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Spontaneous Recombination in *Drosophila melanogaster* Males

YUICHIRO HIRAIZUMI

Department of Zoology, The University of Texas at Austin, Austin, Texas 78712

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ABSTRACT A second chromosome of *Drosophila melanogaster* (symbol *T-007*) isolated from a natural population in Harlingen, Texas, was found to undergo recombination in heterozygous males. Heterozygous males transmit this chromosome with a frequency, *k*, of about 0.4, considerably reduced from the expected value of 0.5. The frequency of male recombination and the *k* value are negatively correlated, indicating that the two phenomena are in some way related. The complementary recombinant products are recovered in equal frequency and the recombination is not restricted to the heterochromatic regions. The time of recombination is not certain, but the distribution of recombinants is more suggestive of meiotic than of premeiotic occurrence. In the natural population of these flies, the frequency of chromosomes with male recombination is 20% or more.

The absence of crossing over in *Drosophila melanogaster* males is one of the most familiar facts of genetics. This is a report of an exception, a chromosome that undergoes a small but definite amount of crossing over in males.

In February 1970, about 150 second chromosomes were isolated from males that had been collected in a wild population at Harlingen, Texas. Since that time, each chromosome has been maintained in a heterozygous condition by successive backcrosses to females marked with recessive mutants. At the time of stock changing one line, *T-007*, was found to have a reduced proportion of progeny with the wild type chromosome. The result was consistently repeatable, giving a *k* value of about 0.40, where *k* is the proportion of the wild type chromosome from the heterozygous male parent.

During these experiments it was noticed that recombinations had occurred in the right arm of chromosome 2, although at very low frequencies. Crossing over and the segregation ratio are both normal in females.

MATERIALS AND METHODS

The following lines of *D. melanogaster* were used.

(*1*) *cn bw*. A standard second-chromosome line marked with two recessive mutants, *cn* (cinnabar eyes, 2R-57.5) and *bw* (brown eyes, 2R-104.5). The *cn bw* phenotype is white eyed. This is the standard stock used in our laboratory, especially for studies of segregation distortion. The line is kept in small mass cultures.

(*2*) *cn* L^2 *bw*. A line carrying the additional marker, L^2 (dominant lobed eyes, 2R-72.0). Established many years ago as a double crossover from a *cn bw*/L^2 female, this line has been maintained since by backcrossing to standard *cn bw* females.

(*3*) *T-007*. A second-chromosome line isolated from a natural population in Harlingen, Texas, and maintained by backcrossing heterozygous males to standard *cn bw* females.

All experiments were done at a temperature of about 23–24°C. Progeny counts were completed on the 18th or 19th day after mating.

EXPERIMENTS TO DEMONSTRATE RECOMBINATION IN MALES

Experiment 1. After at least four generations of backcrossing to *cn bw* females, 206 *T-007*/*cn bw* males, 2–5 days old, were individually mated with 2–3 *cn bw* females. Parents were kept in a culture vial for 1 week and then discarded.

Experiment 2. In order to obtain a larger number of progeny, 42 one-day-old *T-007*/*cn bw* males were mated individually to 5 *cn bw* females. After 4 days the parents were transferred to a fresh vial. The procedure was repeated every 4 days to produce seven broods.

Experiment 3. Eleven males, less than one day old, were mated to 5 *cn bw* females. After 4 days, each male was transferred to a new group of 5 females, and the process repeated after another 4 days. Each group of females was transferred to fresh food every 4 days until they stopped laying eggs. The purpose was to produce as many progeny from one male as possible.

Apparent crossover progeny, showing the *cn* or *bw* phenotype, were tested to confirm that they had the indicated genotype. No exceptions have thus far been found.

The results of the three experiments are shown in Table 1.

The recombination frequency does not differ significantly among the three experiments ($X_2^2 = 5.83$, $0.05 < P < 0.10$). The average *k* values are also not significantly different. The sex ratio among the recombinants is not significantly heterogeneous ($X_2^2 = 5.80$, $0.05 < P < 0.10$) and the overall sex ratio does not differ significantly from 1:1 ($X_1^2 = 0.04$, $0.75 < P < 0.90$). The relative frequencies of the two complementary recombinants were homogeneous among the three experiments ($X_2^2 = 0.39$, $0.75 < P < 0.90$) and the overall ratio of *cn* to *bw* does not differ significantly from 1:1 ($X_1^2 = 3.33$, $0.05 < P < 0.10$). Furthermore, when there are multiple crossovers from a single male, these are consistent with the binominal distribution of *cn* and *bw* types.

Table 2 shows the distribution in the number of recombinants per male parent, along with the Poisson expectations. In each experiment the variance is greater than that expected with the Poisson distribution, as indicated by the significant Index of dispersion X^2. However, the departure, though significant, is not large, and it seems more probable that this

TABLE 1. *Frequency of recombination in T-007/cn bw males. The mating was cn bw ♀ ♀ × T-007/cn bw ♂*

Expt.	No. of males tested	cn bw	T-007	cn female	cn male	bw female	bw male	Total no. of flies	Recomb. frequency	Average k (unweighted)
1	206	8,956	6,448	20	20	20	26	15,490	0.0056	0.413
2	42	12,703	9,741	20	20	25	30	22,539	0.0042	0.426
3	11	5,945	4,407	10	6	16	6	10,390	0.0037	0.411
Total	259	27,604	20,596	50	46	61	62	48,419	0.0045	0.419

TABLE 2. *Distribution of the number of recombinant progeny per male parent and Poisson expectations*

Number of recombinants per male	Number of males					
	Expt. 1		Expt. 2		Expt. 3	
	Observed	Expected	Observed	Expected	Observed	Expected
0	147	135.69	8	4.37	1	.35
1	42	56.65	10	9.89	0	1.20
2	11	11.82	6	11.19	4	2.07
3	3	1.65	5	8.44	2	2.39
4	2	.17	11	4.77	1	2.06
5	1	.01	0	2.16	2	1.43
6			1	.81	0	.82
7			0	.26	0	.40
8			1	.07	0	.17
9					0	.07
10					1	.02
Total	206	206.0	42	42.0	11	11.0
Mean ($\bar{x}$)	.417		2.262		3.455	
Variance (V_x)	.654		3.418		6.873	
X^2	321.2		61.9		19.9	
D.F.	205		41		10	
Probability	<0.001		~0.02		~0.03	

is caused by variability in number of progeny per male, variability in the recombination frequency from male to male, and other extraneous sources, rather than to premeiotic recombination. The clustering is not nearly as extreme as is found with gonial crossing over. For example, Meyer (1) reported 15 spontaneous recombinants in the right arm of chromosome 2 among 98,600 progeny; but these were only two events, a cluster of 11 among 181 progeny of one male and 4 among 118 progeny of another. If any appreciable number of events are gonial, they must be very late.

RELATIONSHIP BETWEEN RECOMBINATION FREQUENCY AND THE *k* VALUE

That the two phenomena found in males heterozygous for the *T-007* chromosome, reduced transmission frequency and recombination, are related is shown by the data in Table 3. The table shows a highly significant negative regression of recombination frequency on *k* value. Also, when the males are grouped into those from which a recombinant appeared and those from which no recombinant was found, the latter group transmitted the *T-007* chromosome in a significantly high proportion.

POSITION OF THE RECOMBINATION

In a fourth experiment, 29 *T-007/cn L^2 bw* males were mated to *cn bw* females to determine whether the recombinants are restricted to a particular part of the chromosome. Three males had recombinant progeny. Two produced *cn L^2* recombinants and one produced a *L^2 bw* recombinant, thus showing that recombination can occur both to the left and right of L^2.

The total number of recombinants in these experiments was 3 out of 7,793, or 0.0004. This is only about 10% of the value in the experiments with *cn bw*, showing that the frequency is not the same in different genotypic backgrounds.

TABLE 3. *Regression coefficient, b, of the frequency of recombination (after arcsin transformation) on k*

Expt.	b	P	Average k for males which produced: No recomb.	(No. male)	At least one recomb.	(No. male)
1	−9.6524	<0.01	0.424	(147)	0.385	(59)
2	−23.2132	<0.01	0.479	(8)	0.413	(34)
3	−35.6554	<0.01	0.453	(1)	0.407	(10)
Total			0.427	(156)	0.396	(103)

FREQUENCY OF MALE RECOMBINANT CHROMOSOMES IN A NATURAL POPULATION

A total of 154 second chromosome lines from the same population were tested for recombination. So far 29 have been found to show recombination, so the frequency of this chromosome type is at least 20% in that population. Furthermore, the average k value was 0.424 for lines that showed at least one recombinant and 0.497 for those that did not, again showing the relationship between male recombination and distorted segregation ratios.

Some of these lines were made homozygous for the second chromosome. Although some were lethal, there were male crossover chromosomes both with and without lethals, so the male recombination property is independent of recessive lethality.

DISCUSSION

It has been believed for a long time that crossing over is absent in male *Drosophila* except in *D. ananassae*, a species quite closely related to *melanogaster*. The equality of complementary classes, the occurrence of crossing over on both sides of L^2, and the rough fit to a Poisson distribution all suggest that the event is meiotic, but the possibility of a premeiotic contribution cannot be eliminated. Mapping of the element (or elements) causing this effect is in progress.

The association of male recombination with low k value and the surprisingly high frequency of this chromosome in the natural population despite the distorted segregation are puzzling properties that are as yet unexplained.

Finally, it is perhaps worth mentioning that the standard *cn bw* chromosome has been used in many genetic studies in the past, particularly to study segregation distortion. Most of these experiments were crosses of the same type as reported in this paper and afforded an easy opportunity for the detection of male recombination; yet no cases have thus far been reported.

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1. Meyer, H., *Genetics*, **39**, 988 (1954).