A CONSIDERATION OF THE NEGATIVE CORRELATION BETWEEN
TRANSMISSION RATIO AND RECOMBINATION FREQUENCY
IN A MALE RECOMBINATION SYSTEM OF
*DROSOPHILA MELANOGASTER*¹⁾

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It is now evident that the genetic system controlling male recombination observed in certain wild second chromosome lines of *Drosophila melanogaster* (Hiraizumi 1971) is rather complex. Mapping experiments have indicated that an element responsible for a major portion of male recombination in the *T-007* chromosome line locates between the *pr*⁺ (2L-54.4) and *c*⁺ (2R-75.5) loci (near *pr*⁺) in *T-007* (Slatko and Hiraizumi 1975). In addition to this major element (symbol: *Mr*, for Male recombination), there appear to be other elements present, as some chromosome lines from which *Mr* had been removed through recombination were still able to induce male recombination, although at lower levels than the original *T-007* chromosome. There was some indication that a male recombination element with a minor effect was present in the left arm of the *T-007* chromosome, but the data were inconclusive.

For these mapping experiments, Slatko and Hiraizumi (1975) utilized only male recombination as an assay for *Mr*, but the *Mr* element also causes distorted transmission frequency from a *Mr* heterozygous male parent. For simplicity, the transmission frequency—the frequency of chromosomes carrying the male recombination element or elements among non-recombinant progeny from a heterozygous male parent—is referred to as the *k* value. If the transmission frequency is Mendelian, half of the progeny will receive one homologue, while the other half will receive the other homologue, resulting in a *k* value of 0.50. Hiraizumi (1971) noted a relationship between male recombination and *k* values in his initial report of the *T-007* male recombination line. He found that the *k* value for the *T-007* chromosome, when transmitted from a heterozygous male parent, was reduced to about 0.42. This relationship was recently studied in more detail by Hiraizumi (1977). He established the presence of a negative correlation between *k* and recombination frequency for the original *T-007* chromosome line containing the *Mr* element. Reduced *k* has also been observed by Kidwell and Kidwell (1976) in male recombination strains which were isolated from natural popu-

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lations in New England.

The purpose of the present study is to examine the relationship between k and male recombination frequency for various second chromosome genotypes derived from the *T-007* chromosome, from which the *Mr* element had been removed through recombination. By using this procedure, the nature of minor elements influencing male recombination and k values can be more effectively analyzed.

MATERIALS AND METHODS

The following chromosome lines of *D. melanogaster* were used in the present study (for full description see Lindsley and Grell, 1968):

(1) *al dp b pr c px sp*: A second chromosome line marked with seven recessive markers, *al* (aristaless, 2L-0.01), *dp* (dumpy, 2L-13.0), *b* (black, 2L-48.0), *pr* (purple, 2L-54.4), *c* (curved, 2R-75.5), *px* (plexus, 2R-100.5), and *sp* (speck, 2R-107.0). This line will be abbreviated as *apl* in this report.

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

(3) *al dp cn bw*: A standard second chromosome line marked with four recessive mutants, *al*, *dp*, *cn*, and *bw*. This line will be abbreviated as *alpha* in this report.

(4) *In(2L+2R)Cy, cn² bw*: A second chromosome line with two large inversions, one in the left arm and one in the right. This chromosome carries the dominant marker *Cy* (Curly, 2L-6.1) and two recessive eye color mutants, *cn²* (an allele of *cn*) and *bw*; it is homozygous lethal. This line will be abbreviated as *Cy* in this report.

(5) *T-007*: A second chromosome line which gives a male recombination frequency of about 1% between *cn* and *bw*. This line was isolated from a natural population at Harlingen, Texas in 1970.

Isolation of the various "recombinant" genotype chromosomes used in the following experiments was achieved essentially as described in Slatko and Hiraizumi (1975) (see Fig. 1): Initially, *T-007/Cy* males were mated to *apl* females. From this mating, virgin F_1 *T-007/apl* females were selected and backcrossed to homozygous *apl* males. Among the progeny of these matings were individuals bearing various recombinant chromosomes which were generated in the heterozygous F_1 females. Those recombinant chromosomes which carried the *pr* region (and were therefore *Mr⁺*) were then assayed for the ability to induce male recombination between the two recessive second chromosome markers *cn* and *bw*. This was done as follows: F_2 males of the appropriate recombinant genotypes were individually mated to *cn bw* females, and from each of these matings, F_3 males were isolated. All F_3 males collected from each F_2 mating were phenotypically "wild type", but genotypically either *apl/cn bw* or *recombinant chromosome type/cn bw*. It was therefore necessary to double-mate individual F_3 male progeny (from each original F_2 recombinant male) first to *apl* females (F_{3a} in Fig. 1(A)) to select males of the appropriate genotypes and then to *cn bw* females (F_{3b} in Fig. 1(A)) to isolate a large number of *recombinant chromosome type/cn bw* males. The resulting F_4 males were genotypically heterozygous for *cn bw* and the identical *recombinant*

Fig. 1. (A) Mating scheme used to isolate recombinant genotypes. (B) Mating scheme used to isolate presumptive non-recombinant *apl* genotype.

chromosome type present in the F₂ parental males. These heterozygous F₄ males were then mated to *cn bw* females to test for the ability to induce male recombination. By this scheme, each of the individually isolated recombinant genotypes could be tested for the ability to induce male recombination between *cn* and *bw*, and in addition, it was possible to obtain a fairly large number of F₄ males (i.e., replications) for each individually isolated recombinant chromosome line. It was impossible to isolate the presumptive non-recombinant *apl* chromosome type from this mating scheme since, in the F₂ matings in Fig. 1(A), it was impossible to distinguish the *apl* chromosomes derived from the F₁ females from the chromosomes derived from the *apl* stock. The mating scheme shown in Fig. 1(B) was used to isolate the presumptive non-recombinant *apl* chromosome lines. The average number of replications (number of matings) per line was 26.

A standard cornmeal-agar food was used throughout these experiments. Parents were kept in vials for seven days, then discarded. For all experimental matings, two females were crossed to an individual male. Progeny counts were completed on the nineteenth day from the date of mating. All experiments were carried out at room temperature, 23°-24°C.

EXPERIMENTS AND RESULTS

For simplicity, in the following sections the male recombination frequency measured between the *cn* and *bw* loci will be designated as θ . Throughout this report, all of the statistical significance tests were performed after applying arcsin transformation to k and θ values.

The relationship between k and θ : A preliminary analysis

The data were first analyzed to determine whether the negative correlation between k and θ observed by Hiraizumi (1971, 1977) for *Mr* genotypes held for all lines of various non-*Mr* recombinant genotypes. An average value of k and θ was computed for each line, and the correlation coefficient between them, r , was computed. The correlation coefficient was highly negative: $r = -0.6522$ (d.f.=94, $t=8.34$, $p<0.001$). Thus, it appears that each line (or group of lines), or each genotype (or group of genotypes) has its own characteristic k and θ values, and these two parameters are correlated negatively. More detailed analyses were performed as shown in the following sections.

The relationship between k and θ among males within lines

Since the number of progeny counted from each F_4 male was not large (on the average, 65), and the number of males within a line which gave recombinant progeny was generally small (only 1 male in several lines), it was unfeasible to work with the correlation coefficient between k and θ among males within lines. Therefore, analysis was carried out in the following manner: for each *recombinant chromosome type*, those lines in which none of the males produced recombinant progeny ($\theta=0.0000$) were excluded from further analysis since they offered no information about the relationship between k and θ . For the remaining lines, males within each line were divided into two groups: Group A included males giving some recombinant progeny, and Group B included males from which no recombinant progeny were observed. The average k value, $\bar{k}$, was computed for each group of each line, and the difference between the groups, D (where $D = \bar{k} \text{ (Group A)} - \bar{k} \text{ (Group B)}$), was computed for each line. The average values of D ($\bar{D}$) among the lines of each genotype are shown in Table 1, and the results of statistical analyses are summarized in Table 2. It can be seen from Table 2 that the value of $\bar{D}$ is homogeneous among lines within genotypes, and also among genotypes. The deviation from zero of the overall average of $\bar{D}$ was highly significant. Thus, for all males tested for any given line, those which gave recombinant progeny had a lower k than those which had no (observed) recombination, and furthermore, the reduction in k was of the same magnitude among lines within a genotype, and also among genotypes.

The following points should be considered here. First, since k value was defined for the second chromosome, and is chromosome specific (not both homologues are

Table 1. Average of D values for lines of 18 genotypes analyzed for the relationship between k and θ among males within lines within genotypes

Genotype	Number of lines analyzed	Average of D	p
<i>al dp b pr c px sp</i>	13	-0.032	*
<i>al dp b pr c px +</i>	3	-0.035	
<i>+ dp b pr c px sp</i>	4	-0.059	*
<i>al dp b pr c + +</i>	3	-0.031	
<i>al dp b pr + + +</i>	4	-0.030	
<i>+ + b pr c + +</i>	3	+0.001	
<i>+ + + pr + + +</i>	2	+0.003	
<i>al + b pr c px sp</i>	1	-0.004	
<i>al dp + pr c px sp</i>	2	-0.016	
<i>+ + + pr c px sp</i>	10	-0.034	*
<i>al dp b pr + px sp</i>	2	-0.061	
<i>+ + b pr c px +</i>	1	+0.094	
<i>+ + b pr + + +</i>	2	-0.033	
<i>+ + + pr c + +</i>	6	-0.019	
<i>+ dp b pr c + +</i>	1	+0.004	
<i>+ + b pr c px sp</i>	8	-0.048	**
<i>al dp + pr + + +</i>	1	-0.135	
<i>al dp + pr c + +</i>	1	+0.029	

* significant at the 5% probability level.

** significant at the 1% probability level.

Table 2. The results of variance analysis of D values after arcsin transformations of k

Source of variation	S.S.	d.f.	M.S.	F	p
Overall D (difference between two groups)	471.4608	1	471.4608	20.73	***
Homogeneity of D					
Among genotypes	386.7023	17	22.7472	1.11	
Among lines within genotypes	1220.0660	49	24.8993	1.22	
Error	32291.8800	1581	20.4250		

*** significant at the 0.1% level.

affected), an element, or elements, involved in some aspect of distortion must locate on the male recombination second chromosome. Second, the homogeneous $\bar{D}$ value among genotypes (including the presumptive non-recombinant genotype, *al dp b pr c px sp*) implies that the element, or elements, responsible for the negative correlation within lines should be located in a region common to all of the genotypes examined, i.e., the *pr* region. This is somewhat surprising since the *pr* region was derived from a laboratory strain, *apl* (*al dp b pr c px sp*), and not from the *T-007* chromosome. This matter will be discussed in more detail later.

Table 3. Correlation coefficients, r , between k and θ among lines within genotypes

Genotype analyzed	r	d.f.	p
<i>al dp b pr c px sp</i>	-0.433	24	*
<i>al dp b pr c px +</i>	-0.693	3	
<i>+ dp b pr c px sp</i>	-0.378	2	
<i>al dp b pr c + +</i>	-0.868	4	*
<i>al dp b pr + px sp</i>	-0.390	2	
<i>+ + b pr + + +</i>	+0.912	1	
<i>+ + + pr c + +</i>	-0.501	4	
<i>+ + b pr c px sp</i>	-0.650	8	*
<i>al dp b pr + + +</i>	-0.697	8	*
<i>+ + b pr c + +</i>	-0.126	1	
<i>+ + + pr c px sp</i>	-0.801	8	**

* significant at the 5% probability level.

** significant at the 1% probability level.

The relationship between k and θ among lines within genotype

Since all the lines within each genotype were independently isolated, the lines were expected to have somewhat different genotypes due to different exchange points between the two appropriate markers. Such differences could result in each line having characteristic k and θ values. If the original *T-007* chromosome, in addition to *Mr* in the *pr*⁺ region, carried another male recombination element, say *X*, located between two particular markers, then some of the recombinant chromosomes generated between those two markers should carry *X*, while others would not, thus generating two "classes" of recombinants within the same genotype. For example, if *X* located halfway between *dp* and *b*, 50% of the lines of the genotype *+ + b pr c px sp* would carry *X*, while the other 50% would not. Assuming that *X* influences both θ and k , these two classes of lines would show different k and θ values. This would result in a correlation (perhaps negative) between k and θ among lines within this genotype. On the other hand, there would be no such correlation between k and θ for such genotypes as *al dp b pr c + +* or *+ + + pr c px sp*, since all lines of the former genotype do not carry *X*, while all lines of the latter genotype carry *X*, regardless of the positions of the exchange points between the two appropriate markers.

Accordingly, the correlation coefficient between k and θ among lines was computed for each genotype with at least three lines. Results are shown in Table 3. It can be seen from Table 3 that r was negative for all genotypes except one (which was not statistically significant). Statistical tests indicated that the r values were homogeneous among genotypes. Thus, all genotypes showed the same degree of negative association between k and θ among lines, making a single element model unlikely, and suggesting the presence of multiple elements distributed along the *T-007* chromosome.

The relationship between k and θ among genotypes

If the hypothesis of multiple elements scattered along the chromosome arms of

Table 4. Recombinant genotypes, their average k and θ values, and the estimated length (in centimorgans) of left arm (L) and right arm (R) segments derived from the *T-007* chromosome

Genotype	Number of lines	Average k	Average θ	L	R
<i>al dp b pr c + +</i>	6	0.485	0.0005	0.0	19.0
<i>+ dp b pr c + +</i>	2	0.485	0.0009	6.5	19.0
<i>al dp b pr c px sp</i>	26	0.481	0.0008	0.0	0.0
<i>al dp b pr + px sp</i>	3	0.478	0.0032	0.0	23.0
<i>al dp b pr + + +</i>	10	0.477	0.0019	0.0	42.0
<i>+ dp b pr c px sp</i>	4	0.468	0.0019	6.5	0.0
<i>al dp b pr c px +</i>	5	0.462	0.0012	0.0	3.25
<i>al dp + pr + + +</i>	1	0.458	0.0009	20.75	42.0
<i>al + b pr c px sp</i>	1	0.454	0.0009	24.25	0.0
<i>+ + b pr c px sp</i>	10	0.445	0.0019	30.75	0.0
<i>al dp + pr c px sp</i>	2	0.441	0.0029	20.75	0.0
<i>+ + b pr c + +</i>	3	0.435	0.0045	30.75	19.0
<i>al dp + pr c + +</i>	1	0.428	0.0025	20.75	19.0
<i>+ + + pr c px sp</i>	10	0.426	0.0028	51.5	0.0
<i>+ + b pr + + +</i>	3	0.424	0.0021	30.75	42.0
<i>+ + b pr c px +</i>	1	0.415	0.0043	30.75	3.25
<i>+ + + pr + + +</i>	2	0.406	0.0041	51.5	42.0
<i>+ + + pr c + +</i>	6	0.392	0.0059	51.5	19.0

T-007 is correct, as was suggested in the previous section, the correlation coefficient between k and θ among genotypes would probably, although not necessarily, be negative also. Since it is possible to have an estimate of the length of the chromosome segment derived from the original *T-007* chromosome for each of the recombinant chromosome genotypes, the "among genotype" analysis will provide some information about the relationship between the length (and position) of chromosome segments from *T-007* and the k and θ values.

Accordingly, a total of 18 genotypes were analyzed. For each genotype, average k and θ values were computed; the correlation coefficient between them (weighted by the number of lines tested for each genotype) was calculated as $r=0.9025$ (d.f.=16, $p<0.01$). Thus, there was a strong negative correlation between k and θ among genotypes.

Further analyses were undertaken to examine the relationship between the length of the chromosome segment derived from the *T-007* chromosome, and the value of k , or θ . To calculate the length of the segment derived from the *T-007* chromosome, it was assumed that each recombinant genotype was the result of a cross-over event occurring halfway between the two appropriate markers. The recombinant genotypes, the number of lines tested for each genotype, average values of k and θ , and the segment length (in centimorgans) originating from *T-007* (where L indicates segment length from the left arm of *T-007*, and R indicates segment length from the right arm of *T-007*) are summarized in Table 4. The genotypes listed in this table are

Table 5. (A) Regression coefficients, b , of k on L (b_k) and θ on L (b_θ) with fixed right arm genotypes
 (B) Regression coefficients, b , of k on R (b_k) and θ on R (b_θ) with fixed left arm genotypes

(A) Right arm fixed

Genotype of right arm	b_k	p	b_θ	p	d.f.
$+/+/+$	-0.00147	**	+0.000031		14
$c+/+$	-0.00183	**	+0.000108	**	16
$cpx/+$	-0.00152		+0.000101		4
$cpxsp$	-0.00137	**	+0.000037	**	51
overall	-0.001496	**	+0.000054	**	85

(B) Left arm fixed

Genotype of left arm	b_k	p	b_θ	p	d.f.
$+/+/+$	-0.00082		+0.000065		16
$+/+b$	-0.00045		+0.000016		15
$+dpb$	+0.00090		-0.000057		4
$al dpb$	-0.00003		+0.000027		48
$al dp+$	+0.00034		-0.000045		2
overall	-0.000187		+0.000025		85

** significant at the 1% probability level.

arranged according to the average k value proceeding from largest to smallest.

It is evident from the table that as k decreases, L increases and θ tends to increase. There is no apparent association between k and R , or θ and R . To further examine this relationship, the regression coefficient of k , and of θ , on L was computed with fixed right arm genotypes. All genotypes were divided into 4 groups according to the genotype of the right arm, and the regression coefficient, b , was computed for each group. Results are shown in Table 5A. For both k and θ , the regression coefficients among the 4 groups were homogeneous, and the overall b value was highly significantly negative for k , and positive for θ . Thus, when L increased, k tended to decrease and θ tended to increase.

The same procedure was applied to R , keeping the left arm genotype constant. Each genotype was relegated to one of five groups according to the genotype of the left arm, and the correlation coefficient was computed for each group. Results are shown in Table 5B. For both k and θ , the value of b was homogeneous among groups, but the overall b was not significantly different from zero. It should be noted, however, that the signs of the overall b of k , and b of θ , on R were the same as those found for L . Apparently factors in the left arm of the $T-007$ chromosome are responsible for more of the variation in k and θ than factors in the right arm.

A set of matings which may serve as a non- Mr control were also made. Several apl females were crossed to a single $cn bw/Cy$ male (parental mating). A single F_1

male, *apl/Cy*, was recovered from this cross and mated to *cn bw* females. From this cross, a number of F_2 *apl/cn bw* males were selected and individually back-crossed to *cn bw* females (F_2 mating). The progeny from this F_2 mating were scored for recombination between *cn* and *bw*. A total of 43 parental matings were scored, with about 10 replications made for each at F_2 . The total number of progeny counted was 26,080; mean k value was 0.507, and the total recombination frequency was 0.00008, representing two males which gave one recombinant progeny each. We shall discuss the results of these control matings later.

DISCUSSION

Two main points have emerged from the present observations. First, in addition to the major element *Mr*, the original *T-007* chromosome contains several male recombination elements (each with relatively minor effects) distributed along the chromosome arms, perhaps being more concentrated in the left arm. These elements cause a decrease in k , and an increase in θ . Second, and of greatest interest, the *pr* region of these genotypes, derived from the *apl* laboratory stock, now appears to have acquired some male recombination activity, causing a negative correlation between k and θ among males within each line.

In order to study this second point in more detail, additional experiments were conducted as follows. In much the same way as shown in Fig. 1, two recombinant chromosome types, $+ + b pr c + +$ and $+ + + pr c + +$, were isolated from *T-007/apl* females. These chromosome lines were back-crossed to *cn bw* females for two to three generations, and then made heterozygous with *alpha* (*alpha=al dp cn bw*) second chromosomes. These heterozygous females were mated to *alpha* males to generate various re-recombinant genotypes.

From the $+ + b pr c + +$ line, 12 re-recombinant chromosome types were isolated and tested. A summary of the data is presented in Table 6. The $\bar{D}$ value (see within line analysis) was heterogeneous ($p=0.025$) among genotypes when all of the genotypes were tested simultaneously, but this was due to heterogeneity between the two groups. Within Group 1 (those genotypes which contained the *pr* region from the $+ + b pr c + +$ chromosome), $\bar{D}$ was homogeneous among genotypes, and also among lines within genotypes. $\bar{D}$ was also homogeneous both among genotypes and among lines within genotypes for Group 2, which consisted of those genotypes without the *pr* region from the $+ + b pr c + +$ chromosome. The overall average value of $\bar{D}$ for Group 1 (-0.029) was highly significantly different from zero (i.e., there was a negative correlation between k and θ among males within lines), while that for Group 2 ($+0.031$) did not significantly deviate from zero (i.e., there was no correlation within lines).

For the second genotype used to isolate re-recombinants, $+ + + pr c + +$, both the number of genotypes and the number of lines isolated and tested were very small, so the amount of information available is limited. Nevertheless, it is interesting to examine this set of data for confirmation of the previous results. Seven genotypes were tested, and their $\bar{D}$ values computed. The results are shown in Table 7. The $\bar{D}$ values within both Group 1 and Group 2 were homogeneous among genotypes and

Table 6. Average D values of re-combinant genotypes generated from $a + + b pr c + +/al dp cn bw$ female

Genotype	Average D	p
<i>Group 1</i>		
$+ + b pr + c +$	-0.022	
$al + b pr + c +$	+0.008	
$al dp b pr + c +$	-0.050	**
$al dp + pr + c +$	-0.024	
$+ + b pr cn + bw$	-0.016	
$+ + b pr + + bw$	-0.003	
$+ + b pr + c bw$	-0.058	**
overall	-0.029	***
<i>Group 2</i>		
$al dp + + + c +$	+0.032	
$al dp + + cn c +$	+0.056	
$al dp + + cn + +$	+0.051	
$+ + + + cn + bw$	+0.015	
$+ + b + cn + bw$	+0.022	
overall	+0.031	

** significant at the 1% probability level.

*** significant at the 0.1% probability level.

Table 7. Average D values of re-combinant genotypes generated from $a + + + pr c + +/al dp cn bw$ female

Genotype	D	p
<i>Group 1</i>		
$+ + + pr + c +$	-0.070	*
$+ + + pr + c bw$	+0.021	
$+ + + pr + + bw$	-0.060	
$al dp + pr + c +$	-0.014	
$al + + pr + c +$	-0.061	
overall	-0.035	
<i>Group 2</i>		
$+ + + + cn + bw$	+0.097	
$al dp + + cn c +$	-0.008	
overall	+0.066	

* significant at the 5% probability level.

among lines within genotypes, but the overall average value of $\bar{D}$ was not significantly different from zero for either group. However, it is perhaps worth noting that the trend is the same as that for the previous set.

The results mentioned above suggest that Mr is able to induce some change in its homologue which renders the pr region capable of some male recombination activity, but this altered region, designated the Mr' region, seems unable to induce such a change in its subsequent homologue. This is similar to some of the *paramutation* phenomena described in maize (Brink 1973). By analogy to paramutation, Mr would be classified as paramutagenic, while Mr^+ would be classified as paramutable. However, in this case, the paramutant, Mr' does not appear to be paramutagenic.

The negative correlation between k and θ "among males within lines" deserves further consideration. Since males within lines should have the same genotype for the second chromosome, the differential k values between the two groups of males within lines (one group including males giving some recombinants and the other group including males giving no recombinants) could be due to either 1) segregating modifiers of Mr' located outside of the second chromosome, or 2) Mr' showing some sort of variegation-like phenomenon.

As mentioned previously, a negative correlation between k and θ was observed

among males within the original *T-007* chromosome line (Hiraizumi 1977). For each of the non-*Mr* (but with *Mr'*) recombinant lines, the negative correlation tends to disappear when *Mr'* is removed by further recombination, even though the ability to induce male recombination may remain (present study). Thus, the "negative correlation" seems to be a characteristic of the *Mr*, or *Mr'*, region, but not of the "minor" elements located outside of this region. The fact that the position of *Mr*, or *Mr'* (i.e., the *pr* region), is adjacent to the centromeric heterochromatin seems to suggest that the second possibility, variegation, is an appropriate explanation. It may be that the male recombination elements are distributed along the second chromosome, and when they locate in the "euchromatic" region, their expression is "stable", but when they are in the vicinity of heterochromatin, they may become "unstable".

Finally, it is perhaps worth considering the data obtained for the presumptive non-recombinant genotype, *al dp b pr c px sp*. A total of 26 independent lines of this genotype were analyzed, of which 13 lines (Group 1) gave some recombinants, while from the other 13 lines (Group 2) none were observed. The overall average *k* value for Group 1 was 0.471, while it was 0.491 for Group 2, and the difference in *k* between these two groups was statistically significant. The average *k* of 0.491 is somewhat smaller than that for the control matings, 0.507, but they are very close. This suggests that the majority of lines, if not all, in Group 2 were those which did not experience the "*Mr*-related mutational change". The "instability" mentioned above suggests that the variance in *k* among males within lines would be increased in those lines where the presumptive *Mr*⁺→*Mr'* change had taken place. Thus, this hypothesis predicts that the variance in *k* among males within a line will increase for those lines in Group 1, while the variance for Group 2 males would stay more or less the same, as in the control matings (presented earlier). Accordingly all of the individual *k* values were subjected to arcsin transformation, and the variance (weighted by the number of progeny per male) was computed for each line. The variances for the 43 control male lines were homogeneous ($X^2_{12}=52.32$, $0.10 > p > 0.05$), giving an overall average variance of 1063 (the expected variance due to binomial distribution is about 821 in this case). The variances for the 13 lines in Group 2 were homogeneous ($X^2_{12}=13.83$, $0.50 > p > 0.25$), and the average was 901, which is close to that of the control; the difference between these two was not statistically significant. The variances for the 13 lines in Group 1 were homogeneous ($X^2_{12}=19.46$, $0.10 > p > 0.05$), and the average was 1314, which is significantly larger than those of control ($F=1.24$, $n_1=360$, $n_2=375$, $p<0.01$), and of Group 2 ($F=1.46$, $n_1=360$, $n_2=240$, $p<0.01$). The average variance of control and Group 2 is about 1000. Comparing this value with the variance for Group 1 indicates that Group 1 has an increase in variance of approximately 31%.

It is very likely that the 13 lines in Group 1 were *Mr'* chromosomes, while the majority of lines in Group 2 were chromosomes which did not experience such a change. This gives a crude estimate of the "*Mr*-related mutation rate" of about 50%. Confirmation of the estimation of this rate comes from the following analysis. The variances in *k* among males within lines were computed in the same way as before for all of the remaining 17 genotypes studied. The overall average value of variances for the group composed of those lines which did not give any recombinant progeny was com-

puted to be 943, while it was 1188 for the group including lines which produced some recombinants (weighted average of the two groups=1119). The former value, 943, is very close to that found for the control lines. The variance for the latter group, 1188, appears to be smaller than that found for Group 1 of the presumptive non-recombinant *apl* genotype (variance=1314). However, this is to be expected since male recombination in some of the lines included in this analysis may not be due to *Mr'*, but instead be the result of the presence of (euchromatic) segments from the original *T-007* chromosome. Let x be the rate of the mutational event, $Mr^+ \rightarrow Mr'$. Then the variance expected from the presumptive non-recombinant *apl* lines, when both *Mr'* and *Mr*⁺ are mixed, would be approximately $1314x + 1000(1-x)$. Equating this to 1119 (average of the two groups, see above), we obtain the value of x approximately equal to 0.4, or 40%. This value is comparable to that estimated based on the presumptive *apl* genotype.

It was of special interest to examine the variances in k among males for those lines in which *Mr'* was removed by recombination (some of the re-recombinants presented earlier). Since the k values, and their variances, are affected largely by the viabilities of the segregating progeny phenotypes, only the matings which gave segregants of *al dp* and *cn bw* phenotypes were selected, and the variances were compared between the *Mr'* and the *Mr*⁺ groups (*Mr*⁺=those genotypes from which *Mr'* had been removed by further recombination). The *Mr'* group gave an average variance within lines of 2100, while the *Mr*⁺ group gave the value of 1452; thus, an increase in variance of about 45% occurs when *Mr'* is present. Note that this value is comparable to that presented previously, i.e., 31%.

SUMMARY

The negative correlation between transmission ratio (k) and male recombination frequency (θ), which was found in the presence of the "major" male recombination element (*Mr*) in the *pr*⁺ region of the *T-007* chromosome, was examined for hypothesized "minor" male recombination elements associated with *T-007*. The data indicate that many minor male recombination elements are distributed along the arms of the *T-007* chromosome. They generally cause a reduction in k and an increase in θ , and their expression seems to be constant among males within each line.

More interestingly, the presence of another type of male recombination element is also suggested by the present observations. It appears that a "*Mr*-related mutational change" (analogous to paramutation) occurs in the *pr* region (therefore, the *Mr*⁺ region) of previously non-male recombination second chromosomes. This change, designated *Mr'*, enables those chromosomes which carry it to induce some male recombination and a distorted transmission ratio. In this case, the expression of *Mr'* seems to vary among males within each line.

It is suggested that the relationship between k and θ among males within the same line is related to the position of the elements in the chromosome. Specifically, minor elements, which locate in largely euchromatic regions of the chromosome, appear to be "stable" in their expression, while the *Mr* and *Mr'* elements, which locate in or

adjacent to the centric heterochromatin, appear to be "unstable". The expression of these elements may be variable among males within each line such that the activities of *Mr* or *Mr'* are stronger (lower *k*, higher *θ*) for some males, while they are weaker (higher *k*, lower *θ*) for other males.

Finally, it is estimated that the rate of the "*Mr*-related mutational change", $Mr^+ \rightarrow Mr'$, is roughly 0.50.

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