MUTATION INDUCTION IN THE MALE RECOMBINATION STRAINS OF *DROSOPHILA MELANOGASTER*[1,2]

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ABSTRACT

One group of the second chromosome lines isolated from a southern Texas population of *Drosophila melanogaster*, which has been known to show relatively high frequencies of male recombinations, was found to increase the frequency of sex-linked recessive lethal mutations from a control frequency of 0.18% to 1.63%. The second group, which showed a very much reduced frequency of male recombinations, was found to cause a slight increase to 0.48%, although it was not statistically significant. The first group was also tested for the recessive lethal mutation frequency in the second chromosome; the frequency increased from a control frequency of 0.28% to 2.82%. Mapping of a portion of the sex-linked lethals indicated a distribution along the entire *X* chromosome, although there was a tendency of clustering towards the tip of the *X* chromosome. One sex-linked lethal line so far tested was found to be associated with an inversion (approximate breakpoints, 14A–18A). It was suggested that the element causing male recombination might be similar to the *hi* mutator gene studied earlier by IVES (1950).

HIRAIZUMI (1971) reported that many of the strains of *Drosophila melanogaster* isolated from a natural population in southern Texas showed recombinations in the males, although the frequencies were much reduced as compared with those in the females. In a recent paper, HIRAIZUMI *et al.* (1973) suggested that the strains which showed male recombinations seemed also to exhibit an increased frequency of mutations. This suggestion originally came from the observations that various kinds of visible mutants were recovered fairly frequently in some of the matings involving the male recombination lines. The absolute frequency of such mutations was rather low, but it seemed to be much higher than what would be expected by the "spontaneous" mutation rates, and the occurrences of such mutations were restricted to the male recombination lines.

The purpose of the present report is to provide an experimental basis supporting this suggestion.

MATERIALS AND METHODS

Strains of *Drosophila melanogaster* used for the present study are listed as follows.

[1] This paper is based in part on the Masters thesis (University of Texas at Austin, 1973) of BARTON E. SLATKO.

[2] This work was supported by research grants, NSF USDP GU-1598 and NIH GM-19770.

1) *cn bw:* A standard second chromosome line marked with two recessive eye color mutants, *cn* (cinnabar eye color, 2R-57.5) and *bw* (brown eye color, 2R-104.5).

2) *Tokyo:* A standard wild-type second chromosome line which has been maintained by backcrossing, through males, to the standard *cn bw* females for more than ten years.

3) *Canton-S:* A standard wild-type laboratory stock which has been kept in this laboratory in a large mass culture.

4) y^2 *cv v f car:* An *X* chromosome line carrying five recessive mutants, y^2 (an allele of *y*, yellow body color, 1–0.0), *cv* (crossveinless wing, 1–13.7), *v* (vermilion eye color, 1–33.0), *f* (forked bristle, 1–56.7), and *car* (carnation eye color, 1–62.5).

5) *In(2LR) Cy;* cn^2 *bw:* A second chromosome line with two large inversions, one in the left, and the other in the right arm. This chromosome carries dominant marker *Cy* (Curly wing) and two recessive eye color mutants, cn^2 (an allele of *cn*) and *bw*. This line will be abbreviated as *Cy*.

6) *Muller-5* (= *Basc*). *In(1)* sc^{S1L} sc^{8R}+*S*, sc^{S1} sc^8 w^a *B:* An *X* chromosome line carrying complex inversions, a dominant marker *B* (Bar eye, 1–57.0), and a recessive marker w^a (white apricot eye color, 1–1.5).

7) *FM-7. In(1)* sc^8+*15D-E; 20A-E* + *dl 49*, y^{31d} sc^8 w^a v^{Off} *B:* An *X* chromosome line carrying complex inversions, a dominant marker *B*, and recessive markers y^{31d} (an allele of yellow body colors), w^a, and v^{Off} (an allele of vermilion eye color).

8) *T-007, T-032, T-037, T-066:* The second chromosome lines isolated from a natural population in Harlingen, southern Texas. All of these lines carry the male recombination element, showing an average recombination frequency of about 0.7% between the *cn* and the *bw* loci. These lines have been maintained by backcrossing, through males, to the standard *cn bw* females.

9) *T-043, T-603, T-122:* The second chromosome lines isolated from the same southern Texas population. These lines seem to carry the male recombination elements, but the frequencies of recombinations are much reduced (about 0.09% between *cn* and *bw*). These lines have been kept in the same way as above.

10) *SD-5, SD(NH)-2:* The second chromosome lines carrying the Segregation Distorter complex system. They were isolated from a natural population in Madison, Wisconsin, and in Odate, Japan, respectively. These lines have been kept by backcrossing to the standard *cn bw* females for more than ten years.

A standard cornmeal food was used throughout the present study, and the room temperature was about 23–24°.

EXPERIMENTS AND RESULTS

Sex-linked recessive lethal mutations: Seven *Texas* lines (*T-007, T-032, T-037, T-066, T-043, T-063* and *T-122*) and three control lines (*cn bw, Tokyo* and *Cy*) were examined for the frequency of sex-linked recessive lethal mutations. The *X* chromosomes tested for lethal mutations were those from the standard *cn bw* stock (= X^{cb}). Each of the second chromosome lines except, of course, the standard *cn bw*, was made heterozygous with the *cn bw* chromosomes. The heterozygous males, carrying the X^{cb} chromosome, were then individually mated to the *Muller-5* or *FM-7* homozygous females. The number of heterozygous parental males examined for each line was as follows: 28 for *T-007*, 30 for *T-032*, 26 for *T-037*, 40 for *T-066*, 30 for *T-043*, 29 for *T-063*, 27 for *T-122*, 15 for *cn bw*, 31 for *Tokyo* and 17 for *Cy*. The F_1 females of the genotype, X^{cb}/*Muller-5* or *FM-7* (about 25 F_1 females per parental mating), were then mated individually to males to examine for the absence of wild-type males, indicating recessive lethal mutations in the X^{cb} chromosomes. Results are summarized in Table 1.

The frequency of mutations was highly significantly heterogeneous among the

TABLE 1

Relationships among recombination, mutation and segregation frequencies

Group	Strain	c.o. %*	(N)†	No. of mutation	(%)	(N)‡	k§	(N)†
	cn bw	...	...	1	(0.28)	(356)	....	...
Control	Tokyo	0.00	(100)	1	(0.13)	(782)	0.550	(100)
	Cy	0.00	(66)	1	(0.22)	(464)	0.543	(66)
	Mean	0.00	(166)	3	(0.19)	(1,592)	0.547	(166)
	T-043	0.05	(108)	6	(0.79)	(755)	0.556	(108)
Group A	T-063	0.11	(105)	4	(0.51)	(780)	0.528	(105)
	T-122	0.11	(108)	0	(0.00)	(535)	0.525	(108)
	Mean	0.09	(321)	10	(0.48)	(2,070)	0.536	(321)
	T-007	0.80	(697)	7	(1.15)	(607)	0.338	(697)
Group B	T-032	0.38	(117)	8	(1.63)	(490)	0.447	(117)
	T-037	0.49	(107)	7	(1.00)	(703)	0.408	(107)
	T-066	0.75	(81)	25	(2.29)	(1,092)	0.404	(81)
	Mean	0.71	(1,002)	47	(1.63)	(2,892)	0.367	(1,002)

* [(Number of recombinant flies recovered)/(Total number of progeny flies)] × 100.
† Number of males tested for recombination frequency.
‡ Number of X^{cb} chromosomes examined for lethality.
§ Frequency of the second chromosome in question, *x*, recovered among progeny of the mating, *cn bw* ♀♀ × *x*/cn bw ♂.

three groups ($\chi^2_2 = 29.83$, $p < 0.001$), but this heterogeneity was mainly due to the high frequency in group B (the group including lines showing relatively higher frequencies of male recombinations); there was no significant difference between the control and the group A (the group including lines showing relatively lower frequencies of male recombinations. $\chi^2_1 = 2.21$, $0.25 > p > 0.10$). It is interesting to note, however, that group A showed about three times more mutations than the control lines. The difference between the groups A and B was highly significant ($\chi^2_1 = 13.86$, $p < 0.001$). It should be noted that the above statistical results remain essentially the same even after removing the *T-066* line from the group B.

Except for one male which gave two lethals, all the remaining lethal chromosomes were recovered independently. There was therefore no indication of the clustering occurrence of lethal mutations, although the present data were not large enough to detect the presence of small clustering occurrences. The lethal frequencies of the control lines, which average 0.19%, are comparable values to those reported by other investigators (for example, WALLACE 1970; 0.24%).

HIRAIZUMI (1971) reported that the *k* value, the frequency of *Texas-x* second chromosomes recovered among progeny of the mating, *cn bw* ♀♀ × *Texas-x*/*cn bw* ♂, was considerably lower for the male recombination line than that of the control, and furthermore, that the *k* value and the male recombination frequency were negatively correlated. This relationship can be clearly seen in Table 1. Note that the average *k* value for the group A (0.536) is close to, but slightly less than, the 0.547 of the control line. It is not certain at this moment whether the lines in

TABLE 2

Distribution of recessive lethal mutation loci in the X *chromosome*

Region in standard map	Obs. No. of lethals*	(%)
0–10	6	(26.1)
10–20	5	(21.7)
20–30	3	(13.0)
30–40	2	(8.7)
40–50	1	(4.3)
50–60	4	(17.4)
60–	2	(8.7)
Total	23	(99.9)

* One line was found to be associated with an inversion (approximate break positions, 14A–18A). This line was not included in the tabulation.

the group A are those free of male recombination element, or are those carrying the element but are associated with genetic backgrounds which partially suppress the function of the element. In any event it is clear that the male recombination frequencies correlate positively with mutation frequencies, thus indicating that these two phenomena are interrelated.

Mapping of most lethal mutations, except those induced by the *T-066* line, was undertaken by using a chromosome line carrying y^2 *cv v f car*, and the results are shown in Table 2.

As can be seen from this table, the positions of lethals were distributed along the entire X chromosome, but there was a suggestion that the distribution was not quite at random; relatively more lethals seemed to be located near the tip of the *X* chromosome. It should be mentioned that one lethal *X* chromosome line was found to carry an inversion, with approximate breakpoints 14A–18A.

It is interesting to compare the distribution of the lethals in the present study with those studied by IVES (1959). Comparing with the distribution of radiation-induced mutations, IVES found that the *hi* mutator gene seemed to work more specifically in the interstitial region, while the present element seemed to operate relatively more specifically to the region towards the tip of the chromosome. The number of lethals mapped in this study was, however, very small and the authors wish to open this subject for future studies. It should be noted, however, that GREEN and LEFEVRE (1972) studied the locations of sex-linked recessive lethal mutations induced by their mutator gene, *mu*, and found a tendency of clustering toward the tip of the chromosome, a result similar to that found in the present study.

Recessive lethal mutations in the cn bw *second chromosome:* It is extremely tedious to work with the frequency of autosomal recessive lethal mutations, and therefore only one male recombination line, *T-066*, and one control line, *Tokyo*, were examined. The *T-066* and the *Tokyo* second chromosomes were made heterozygous with lethal free *cn bw* chromosomes, and the heterozygous males (12 males for *T-066* and 14 for *Tokyo*) were individually mated to *cn bw/Cy* females.

The F_1 *cn bw/Cy* males (about 30 F_1 males per parental mating) were then mated individually to *+/Cy* females, and the F_2 sibs of the genotype *cn bw/Cy* were mated together for detecting recessive lethal mutations in the *cn bw* chromosomes.

A total of 319 *cn bw* chromosomes were tested in the experimental set, of which 9 (2.82%) were found to carry recessive lethals, while only 1 out of 356 (0.28%) *cn bw* chromosomes tested in the control set was found to show the presence of a recessive lethal. All of the lethals were of independent origin; no parental male produced more than one lethal *cn bw* chromosome. The control frequency of 0.28% is slightly lower than, but comparable to, the values found in other laboratories (see summary table by CROW and TEMIN 1964; about 0.5% on the average). The frequency of lethal mutations with the *T-066* line is thus seen to be about ten times as high as the values obtained in the controls.

Of the nine *cn bw* lethal chromosomes, one was lost accidentally before further tests could be made, and the remaining eight were tested for allelism; two out of the eight were found to be allelic, suggesting non-random occurrence of the lethal induction. It is also interesting to note that the induced mutation frequency in the second chromosome is roughly twice that in the *X* chromosome, which is consistent with the relative length of these two chromosome pairs. This, along with data presented previously (HIRAIZUMI *et al.* 1973) showing that the male recombinations occurred in comparable frequencies between the second and the third chromosomes, indicates that the element operates among chromosome pairs with approximately the same intensity.

"Visible" mutations at the cn *and the* bw *loci:* This experiment was done with the *T-066* line only. The *T-066* chromosome was made heterozygous with a wild-type laboratory second chromosome, and the heterozygous males were mated individually to the standard *cn bw* females.

A total of 14,908 F_1 progeny flies of the above mating were examined, of which 2 showed *cn* and 3 *bw* mutant phenotypes (these 5 mutants were recovered independently). Thus, the mutation rate of bw^+ to *bw* and of cn^+ to *cn* was approximately 2×10^{-4} and 1.3×10^{-4}, respectively. No control mating was made in this experiment, but the above rates seemed to be much higher than those expected by the spontaneous mutation rates. Unfortunately all of these mutant flies recovered were completely, or almost completely, sterile and no further tests could be made.

DISCUSSION

The present observations have provided a strong basis to conclude that the male recombination lines operate similar to those carrying "mutator genes." In fact, it might be that the "male recombination element" is a similar or even an equivalent element to a mutator gene, such as *hi* reported by IVES (1950) from a Florida population, and this mutator gene was reisolated by one of the present authors (HIRAIZUMI 1971), but this time as an element which caused male recombinations. There were many similarities between the present element and the *hi* mutator gene of IVES; i.e., *hi* was mapped to the second chromosome; it caused sex-linked recessive lethal mutations in the frequency of approximately

1.1% and in the second chromosome, from 0.4% to 7.2%; its frequency in natural population seemed to be fairly high, and finally, the *hi* mutator and the present element both induced inversions. In this connection, it is extremely interesting to examine *hi* as well as other mutator genes for the ability to induce male recombinations.

It is perhaps worth mentioning here that the male recombination lines seem to induce dominant lethals as well. A small set of egg hatchability tests was performed for the *T-007* and the *Tokyo* lines. Females from a standard wild-type stock, *Canton-S*, were mated individually with the *T-007/cn bw* and the *Tokyo/cn bw* males, and after ensuring the copulation, the eggs were sampled and the "hatchabilities" were examined by counting the number of adult progeny recovered. Of a total of 1,072 eggs sampled from the control *Tokyo* male mating, 954 adult progeny flies (89%) were recovered, while in the *T-007* male mating, a total of 1,469 sample eggs produced 786 progeny (54%). This test can not exclude the possibility that the number of functional sperms produced by the *T-007/cn bw* males was extremely small and that the reduced "hatchability" was in fact due to an increased frequency of unfertilized eggs. It seems reasonable to consider, however, that the above result strongly suggests that the male recombination lines cause dominant lethality in a fairly high frequency.

Mutator genes in Drosophila have been reported by many investigators (DEMEREC 1937; IVES 1950; GREEN 1970; WOODRUFF, BOWMAN and SIMMONS 1972; *et al.*), yet their frequencies in natural populations have not yet been satisfactorily estimated. In fact, not even a crude estimate has been made. One obvious reason for this would be the tediousness of the experiments; it might be practically impossible to work with 1,000 or more replications for a *single* chromosome or genotype collected from a natural population in order to see the presence or absence of the mutator gene in it. The present result, positive association between male recombination and mutation induction, offers an excellent and much easier way to estimate the frequencies of mutator genes in natural populations of Drosophila; i.e., presence of a mutator could be examined by testing the ability of male recombination inductions, and male recombination frequencies could be examined much more easily than those of the mutation frequencies.

As was stated earlier, the frequency of the male recombination element in the Harlingen population was very high (50% or more). A later study for another southern Texas population, at Brownsville (unpublished), also showed a high frequency (50% or more) of the male recombination element. Recently this element was discovered also in a Bowling Green, Ohio, population in a fairly high frequency of 20% or more (DRS. WADDLE and OSTER, personal communication), suggesting that the element is widely spread among natural populations. It is extremely interesting and important to examine the distributions and the frequencies of male recombination elements in many natural populations of *Drosophila melanogaster*, as well as those of the previously reported mutator genes.

HIRAIZUMI (1961) reported that the Segregation Distorter (*SD*) of *Drosophila melanogaster* might operate like a mutator gene inducing recessive lethal mutations in the second chromosome, in the vicinity of the SD^+ region, although the number of replications in his report was too small to reach any firm conclusion.

It was decided to explore whether or not the *SD* element could induce mutations in the *X* chromosome. Two *SD* lines, *SD-5* (isolated from a Madison, Wisconsin population. See SANDLER, HIRAIZUMI and SANDLER 1959), and *SD(NH)-2* (isolated from an Odate, Japan, population; see HIRAIZUMI and NAKAZIMA 1967), were tested for the sex-linked recessive lethal mutations. Six hundred and sixty-nine X^{cb} chromosomes from the *SD-5*, and 532 from the *SD(NH)-2* male parents were examined; there were no lethals (0%) produced. Thus the *SD* system seemed to be different from the male recombination system, although *SD* may possibly induce mutations in its homologous chromosome.

Finally, the fact that the *Texas* second chromosome lines showed different recombination and mutation frequencies suggests that the system of male recombination-mutation is a complex system involving many modifying factors. In fact, a recent study showed that the male recombinations were under a polygenic control (manuscript in preparation), somewhat similar to the male recombination system in *Drosophila ananassae* (MORIWAKI, TOBARI and OGUMA 1970). The evolutionary significance of such a system in natural populations, is still an open question for future studies.

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