VARIATION OF SPONTANEOUS OCCURRENCE RATES OF CHROMOSOMAL ABERRATIONS IN THE SECOND CHROMOSOMES OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

After accumulating mutations by the aid of marked inversions, spontaneous occurrence rates of chromosome aberrations were estimated for 1148 chromosome lines that originated from five stem line second chromosomes of *Drosophila melanogaster*. In chromosome lines originating from three stem chromosomes (*CH*, *PQ*, and *RT*), mutations were accumulated for 7550, 7252, and 7256 chromosome generations, respectively, but no structural change was detected. For the chromosome lines that originated from the other two stem chromosomes, the situation was different: Twenty aberrations (19 paracentric inversions and 1 translocation between the second and the third chromosomes) during 45990 chromosome generations took place in the 500 chromosome lines derived from stem line chromosome (*AW*), and 92 aberrations (83 paracentric inversions, 6 pericentric inversions, 2 translocations between the second and the third chromosomes and 1 transposition) arose during 45006 chromosome generations in the 500 chromosome lines derived from stem line chromosome (*JH*). For the *AW* group the occurrence rate becomes 0.00043 per chromosome per generation for all aberrations and 0.00041 for inversions. For the *JH* group the corresponding rates are 0.00204 and 0.00198, respectively.—A non-random distribution of the breakpoints on the salivary gland chromosome was observed and the breakpoints were concentrated in the regions 26, 29, 33, and 34.—The cytoplasms and the chromosomes (other than the second chromosomes) were made approximately uniform throughout the experiments. Thus, this remarkable variability in the occurrence rate is most probably due to the differences in one or more chromosomal elements on the original five stem chromosomes. The mutable chromosomes (*AW* and *JH*) appear to carry a kind of mutator factor such as *hi* (IVES 1950).

MORE than two decades have passed since HINTON, IVES and EVANS (1952) reported that the mutator factor *hi* (IVES 1950) induced chromosomal aberrations, especially inversions, in *D. melanogaster*. Recently, CHIGUSA, METTLER and MUKAI (1969) have predicted the existence of a kind of mutator in the Raleigh, North Carolina population, which may not be geographically isolated to a great extent from the Florida population where the *hi* gene was originally

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discovered (IVES 1950). The bases of the above prediction were as follows: (1) the magnitudes of the lethal and detrimental loads are, respectively, greater than those in the northern population (e.g., Madison, Wisconsin; cf. GREENBERG and CROW 1960), and (2) an appreciable frequency of inversions including "unique" ones were found (METTLER, CHIGUSA and MUKAI 1974). In fact, unique inversions were observed in the 1968, 1969, and 1970 populations (MUKAI and YAMAGUCHI 1974). WOODWARD (1971) detected several unique inversions in the second chromosome lines, which were established and used by CHIGUSA, METTLER and MUKAI (1969). These lines had been inversion-free and were maintained in the stock room for more than one year.

Thus, two questions were raised: (1) Were these newly arisen "unique" inversions induced by a kind of mutator or is the spontaneous occurrence rate of inversions generally high? Indeed, IVES (1950) concluded that mutator genes are probably the major cause of spontaneous gene mutations and chromosomal rearrangements in natural populations. (2) If these new inversions were due to the mutator gene, would the mutators exist only in a restricted geographical area or over a wide area? Also what is the mechanism of the maintenance of mutator factors of this type which cause deleterious mutations including chromosome aberrations? Fortunately, we had appropriate materials for examining some of the above questions: 1148 second chromosome lines which originated from five stem chromosomes and were maintained at a minimum pressure of natural selection for more than 90 generations. The salivary gland chromosomes of these chromosome lines were examined after making crosses to a line with the standard gene arrangement. The results are reported in this short article.

MATERIALS AND METHODS

A total of five stem second chromosomes (*CH*, *PQ*, *RT*, *AW*, and *JH* in abbreviations) of *Drosophila melanogaster* were utilized in the present experiment. The origin of three of these stem chromosomes (*CH*, *PQ*, and *RT*) and the procedure for accumulating mutations were described in detail by MUKAI *et al.* (1972) but will be described briefly here.

CH *chromosome:* This was extracted from a Madison, Wisconsin population in 1965 by DR. R. G. TEMIN. A single *Cy*/+ male from the cross between C-160 [*In(2LR)SM1(Cy)*/*In(2LR)bw*^{V1}*(Pm)*; the genetic background is isogenic] female and an isogenic wild-type male (*CH* chromosome) which was near normal in viability was chosen, and mated to several *Cy*/*Pm* females (C-160). Fifty *Pm*/+ (or if necessary *Cy*/+) males of the offspring were individually mated to 3 *Cy*/*Pm* females to establish 25 chromosome lines numbered C-1, C2, . . . , C-25 (low density subgroup). The remaining 25 males were individually mated to 10 *Cy*/*Pm* females, and 25 more lines were established: H-1, H-2, . . . , H-25 (high density subgroup). In each C line a single *Pm*/+ male was chosen in each generation and mated to 3 *Cy*/*Pm* females. This process was repeated for 150 generations. The wild-type second chromosome in every line was descended from that of the original *Cy*/+ male: therefore, there was no opportunity for crossing over since this chromosome was always kept heterozygous with a *Pm* chromosome in males. Since a single male was used in each generation and the chromosome was never allowed to become homozygous, there was minimum opportunity for selection. In each *H* line, 10 *Pm*/+ males (if necessary, some *Cy*/+ males) were mated to 10 *Cy*/*Pm* females per vial in each of the ensuing generations. The gene arrangement of this original chromosome was determined later to have been the standard type (BRIDGES 1935).

PQ and RT chromosomes: These were derived from the same population as *CH*, and allowed to accumulate mutations in the same way as described above for about 150 generations. *P* and *R* lines (each subgroup consisted of 25 chromosome lines) were maintained in the same manner as *C*. *Q* and *T* were maintained in the same manner as *H*. The original *PQ* chromosome had a polymorphic inversion [*In(2R)NS*] (cf. LINDSLEY and GRELL 1967) and the *RT* had a standard gene arrangement.

The genetic background (*X*, third and fourth chromosomes) of C-160 was originally isogenic (cf. MUKAI 1964) and originated from an Erie, Pennsylvania population. The cytoplasm was from stock H-41 of DR. A. B. BURDICK (cf. MUKAI and BURDICK 1959). Thus, the genetic background of *C*, *H*, *P*, *Q*, *R*, and *T* lines, with the advances in generation numbers, essentially became that of C-160, an almost completely homozygous one.

AW *chromosome:* This was derived in 1967 from a cage population (W-1) which was initiated with stock W-1 of DR. BURDICK (this stock was maintained in a half-pint milk bottle after being collected from an Erie, Pennsylvania population in 1954). A lethal-carrying chromosome was extracted from the cage population with a marked inversion technique using C-160. This chromosome is denoted as *l(AW)*. A single male, which was a heterozygote for *SM1(Cy)* and *l(AW)*, was mated to a single C-160 female. To establish the chromosome lines from the offspring, *Cy/l* males and females were collected and many single-pair matings were made between them. This generation was counted as generation 1. In the offspring only *Cy/l* heterozygotes can survive since *Cy/Cy* and *l/l* are lethal. Using these flies again, a single-pair mating was made in each line for maintaining it. The remainder were used for making as many additional single-pair matings as possible and the number of lines was increased. By generation 3, 500 lines were established (AW-1, AW-2, . . . , AW-500), and each line was maintained by a single-pair mating and a five-pair mating. Whenever the single-pair mating was successful, its offspring were used to make a single-pair mating and a five-pair mating to produce the next generation. When the single-pair cross was not successful, the five-pair mating was used as a substitute source of flies to produce the next generation. These mating schemes are presented in Figure 1. It is noteworthy that using this method it is possible to find any contamination since, if it occurs, phenotypically wild-type flies appear. However, no contamination was seen, at least before the examination of the salivary gland chromosomes was initiated. The gene arrangement of the original *l(AW)* chromosome was the standard sequence. At generation 0 when this chromosome was extracted from the cage population, the genetic background was on an average 25% heterozygous for the chromosome set of C-160 (Erie, Pa. origin) and the original chromosome set including the *l(AW)* second chromosome, and the remaining 75% was homozygous for the genetic background of C-160. However, it is expected to approximate 100% homozygosity with the increase in generation number. An average of 12.5% of the genetic background was from the original flies sampled from the cage, but there is a variation between the chromosome lines.

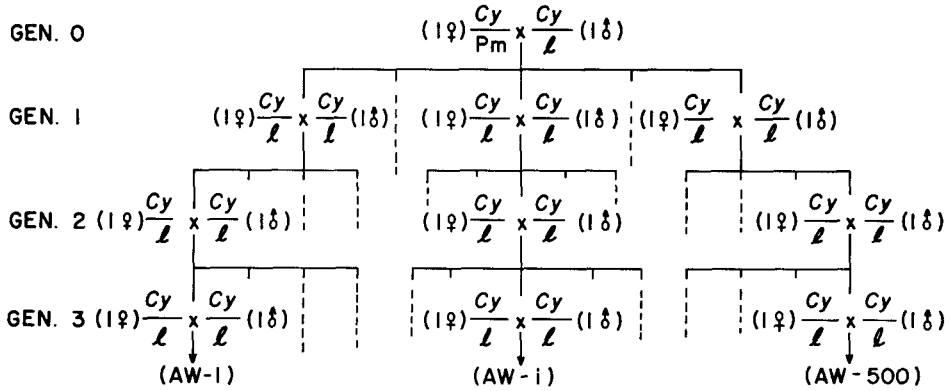

FIGURE 1.—Mating scheme for the accumulation of mutations (*AW*).

JH *chromosome:* This was derived at the same time as the *AW* chromosome from a cage population (W-1). This chromosome also carried a recessive lethal which was not allelic to *l(AW)* and was identified as *l(JH)*. Five hundred lines [*Cy/l(JH)* × *Cy/l(JH)*] were established and maintained as in the case of *AW* and the lines were numbered from JH-1 to JH-500. The nature of the genetic backgrounds of these lines is the same as that of the *AW* lines. It should be noted that the cytoplasms of all the lines of *C*, *H*, *P*, *Q*, *R*, *T*, *AW*, and *JH* were the same, i.e., that of C-160. The genetic backgrounds of *C*, *H*, *P*, *Q*, *R*, and *T* are the same, i.e., that of C-160, and on the average 87.5% of the genetic backgrounds of *AW* and *JH* lines were the same as those of *CH*, *PQ*, and *RT*.

Throughout the experiment the lines were raised at 25° in 2 × 10 cm vials on cornmeal molasses-yeast culture media with two drops of heavy suspension of live yeast. For the salivary gland chromosome analysis, a single male was picked out from each line and mated to W-507 females (a stock carrying the standard sequence in all chromosomes). The *l(AW)* and *l(JH)* chromosomes carried the standard gene-arrangement.

Salivary gland preparations were made by dissecting the third instar larvae of the crosses and by staining with 1% acetic-lactic orcein.

RESULTS

Occurrence rates of chromosome aberrations: The types of chromosome aberrations and their frequencies for each original stem chromosome are presented in Table 1. As the table shows, no chromosome aberration was detected in the chromosomes which were extracted from the Madison, Wisconsin population. In this case, the total number of chromosome generations is 22058. On the contrary, appreciable frequencies of chromosome aberrations were detected in the *AW* and *JH* chromosome lines. In the *AW* lines, a total of 19 paracentric inversions and 1 translocation between the second and third chromosomes were discovered. The total number of chromosome generations is 45990. In the *JH* lines, a total of 89 inversions (83 paracentrics and 6 pericentrics), 2 translocations between the second and third chromosomes and 1 transposition were detected. The total number of chromosome generations is 45006. The rates of all types of aberrations are 0.00043 per second chromosome per generation for the *AW* lines and 0.00204 per second chromosome per generation for the *JH* lines. These rates are significantly larger than those for the *CH*, *PQ*, and *RT* lines. The rates of inversions are 0.00041 for the former and 0.00198 for the latter.

TABLE 1

Occurrence rates of spontaneous chromosome aberrations in the five stem second chromosomes

Chromosomes:	*CH*	*PQ*	*RT*	*AW*	*JH*
No. of lines	50	49	49	500	500
No. of chromosome generations	7550	7252	7256	45990	45006
No. of inversions	0	0	0	19(0)	89(6)
No. of translocations	0	0	0	1	2
No. of transpositions	0	0	0	0	1
Rate of inversions*	0	0	0	0.00041	0.00198
Rate of aberrations*	0	0	0	0.00043	0.00204

The figures in parentheses indicate the numbers of pericentric inversions, and are included in the numbers outside the parentheses.

* Per second chromosome per generation.

TABLE 2

List of newly arisen chromosomal aberrations

Designation	Breakpoints	Designation	Breakpoints	Designation	Breakpoints
Paracentric inversion					
In(2L)AWI1	22D;30B	*In(2L)JHI21*	26A;31D	*In(2L)JHI60*	31C;36D
In(2L)AWI2	22E;24C	*In(2L)JHI22*	26A;32A	*In(2L)JHI61*	32B;40D
In(2L)AWI3	23B;29F	*In(2L)JHI23*	26A;32D	*In(2L)JHI62*	32C;36C
In(2L)AWI4	23E;26B	*In(2L)JHI24*	26A;33B	*In(2L)JHI63*	32D;34A
In(2L)AWI5	25C;32C	*In(2L)JHI25*	26A;34A	*In(2L)JHI64*§	32D;34A
In(2L)AWI6	26A;30D	*In(2L)JHI26*	26A;34A	*In(2L)JHI65*	32D;36A
*In(2L)AWI7**	26A;31B	*In(2L)JHI27*	26A;35A	*In(2L)JHI66*	33E;38D
In(2L)AWI8	26A;33B	*In(2L)JHI28*†	26A;35F	*In(2L)JHI67*¶	34A;35B
In(2L)AWI9	27D;29F	*In(2L)JHI29*	26A;38D	*In(2L)JHI68*	34A;39A
In(2L)AWI10	29F;33B	*In(2L)JHI30*	26B;28D	*In(2L)JHI69*	34D;38F
In(2L)AWI11	33A;37B	*In(2L)JHI31*	26B;29C	*In(2L)JHI70*	34E;38E
In(2L)TWI12	33B;35F	*In(2L)JHI32*	26E;29B	*In(2L)JHI71*	35A;40D
In(2L)AWI13	34A;36A	*In(2L)JHI33*	26F;32D	*In(2L)JHI72*	35F;39E
In(2L)AWI14	35B;39E	*In(2L)JHI34*	27B;27C	*In(2R)JHI1*	42C;47F
In(2R)AWI1	42F;50C	*In(2L)JHI35*	27B;29C	*In(2R)JHI2*	46C;48F
In(2R)AWI2	42F;50E	*In(2L)JHI36*	27C;34C	*In(2R)JHI3*	50C;57C
In(2R)AWI3	43A;45D	*In(2L)JHI37*‡	27D;28F	*In(2R)JHI4*	51B;59A
In(2R)AWI4	50A;60B	*In(2L)JHI38*	27D;29D	*In(2R)JHI5*	51E;52C
In(2R)AWI5	57C;59D	*In(2L)JHI39*	27D;34C	*In(2R)JHI6*	52A;56C
In(2L)JHI1	21C;26F	*In(2L)JHI40*	28B;30A	*In(2R)JHI7*	54D;59C
In(2L)JHI2	22C;26A	*In(2L)JHI41*	28B;34D	*In(2R)JHI8*	54E;59D
In(2L)JHI3	22F;26A	*In(2L)JHI42*	28C;31E	*In(2R)JHI9*	56A;57B
In(2L)JHI4	22F;26A	*In(2L)JHI43*	28D;30B	*In(2R)JHI10*	57B;59D
In(2L)JHI5	24A;29F	*In(2L)JHI44*	29B;34D	*In(2R)JHI11*	57F;59D
In(2L)JHI6	24D;26B	*In(2L)JHI45*	29C;34A		
In(2L)JHI7	25E;26B	*In(2L)JHI46*	29D;34C	Pericentric inversion	
In(2L)JHI8	26A;27C	*In(2L)JHI47*	29E;30F	*In(2LR)JHI1*	22F;60A
In(2L)JHI9	26A;27D	*In(2L)JHI48*	29E;35C	*In(2LR)JHI2*	24D;56F
In(2L)JHI10	26A;27E	*In(2L)JHI49*	29E;35F	*In(2LR)JHI3*	26A;56D
In(2L)JHI11	26A;28B	*In(2L)JHI50*	29F;34A	*In(2LR)JHI4*	26A;59D
In(2L)JHI12	26A;28F	*In(2L)JHI52*	30A;36B	*In(2LR)JHI5*	29B;50B
In(2L)JHI13	26A;29B	*In(2L)JHI52*	30B;34A	*In(2LR)JHI6*	33B;60B
In(2L)JHI14	26A;29C	*In(2L)JHI53*	30B;38A	Translocation	
In(2L)JHI15	26A;29D	*In(2L)JHI54*	30B;39E	*T(2;3)AWT1*	29F;94B
In(2L)JHI16	26A;29E	*In(2L)JHI55*	30B;39F	*T(2;3)JHT1*	34C;96F
In(2L)JHI17	26A;29E	*In(2L)JHI56*	30C;36D	*T(2;3)JHT2*	56F;97B
In(2L)JHI18	26A;29F	*In(2L)JHI57*	31B;34A		
In(2L)JHI19	26A;30D	*In(2L)JHI58*	31B;34D	Transposition	
In(2L)JHI20	26A;31B	*In(2L)JHI59*	31B;38E	*Tp(2L)JHTP1*	26A;29B;34D

* Overlapped *In(2L)AWI8.*
† Overlapped *In(2L)JHI62.*
‡ Overlapped *In(2L)JHI38.*
§ Overlapped *In(2L)JHI26.*
¶ Overlapped *In(2L)JHI64.*

Under the assumptions that (1) chromosome or chromatid breaks occur on the chromosomes according to a Poisson distribution and (2) inversions (or transpositions) occur whenever more than 1 (or 2) breaks occur in the same generation, the average frequency of breaks per chromosome per generation (μ) can be obtained from the following relationship:

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

where p is the occurrence rate of inversions and transpositions per chromosome per generation. Since, in assumption (2), the ratio of p to μ is assumed to be much larger than the actual value, the estimate μ is a minimum value. From Table 1, p values become 0.00041 for the *AW* group and 0.00200 for the *JH* group, respectively. Accordingly, μ values become 0.029 for the *AW* and 0.064 for the *JH* group, respectively.

From the above results, it can be seen that there is a large amount of variability between stem chromosomes with respect to the rates of chromosome aberrations. Thus, it appears meaningless to calculate an average inversion occurrence rate in a population using a few original chromosomes.

Characteristics of new chromosome aberrations: Newly arisen chromosome aberrations are tabulated in Table 2 in terms suggested by BRIDGES and BREHME (cf. LINDSLEY and GRELL 1967). Included were 102 paracentric inversions, 6 pericentric inversions, 3 reciprocal translocations between the second and third chromosomes, and 1 transposition. A transposition can be considered to have been produced by three breakpoints. *Tp(2L)JHTP1* had breakages at 26A, 29B, and 34D on the salivary gland chromosomes. The segment between 26A and 29B is inserted into the breakage at 34D. Some of the typical chromosomal aberrations are presented in Figure 2. The characteristics of the newly arisen chromosome aberrations can be summarized as follows: (1) None are identical to any previously reported chromosomal aberrations. (2) These aberrations are transmissible. Indeed, at the time of final karyotype analyses, at least 5 larvae were examined for the salivary gland chromosomes in each line, which descended from a single male parent (*Cy/l*). The same chromosome aberrations were observed in the preparations from all larvae examined. (3) The distributions of the breakpoints are not random on the second chromosome: There were 39 breakpoints in the *AW* group and 183 in the *JH* group. Their distribution patterns on the salivary gland chromosome map are shown in Figure 3. The locations of the breakpoints were made on numbered segments of the salivary gland chromosome (left arm: 21–40, and right arm: 41–60). Although each segment is further subdivided by letters, this was not considered in the present work. It is obvious, especially in the *JH* chromosome, that the distribution of the breakpoints is not at random on the chromosome. The numbers of the breakpoints in the left and right arms are shown in Table 3. A clear difference can be seen between the two arms (AW: $\chi^2_{df=1} = 9.26$, $0.001 < P < 0.005$; JH: $\chi^2_{df=1} = 85.38$, $P \ll 0.001$) in each case. The difference in distribution patterns of the breakpoints between the *AW* and *JH* chromosomes was tested by the Kolmogorov-Smirnov non-parametric tests, although there was a great difference in the total frequencies of

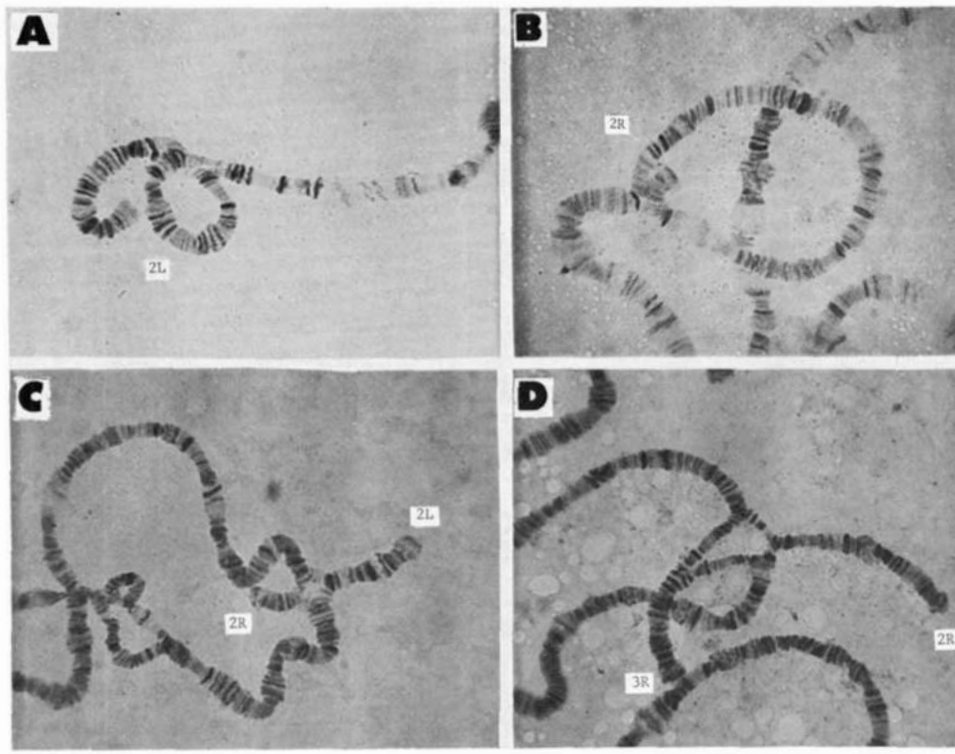

FIGURE 2.—Salivary gland chromosomes of individuals heterozygous for standard sequence and:

In(2L)AW15 (A)
In(2R)AW14 (B)
In(2LR)JH11 (C)
and
T(2;3)JHT2 (D)

breakpoints between the two stem chromosomes. However, a significant difference was not detected.

In connection with a hypothesis proposed by NOVITSKI (1946) concerning the induction of "mimic" inversions by pre-existing inversions, it should be noted here that the chromosomal regions with higher frequencies of breaks (regions 26, 29, 33, and 34) do not always overlap the regions where breaks occurred in the

TABLE 3

The numbers of breakpoints in the left and right arms in the AW *and* JH *chromosomes*

Chromosome	Left arm	Right arm
AW	29(1)	10
JH	154(1)	29(1)

The figures within parentheses are due to translocation.
The figures outside the parentheses include the numbers within the parentheses.

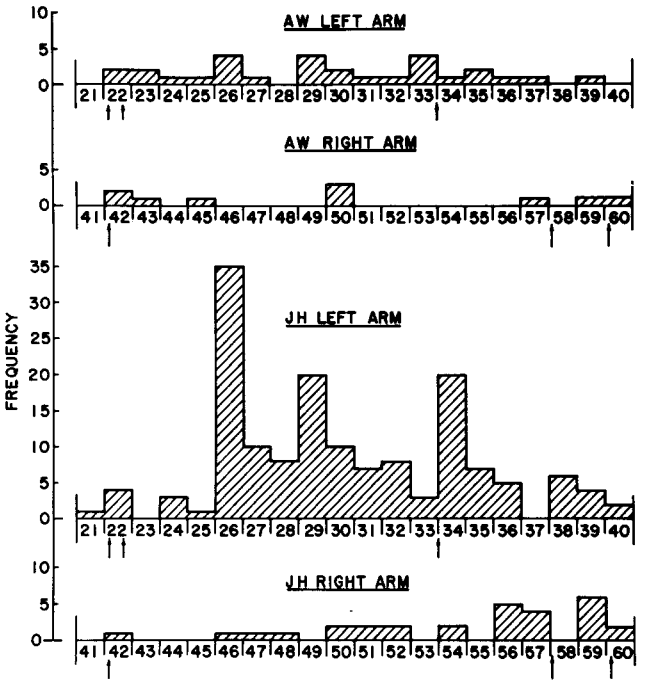

FIGURE 3.—Distribution of the breakpoints on the *AW* and *JH* chromosomes.

formation of *In(2LR)SM1(Cy)* (22A, 22D, 35F, 42A, 58A, and 60B which are shown using arrows in Figure 3). A detailed consideration will be made in the DISCUSSION.

DISCUSSION

Characteristics of the mutator factor: Since there is much variation in occurrence rates of chromosomal aberrations among the five stem chromosomes studied (in fact, no aberration occurred in the *CH*, *PQ*, and *RT* chromosome groups), it is reasonable to assume mutator factors in the *AW* and *JH* lines. At present, it is not known whether the mutators in the *AW* and *JH* lines are the same or not. However, we tentatively consider that they are the same, since the distribution patterns of the breakpoints in the chromosomes are not significantly different from each other even though both of them showed deviations from random distributions. Their absolute frequencies of breakages were different. This might be due to different modifiers of the mutator factor (cf. MINAMORI and SUGIMOTO 1973). This mutator factor is *not* a cytoplasm-specific substance since some stem chromosomes had high frequencies of chromosome aberrations but the others did not have any, in spite of the fact that their cytoplasms were originally the same.

There is one alternative hypothesis to the above explanation for the variability in the occurrence rate of chromosome aberrations: The hypothesis states that, in general, chromosome or chromatid breaks occur at a much higher frequency in females than in males, and that this is the cause for the variability of occurrence

observed in the present experiment. This hypothesis might seem tenable because, in the *AW* and *JH* chromosome lines, the second chromosomes tested were transferred to the next generation through males or females, while, in the *CH*, *PQ*, and *RT* lines, all the second chromosomes tested were transferred to the next generation only through males. This hypothesis will, however, be rejected later.

The mutator factor is most probably located in the second chromosome as *mu-F* (DEMEREC 1937) and *hi* (IVES 1950). In fact, CARDELLINO and MUKAI (1975) found a mutator factor located in the second chromosome in materials extracted from a Raleigh, North Carolina population. However, there is an extremely small chance that it is located in the third chromosome, since, as indicated above, on an average 12.5% of the third chromosomes of the *AW* and *JH* chromosome lines were different from those of C-160 or the genetic background of the *CH*, *PQ*, and *RT* chromosome lines. This portion of the third chromosomes might have had a mutator such as found by GREEN (1970) and by KIDWELL, KIDWELL and NEI (1973), but this probability is negligible.

From the present results, it was difficult to determine whether these chromosome aberrations occurred in males, in females, or in both. Recently, HIRAIZUMI (1971) reported male recombination due to a factor located in the second chromosome. In fact, male recombinations were first reported by MORIWAKI (1937) and KIKKAWA (1937) in *D. ananassae*. In order to examine whether or not inversions occur in males and in order to test the similarity of the present mutator to the male recombination factor of HIRAIZUMI (1971), a small scale experiment was carried out. Male recombination in 6 inversion-free lines of the 500 *JH* lines was tested. A single male [*Cy/l(JH)*] was sampled from each of the six *JH* lines and individually mated to homozygous *cn bw* females (supplied by DR. Y. HIRAIZUMI). In each line, a single F_1 male [*cn bw/l(JH)*] progeny was backcrossed to homozygous *cn bw* females, and an average of ten replications (9–11) was made. Male recombination was detected in only one out of the 60 lines. In this cross, the following segregation took place: phenotypically white (*cn bw*): 164; phenotypically wild: 138; and phenotypically brown: 13. Three females out of these 13 brown individuals were individually mated to homozygous *cn bw* males and phenotypically cinnabar flies and brown flies segregated in the offspring in each cross. Thus, the genotypes of parental flies were proved to be *cn bw*/+ *bw*, and male recombination was demonstrated. The three brown males carrying possible recombinant chromosomes were all sterile. From the vial, in which male recombination was demonstrated, 10 *cn bw*/+ + male flies were taken and individually crossed to homozygous *cn bw* females. In one cross there appeared a single mosaic fly whose eye color was brown in one eye and was red (or wild-type) in the other. This may indicate that chromatid breakage and somatic (mitotic) crossing over occurred in the process of development after fertilization, although the possibility of somatic mutation cannot be ruled out. These experimental results can be summarized as follows: (1) Male recombination occurred at a very low frequency. This can be understood in light of the fact that the chromosome aberration breaks occurred at a much higher frequency in the left arm than in the right arm and *cn* and *bw* are located in the

right arm. (2) Unequal crossing over might have occurred (male recombinants were sterile). (3) Complementary gametes (*cn* +) either were not produced or were not viable. (4) Male recombination can occur at least at a mitotic stage (since one mosaic appeared) or its occurrence is possible at a premeiotic stage. (2) and (3) strongly suggest that breakages and reunions of the chromatids occurred, and (2), (3), and (4) show that the mechanism of male recombination is different from that of ordinary female recombination. These results are similar, but not identical, to those reported by HIRAIZUMI *et al.* (1973) and clearly show that, at least in males, chromatid breakages occur which can cause inversions (at this moment, we do not know whether or not breakages occur in females). Therefore, the above 'hypothesis' that the difference in the occurrence of inversions between the group of *CH*, *PQ*, and *RT*, and the group of *AW* and *JH* is due to the fact that the chromosome or chromatid breaks occur only in females can be rejected. The above experimental results also suggest a similarity of the present mutator to HIRAIZUMI's (1971) male recombination factor. From the finding that a chromatid break occurred at a stage of mitosis (i.e., a mosaic appeared), it may be tentatively concluded that inversions occurred at a premeiotic stage. but the present experimental results cannot exclude the possibility of the occurrence at a stage of meiosis.

Locations of breakpoints: HINTON, IVES and EVANS (1952) reported that the breakpoints induced in the *X* chromosomes by the gene *hi* were more frequently located in the proximal heterochromatic region (region 20), and this phenomenon coincided with the breakpoints induced by X-rays (KAUFMAN 1939). In the present experiment, the breaks occurred most frequently in the regions 26, 29, and 34 in the *JH* group and in regions 26, 29, and 33 in the *AW* group; breaks did not occur frequently in the proximal heterochromatic region. We do not know whether regions 26, 29, 33, and 34 contain much heterochromatin.

On the basis of the location of breakpoints of inversions in *D. pseudoobscura* and *D. athabasca*, NOVITSKI (1946) proposed a hypothesis that an original inversion is able to induce "mimic" inversions in the homologous chromosome with the standard gene arrangement owing to their structural heterozygosity. That is to say, in the meiotic division the stretched asynaptic portions of the homologous chromosomes are subject to mechanical stress and tend to break easily. If breaks occur in two points in the same strand of the stretched region of the normal chromosome, and their reunions take place in the new combination between the break ends, a new inversion will be formed. The probability of this type of reunion is high because of the close proximity of the two breaks in the loop configuration. As presented in Figure 3, the breakpoints in *In(2LR)SM1(Cy)* are not always located in the regions where the breaks occurred most frequently in the *AW* and *JH* groups. Thus, the mechanisms of the chromosome or chromatid break due to the present mutator factor is different from that suggested by NOVITSKI (1946). Incidentally, a hypothesis similar to the above was proposed by THOMPSON (1960) with respect to the induction of recessive lethals. However, WATANABE and OSHIMA's (1966) data for newly arisen recessive lethals (cf.

MUKAI 1964) does not support this hypothesis. (See also KIDWELL, KIDWELL and NEI 1973).

Comparison with other mutators: In the present experiment, we investigated chromosomal aberrations and male recombination. Insofar as chromosome breaks occur at a high rate, it is naturally expected that recessive lethal mutations are also induced by the present mutator factor.

Recently, factors similar to the present mutator factor were detected in natural populations (MINAMORI and SUGIMOTO 1973 and earlier; HIRAIZUMI 1971; CARDELLINO and MUKAI 1975; VOELKER 1974) and in laboratory stocks of *D. melanogaster* (GREEN 1970; KIDWELL, KIDWELL and NEI 1973). MINAMORI and ITO (1971) hypothesized that extrachromosomal elements (like delta detected by them) transcribed by a chromosomal gene (or genes) or by an integrated agent cause mutations, and in such a case chromosomal elements may be considered as the mutators. They considered that high mutabilities observed by DEMEREC (1937), by NEEL (1942), and by IVES (1950) appear to be due to such extrachromosomal elements as described above. KIDWELL, KIDWELL and NEI (1973) hypothesized a controlling element such as that described by MCCLINTOCK (1951) in corn and by GREEN (1967) in *D. melanogaster*. They have assumed transposability of the controlling element in order to explain that lethal mutations occurred at a restricted number of loci. There is some possibility that mutations or chromosome breaks were caused by virus infections (cf. MAMPELL 1946).

It appears that our mutator factor may be different from those of MINAMORI and ITO (1971), of HIRAIZUMI (1971) and of VOELKER (1974) in that the distribution of breakpoints in the present experiment is different from that of recessive lethals (MINAMORI and ITO 1971) and of the breakpoints in male recombination (HIRAIZUMI et al. 1973 and VOELKER 1974). But this speculation is not valid if the distributions of breakpoints in the chromosomes change following the transposition of the mutator factors (assuming that transposition occurs). The mutator factor of GREEN (1970) is definitely different from the present one in that it is located in the third chromosomes and does not cause chromosome breaks in males. At present, we do not know at all about the mechanism of the induction of chromosome breaks of our mutator.

Mutators in natural populations: From the present experimental results, it is clearly speculated that some of the chromosomes in natural populations carry mutator factors. The unique inversions detected in the Raleigh population (METTLER, CHIGUSA and MUKAI (1974); MUKAI and YAMAGUCHI 1974) were most probably induced by mutator factors. This is the answer to the first question raised in the introduction.

Recently, mutator factors or male recombination factors have been discovered several natural populations: In Pennsylvania (present experiment), Florida (VOELKER 1974), North Carolina (CARDELLINO and MUKAI 1975), Texas (HIRAZUMI 1971; SLATKO and HIRAZUMI 1973), and Japan (MINAMORI *et al.* 1970, although they later pointed it out as a mutator; MINAMORI and ITO 1971).

HIRAIZUMI (1971) reported that at least 20% of the second chromosomes in the Harlingen, Texas population carry male recombination factors.

These mutators or male recombination factors are harmful to the populations, because they increase the genetic load. In spite of this fact, they may have been maintained for a long time, at least since their detection in Florida populations by DEMEREC (1937) and IVES (1950). It is important to find the frequency of mutator-carrying chromosomes and the mechanisms of their maintenance over a wide area of populations in order to understand the population structure.

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