>
<tr>
<td>III</td>
<td>0.176</td>
<td>0.506</td>
</tr>
</tbody>
</table>

The increase in the frequency of lethal third chromosomes for the no-EMS groups significantly deviates from the exponential curve ($P < .005$). Although the others do not, the number of generations examined is small and thus the probability of detecting a small but real deviation is low. All groups have a consistent pattern of fewer than expected lethal chromosomes in the early generations and more than expected in the later generations. This pattern is particularly pronounced in the third chromosome no-EMS line. Later, we will discuss the reason for this pattern. Here attention is directed to the very high rate of increase of lethal chromosomes in the control lines. Crow and Temin (1964) reported an average spontaneous mutation rate of approximately 0.5% per generation to lethal second chromosomes. Wallace (1968) obtained a similar rate of 0.6% per generation for both second and third chromosomes. Thus the values observed for the no-EMS second and third chromosomes are, respectively, about 24 and 30 times larger than expected. There is a possibility of overestimation due to the small progeny numbers per line and the non-lethal deleterious mutations which will be discussed later. However, a high mutation rate has been confirmed in four
subsequent independent tests, which involved similar techniques and are described below. Three of these experiments were designed to test at least one additional hypothesis concerning the nature and location of the mutator mechanism.

**Further evidence of high mutation rate:** In order to test the repeatability of the high mutation rate in independently-derived chromosomes from the same isogenic source and to examine the viability distribution after one generation of accumulation, Experiment 2 was carried out. Only chromosome II was examined and the use of a *Cy/Pm* rather than a *Cy/Pm;Sb/Ubx* balanced marker stock decreased the expected loss of progeny due to homozygous lethal markers from 7/16 to 1/4. Thus the progeny number was increased to give a more reliable test.

A one-generation viability test of 66 second chromosomes from the original isogenic line was made. The distribution of viabilities is indicated by the dotted line in Figure 4. An additional independent test of the viability of 45 second chromosomes after one generation was provided by Experiment 4, described below. The viability distribution in this test is indicated by the solid line in Figure 4. There is close agreement between the two distributions. The mean homozygous viability of non-lethal lines is clearly below the 33.3% expected with chromosomes of normal viability. Also there is no discontinuity in the distribution which would enable a natural separation between lethal and non-lethal lines. The frequency of completely lethal second chromosomes was 9.1% and 13.3%, respectively, in the two tests described. Both estimates are higher than that of 6.8% for chromosome II with no EMS after one generation of accumulation in Experiment 1, but appear to be more in agreement with the least squares estimate of $U$ of 11.9% in that experiment.

![Figure 4](image_url)

*Figure 4.*—Second chromosome viability frequency distributions after one generation of accumulation of mutations in Experiments 2 and 4.
Extensive previous work with the marker stocks used in this experiment had given no suggestion of an abnormal mutation rate. This strongly suggested that the high mutation rate in the present case was attributable to the isogenic line or was due to an interaction between the isogenic line and the marker stock. The consistency of mutation rates among experiments, despite the use of several different marker stocks, adds support to this argument. However, the possible effect of heterozygous inversions in increasing lethal frequency (Thompson 1960) had to be tested.

The design of Experiment 1 virtually excluded the isogenic X and fourth chromosomes as carriers of the mutator mechanism. The X chromosome of experimental males was always derived from the marker stock, and never from the isogenic line. Assuming normal segregation and equal viabilities the probability that an isogenic fourth chromosome was carried by an experimental male was \((1/2)^n\) where \(n\) is the generation number. Mutation rates did not decrease with time. This makes it very unlikely that the fourth chromosome carried a mutator gene. The possibility that high mutation rate was associated with the Y chromosome was ruled out by a test in which accumulation of lethals was permitted in unmarked second chromosomes of males in which only the Y chromosome was derived from the isogenic line. No lethal chromosomes were observed after three generations of accumulation in 30 male lines. Consequently, attention has been directed to the second and third chromosomes as the most likely carriers of the mutator mechanism.

Effects of inversion chromosomes: In order to test Thompson’s (1960) hypothesis that the presence of a marked inversion chromosome in heterozygous condition was responsible for the high mutation rate, Experiment 3a was performed. The \(C_y/P_m; S_b/U_bx\) marked-inversion stock was crossed with males from an inbred line, 16A1, known to carry non-lethal, unmarked second and third chromosomes with standard gene arrangements (Kidwell 1972). Otherwise the mating scheme was identical with Figure 1. A homozygous viability test of 51 second and 51 third chromosomes was performed after two generations of lethal accumulation. The distribution of viabilities is illustrated in Figure 5. Only one lethal chromosome was observed, which indicated a lethal mutation rate remarkably close to the normal spontaneous rate of 0.5 to 0.6% per generation. Apart from this there was no line with a viability less than 15%. The two distributions are very close to those expected of chromosomes with quasi-normal viability and are in marked contrast to the distributions of Figure 4. The results not only suggest that the marker stock, alone, was not a direct source of high mutability, but they also provide a check on technique.

Experiment 3b provided a direct test of whether a high rate of accumulation of lethals occurs in the absence of inversion chromosomes. It was designed to shelter an isogenic-derived third chromosome paired with a multiply-marked third chromosome \(r u c u c a\) having a standard, rather than an inverted, gene sequence. These two chromosomes were carried together in the male, thus shelter-
ing from recombination was ensured by the normal absence of crossing over in this sex. The *rucuca* chromosome carried eight recessive markers: roughoid, *ru* (3-0.0); hairy, *h* (3-26.5); thread, *th* (3-43.2); scarlet, *st* (3-44.0); curled, *cu* (3-50.0); stripe, *sr* (3-62.0); ebony-sooty, *es* (3-70.7); and claret, *ca* (3-100.7). Examination of salivary chromosomes from three EMS and three no-EMS lines in Experiment 1 had indicated only one small chromosomal abnormality in the isogenic line \( \text{IN}(ZL)31B-38C \). The absence of any but very small inversions in the *rucuca* chromosome was inferred from normal frequencies of recombination in each of the seven marked intervals in earlier experiments. Thus it was assumed with confidence that *rucuca* and isogenic-derived third chromosomes were essentially homosequential.

The mating scheme was similar to that in previous experiments. Each line was maintained through five generations by one male, heterozygous for an isogenic-derived third chromosome and a *rucuca* chromosome, mated with four homozygous *rucuca* females. Marked inversion chromosomes were only used peripherally for the lethal tests to ensure no recombination in females. After one generation of accumulation 5.2% of third chromosomes tested were completely lethal. Of the 49 lines tested after 5 generations, 23 (46.9%) produced no wild-type progeny. In comparison, 6.8% and 42.9% of third chromosomes carried complete lethals at generations 1 and 5, respectively, in Experiment 4, which will be described in the next subsection. This provides very good evidence that the mutator mechanism is not dependent on the presence of an inversion to exert its effect.

**Second chromosome:** Experiment 4 was designed to determine whether the high mutation rate in the third chromosome was dependent on the presence in the same genome of an isogenic second chromosome. The mating scheme was a modification of that shown in Figure 1, starting with 114 male lines. After gen-
### TABLE 2

Mean percentage viabilities of third chromosomes in Experiment 4

<table>
<thead>
<tr>
<th>Generation no.</th>
<th>Mean viability All lines</th>
<th>Mean viability Non-lethal lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.7 (102)*</td>
<td>23.3 (90)</td>
</tr>
<tr>
<td>3</td>
<td>7.3 (106)</td>
<td>12.1 (59)</td>
</tr>
<tr>
<td>5</td>
<td>3.7 (105)</td>
<td>9.7 (33)</td>
</tr>
<tr>
<td>7</td>
<td>2.2 (90)</td>
<td>7.2 (19)</td>
</tr>
<tr>
<td>9</td>
<td>0.5 (89)</td>
<td>7.4 (2)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses denote number of lines tested.

In generation 2 all chromosomes from the isogenic line, except one third chromosome and the $Y$ chromosome, were replaced with unmarked chromosomes from several balanced marker stocks. Third-chromosome viability tests were made at generations 1, 3, 5, 7 and 9. A total of 35,340 flies were counted and classified. Mean viabilities of non-lethal lines and of all lines are given in Table 2.

The increase in the percentage of lethal-bearing chromosomes with generation number is illustrated in Figure 6. Considering complete lethals, the one-generation test of 102 third chromosomes gave a frequency of 6.82%, which is very close to the estimate of 5.5% from Experiment 1. The least-squares estimate of $U$ calculated for complete lethals from the five tests in this experiment is 12.8%, compared with 17.6% in the no-EMS lines in Experiment 1. However, on average, the number of progeny per line examined was approximately double that in

![Figure 6](image-url)

**Figure 6**—Percentage of third chromosomes with at least one lethal in Experiment 4. Theoretical curves are indicated by dashed lines.
the original experiment, thus giving a more precise estimate of viability. In this context, if we redefine a lethal line as that having a homozygous viability less than 5%, then the least-squares estimate is 20.8%. Thus, using this definition, the frequency and rate of accumulation of lethals over time is in quite close agreement in the two experiments. It therefore appears that the presence of an isogenic second chromosome is not a necessary condition for a high lethal mutation rate on the third chromosome.

Third chromosome: The purpose of Experiment 5 was to test whether mutation on the second chromosome was controlled by a factor which was linked to the isogenic third chromosome. The rate of accumulation of viability mutations was compared in structurally heterozygous isogenic second chromosomes with two different third chromosome complements. This was achieved by the scheme illustrated in Figure 7. Set A males carried Sb/Ubx whilst all group B males, after generation 2, were heterozygous for an isogenic third chromosome and either a Sb or Ubx marked inversion chromosome. For each line the second chromosome carried by sets A and B had a common origin in generation one. If chromosome II mutation rate is dependent on the presence of an isogenic third chromosome in

![Diagram of mating scheme for Experiment 5](image)

**Figure 7.**—Mating scheme for Experiment 5 in which mutations are accumulated in isogenic-derived second chromosomes. In set A only one second chromosome is from the isogenic line. In set B one second and one third chromosome are isogenic-derived.
Table 3

Mean percentage viabilities of second chromosomes in Experiment 5

<table>
<thead>
<tr>
<th>Generation no.</th>
<th>Mean viability All lines</th>
<th>Mean viability Non-lethal lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>20.0 (61)*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16.3 (71)</td>
<td>16.5 (60)</td>
</tr>
<tr>
<td>3</td>
<td>9.9 (68)</td>
<td>11.6 (71)</td>
</tr>
<tr>
<td>4</td>
<td>8.9 (68)</td>
<td>11.2 (66)</td>
</tr>
<tr>
<td>5</td>
<td>9.8 (65)</td>
<td>11.6 (72)</td>
</tr>
<tr>
<td>7</td>
<td>9.0 (65)</td>
<td>8.2 (67)</td>
</tr>
<tr>
<td>9</td>
<td>9.5 (67)</td>
<td>5.4 (69)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses denote number of lines tested.

The same zygote, then it is expected that after the second-generation test, at the latest, the rate of accumulation of mutations in set A will decline whilst that in set B will continue as before. Viability tests of chromosome II were carried out every generation for the first five generations and in alternate generations thereafter. Mean viabilities of all lines and of non-lethal lines are given in Table 3 for sets A and B. The mean number of flies examined per line exceeded fifty in all except the first generation. The increase in frequency of lethal-bearing chromo-

Figure 8.—Percentage of second chromosomes with at least one lethal in Experiment 5. The theoretical curve for sets A and B combined is indicated by the dashed line.
somes with generation number is illustrated in Figure 8. Preliminary least-squares estimates of $U$ based on the results up to nine generations are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Set A</th>
<th>Set B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete lethals</td>
<td>0.105</td>
<td>0.120</td>
</tr>
<tr>
<td>Viability $&lt; 5%$</td>
<td>0.121</td>
<td>0.130</td>
</tr>
</tbody>
</table>

The estimates are very similar to each other and are very close to that of 0.119 for completely lethal second chromosomes (no-EMS) in Experiment 1. This suggests that the second chromosome alone is capable of producing a high rate of mutations on the second chromosome.

An examination of viabilities of sets A and B within individual lines in succeeding generations indicated that new mutations were accumulated independently in the two sets. In generation three, for example, eight lines carried new mutations to complete lethals in set A but their sister lines in set B did not show a significant reduction in viability. Likewise there were eleven lines with new lethals in set B but no new lethals appeared in comparable lines in set A. In one case there were new lethals in both sets of lines. This independence of mutations in the two sets, together with the close agreement of the lethal accumulation rate in sets A and B of Experiment 5 and Experiment 1 (chromosome II no-EMS), indicates that the presence of an isogenic third chromosome is not a necessary condition for the production of a high rate of viability mutations on the second chromosome. Clearly, the mechanism responsible for the high mutation rate is associated with both second and third chromosomes.

**Some Properties of the Mutator Mechanism**

*Patterns of accumulation of lethal second and third chromosomes:* As mentioned in an earlier section, in Experiment 1 the increase in frequency of lethal chromosomes with generation number deviates from the exponential curve only in the case of no-EMS third chromosomes. With respect to Experiment 4 comparison of the observed and expected curves is made in Figure 6. This indicates that, as in the first experiment, there is a tendency toward a deficiency of observed lethals in the early generations compared with the number expected if accumulation follows a Poisson distribution. There is also a marked excess of observed over expected lethals in the ninth generation. A test for goodness of fit of the observed values to the exponential curve gave a chi square value of 5.367 ($0.25 > P > 0.10$, 3 df). In contrast to the first experiment the data did not provide evidence for deviation from the exponential curve, but as in the earlier experiments data were obtained from only a few generations and therefore the power of the test to detect small deviations is limited.

With respect to Experiment 5, reference to Figure 8 shows that there is very close agreement between the observed and expected frequencies of lethal second chromosomes except at generation 9 in set A. In this case the theoretical curve shown was constructed for the two sets together because of the close similarity of
FIGURE 9.—Third chromosome viability frequency distributions in Experiment 4. The expected viability of 'normal' chromosomes is 33\%.

the two separate curves. Independent tests of goodness of fit again provided no evidence of deviation of the observed values from the exponential curve (Set A, $x^2 = 1.935, .90 > P > .75, 5$df; Set B, $x^2 = 0.547, P > .99, 5$df).

Third chromosome viability distribution: The frequency distributions of homozygous viabilities for the five generations tested in Experiment 4 are shown in Figure 9. It is seen immediately that not only is there a high rate of mutation to complete lethals, but there is also an extremely high frequency of mutations affecting viability to a variable degree short of complete lethality (subvital or semilethal mutations). This is also reflected in the rapid reduction of mean viability with time indicated by Table 2. Virtually no lines appeared to be immune to drastic viability reduction. At generation 9 only two lines had a viability exceeding 5\% and the mean of the two was 7.4\%.

With a high rate of viability mutations (subvitals and semilethals), it is expected that the probability of a lethal mutation is higher in a line in which a viability mutation has already occurred than in a line in which no mutation has occurred, since in the former line a second viability mutation as well as a true lethal mutation may result in complete lethality. That this is in fact so can be seen by comparing individual line viabilities between generations one and three. Of those lines with a homozygous viability ranging between 5\% and 15\% in generation 1, 50\% had mutated to complete lethals in the next test (generation 3). In comparison, of those lines with a homozygous viability above 15\% in the first test, 23.3\% carried a completely lethal third chromosome in the third generation test. The same trend continues in later successive test comparisons, but the
paucity of lines in the higher viability classes very quickly renders comparisons less reliable and eventually impossible.

Second chromosome viability distribution: Figure 10 shows the frequency distributions of homozygous viabilities for the pooled data of sets A and B in Experiment 5 for the first, fifth and ninth generations. For clarity of presentation the distributions for the remaining generations were omitted. There are several marked differences between the distributions illustrated in this figure and those of third chromosomes, as illustrated in Figure 9. With increase in generation number non-lethal second chromosomes remained distributed throughout the original viability range, whilst the distribution of non-lethal third chromosomes shifted very quickly to the lower end of the viability scale, resulting in a rapid reduction in variance. Related to this was a very low frequency of second chromosome lines in the 0–5% viability range, suggesting almost a natural discontinuity between lethal and non-lethal lines. Again this is in sharp contrast to the third chromosome distribution where the frequency of lines in this viability range was quite high. The mean viability of non-lethal lines (Table 3) appears to decrease over the first three or four generations and then to increase again in succeeding generations up to the first or second generation level. These results might be explained by systematic environmental effects in conjunction with a negligible rate of non-lethal viability mutations. Alternatively they could be explained by a higher lethal frequency in the early generations among those lines which had already accumulated less drastic viability mutations. Back mutation to higher viability is possible but is considered to be unlikely. Detailed examination of the data revealed no differential lethal mutation rate in low viability lines compared
with high viability lines. These findings strongly favor systematic environmental effects as the explanation for the change in mean viability with increasing generations.

**Sex-linked lethal mutations:** In order to determine whether the mutation rate of isogenic X chromosomes was elevated, one-generation lethal and viability tests were carried out in Experiment 6 at three separate times in the period March to May 1972. The standard Basc technique for males was used. In the first test, only the frequency of complete lethals was estimated. In the third test all F₂ progeny were counted and classified; the viability of males carrying an X chromosome from the isogenic line was estimated in comparison with the number of female progeny which were heterozygous for a Basc and an isogenic-derived chromosome within the same progeny group. (Hemizygous and homozygous Basc chromosomes tend to have lower viability than wild type.)

In the first test 3 out of 84 lines (3.57%) had mutated to complete lethals, as judged by the absence of wild-type males in the F₂ generation. Comparable figures for the second and third tests, respectively, are 8 lethals out of 119 (6.72%) and 1 lethal out of 130 (0.77%). Whilst mutation rates differ markedly from test to test they all appear to be significantly higher than the normal range of approximately 0.001 to 0.002 (DOBZHANSKY 1970; WALLACE 1970). The mean frequency of lethals for all three tests is 3.6% which is 18–36 times the normal rate and is an increase of about the same magnitude as that in the major autosomes. The reason for the large difference in mutation rate from test to test is unclear. Further examination of this question is indicated.

The frequency distribution of viabilities in the third test is illustrated in Figure 11. It appears to differ from the distribution in the one-generation tests of chromosomes II and III in three main respects: (i) There was less displacement of the mean and mode from those expected with normal viability (in this case

![Figure 11](image-url)
50%); (ii) the variance of viability was smaller; (iii) there was a discontinuity in the distribution between the lethal and non-lethal lines. No lines other than complete lethals had a viability less than 25%, i.e., half of that of the expected value of 50% for normal chromosomes.

**Allelism of lethal chromosomes:** The distribution of lethal loci on a chromosome may give some clues as to the mode of action of a mutator mechanism. An approach to this question was made in Experiments 1 and 4. In Experiment 1, at generation 17 there were 53 no-EMS lines available which carried both lethal second and third chromosomes. The number of possible pair-combinations is \(\frac{53 \times 52}{2} = 1378\). Of these, as many random combinations as possible were tested in generations 17 through 25. \(Cy^+/-; Ubx^+/-\) males of one line were mated with females of the same genotype in the second line. Lethals were defined as allelic when the frequency of wild-type progeny was less than three percent.

The allelism of lethals on second and third chromosomes was scored independently. On the basis of 326 crosses, which yielded between 15 and 30 progeny per cross, 14.1% of lethal second chromosomes were estimated to be allelic. Comparable figures for the third chromosome were 50.1% allelism based on 307 crosses. As expected, the lethal allelism rate was higher when computed among lines which became lethal in the first three generations (24.2 and 72.9% for second and third chromosomes, respectively) than when computed over all lethal lines. Apart from the large difference in allelism rate between the two chromosomes, it was noted that even in line crosses which proved to carry non-allelic lethals, the viability of wild-type progeny for both chromosomes was low. This suggests a high rate of allelism for detrimental as well as lethal mutations.

In Experiment 4 a less extensive test was made of the third-chromosome allelism rate at generation 10. Seventy pair combinations of lethal chromosomes were tested, with an average of 36 progeny per mating. Of these 18.6% were allelic for lethals. As expected, this value is considerably lower than that at a later generation in Experiment 1 but higher than that for the second chromosome in the same experiment.

These high rates of allelism suggest that the number of loci at which lethal mutations occurred with a high frequency is rather small. A rough estimate of this number may be obtained by using the formula for the effective number of lethal loci on the assumption that the mutation rate is the same for all lethal loci. The formula for this number is given in the APPENDIX. If we use the data from Experiment 1, the estimate of effective number of lethal loci becomes 36.5 for the second chromosome and 14.9 for the third chromosome (see the APPENDIX). On the other hand, if we use the allelism rate from Experiment 4, the effective number for the third chromosome is estimated to be 22.7. These numbers are both much smaller than the generally accepted value of at least 400–500 for these chromosomes. This suggests that the mutator mechanism in our strain affects a rather limited number of loci, a conclusion which is in agreement with that of WALLACE and MADDEN (1965).
The characteristics of the present case of high mutation which have been discovered so far are summarized as follows:

a) Lethal and viability mutations occur with a very high frequency in second and third chromosomes derived from an isogenic line. A high rate of lethal mutations also occurs in the X chromosome.

b) The mutator mechanism responsible for the high rate of mutation seems to be present in both the isogenic second and third chromosomes.

c) A high mutation rate attributable to either a direct or an indirect effect of heterozygous inversions (Thompson 1960) can be ruled out due to the results of Experiment 3.

d) The pattern of accumulation of lethal genes on sheltered chromosomes deviates from that which is expected from the Poisson process in the case of the third chromosome. There is closer agreement between observed and expected values in the second chromosome.

e) There appear to be significant differences between the second and third chromosomes with respect to the frequency distribution of lethal and viability mutations. In the third chromosome, mutations result in a wide range of detrimental viability effects including complete lethality. In the second chromosome, detectable reductions in viability were largely due to completely lethal mutations.

f) The mutator mechanism involved appears to affect a rather limited number of loci in the second and third chromosomes.

In Experiments 1 and 4 we noted that the frequency of lethal chromosomes in early generations was lower than the exponential curve, while in later generations it was higher; this was particularly so with the third chromosome. On the other hand, in Experiment 5 in which second-chromosome lethals were tested, no such tendency was observed. Clearly, the accumulation of lethals is closer to the Poisson process in the second chromosome than in the third chromosome. This difference between chromosomes may be explained by the difference in the pattern of viability mutations. In the third chromosome many subvital or semilethal mutations occurred in addition to complete lethals, while the majority of mutations occurring in the second chromosome were complete lethals. In the third chromosome, therefore, the rate of new lethal chromosomes occurring in later generations is expected to be higher than that in early generations, since two or more subvital or semilethal mutations occurring in the same chromosome could have the same effect as a completely lethal mutation.

In Experiments 4 and 5, the least-squares estimate of mutation rate per chromosome (U for less than 5% viability) was higher in the third chromosome than in the second chromosome, though the rate of completely lethal mutations was almost the same for the two chromosomes. The results for Experiment 1 are not inconsistent with this if we consider that, due to differences in method of scoring and numbers sampled, "lethal chromosomes" are more closely comparable to chromosomes carrying mutations which resulted in less than five percent viability rather than in complete lethality in Experiments 4 and 5. The fact that there
occur many subvital and semilethal mutations in the third chromosome also sug-

gests that the value of $\bar{U}$ for the third chromosome in Experiment 1 is an over-
estimate for complete lethals. Thus the rate of completely lethal mutations may
be the same order of magnitude for both chromosomes in Experiment 1 also.

In plotting the frequencies of lethal chromosomes against generation number
(Figures 2, 6 and 8), for simplicity, the point of origin was assumed to be zero;
i.e., it was assumed that all lethals which appeared in the first viability test had
arisen in the first generation of accumulation. However, the first-generation
lethal frequency is theoretically made up of two components: (1) new mutations
occurring in the wild-type isogenic male progenitors of each line and (2) lethals
present due to residual heterozygosity of mutants occurring earlier in the isogenic
line. The relative proportions due to each component are in fact not known, but
the first-generation rate does provide an upper limit for either component alone.

Haldane (1936) showed that for a single completely recessive autosomal
lethal in diploids, with brother-sister mating, the expected frequency of lethal
genes at equilibrium is in the range $4.6u - 6.3u$ where $u$ is the lethal mutation
rate per locus per generation. In the present case $u$ should be replaced by $U$
to get an approximate frequency of lethal chromosomes at equilibrium. If we use
the least-squares estimate of $U$, the expected frequency of lethal chromosomes
in the first generation, which is equal to $4.6U - 6.3U$, is much higher than that
observed. This may be due to a number of factors such as (1) a lower mutation
rate in homozygous chromosomes in the isogenic line than in heterozygous chro-
mosomes, (2) incomplete recessiveness of viability mutations resulting in a lower
fitness in heterozygous condition and consequent stronger selection against them
(it is necessary to practice strong inter-subline selection in the maintenance of
the isogenic line), and (3) overestimation of $U$ discussed in a foregoing para-
graph.

With the limited evidence so far available, it is impossible to draw conclusions
as to the mechanism responsible for the high mutation rate. It is worthwhile,
however, to consider some of the alternative possibilities in order to provide
hypotheses which may be tested experimentally.

In Experiments 4 and 5 it was demonstrated that high mutability was not
associated with the presence of one specific chromosome; both second and third
chromosomes were shown to be independently associated with a mutator, and
the genetic constitution of other chromosomes appears to have little or no effect.
These results suggest that high mutability cannot be attributed to a single muta-
tor gene, as usually defined—i.e. an allele of a gene which causes an increased
mutation rate at other loci in the genome and which (at least in theory) can be
mapped to a specific chromosome region. There is a possibility that a mutator
gene is carried by both chromosomes II and III. Two mutator genes might have
arisen as the result of independent mutations or they may have had a common
origin. Mutator genes have been shown to cause lethal mutations; e.g. Green
and Lefevre (1972) have demonstrated that the action of a mutator gene in
D. melanogaster can result in the production of X-linked lethals, some of which
are associated with deficiencies of several salivary chromosome bands.
Our finding that high mutability is associated with more than one chromosome is not inconsistent with a controlling-element hypothesis such as that described by McClintock (1951) in corn and Green (1967) in D. melanogaster. A controlling element is defined as a genetic element which is accessory to structural genes (i.e. genes which code for polypeptides) but which can, in close association, change the action or expression of such genes either autonomously or by interacting with other components of a regulatory system. Most evidence comes from cases in which controlling elements were transposed to different loci in the genome where they replicated coordinately with the chromosome in which they were integrated. The origin of controlling elements is not clear. A number of their properties are similar to those of temperate phage in bacteria but the existence of viruses of this type in a non-integrated state in higher organisms has not been demonstrated.

Whatever their origin there is evidence that controlling elements can give rise to lethal mutations, probably deletions, which are produced when transposition occurs. McClintock (1951) reported that in maize a number of dominant lethals have arisen due to transpositions of the controlling element Dissociator (Ds). Green (1967) showed that deletions in the X chromosome were produced as a result of deintegration of a controlling element. It was not clear whether these deletions affected viability.

Due to the property of transposability, controlling-element-mediated high mutation rates are not necessarily associated with the presence of a single chromosome. For example, in the case of the controlling element Modulator (Mp) in corn described by Barnik (1954), through the processes of reduplication and transposition a number of Mp elements were accumulated in the genome. In the present case controlling elements might become integrated at several loci on the second and third chromosomes. The high allelism rate, particularly in chromosome III, suggests that lethals occur at a restricted number of loci.

All experiments conducted so far have measured mutation in males. The females which contribute most of, if not all, the cytoplasm to the zygote were from a different source. High mutability due to the interaction of isogenic male chromosomes with the cytoplasm from a non-isogenic source is another possibility now under test.

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APPENDIX

Allelism Rate of Lethal Chromosomes Under Mutation Pressure and Effective Number of Lethal Loci

MASATOSHI NEI

The allelism rate of lethal chromosomes is defined as the proportion of random pairs of lethal chromosomes which carry the same lethal gene at least at one locus. If there are \( n \) lethal loci...
in a chromosome and \( q_i \) denotes the frequency of lethal gene at the \( i \)-th locus, the allelism rate of lethal chromosomes is given by

\[
I_c = \frac{1 - \prod_{i=1}^{n} (1 - q_i^2)}{(1 - \prod_{i=1}^{n} (1 - q_i))^2}
\]

where random combination of genes at different loci is assumed. \( I_c \) should not be confused with the allelism rate of lethal genes, \( I_p \), studied by Dobzhansky and Wright (1941) and Nei (1968). The latter author has provided a conversion formula from \( I_c \) to \( I_p \).

Let \( u_i \) be the rate of lethal mutations per generation at the \( i \)-th locus. The frequency of lethal genes at this locus after \( t \) generations is given by \( 1 - e^{-u_i t} \) approximately. Therefore,

\[
I_c = \frac{1 - \prod_{i=1}^{n} (1 - (1 - e^{-u_i t})^2)}{(1 - e^{-u_i t})^2}.
\]

If we assume that \( u_i \) is the same for all loci and \( u_i t \) is much smaller than unity, then \( I_c \) becomes

\[
I_c = \frac{1 - \sum_{i=1}^{n} (1 - e^{-u_i t})}{1 - e^{-u_i t}}.
\]

approximately, where

\[
U = \sum_{i=1}^{n} u_i.
\]

The real number of lethal loci (\( n \)) must be close to the total number of genes in a chromosome, but we are interested only in those loci at which lethal mutations occur with a high frequency. We therefore estimate the effective number of lethal loci (\( n_e \)) on the assumption that \( u_i \) is the same for all loci. Thus, replacing \( n \) in the above formula by \( n_e \), we have

\[
n_e = \frac{(U t)^2}{-\log_e [1 - I_c (1 - e^{-U t})^2]}.
\]

In the present study an allelism test was conducted at the 17th generation in Experiment 1, and \( I_c \) was 0.141 for chromosome II and 0.501 for chromosome III. The estimates of \( U \) for chromosomes II and III were 0.119 and 0.176, respectively. Putting these values into the formula for \( n_e \), we obtain 36.3 and 14.9 for chromosomes II and III, respectively. The allelism rate for chromosome III in Experiment 4 was 0.186 at the 10th generation. The estimate of \( U \) in this experiment appears to be an underestimate. Using the value of \( U \) in Experiment 1, we get \( n_e = 22.7 \). Therefore, the effective number of lethal loci for chromosome III is about a half of that for chromosome II. However, the effective number for the third chromosome is possibly underestimated because of a high rate of subvital and semilethal mutations.