# HIGH RATES OF OCCURRENCE OF SPONTANEOUS CHROMOSOME ABERRATIONS IN *DROSOPHILA MELANOGASTER*[1]

OSAMU YAMAGUCHI,[2] RICARDO A. CARDELLINO[3] AND TERUMI MUKAI[2]

*Department of Genetics, North Carolina State University, Raleigh, North Carolina 27607*

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ABSTRACT

Spontaneous mutations were accumulated for 40 generations in 140 unrelated second chromosomes with the standard gene arrangement. These were extracted from the same population by using the marked inversion technique, and the following findings were obtained: (1) In 42 out of the 140 chromosome lines, chromosome aberrations were detected by examining the salivary gland chromosomes: 40 paracentric and 15 pericentric inversions, 2 reciprocal translocations between the second and the third chromosomes, and 6 transpositions. (2) In 63 out of the 90 originally lethal-free lines, recessive lethal mutations occurred. (3) There were only 3 lines that acquired chromosome aberrations (inversions) with no lethal effects in the homozygous condition. (4) In a comparison of these results with those of the (CH), (PQ), and (RT) chromosomes in which no chromosome aberrations occurred after accumulating mutations for 22058 chromosome·generations (YAMAGUCHI and MUKAI 1974), it was concluded that some of these 140 chromosomes carried a kind of mutator. (5) The frequency of mutator-carrying chromosome lines was estimated to be 0.66 on the basis of the distribution of the break-points on the chromosome lines and the frequency of lines that acquired neither recessive lethal mutations nor chromosome aberrations. Thus, the average number of breaks per mutator-carrying chromosome was estimated to be about 0.19/generation.

On the basis of these estimates, the nature of the mutator factor was discussed.

ALTHOUGH WARTERS (1944) reported a long time ago that several polymorphic inversions were found in many populations of *Drosophila melanogaster* over the U.S.A., recently special attention has been paid to inversions in connection with the mechanisms involved in the maintenance of a large number of isozyme polymorphisms in natural populations (MUKAI, METTLER and CHIGUSA 1971; LANGLEY, TOBARI and KOJIMA 1974; MUKAI, WATANABE and YAMAGUCHI 1974).

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[2] Present address: Department of Biology, Faculty of Science, Kyushu University, Hakozaki, Fukuoka 812, Japan.

[3] Present address: Brazilian Institute of Agricultural Research, CX. Postal 1316, 70.000 Brasilia, D. F., Brazil.

The results of recent investigations (cf. METTLER, CHIGUSA and MUKAI 1976; MUKAI and YAMAGUCHI 1974) show that there are many unique inversions in addition to the several types of polymorphic inversions reported previously, e.g., *In(2L)t* and *In(2R)NS* in the second chromosome (LINDSLEY and GRELL 1967; WARTERS 1944). These polymorphic inversions seem to have occurred many years ago and have been maintained by some forms of balancing selection. However, most unique inversions appear to have been induced recently by mutators [See the *hi* gene of IVES (1950)] or a male recombination factor of HIRAIZUMI (1971) which were recently rediscovered by many investigators in several natural populations of *D. melanogaster* (HIRAIZUMI 1971; YAMAGUCHI and MUKAI 1974; VOELKER 1974 and others).

It is important to estimate the spontaneous occurrence rates of chromosome aberrations (mainly inversions) since the inversions have played a significant role in the speciation of Drosophila species (cf. DOBZHANSKY 1970). Recently, YAMAGUCHI and MUKAI (1974) attempted to estimate the rate of occurrence of chromosome aberrations and found that there was a large variation in the rates among five stem chromosomes employed (0–0.00204/second chromosome/generation) in *D. melanogaster*. They suggested on the basis of these findings that there are mutators in the population from which their experimental materials were taken, and that it is necessary to use many chromosomes in the experiments of this type in order to obtain a good estimate of the rate of occurrence.

The purposes of the present experiment were to estimate an average spontaneous occurrence rate of chromosome aberrations (mainly inversions) using 140 second chromosomes that originally did not carry any aberration (cf. MUKAI and YAMAGUCHI 1974 and CARDELLINO and MUKAI 1975), and to obtain basic information for the estimation of the frequency of mutator-carrying chromosomes in a *D. melanogaster* population.

## MATERIALS AND METHODS

*Accumulation of chromosome aberrations:* The present experimental methods are the same as those used by CARDELLINO and MUKAI (1975). Six hundred and ninety-eight second chromosomes were originally extracted from the Reedy Creek State Park population near Raleigh, North Carolina (MUKAI and YAMAGUCHI 1974). After examining salivary gland chromosomes of these chromosome lines, 140 *inversion-free* chromosome lines were selected at random and used for the present experiment.

Mutations were accumulated on the chromosome of each line independently by repeating the following cross over many generations: *In(2LR)SM1/In(2LR)bw*$^{V1}$ (5 ♀♀) × *In(2LR)-bw*$^{V1}$/+$_i$ (1 ♂) where +$_i$ stands for the second chromosome of line $i$ ($i = 1 - 140$) on which mutations can be accumulated. These chromosome lines were called *RBR-1*, *RBR-2*, . . . , *RBR-150* (10 line numbers are missing). *In(2LR)SM1/In(2LR)bw*$^{V1}$ stock has been called C-160 and abbreviated *Cy/Pm*. In every generation in the process of accumulating mutations, fresh virgin females of *Cy/Pm* were supplied from the original C-160 stock. The genetic background (*X*, third, and fourth chromosomes) of C-160 was originated from an Erie, Pennsylvania population and was isogenic (cf. MUKAI 1964). The cytoplasm of C-160 was from stock H-41 of DR. A. B. BURDICK (cf. MUKAI and BURDICK 1959). Thus, the genetic backgrounds of these 140 chromosome lines with the advance in generation number essentially become that of C-160, an almost completely homozygous one. It has also been known that C-160 does not carry any mutator factor. For details, see YAMAGUCHI and MUKAI (1974) and CARDELLINO and MUKAI (1975).

*Cytological examination*: After accumulating mutations in these chromosome lines for 40 generations, 5 $Cy/+_i$ males from each chromosome line were crossed to 5 *cn bw* (cinnabar, brown) females with the standard gene-arrangement. Salivary gland chromosomes of 5 *cn bw*/$+_i$ larvae in the progeny were observed by staining with 1% acetic-lactic orcein. The detected aberrations were described on the salivary gland chromosome map (BRIDGES 1935). A total of 5600 chromosome generations (140 chromosomes × 40 generations) was examined.

Throughout these experiments, the lines were raised at 25° in 2 × 10 cm vials on cornmeal-molasses-yeast medium.

## RESULTS AND ANALYSES

*Frequencies of chromosome aberrations*: After 40 generations of accumulating mutations, a total of 63 new chromosomal aberrations was detected in 42 out of the 140 chromosome lines: 55 inversions (40 paracentrics and 15 pericentrics), 2 reciprocal translocations between the second and the third chromosomes, and 6 transpositions. All of them were germinal mutations since they were observed in all 5 *cn bw*/$+_i$ larvae examined from each chromosome line. Consequently, the total observed rate of occurrence of chromosome aberrations is 63/140/40 or 0.0112 per second chromosome per generation. The observed rates of occurrence of inversions, translocations, and transpositions were 0.0098 (0.0071 for paracentric and 0.0026 for pericentric inversions), 0.00036 and 0.0011 per second chromosome per generation, respectively. These results are tabulated in Table 1. These figures are much greater than those of the *AW* and *JH* chromosomes which were considered to carry a mutator factor(s). In fact, the rates of chromosome aberrations were 0.00043 and 0.00204 per second chromosome per generation for the *AW* and *JH* chromosomes, respectively (YAMAGUCHI and MUKAI 1974). Thus, it is concluded that some (or all) of the second chromosome lines used in the present experiment carry mutator factors. Since the cytoplasms and the genetic backgrounds (*X*, *III*, and *IV* chromosomes) of the chromosome lines came from C-160 which are mutator-free, the mutator factor(s) found in the present experiment should have been located in the second chromosome.

*Description of newly arisen chromosome aberrations*: All the chromosome aberrations detected in the present experiment are described in Table 2 in terms

TABLE 1

*Occurrence rate of spontaneous chromosome aberrations in 140 second chromosome lines after accumulating mutations for 40 generations*

| Type of aberrations | No. of aberrations | No. of chromosome generations | Occurrence rate (per chromosome per generation) |
|---|---|---|---|
| Inversion | 55 | 5600 | 0.0098 |
| paracentric | 40 | | 0.0071 |
| pericentric | 15 | | 0.0026 |
| Translocation | 2 | 5600 | 0.00036 |
| Transposition | 6 | 5600 | 0.0011 |
| Total | 63 | 5600 | 0.0112 |

TABLE 2

*List of newly arisen chromosome aberrations*

| Line number | Type of aberration | Breakpoints | Line number | Type of aberration | Breakpoints |
|---|---|---|---|---|---|
| *RBR-2* | *In(2L)RBR2-1* | *28E;37E* | *RBR-77* | *In(2R)RBR77-1* | *51B;52B* |
| *-3* | *In(2L)RBR3-1* | *22A;22F* | | *T(2;3)RBR77-1* | *33B;99F* |
| | *Tp(2R)RBR3-1* | *45F-47C;57D* | *-78* | *In(2LR)RBR78-1* | *28C;59B* |
| *-7* | *In(2R)RBR7-1* | *58E;59B* | | *In(2LR)RBR78-2* | *32E;60F* |
| | *In(2LR)RBR7-1* | *34A;50B* | *-91* | *In(2LR)RBR91-1* | *30B;45E* |
| *-8* | *In(2L)RBR8-1* | *23A;33B* | *-93* | *In(2L)RBR93-1* | *26A;34A* |
| *-13* | *In(2R)RBR13-1* | *43A;52F* | | *In(2R)RBR93-1* | *49E;57E* |
| *-24* | *In(2L)RBR24-1* | *22A;30B* | *-100* | *In(2R)RBR100-1* | *50B;58B* |
| | *In(2L)RBR24-2* | *23F;35B* | | *In(2LR)RBR100-1* | *33E;50B* |
| | *In(2R)RBR24-1* | *44B;57C* | *-104* | *In(2LR)RBR104-1* | *22A;59C* |
| *-25* | Complex | *26E;29B;35D;* | *-109* | *In(2L)RBR109-1* | *21B;34D* |
| | Inversion | *46C;49D;56D* | | *In(2L)RBR109-2* | *26B;34D* |
| *-26* | *In(2L)RBR26-1* | *32C;33E* | *-115* | *In(2L)RBR115-1* | *21E;34B* |
| *-31* | *In(2R)RBR31-1* | *44D;47D* | | *In(2L)RBR115-2* | *28E;34B* |
| | *In(2R)RBR31-2* | *50A;57B* | *-116* | *In(2LR)RBR116-1* | *31C;57E* |
| *-33* | *Tp(2LR)RBR33-1* | *28C-32D;57A* | *-117* | *In(2R)RBR117-1* | *42A;59E* |
| *-36* | *In(2LR)RBR36-1* | *30E;42C* | | *Tp(2R)RBR117-1* | *44A-47F;50F* |
| *-37* | *In(2L)RBR37-1* | *26A;28C* | *-118* | *In(2L)RBR118-1* | *22D;34A* |
| | *T(2;3)RBR37-1* | *26A;88F* | *-120* | *In(2LR)RBR120-1* | *35B;58E* |
| *-40* | *In(2L)RBR40-1* | *29F;35F* | *-132* | *In(2L)RBR132-1* | *21C;34D* |
| *-49* | *In(2L)RBR49-1* | *22A;26A* | | *In(2L)RBR132-2* | *31E;34D* |
| | *In(2LR)RBR49-1* | *34A;58E* | *-135* | *In(2R)RBR135-1* | *44E;47C* |
| | *Tp(2LR)RBR49-1* | *26E-34A;57C* | *-137* | *In(2R)RBR137-1* | *51E;57A* |
| *-50* | *In(2L)RBR50-1* | *22E;33E* | *-141* | *In(2L)RBR141-1* | *30E;38F* |
| *-63* | *In(2LR)RBR63-1* | *38A;57B* | | *Tp(2R)RBR141-1* | *42B-47A;57C* |
| *-66* | *In(2R)RBR66-1* | *51F;57F* | *-143* | *In(2R)RBR143-1* | *49D-50F* |
| | *Tp(2LR)RBR66-1* | *35B;45F-51E* | *-144* | *In(2LR)RBR144-1* | *29B;42C* |
| *-68* | *In(2L)RBR68-1* | *22A;23A* | *-145* | *In(2R)RBR145-1* | *43F;54B* |
| | *In(2L)RBR68-2* | *32E;36A* | *-146* | *In(2LR)RBR146-1* | *38F;42C* |
| *-69* | *In(2R)RBR69-1* | *50F;56C* | *-147* | *In(2LR)RBR147-1* | *35D;57B* |
| *-70* | *In(2L)RBR70-1* | *22E;28E* | *-148* | *In(2R)RBR148-1* | *49B;56C* |

utilized in LINDSLEY and GRELL (1967), including their breakpoints. The second chromosome has been separated into 40 numbered regions in the salivary gland chromosome. The left arm is numbered from 21 to 40 and the right arm is numbered from 41 to 60. Each region is subdivided into 6 subregions, from A to F. As shown in Tables 1 and 2, inversions are the most frequent aberrations. In some lines, complicated chromosome aberrations occurred. They are described as follows:

*RBR-3*: The left arm carried a small paracentric inversion with breaks at *22A* and *22F*, *In(2L)RBR3-1*. The right arm carried a transposition, *Tp(2R)RBR3-1*, presented in Figure 1-A. In this case, the segment between *45F* and *47C* has been inserted into *57D*.

*RBR-25*: It was difficult to determine the sequence of the formation of the aberrations in this chromosome, which contains one heterobrachial change and 6 breakpoints (*26E*, *29B*, *35D*, *46C*, *49D*, and *56D*). Thus, it may be reasonable to suppose that one pericentric and two paracentric inversions occurred, since inversions are produced more frequently than any other type of chromosome aberrations.

*RBR-33*: A transposition involving both arms ("heterobrachial shift"), *Tp(2LR)RBR33–1*, occurred as presented in Figure 1-B. The segment between *28C* and *32* was inserted into *57A*.

*RBR-37*: *In(2L)RBR37–1*, a paracentric inversion with breaks at *26A* and *28C* occurred. Successively, a reciprocal translocation between the second and third chromosomes took place with breakpoints of *26A* and *88F*, *T(2,3)RBR37–1*. The third chromosome is separated into 40 numbered segments from 61 to 100 (LINDSLEY and GRELL 1967). The configuration is shown in Figure 1-C.

*RBR-49*: The three aberrations in this chromosome overlapped. A subterminal paracentric inversion with breakpoints of *22A* and *26A* [*In(2L)RBR49–1*] and a pericentric inversion with breakpoints of *34A* and *58E* [*In(2LR)RBR49–1*] occurred independently. After that a transposition, *Tp(2LR)RBR49–1*, took place into the pericentric inversion, i.e., the segment between *26E* and *34A* is inserted into *57C*.

*RBR-66*: A paracentric inversion with breakpoints of *51F* and *57F* [*In(2R)-RBR66–1*] and a transposition [*Tp(2LR)RBR66–1*] occurred independently. The segment between *45F* and *51E* is inserted into *35B*.

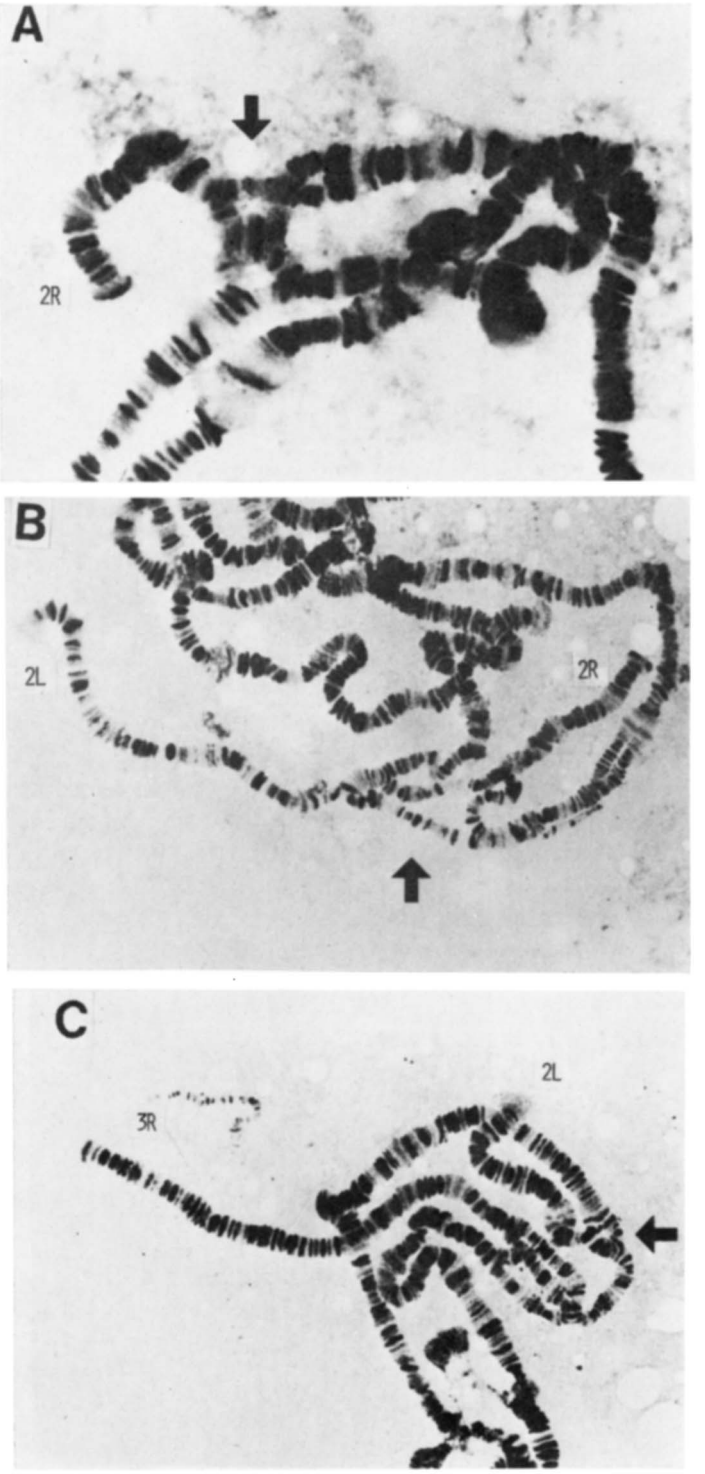
*RBR-77*: A reciprocal translocation between the second and third chromosomes [*T(2;3)RBR77–1*] occurred with breakpoints of *33B* and *99F*. See Figure 1-D.

*RBR-117*: A large paracentric inversion with breakpoints of *42A* and *59E* [*In(2R)RBR117–1*] and a transposition within this inversion [*Tp(2R)RBR-117–1*] occurred. The segment between *45A* and *47F* is inserted into *50F*.

*RBR-141*: A transposition, *Tp(2R)RBR141–1*, occurred in the right arm. The segment between *42B* and *47A* is inserted into *57C*.

None of the chromosome aberrations tabulated in Table 2 are identical to any of the aberrations found in natural or experimental populations. In fact, translocations and transpositions have never been found in natural populations of Drosophila except *D. ananassae* and *D. pseudoobscura* (DOBZHANSKY 1970).

*Characteristics of the breakpoints*: The distribution of the breakpoints was examined. They were localized in the 40 regions (Figure 2) and were examined to determine whether or not they were distributed uniformly. The whole second chromosome is separated into 20 units (two regions adjacent to each other were combined). The observed and the expected frequencies were compared by the $\chi^2$ test. The results, $\chi^2_{df=19} = 53.69$ ($P < 0.001$), indicates that there is no evidence supporting a uniform distribution of the breakpoints over the chromosome, but it should be noted that the frequencies of the breakpoints in the left arm and the right arm are almost the same (68 *versus* 62). This finding is different from the cases of the *AW* and *JH* chromosomes where the left arm acquired more breaks than the right arm, i.e., 29:10 for the *AW* and 154:29 for the *JH* chromosomes,


A
2R
B
2L
2R
C
3R
2L

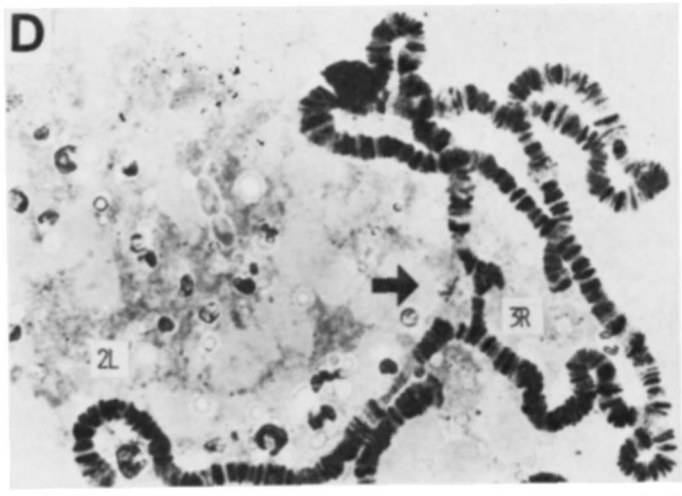



FIGURE 1.—Some configurations of salivary gland chromosomes in individuals heterozygous for newly arisen chromosome aberrations:

A: *Tp(2R)RBR3-1*
B: *Tp(2LR)RBR33-1*
C: *In(2L)RBR37-1* and *T(2;3)RBR37-1*
D: *T(2;3)RBR77-1*

after accumulating chromosome aberrations for about 90 generations. Both ratios are significantly different from the result of the present experiment (*AW*: $\chi^2_{df=1} = 5.97$, $P < 0.05$; *JH*: $\chi^2_{df=1} = 37.38$, P = very small). Thus, the distribution pattern of the points of chromosome- and/or chromatid-breaks is different from those of the *AW* and the *JH* chromosomes. This suggests that the mutator

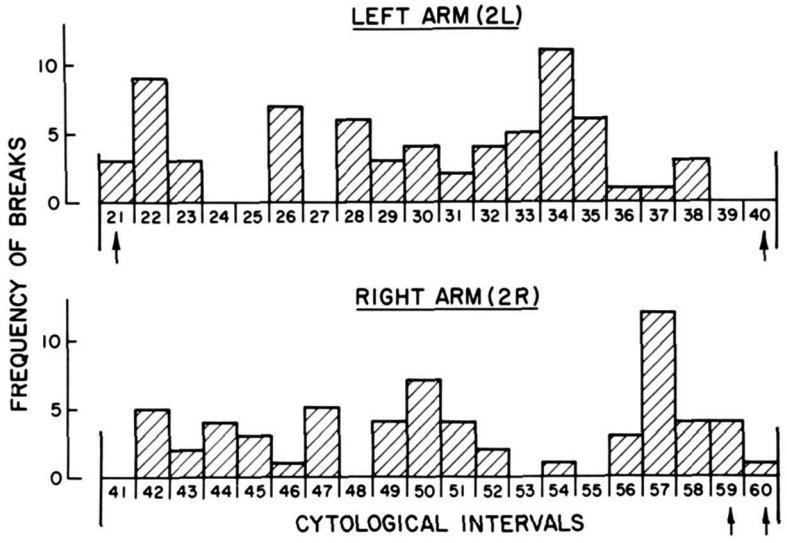



FIGURE 2.—Distribution of the breakpoints on the *RBR* chromosomes.

found in the Raleigh population is different from that found in an Erie, Pennsylvania population from which the original *AW* and *JH* chromosomes were isolated (YAMAGUCHI and MUKAI 1974).

Since the relative lengths of numbered regions in the salivary gland chromosomes are not the same as those in chromosomes in germinal cells, due to shrinkage of heterochromatic parts in salivary nuclei, the rejection of the uniformity of the distribution of breakpoints in salivary gland chromosomes does not necessarily mean that breaks occur nonrandomly in the germinal cells. However, the difference in distribution pattern of breakpoints between the present materials (*RBR*) and the *AW* (or *JH*) chromosomes implies that, at least in either the *RBR* or the *AW* (or *JH*) chromosomes, breaks occurred nonrandomly (most probably in both). Incidentally, the distribution of breakpoints of X-ray-induced chromosome rearrangements in the *X* chromosome is highly correlated with that of repetitious DNA, and the breakpoints concentrated on some restricted regions along the *X* chromosome (LEE 1975).

The Novitski effect with respect to the induction of breaks in chromosomes with the standard gene arrangement in inversion heterozygotes due to the abnormal pairing (NOVITSKI 1946) was examined. The *RBR* chromosomes were maintained in heterozygotes with the chromosomes carrying *In(2LR)bw*$^{V1}$. The breakpoints in this chromosome were shown in Figure 2 by arrows (*21C-D*, *40F*, *59D-E*, and *60D*). It can be seen from Figure 2 that there is no correlation between the frequencies of breakpoints in the *RBR* chromosomes and the position of the breaks in the *In(2LR)bw*$^{V1}$ chromosome. Thus, the breaks observed in the present experiment do not appear to have been primarily caused by the Novitski effect.

*Frequency of the mutator-carrying second chromosomes*: It is quite doubtful that all second chromosomes investigated carried mutators. YAMAGUCHI and MUKAI (1974) attempted to estimate the minimum average number of breaks per chromosome per generation. Under the assumptions that (1) chromosome or chromatid breaks occur on the mutator-carrying chromosome according to a Poisson distribution and (2) inversions (or transpositions) occur whenever more than 1 (or 2) breaks occur in the same generation, then the average frequency of breaks per chromosome per generation ($\mu$) is estimated from the following relationship:

$$1 - e^{-\mu}(1+\mu) = p \qquad \ldots . (1)$$

where $p$ is the occurrence rate of inversions and transpositions per chromosome per generation. From Table 1, $p = 0.4357/(40 \cdot x)$ where $x$ is the proportion of mutator-carrying chromosomes. When $x = 1$, 0.9, 0.8, and 0.7, $\mu$ becomes 0.154, 0.169, 0.175, and 0.188, respectively.

Even when $x = 1$, at least 0.15 breaks can be expected per chromosome per generation, or a minimum number of breaks after accumulating mutations for 40 generations is 6 per chromosome. Under these conditions, it may be expected that almost all mutator-carrying chromosomes acquired either recessive lethal mutations or chromosome aberrations, or both. In fact, 66 out of the 90 lines that were originally lethal-free acquired lethal genes and/or inversions, but 24 lines

got neither lethal mutations nor chromosome aberrations. Furthermore, the average homozygous viability of these 24 lines did not decrease significantly after 40 generations of mutation accumulation [the average homozygous viabilities were 0.6884 ± 0.0420 at generation 0 and 0.6614 ± 0.0464 at generation 40 in the scale that the viability of the *Cy* heterozygote (*Cy*/+) is 1.0000]. It appears that these 24 lines did not carry the mutator factors. Then, it is possible to estimate the $x$ value from the following relationship assuming that (1) lethal mutations and/or chromosome aberrations occurred in all of the mutator-carrying chromosomes and (2) recessive lethal mutations occurred on the initially mutator-free chromosomes according to a Poisson distribution:

$$(1 - x)\, e^{-40m} = \frac{24}{90}$$

where $m$ is the recessive lethal mutation rate per mutator-free second chromosome per generation ($m = 0.006$ is assumed. See MUKAI 1964 and MUKAI *et al.* 1972). From this formula $x$ is estimated to be 0.66. Thus, it may be speculated that about 60–70% of the initially lethal- and inversion-free second chromosomes carried mutator-factors (see also CARDELLINO and MUKAI 1975). This is an astonishingly large fraction about which some comments will be made later.

The above estimate was obtained from chromosomes that were originally inversion-free. In order to examine whether or not mutator factors are randomly distributed over various types of chromosomes, the frequencies of inversion-carrying lines at generation 40 within originally lethal-free chromosome lines and within lethal-carrying chromosomes were estimated. The results are: 0.322 (= 29/90) in originally lethal-free chromosomes and 0.260 (= 13/50) in originally lethal-carrying chromosomes. These two figures are not significantly different. We do not know what proportion of chromosomes with polymorphic inversions carry mutator factors, but we know that at least some chromosomes with polymorphic inversions carried unique inversions. In the Raleigh, North Carolina population, the frequency of second chromosomes with polymorphic inversions is less than 20%, so the above estimate of the frequency of mutator-carrying chromosomes should not be biased a great deal, even if that estimate is considered for all types of chromosomes.

*Fitnesses of homozygotes and heterozygotes for unique inversions*: There were 90 chromosome lines that were lethal-free and inversion-free at the starting generation. Twenty-nine of them acquired unique inversions. Among them only three lines were viable in the homozygous condition. Thus, about 90% of the chromosomes carrying unique inversions were homozygously lethal. This figure is an overestimate since the effects of recessive lethal mutations are confounded. The change in the relative homozygous viabilities of the above three lines with homozygously viable inversions from generation 0 to generation 40 were 0.7740 → 0.7460, 0.8030 → 0.6072, and 0.5526 → 0.2850 (cf. MUKAI and YAMAGUCHI 1974). The decreases in the last two lines are significant. The magnitudes are larger than the value expected on the basis of polygenic viability mutations (ca. 0.16). Thus, it appears that the majority of inversions decrease homozygous viabilities, and most of them are homozygously lethal.

Incidentally, the frequency of nonlethal inversions within all newly arisen inversions (3/29) may be used to speculate as to the approximate proportion of DNA which is not *absolutely* necessary for survival ($p$): $p^2 = 3/29$ or $\hat{p} = 0.32$. This value is, of course, a rough estimate and might be related to the proportion of noninformational DNA in the second chromosome. It should be noted that there is a slight difference between the figures estimated in the present experiment and that obtained in the irradiated $X$ chromosome. According to LEFEVRE (1973), approximately half of the breakpoints of radiation-induced rearrangements in the $X$ chromosomes were associated with lethal or visible mutations and the remaining half were of ostensively nonmutant and may correspond to noninformational "spacer" DNA.

The frequencies of unique inversions in the Raleigh, North Carolina population were investigated from 1968 to 1970. They were 0.0286 (= 9/315), 0.0205 (= 3/146), and 0.0203 (= 14/691) in 1968, 1969, and 1970, respectively (METTLER, CHIGUSA and MUKAI 1976; MUKAI, METTLER and CHIGUSA 1971; MUKAI and YAMAGUCHI 1974). The frequencies appear to have been stable. It should be noted that this estimate of the frequency of unique inversions for the natural population is approximately equal to the sum of the occurrence rates of unique inversions for 2.3 generations. In fact, the frequencies of unique inversions were estimated after extracting chromosomes from the natural population using the *Cy-Pm* inversion technique, and using this method *three generations* are necessary to extract a single chromosome from a natural population (unique inversions that occurred for two generations become homozygous in the established chromosome lines. cf. WALLACE 1956). Thus, the actual frequency of unique inversions should be very low in the natural populations if unique inversions occurred in the process of extracting second chromosomes from the natural population at the same rate as the present estimate (0.0098/second chromosome/generation). This means that the heterozygous effects of inversions are very large (nearly dominant lethal) if inversions arise in the natural population at the same frequency as the present estimate in the laboratory, or that the occurrence rates of inversions in the natural population are smaller than the present estimate. Incidentally, L. E. METTLER (personal communication) found the same type of "unique" inversion more than once (which he calls "endemic") in the present natural population. This is an evidence for the occurrence of unique inversions in the natural population.

## DISCUSSION

*Nature of mutator factors*: It is certain from the present experimental results, together with the others (YAMAGUCHI and MUKAI 1974; CARDELLINO and MUKAI 1975), that the primary action of the mutator(s) is to break the chromatids or chromosomes, which results in recessive lethal mutations, male recombination, and chromosome aberrations.

The studies of mutator genes have been much more advanced in microorganisms than in Drosophila. For example, COUKELL and YANOFSKY (1970) reported that DNA polymerase-deficient mutants in *E. coli* increase the frequency of

deletions. Chromatid or chromosome breaks may be expected as a result of a deficiency of the DNA polymerase in the process of chromosome replication. If chromatid or chromosome breaks occur in normal chromosomes at such a high rate as that found in the present experiment in the process of chromosome replication, but a repair enzyme fixes them, then the mutator genes may be explained as due to the deficiency of the repair enzymes (cf. GREEN and LEFEVRE 1972). However, we do not know whether or not a minimum frequency of chromatid or chromosome breaks is 0.19/second chromosome/generation in normal chromosomes. DNA polymerase might be concerned with the mutator phenomenon. Furthermore, there is a strong hint that DNA polymerase sometimes plays a repair role in *E. coli* (WATSON 1970).

The mutator factor(s) found in the present experiment appear to be different from those found in the second chromosomes extracted from a Pennsylvania population (*AW* and *JH* chromosomes, YAMAGUCHI and MUKAI 1974), but to be the same as those found in Florida and Texas populations (HIRAIZUMI *et al.* 1973; VOELKER 1974) on the basis of the distribution patterns of breakpoints (or recombination points in males) on the second chromosomes. However, this difference might be explained if the mutator genes are transposable as suggested by KIDWELL *et al.* (1973), and if the aberration breakpoints are determined by the positions of mutator factors. This hypothesis must be examined.

If mutator factors induce chromosome or chromatid breaks at a minimum frequency of 0.19/second chromosome/generation, and if about 70% of the chromosome breaks become recessive lethal mutations as the present experimental results suggest, then the recessive lethal mutation rate per mutator-carrying chromosome is expected to be 0.133 $(= 0.19 \times 0.7)$/second chromosome/generation. The indirect estimate of this parameter was 0.066 $(= 0.006 \times 11$, cf. CARDELLINO and MUKAI 1975), which is about one-half the predicted value. This difference might be explained by the accumulation of the errors for various estimates necessary for the above prediction. If 66% of the second chromosomes carry the mutator factors, and if the recessive lethal mutations occur at the above rate, then the recessive lethal mutation rates in the natural population ($\Sigma$m) can be expected to be 0.044 $(= 0.66 \times 0.006 \times 11 + 0.006 \times 0.34)$/second chromosome/generation. Then, the actual frequency of lethal-carrying second chromosomes in the natural population ($Q$) can be estimated to be 0.312 $(= 0.40 - 0.044 \times 2)$ (cf. MUKAI and YAMAGUCHI 1974). The figure 2 in the parenthesis is due to the 2 generations necessary for the extraction of the chromosomes. Thus, the average degree of dominance ($\bar{h}$) of lethal genes must be approximately as follows (cf. NEI 1968): $\bar{h} = \Sigma m/[-ln(1\text{-}Q)] = 0.12$. This value is too large in comparison with the direct estimates. MUKAI and YAMAGUCHI (1974) estimated $\bar{h}$ to be 0.012 for viability and nearly zero for fecundity and their genetic variances are very small. Thus, it may be speculated that the mutation rate of lethals (accordingly, the occurrence rate of unique inversions, too) in the natural population must be lower than the present estimate. K. ITO and C. H. LANGLEY (personal communication) estimated the recessive lethal mutation rate for a number of $X$ chromosomes immediately after their

sample was taken from the same natural population, but they could not find any evidence for an increase in the mutation rate. What is the cause for the above difference?

CARDELLINO and MUKAI (1975) proposed the potential mutator hypothesis for the reason that the mutation load becomes too large if mutations including inversions and recessive lethals occur in the natural population at the same rate as in the laboratory. This hypothesis states that about 70% of the second chromosomes in the natural population carry potential mutators which become actual mutators, perhaps when some kind of virus infects the flies. Otherwise, chromosome breaks are not induced. They presumed that the present experimental materials might have been heavily infected by a virus in the course of the experiment or in the natural population. It was likely that viruses in the bodies of the flies propagated rapidly in the laboratory conditions. The above large estimate of $\bar{h}$ value for lethals supports the potential mutator hypothesis.

*High occurrence rate of inversions and the number of the types of polymorphic inversions in natural populations*: Although unique inversions are occurring at a rather high rate, only six inversions [*In(2L)t*, *In(2R)NS*, *In(3L)P*, *In(3R)P*, *In(3R)C*, and *In(3R)Mo*] can be seen in polymorphic states in many natural populations and over many years (cf. WARTERS 1944; MUKAI, METTLER and CHIGUSA 1971; MUKAI, WATANABE and YAMAGUCHI 1974; LANGLEY, TOBARI and KOJIMA 1974). These polymorphic inversions are considered to have been maintained by some forms of balancing selection or heterozygote superiority. The majority of unique inversions are heterozygously deleterious as shown above, and almost none are heterotic. However, some unique inversions are heterozygously close to neutrality. In the data of the 1970 Raleigh, North Carolina population (cf. MUKAI and YAMAGUCHI 1974), 14 unique inversion-carrying chromosomes were found and their average relative heterozygous viability and fecundity were $1.0113 \pm 0.0205$ and $0.9836 \pm 0.0452$, respectively (relative to the average of random heterozygotes). One cross showed an abnormally low relative fecundity (0.5069). If we exclude this cross, the average relative fecundity becomes $1.0202 \pm 0.0285$. All of these 14 chromosomes were associated with recessive lethals. Indeed, it may be expected that inversions, *per se*, are selectively nearly neutral if both the breaks occur in the parts of noninformational DNA. A minimum estimate of these inversions within all newly occurred inversions was 1/29 in the present experiment. Thus, such inversions must have occurred at a high cumulative frequency since the mutator factors became common in the populations for many years. Hence, all the above results may indicate that the majority of the second chromosomes with standard gene arrangements are not well coadapted with each other in natural populations of this species and do not show heterosis. Approximate linkage equilibrium among isozyme genes in the second chromosomes (MUKAI, METTLER, and CHIGUSA 1971; MUKAI, WATANABE, and YAMAGUCHI 1974) supports this speculation (see MUKAI, WATANABE, and YAMAGUCHI 1974).

Further studies are necessary to estimate the frequencies of mutator-carrying chromosomes over many populations as well as to examine the potential mutator hypothesis.

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