

# RECOMBINATION IN *DROSOPHILA MELANOGASTER* MALE<sup>1</sup>

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## ABSTRACT

*T-007* strain of *Drosophila melanogaster* is known to show recombination in males. The present study established the following points: (1) Clustering occurrence of recombinant, unequal recovery of complementary products of recombination, relatively high frequency of recombination around centromeric region, and relatively frequent occurrence of mosaic phenotypic flies—all of these seem to indicate that a considerable fraction of male recombination in the *T-007* strain is of premeiotic, or somatic origin, although a fraction still could be of meiotic origin; (2) Male recombination occurs in the third as well as in the second chromosomes, and the frequencies of recombinations are comparable between these two chromosome pairs.

IT has been accepted for a long time that spontaneous crossing over is absent in male *Drosophila melanogaster*, although it can be induced by an X-ray or by a chemical treatment (PATTERSON and SUCHE 1934; LEWIS 1957; SOBELS and STEENIS 1957; WHITTINGHILL and LEWIS 1961).

In 1971, HIRAIZUMI reported an instance of spontaneous male recombination in the second chromosome of *D. melanogaster* (Symbol *T-007*) isolated from a natural population in Harlingen, Texas. In the *T-007/cn bw* heterozygous male, the frequency of recombinations between *cn* (2R-57.5) and *bw* (2R-104.5) loci was found to be approximately 0.005 or 0.5%. In that paper he was inclined to suggest the meiotic origin of recombination by stating that "The time of recombination is not certain, but the distribution of recombinants is more suggestive of meiotic than of premeiotic occurrence, although the possibility of premeiotic contribution cannot be eliminated."

Experiments have been continued since then and data have been accumulated. The purpose of the present report is (1) to re-examine the time of recombination in males of the *T-007* strain, and (2) to examine whether recombination could occur in the other major autosome, in the third chromosome, as well.

## MATERIALS AND METHODS

Strains of *D. melanogaster* used for the present study are listed below.

1) *cn bw*. A standard second chromosome line marked with two recessive eye color mutants, *cn*

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(cinnabar eye color, 2R-57.5) and *bw* (brown eye color, 2R-104.5).

2) *b pr c px*. A standard second chromosome line marked with four recessive mutants, *b* (black body color, 2L-48.0), *pr* (purple eye color, 2L-54.4), *c* (curved wing, 2R-75.5), and *px* (plexus wing vein, 2R-100.5).

3) *In(2LR) Cy; cn<sup>2</sup> 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<sup>2</sup>* (an allele of *cn*) and *bw*. This line will be abbreviated as *Cy*.

4) *se ss k e<sup>s</sup> ro*. A third chromosome line carrying five recessive markers, *se* (sepia eye color, 3L-26.0), *ss* (spinless bristle, 3L-58.5), *k* (kidney eye, 3R-64.0), *e<sup>s</sup>* (ebony soothly body color, 3R-70.7), and *ro* (rough eye, 3R-91.1).

5) *T-007*. A second chromosome line isolated from a natural population in Harlingen, Texas. This was the chromosome in which male recombination was first recognized. At the time when this strain was isolated from a natural population, it showed male recombination frequency of about 0.005 between *cn* and *bw*. Since then this strain had been kept in several culture vials by backcrossing to the standard *cn bw* females. Sometime later, some cultures (sublines) were found to show slightly, but consistently higher, recombination frequency of about 0.007–0.008 between *cn* and *bw*. This subline was then made heterozygous with the *Cy* chromosome and the stock has been kept by the *T-007/Cy* ♀ × *T-007/Cy* ♂ matings. This *T-007* chromosome carries a recessive lethal gene. Salivary gland chromosome examination showed that this chromosome carried a small inversion located at the distal end of the left arm, but no detectable structural abnormality could be found in the right arm, and in the proximal region of the left arm.

It should be noted that neither the inversion nor the recessive lethal gene was the factor causing male recombination, since it was found recently that an inversion and lethal free second chromosome line, which was isolated from the same natural population, showed male recombination in a frequency comparable to that in the *T-007* line.

6) *R(T-007; cn bw)*. A second chromosome line marked with *cn* and *bw*. This line was established as follows. A *cn bw<sup>+</sup>* recombinant chromosome from a *T-007/cn bw* male was made heterozygous with *bw* laboratory chromosome. The heterozygote male was then mated to *cn bw/Cy* females. Among *Cy* phenotype progeny of this mating, a male showing *cn<sup>2</sup> bw* phenotype (i.e., its genotype was *cn bw/Cy; cn<sup>2</sup> bw*, in which the *cn bw* chromosome was a recombinant) was selected and was established as a stock. This *cn bw* chromosome strain was found, by later test, to show male recombination—about 0.005 between *cn* and *bw*.

A standard cornmeal food was used throughout the present experiment. The ages of the flies at the time of matings were usually within three days old, sometimes three to five days, but rarely more than five days old. Parents were kept in a vial for 7 days; then they were discarded. Room temperature was about 23°–24°C.

## RESULTS

*Recombination frequencies in the T-007 females:* Table 1 summarizes the results of the matings: (1) *T-007/b pr c px* ♀ × *b pr c px* ♂; (2) *R(T-007; cn bw)/b pr c px* ♀ × *b pr c px* ♂; and (3) a control mating, *cn bw/b pr c px* ♀ × *b pr c px* ♂. Of the 102 recombinants between *pr* and *c* produced from the mating Number 2, 97 could be tested to examine whether crossing over occurred between *pr* and *cn*, or *cn* and *c*.

The control mating Number 3 showed recombination frequencies among loci comparable to those of the standard map distances. Experimental matings Number 1 and Number 2 also showed frequencies more or less similar to the control, with some suggestion of a slight increase in the overall frequencies. This is more pronounced in the mating type Number 2. Note, however, that the frequency between *pr* and *cn* (around the centromere) is comparable to the standard map distance. The number of progenies in the mating type Number 2 are rather small and the discrepancy might well simply be due to chance.

TABLE 1

Recombination frequencies in the T-007 heterozygous females

Mating type (1) = T-007/b pr c px ♀ × b pr c px ♂, (2) = R(T-007; cn bw)/b pr c px ♀ × b pr c px ♂, (3) = cn bw/b pr c px ♀ × b pr c px ♂

Region	Recombination frequencies		
	Mating 1	Mating 2	Mating 3
Total number of chromosomes examined	807	394	779
b — pr	0.0979	0.1015	0.0757
pr — c	0.1896	0.2640	0.1784
c — px	0.2119	0.2411	0.2195
		pr-cn: 0.0191	
		cn- c: 0.2449	

Based upon these observations we may conclude that the frequency of recombination in the T-007 heterozygous female is normal, or at least almost normal.

*Recombination frequencies in the T-007 males:* Table 2 summarizes the results of the matings: (1) *b pr c px* ♀ × T-007/*b pr c px* ♂; (2) *b pr c px* ♀ × R(T-007; *cn bw*)/*b pr c px* ♂; (3) *cn bw* ♀ × T-007/*cn bw* ♂; and (4) a control mating, *cn bw* ♀ × *cn bw/b pr c px* ♂. The mating Number 2 produced a total of 60 recombinants between *pr* and *c*. All of them were tested to determine whether recombination occurred between *pr* and *cn*, or between *cn* and *c*.

It first must be pointed out that the observed distribution in the number of recombinants per male parent does not fit with the Poisson expectations, as is indicated by the significant index of dispersion  $\chi^2$ . The number of progeny was fairly constant among cultures and we feel it unnecessary to make any corrections for the varied number of progeny. For example, in the *cn bw* ♀ × T-007/*cn bw* ♂ mating, the average number of progeny per male parent was 80, 81, 84, and 85 for the group of males producing 0, 1, 2, 3 or more recombinants, respectively.

The frequencies of the two complementary recombinant classes were, when pooled together, close to the expected 1:1. This was not true, however, when the results were examined separately for each of the males which produced clustered recombinants. When there were only two crossovers from a single male, these were consistent with the binomial distribution of the complementary classes. This fit became poor, however, when three or more recombinants were produced from a single male.

It is apparent from Table 2 that the *relative* frequency of male recombination in the centromeric region (*pr-c* for T-007, and *pr-cn* for R(T-007; *cn bw*)) was much higher than would be expected from the standard map distance in the female.

All of these observations described above seem to suggest that a fraction, at least, of the male recombination is of premeiotic origin.

It is interesting to examine the recombinants from a male (in the mating type Number 1) which produced 21 recombinant type flies out of 64 progeny. Of the 21, one was of the double crossover type, *b pr<sup>+</sup> c<sup>+</sup> px*, one was *b<sup>+</sup> pr c px* and the

TABLE 2

*Recombination frequencies in the T-007 heterozygous males. Mating type (1) = b pr c pr ♀ × T-007/b pr c px ♂, (2) = b pr c px ♀ × R(T-007; cn bw)/b pr c px ♂, (3) = cn bw ♀ × T-007/cn bw ♂, (4) = cn bw ♀ × cn bw/b pr c px ♂*

Number recombinations per male	Number of males giving recombinations in the regions indicated								
	Mating type 1			Mating type 2				Mating type 3	Mating type 4
	b-pr	pr-c	c-px	b-pr	pr-cn	cn-c	c-px	cn-bw	cn-bw
0	134	91	126	117	113	113	118	326	326
1	15	33	23	7	12	13	7	157	2
2	4	18	2	4	0	2	2	64	.
3	.	4	1	0	0	0	1	25	.
4	.	3	0	0	0	1	0	1	.
5	.	2	0	1	3	.	0	0	.
6	.	0	0	.	0	.	0	2	.
7	.	0	0	.	0	.	0	0	.
8	.	1	0	.	0	.	0	1	.
9	.	0	0	.	0	.	0	0	.
10	.	0	0	.	0	.	0	0	.
11	.	0	0	.	0	.	0	0	.
12	.	0	0	.	1	.	0	1	.
13	.	1	0	.	.	.	0	.	.
14	.	.	0	.	.	.	0	.	.
15	.	.	0	.	.	.	1	.	.
20	.	.	1	.	.	.	.	.	.
Total	153	153	153	129	129	129	129	577	328
Total recombinations	23	124	50	20	39	21	29	396	2
Total progeny	11831	11831	11831	9012	9012	9012	9012	46946	29399
Crossover frequency	0.0019	0.0105	0.0042	0.0022	0.0043	0.0023	0.0032	0.0084	0.00007
Mean	0.1503	0.8105	0.2941	0.1550	0.3023	0.1628	0.2248	0.6863	.....
Variance	0.1818	2.4441	2.8076	0.3508	1.7126	0.2624	1.8944	1.1497	.....
Index of dispersion $\chi^2$	183.2	458.4	1451.1	289.7	725.1	206.3	1078.7	964.9	.....
Degrees of freedom	152	152	152	128	128	128	128	576	...
Probability	p>0.05	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	.....

rest were the  $b^+ pr^+ c^+ px$  type. This double crossover type (the only double crossover type found in this laboratory during the past two years) could be understood as a result of two events: the first was a gonial crossing over between  $c$  and  $px$ , resulting in a cluster of  $b^+ pr^+ c^+ px$ ; and the second, which occurred at a later stage, was a crossing over between  $b$  and  $pr$  involving the  $b^+ pr^+ c^+ px$  product.

In the control mating, two males out of 328 produced recombinants, one recombinant for each. The frequency seems to be comparable to those obtained by others. For example, WHITTINGHILL and LEWIS (1961) found that one out of 111 control males gave one recombinant. Surely the  $cn bw$  and the  $b pr c px$  stocks used in the present study are "normal."

*Recombination in the third chromosome:* Females homozygous for the third chromosome markers, *se ss k e<sup>s</sup> ro*, were mated with *R(T-007; cn bw)Cy* males. Three initial matings, A, B, and C, were made. Non-*Cy*  $F_1$  progeny males from each of the above matings (genotype = *R(T-007; cn bw)/+*; *se ss k e<sup>s</sup> ro/+*) were then individually doubly mated to (1) *cn bw* and (2) *se ss k e<sup>s</sup> ro* females to examine the recombination in the second and the third chromosomes simultaneously. Results are summarized in Table 3.

It is clear from this table that crossing over does occur in the third chromosome as well as in the second, and the relative frequencies of recombination in the two chromosome pairs are comparable.

## DISCUSSIONS

As was stated earlier, HIRAZUMI (1971) suggested the meiotic occurrence of male recombination in the *T-007* strain. With the present results the authors are now inclined to the opposite. A considerable fraction of male recombinations seems to be of premeiotic origin—as evidenced by the clustering occurrences of recombinants, unequal recovery of the complementary recombinant classes for such clustered cases, and a much higher frequency of recombination around the centromeric region—although a fraction of the recombinants could be of meiotic origin.

It is, perhaps, worth mentioning that from time to time, in the matings involving *T-007* strain, eye color (for *cn* and *bw* markers) mosaic flies were discovered. The frequency of mosaic flies can not be estimated accurately at present, but 11 mosaics found in about 900 cultures (about 80 progeny per culture) will provide some idea about the minimum estimate of the frequency. Among the 11 mosaics, some of the phenotypes could not be understood by a simple somatic recombination mechanism, but relatively frequent occurrences of mosaic flies seem to suggest that recombination and/or some other event (mutation, for example) is also occurring during the developmental cell divisions in the *T-007* strain. Presum-

TABLE 3

*Male recombinations in the second and the third chromosomes.  
The mating schemes are described in the text.*

Set	Third chromosome			Second chromosome		
	Number recombinant	Number parental	Total	Number recombinant	Number parental	Total
A	4 (0.0078)	506	510	5 (0.0072)	687	692
B	3 (0.0078)	388	391	1 (0.0021)	477	478
C	2 (0.0049)	410	412	2 (0.0042)	473	475
Total	9 (0.0069)	1,302	1,311	8 (0.0049)	1,637	1,645

ably, recombinations occur during the developmental, gonial and meiotic cell divisions, although the frequency could be different from stage to stage.

Finally it should be noted that the *T-007* strain shows, besides male recombination, several other interesting properties, such as (1) distorted segregation (HIRAIZUMI 1971), (2) induction of mutations (it acts like a mutator element, unpublished), and (3) possible transposability, or non-chromosomal nature of the element (unpublished). Probably all or some of these properties are related, and the simplest mechanism which could be related to those properties is, perhaps, chromosome breakage.

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