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The Modification of Crossing over in Maize by Extraneous Chromosomal Elements[1]

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Summary. Five regions of the maize genome were tested for their response to endogenous factors influencing recombination. These included heterochromatic B chromosomes and abnormal chromosome 10 as well as the sex in which recombination occurred.

The frequency of recombination in the proximal A_2-*Bt* and *Bt-Pr* segments of chromosome 5 was increased in the presence of B chromosomes, with the male meiocytes showing a greater response than the female meiocytes. In addition, experiments involving 0, 1, 2 and 4 B's revealed a dosage effect of B chromosomes on crossing over in chromosome 5. Recombination in the proximal *Wx*-Gl_{15} interval of chromosome 9 was found to be slightly higher than normal in male flowers when two B chromosomes were present. This increase was accompanied by a decrease in the adjacent *Sh-Wx* segment. Crossing over in the distal *C-Sh* segment and in the *C-Sh-Wx*-Gl_{15} regions of female flowers was unaffected by B's.

Comparisons of plants heterozygous for abnormal chromosome 10 (K10 k10) and homozygous for the standard chromosome 10 (k10 k10) showed that abnormal 10 greatly enhances crossing over in the A_2-*Bt* and *Bt-Pr* segments of chromosome 5. In contrast to the finding with B's, the effect is greater in female than in male sporocytes. K10 showed no significant effect on recombination in the *C-Sh-Wx*-Gl_{15} region of chromosome 9 except in male sporocytes, where there was a slight increase in the *Sh-Wx* region of 0 B K10 k10 plants and a possible interaction with B chromosomes to raise the level of recombination between *Wx* and Gl_{15}. The fact that the regions adjacent to the centromere of chromosome 9 show little or no response to the presence of K10 indicates that the proximal heterochromatin of this chromosome differs qualitatively from that of other maize chromosomes. This conclusion is supported by a comparison of the effects of B chromosomes, K10 and sex on crossing over in chromosomes 5 and 9.

Introduction

Supernumerary chromosomal elements can modify crossing over in a number of different organisms. In recent years, B chromosomes have been reported to alter chiasma frequencies, the variation in chiasma frequencies and/or the distribution of chiamata in the A chromosomes of *Myrmeleotettix maculatus* (Barker 1960; John and Hewitt 1965 a, b; and Hewitt and John 1967), *Melanoplus differentialis differentialis* (Abdel-Hameed *et al.* 1970), rye (Jones and Rees 1967; Zečević and Paunović 1969), *Lolium* (Cameron and Rees 1967), *Puschkinia libanotica* (Barlow and Vosa 1968), maize (Ayonoadu and Rees 1968) and *Triticum speltoides* (Simchen *et al.* 1971).

The use of linkage experiments to study the effects of B chromosomes on crossing over has so far been confined to maize. Hanson (1965, 1969) discovered that B's caused a slight increase in the amount of recombination in the Gl_6–Lg_2–A_1 region of chromosome 3 and the Yg_2–C_1–Sh_1–*Wx* region of chromosome 9. A more striking effect of B chromosomes was reported by Rhoades (1968). He tested recombination in plants homozygous for a transposition (Tp9), in which a piece of chromosome 3 was inserted into the short arm of chromosome 9, and heterozygous for the flanking markers *C* and *Wx*. When B's were present, the amount of recombination between *C* and *Wx* was more than doubled, the increase being correlated with a reduction in crossing over in the adjacent *Yg–C* segment. Ward (1972) has recently obtained evidence that this enhancement is caused by both euchromatic and heterochromatic regions of the B chromosome.

Chromosomes possessing an extra piece of chromatin have been found to enhance crossing over. Generally, the supernumerary segment is partially or completely heterochromatic and is located at or near the end of the chromosome. Of these chromosomes, the most intensively studied is the abnormal chromosome 10 (K10) which is found in some stocks of maize. It differs from the normal chromosome 10 (k10) in the distal part of the long arm, where it has an additional, largely heterochromatic, segment. Abnormal 10 increases recombination in the proximal Gl_6–Lg_2 segment of chromosome 3 (Rhoades and Dempsey 1957, 1966) while in chromosome 10 itself, enhanced crossing over in the centromere regions (Miles 1970; Rhoades and Dempsey 1970) is accompanied by a decrease in the distal segment of the long arm (Rhoades 1942, 1952; Kikudome 1959). Marked increases in chiasma and recombination frequencies due to K10 were observed in plants heterozygous for structural rearrangements by Dempsey and Rhoades (1961) and Rhoades and Dempsey (1966). In addi-

[1] Dedicated to Dr. M. M. Rhoades on the occasion of his seventieth birthday.

tion, interaction between abnormal 10 and heterochromatic knobs can influence crossing over in the vicinity of the knobs (Kikudome 1959; Rhoades and Dempsey 1966).

Extra chromosomal segments have also been reported to increase recombination in *Drosophila melanogaster* (Suzuki 1963), *Phryne cincta* (Wolf 1963, 1968) and *Chorthippus parallelus* (John and Hewitt 1966; Hewitt and John 1968; and Westerman 1969). In each case, the effect was an interchromosomal one.

The purpose of the study reported here was to determine the effects of B chromosomes and abnormal 10 on crossing over in the proximal regions of chromosomes 5 and 9.

Materials and Methods

Genetic Markers

The A_2–*Bt*–*Pr* region of chromosome 5 spans the centromere, with A_2 at a position between 0.13 and 0.23 in the short arm (Phillips 1969); *Bt* is in the long arm and close to the centromere (Rhoades 1936), while the cytological position of *Pr* is approximately 0.4 in the same arm (Phillips 1969).

The chromosome 9 markers C_1, Sh_1 and *Wx* are located distally on the short arm, with *C* at approximately 0.75 (in the fifth of 20 chromomeres, McClintock 1943), *Sh* distal to 0.7 (Li 1950) and *Wx* approximately in the middle of the arm (McClintock 1951); Gl_{15} is in the long arm distal to 0.1 (Dempsey and Smirnov 1964).

Determination of Recombination Values

The control and experimental plants used in all testcrosses were sibs. Recombination was tested in both male and female flowers. To ensure sufficiently large progenies when the less vigorous tester stocks were used as female parents in testcrosses, each F_1 plant was usually crossed to two tester plants; in such cases the recombination frequency was based on the combined data from the two ears. In chromosome 5 tests, recombination in the *Bt*–*Pr* region was calculated from the A_2 classes only, since a_2 kernels lack aleurone color and cannot be directly classified for *Pr* and *pr*.

It was necessary to correct the recombination values when *C*–*Sh*–*Wx*–Gl_{15}/*c*–*sh*–*wx*–gl_{15} plants were used as males in testcrosses. Sprague (1932) found that occasionally sperm from one pollen tube fertilizes the egg, while sperm from another pollen tube may unite with the polar nuclei. As a result, the genetic constitution of embryo and endosperm may differ. The pollen grains from *C*–*Sh*–*Wx*–Gl_{15}/*c*–*sh*–*wx*–gl_{15} plants include a mixture of genotypes. Since Gl_{15} is a seedling character, *C* and *Sh* endosperm traits, and *Wx* more easily scored in the endosperm than in mature plants, heterofertilization could cause errors in the calculation of recombination percentages when one trait is classified in the endosperm and the other in the seedling. Recombination between *C*, *Sh* and *Wx* is not affected by heterofertilization because all three characters are scored as endosperm phenotypes.

Recombination values for the *Wx*–Gl_{15} segment were corrected by the following method. The endosperms of kernels produced in testcrosses of *Wx*–Gl_{15}/*wx*–gl_{15} male parents were classified for *Wx*. After germination, the apparent recombinant plants were marked in the seedling stage, i.e. Gl_{15} seedlings in the *wx* class and gl_{15} seedlings in the *Wx* class. The number of actual recombinants was obtained at maturity by scoring the pollen for *Wx*. The difference between the apparent and actual recombinants is a means of obtaining the frequency of plants misclassified as recombinants because of heterofertilization. The correction was carried out by subtracting the misclassified plants from the recombinant classes and adding them to the parental classes. The proportion (p) of parental types erroneously classified as recombinants was then calculated according to the formula

$$p = \frac{\text{No. of misclassified plants}}{\text{Total no. of apparent parentals} + \text{no. of misclassified plants}}.$$

On the assumption that the same proportion of crossover plants was incorrectly scored as being nonrecombinant, the remaining plants in the recombinant classes represent a fraction $(1 - p)$ of the true recombinants. The crossover classes were divided by $(1 - p)$ to give corrected numbers. The value of p is a slight underestimate but the error is so small as to have a negligible effect on recombination frequencies.

Statistical Analyses

Within groups, the homogeneity of the recombination fractions for each region was determined by means of chi-square contingency tables, comparing the numbers of recombinant vs. nonrecombinant individuals. In the absence of significant heterogeneity, the data were pooled. Differences between groups were tested for significance using 2×2 chi-square contingency tables.

If one or both groups showed significant heterogeneity for recombination in a particular region, the following procedure was adopted. The recombination percentages for the individual families within each group were converted to angles, using the arcsin transformation. The values were not weighted because no correlation was observed between family size and recombination frequencies, and also because the family totals from which they were calculated were large, being greater than 100 in nearly all cases. Where the variances calculated from the transformed data were homogeneous according to the *F*-test, means were compared using the formula

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}},$$

where s is an estimate of the standard deviation based on both samples jointly, $\bar{x}_1$ and $\bar{x}_2$ are the means and n_1 and n_2 the numbers of recombination values in the first and second groups, respectively.

If the variances differed significantly, the formula used was

$$t' = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where s_1^2 and s_2^2 are the variances of the two groups. Values of t' larger than

$$\frac{\frac{s_1^2}{n_1} t_1 + \frac{s_2^2}{n_2} t_2}{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

were judged to be significant, t_1 and t_2 being the values of Student's t for $n_1 - 1$ and $n_2 - 1$ degrees of freedom at the chosen level of significance.

Recombination percentages in bold print in the tables of results represent the pooled values for the families concerned but signify that heterogeneity was present and significance was tested by calculating t or t'.

Results and Discussion

Effect of B chromosomes

The effects of B chromosomes on crossing over in the proximal regions of chromosome 5 are summarized

Table 1. *Crossover values from an F_1 family having the genotype A_2-Bt-Pr/a_2-bt-pr and segregating for the presence of B chromosomes and abnormal 10*

Chromosome constitution	No. of plants	No. of progeny	Percent recombination	
			A-Bt	*Bt-Pr*
F_1 used as female parents				
0 B's k10 k10	9	3721	6.1	18.1
2 B's k10 k10	9	3848	**8.1***	21.1*
0 B's K10 k10	9	3368	**16.9****	**32.9****
2 B's K10 k10	9	3882	18.8**	**36.2****
F_1 used as male parents				
0 B's k10 k10	9	3069	**12.1**	**27.5**
2 B's k10 k10	9	3437	**18.8****	**31.5**
0 B's K10 k10	9	3448	**25.1****	38.1**
2 B's K10 k10	9	3329	**25.5****	40.7**

*/** Significantly different from 0 B's k10 k10 value for the same sex, at 5% and 1% levels, respectively.

Table 2. Effect of B chromosomes on crossing over in the A_2-*Bt* and *Bt-Pr* regions of chromosome 5

Family No.	No. of B chromosomes	No. of plants	No. of progeny	Percent recombination	
				A-Bt	*Bt-Pr*
F_1 used as female parents					
537	0	8	2897	5.9	15.9
	1	8	2797	**6.9**	18.1
	2	10	3197	**8.8***	21.7**
536	0	4	1529	8.2	23.0
	2	10	3493	**9.6**	**22.3**
	4	10	3527	**10.7**	**25.3**
F_1 used as male parents					
537	0	10	4675	**11.1**	**26.6**
	1	8	4505	**15.1**	**31.8**
	2	10	5814	**17.7***	**34.6***
536	0	5	2764	13.4	26.0
	2	10	4812	**18.1***	**35.8****
	4	10	4829	**23.6****	**37.6****

*/** Significantly different from no-B value for the same sex, at 5% and 1% levels, respectively.

in Tables 1 and 2. A comparison of the data for the B vs. no-B classes shows clearly that the presence of B chromosomes results in increased crossing over, despite the presence of heterogeneous crossover values within most of the classes and the fact that some of the differences are not statistically significant. It also appears that the increases are proportionally larger in the short A_2–*Bt* segment than in the longer *Bt–Pr* interval. The proximal heterochromatin surrounding the *Bt* locus constitutes a larger proportion of the A_2–*Bt* than of the *Bt–Pr* region, which includes a greater amount of more distally located chromatin. Thus, the effect of the B's may be more pronounced in the proximal regions close to the centromere.

Rhoades (1941) discovered that recombination in the proximal regions of chromosome 5 is higher in male than in female gametes; the values for the no-B classes agree with this finding. Upon the introduction of B chromosomes, the absolute increases in recombination are greater in the male sporocytes, resulting in an accentuation of the differences between the values for the two sexes.

An examination of Table 2, where the number of B chromosomes ranges from none to four, reveals a dosage effect of B's on crossing over in two closely related families. The total amounts of crossing over for both regions in plants used as males are: no-B, 38—39%; 1 B, 47%; 2 B's, 52–54%; and 4 B's, 61%, while the increases in plants used as females are smaller. Dosage effects of B chromosomes have been found for the *C–Wx* region of Tp9 homozygotes (Rhoades 1968) and the Gl_6–Lg_2 segment of chromosome 3 (Rhoades, unpublished).

The dosage effects on chiasma frequencies in microsporocytes reported by Ayonoadu and Rees (1968) and on recombination in the Gl_6–*Lg*–A_1–*Et* (chromosome 3) and *Yg–C–Sh–Wx* (chromosome 9) regions studied by Hanson (1965, 1969) were comparatively small.

Increasing numbers of B chromosomes have been observed to have a zigzag effect on recombination in rye (Jones and Rees 1967), *Listera* (Vosa and Barlow 1970) and maize (Chang and Kikudome 1971a, b). In the latter case, crossing over was increased in the *Bz–Wx* region and decreased in the distal *Yg–Sh* segment when odd numbers of B's were present and chromosome 9 was homozygous for a small terminal knob. Even numbers of B chromosomes had no effect. When chromosome 9 was heterozygous for terminal knobs of different sizes, odd numbers of B's

Table 3. *Crossover values from an F_1 family having the genotype C–Sh–Wx–Gl_{15}/c–sh–wx–gl_{15} and segregating for the presence of B chromosomes and abnormal 10. Male values for the Wx–Gl_{15} region are corrected for heterofertilization. Uncorrected values are shown in parentheses*

Chromosome constitution	No. of plants	No. of progeny	Percent recombination		
			C-Sh	*Sh-Wx*	*Wx-Gl*
F_1 used as female parents					
0 B's k10 k10	8	2735	4.3	18.8	**6.7**
2 B's k10 k10	8	2744	3.5	19.1	**8.9**
0 B's K10 k10	8	2690	4.2	19.7	7.1
2 B's K10 k10	8	2667	4.6	21.0*	**9.1***
F_1 used as male parents					
0 B's k10 k10	8	2468	4.2	23.3	8.9 (9.8)
2 B's k10 k10	8	2291	4.6	19.6**	14.1** (15.3)
0 B's K10 k10	8	2025	4.3	26.6*	**10.5** (11.5)
2 B's K10 k10	8	2448	4.5	21.0	17.7** (19.2)

*/** Significantly different from the 0 B k10 k10 value for the same sex, at 5% and 1% levels, respectively.

Table 4. *Analysis of recombinants from the testcross c–sh–wx–gl_{15} ♀× × C–Sh–Wx–Gl_{15}/c–sh–wx–gl_{15} ♂. Frequencies of nonrecombinants classified as recombinants when scoring endosperms for Wx and seedlings for Gl_{15}*

Chromosome constitution	No. of families tested	Apparent No. of recombinants*	Actual No. of recombinants**	No. of non-recombinants in column 3
0 B's k10 k10	7	121	108	13
2 B's k10 k10	6	169	151	18
2 B's K10 k10	6	236	216	20
Total	19	526	475	51

* Based on classification for *Wx* in endosperms of progeny kernels.
** Based on classification for *Wx* in pollen of progeny grown to maturity.

enhanced recombination in both regions. However, the chromosome 5 results presented in Table 2 do not show any clear evidence of a zigzag effect.

The results from the chromosome 9 testcrosses are summarized in Table 3. There is no evidence from either the male or female gametes that B's have any effect on recombination in the *C–Sh* region, which is the most distal of the three studied. A similar statement can be made for the *Sh–Wx* region in the female gametes. Plants with 2 B chromosomes showed lower crossover values for *Sh–Wx* in the male gametes than did their 0 B sibs. This may represent a compensatory decrease for the enhanced crossing over in the proximal *Wx–*Gl_{15} segment since the total recombination in the *Sh–*Gl_{15} interval is the same in 0 B and 2 B plants within the k10 and K10 groups.

Where F_1 plants were used as males, recombination between *Wx* and Gl_{15} appeared to be higher in plants with B chromosomes (2 B's k10 k10 and 2 B's K10 k10) than in no-B sibs. Since the differences could have been caused by a change in the frequency of heterofertilization induced by B chromosomes rather than by modification of crossing over, plants were grown in the field and the frequencies of apparent and actual recombinants were determined as described in the section on Materials and Methods. A 2×2 chi-square test failed to reveal any significant difference between the proportions of misclassified nonrecombinants to actual recombinants in the 0 B k10 k10, 2 B k10 k10 and 2 B K10 k10 classes (Table 4). The data were therefore pooled to obtain a common correction factor and the recombination values for the *Wx–*Gl_{15} region were reduced by the fraction 51/526 representing the proportion of non-recombinants among plants originally classified as recombinants on the basis of endosperm and seedling traits.

Table 3 reveals that B chromosomes do in fact enhance crossing over in the *Wx–*Gl_{15} region by about 58% in male sporocytes of the 2 B k10 k10 class and that the increase is not the result of heterofertilization. As was true for chromosome 5, the megasporocytes show little, if any, increase attributable to B's. Hanson's (1965, 1969) data also show a differential effect in the two sexes; in plants with one to four B's recombination was increased in the proximal Gl_6–*Lg* region of chromosome 3 by 6% and 25% in female and male sporocytes, respectively. Another feature of Table 3 is that there is considerable heterogeneity of crossover values within groups for the proximal *Wx–*Gl_{15} interval but none in the *C–Sh* and *Sh–Wx* segments.

From the above experiments and from the recombination studies of Hanson and Rhoades, it may be concluded that B chromosomes increase the frequency of crossing over in the proximal regions of three chromosomes (3, 5 and 9) and that they have a dosage effect. In the more distal regions which have been tested so far, B's influence recombination to a lesser degree or not at all. The *C–Wx* region in the distal half of 9 S appears to be an exception when the effect of B's is followed in Tp9 homozygotes (Rhoades 1968). It is true that B's more than doubled the amount of recombination in the *C–Wx* region of Tp 9 homozygotes and caused a compensatory reduction in the adjacent Yg_2–*C* interval at the tip of the short arm. However, in these plants the *C–Wx* region is expanded by an insertion of chromatin from chromosome 3 and the region cannot be considered typical. A B chromosome-induced redistribution of exchanges from distal to more centrally located regions in the short arm of chromosome 9 has been noted by Hanson, Rhoades and Chang and Kikudome. Shifts in the distribution of chiasmata have also been reported in rye (Jones and Rees 1967) and *Puschkinia libanotica* (Barlow and Vosa 1970). To what extent the increases caused by B's in the proximal regions of maize are accompanied by corresponding decreases in other portions of the genome, is not known at present. The increased chiasma frequencies in pollen mother cells when B chromosomes are present (Ayonoadu and Rees 1968) do not favor compensation alone; increased chiasmata would be expected following enhancement in proximal regions only if crossing over remains essentially unchanged in other regions.

Effect of Abnormal Chromosome 10

The effects of abnormal 10 on crossing over in chromosome 5 are more striking than those of B chromosomes. In the presence of K10, crossing over is greatly increased in both the A_2–*Bt* and *Bt-Pr* regions in the sporocytes of both sexes (Table 1). A similar enhancement has been reported by Robertson (1968). However, in contrast to the findings with B's, abnormal 10 is more effective in female than in male cells, the increases in the total amount of recombination between A_2 and *Pr* being approximately 106% and 60% respectively. Here, as before, the A_2–*Bt* segment is the more responsive of the two intervals. When B chromosomes and abnormal 10

are both present, the increase in recombination found in the female gametes is the sum of the increments contributed by each factor, i.e. the enhancement is additive. A contrary situation apparently exists in the male gametes where the increase in recombination is less than additive in plants with K10 and B's. However, too much reliance cannot be placed on slight differences in recombination values, which are subject to sampling errors as well as variation in exchange frequency.

The results obtained for chromosome 9 (Table 3) differ strikingly from the chromosome 5 data. Surprisingly, abnormal 10 has no significant effect on crossing over except in male sporocytes, where there is a small (14%) increase in the *Sh–Wx* region of 0 B K10 k10 plants and a possible interaction with B chromosomes in 2 B K10 k10 plants to raise the level of recombination between *Wx* and Gl_{15}. No information is available on the frequency of heterofertilization in 0 B K10 k10 plants used as males. Recombination percentages for the Wx–Gl_{15} region were corrected for heterofertilization by means of the factor obtained from the 0 B k10 k10, 2 B k10 k10 and 2 B K10 k10 sibs, on the assumption that it did not differ greatly from the corresponding value in the 0 B K10 k10 class.

In Kikudome's (1959) study of the short arm of chromosome 9, plants heterozygous for the terminal *wd* deficiency and a small, medium-sized or large terminal knob were used. Upon the addition of K10, recombination between *wd* and *wx* was raised from 26.9%, 17.7% and 12.7% to 31.5%, 26.8% and 30.3%, respectively, for the three kinds of knobbed-knobless heterozygotes. There was thus an interaction between K10 and the knobs, with the heterozygote containing the smallest knob showing the least increase in recombination. An increase of only 16% in the total amount of recombination in the *Yg–C–Sh–Wx* region was induced by K10 plants heteromorphic for the medium-sized and small knobs. It is possible that K10 has no effect on crossing over in the distal half of 9 S except when the homologues are heteromorphic. There is relatively little change in recombination when the homologues differ only slightly in knob size. Presumably in the latter compounds crossing over may already occur at a maximum rate which cannot be further enhanced by K10. Sporocytes from five plants of the F_1 family in Table 3 were examined and the two homologues proved to be very similar morphologically, being heterozygous for a small and a very small terminal knob or chromomere. Thus, the absence of enhancement by K10 in the distal regions of 9 S may be due to the structural similarity of the two homologues.

Since abnormal 10 has been shown to increase crossing over in the centromere regions of 3 L (Rhoades and Dempsey 1957, 1966), chromosome 10 (Miles 1970, Rhoades and Dempsey 1970) and chromosome 5, the question arises as to why the proximal regions of chromosome 9 show little or no response to the presence of K10. The regions surrounding the centromeres of maize chromosomes are heterochromatic and normally show low rates of crossing over per unit of pachytene length. When the homologous chromosomes are structurally similar, the proximal regions are the most responsive to abnormal 10 and considerable enhancement of recombination occurred in these segments in chromosome 5. The heterochromatic centromere regions of Drosophila are also susceptible to factors affecting crossing over. The influence of environmental agents such as temperature, nutrition, aging of the female, and irradiation is greatest on exchanges adjacent to the centromere (Swanson 1957). The consistent behavior of proximal heterochromatin in response to crossover inducers in both maize and Drosophila emphasizes the uniqueness of the Wx–Gl_{15} region of chromosome 9. It is proposed that the heterochromatin of chromosome 9 differs qualitatively from that of other maize chromosomes and a review of the effects of B's, K10 and sex on crossing over in different parts of the genome supports this contention.

A summary of the results on crossing over in chromosomes 5 and 9 is presented in Table 5. In each of the regions tested, three variables are present: the number of B chromosomes (0 vs. 2), inclusion of abnormal 10 in the genome, and the sex in which recombination occurs. All of these may affect crossing over and the data indicate that different parts of the genome respond differently to these factors. Crossing

Table 5. *Summary of crossing over in chromosomes 5 and 9 as influenced by B chromosomes, abnormal 10 and sex. From Tables 1 and 3*

Factor affecting recombination	Percent increase in recombination									
	A_2-*Bt*		*Bt-Pr*		*C-Sh*		*Sh-Wx*		*Wx-Gl*	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
B's*	32.8	55.4	16.6	14.5	−18.6	9.5	1.6	−15.9	32.8	58.4
K10*	177.0	107.4	81.8	38.5	− 2.3	2.4	4.8	14.2	6.0	18.0
Male vs. female meiocytes**	98.4		51.9		−2.3		23.9		32.8	

* 0 B k10 k10 class for the same sex used as standard.
** For 0 B k10 k10 plants.

over in the *C–Sh* region in a euchromatic segment of 9S is not markedly affected by addition of B's or K10; the same rate of exchange was found in both sexes. Thus, the chromatin lying between the *C* and *Sh* genes is stable and insensitive to the three variable factors. Only when structural differences cause a physical interruption in pairing is crossing over in the *C–Sh* interval susceptible to modification.

An intermediate degree of responsiveness to crossover inducers is exhibited by the *Wx–Gl*$_{15}$ proximal region. Crossover values are higher in the presence of B's and in the male gametes, and there is also a somewhat higher rate of exchange in plants with K10. The proximal regions of chromosome 5 were by far the most sensitive. There were sizeable increases in crossing over in the male gametes and in K10 k10 plants, and consistently higher values were obtained when B's were present. The correlation of the degree of response to all three factors influencing crossing over indicates that qualitative differences in chromatin exist and that at least three types of response can be distinguished.

It has been suggested that the increased recombination in the proximal regions induced by K10 is due to a relaxation of chromatin which is normally tightly coiled and unable to undergo close synapsis. More intimate pairing is found between structurally dissimilar homologues upon introduction of K10 into the genome and a higher rate of exchange follows (Rhoades and Dempsey 1966). No cytological studies are available on the mechanism by which B's operate to increase chiasma frequencies, but there is some evidence that the role of B's may be different from that of K10. Studies of crossover enhancement in chromosome 5 indicate that B's are more effective in the microsporocytes while K10 produces higher values in the megasporocytes. In addition, B chromosomes have a dosage effect whereas the available data indicate that K10 does not (Rhoades and Dempsey 1966).

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Literature

1. Abdel-Hameed, F., Rootham, D. L., Flinn, R. R.: Structural and numerical aberrations in natural populations of the grasshopper, *Melanoplus differentialis differentialis* (Abstract). Genetics **64**, Suppl., 1 (1970). — 2. Ayonoadu, U., Rees, H.: The influence of B chromosomes on chiasma frequencies in Black Mexican sweet corn. Genetica **39**, 75–81 (1968). — 3. Barker, J. F.: Variation of chiasma frequency in and between natural populations of Acrididae. Heredity **14**, 211–214 (1960). — 4. Barlow, P. W., Vosa, C. G.: Synthesis of DNA and chiasma formation in the chromosomes of *Puschkinia libanotica* (Abstract). Heredity **23**, 625 (1968). — 5. Barlow, P. W., Vosa, C. G.: The effect of supernumerary chromosomes on meiosis in *Puschkinia libanotica* (*Liliaceae*). Chromosoma **30**, 344–355 (1970). — 6. Cameron, F. M., Rees, H.: The influence of *B* chromosomes on meiosis in *Lolium*. Heredity **22**, 446–450 (1967). — 7. Chang, C. C., Kikudome, G. Y.: Is the effect of the B chromosome on recombination primarily an additive one? Maize Genet. Coop. News Letter **45**, 134–136 (1971a). — 8. Chang, C. C., Kikudome, G. Y.: Cytogenetic studies on a maize chromosome 9 knob of Mexican origin (Abstract). Genetics **68**, Suppl., s11 (1971b). — 9. Dempsey, E., Rhoades, M. M.: Evidence for the chiasma theory of metaphase pairing. Maize Genet. Coop. News Letter **35**, 65–66 (1961). — 10. Dempsey, E., Smirnov, V.: Cytological location of gl_{15}. Maize Genet. Coop. News Letter **38**, 71–73 (1964). — 11. Hanson, G. P.: Some studies of crossing over in maize as influenced by accessory chromosomal elements. Ph. D. thesis, Indiana University (1965). — 12. Hanson, G. P.: B-chromosome-stimulated crossing over in maize. Genetics **63**, 601–609 (1969). — 13. Hewitt, G. M., John, B.: The B-chromosome system of *Myrmeleotettix maculatus* (Thunb.). III. The statistics. Chromosoma **21**, 140–162 (1967). — 14. Hewitt, G. M., John, B.: Parallel polymorphism for supernumerary segments in *Chorthippus parallelus* (Zetterstedt). I. British populations. Chromosoma **25**, 319–342 (1968). — 15. John, B., Hewitt, G. M.: The B-chromosome system of *Myrmeleotettix maculatus* (Thunb.). I. The mechanics. Chromosoma **16**, 548–578 (1965a). — 16. John, B., Hewitt, G. M.: The B-chromosome system of *Myrmeleotettix maculatus* (Thunb.). II. The statics. Chromosoma **17**, 121–138 (1965b). — 17. John, B., Hewitt, G. M.: A polymorphism for heterochromatic supernumerary segments in *Chorthippus parallelus*. Chromosoma **18**, 254–271 (1966). — 18. Jones, R. N., Rees, H.: Genotypic control of chromosome behaviour in rye. XI. The influence of *B* chromosomes on meiosis. Heredity **22**, 333–347 (1967). — 19. Kikudome, G. Y.: Studies on the phenomenon of preferential segregation in maize. Genetics **44**, 815–831 (1959). — 20. Li, C. H.: A pericentric inversion in chromosome 9. Maize Genet. Coop. News Letter **24**, 32–36 (1950). — 21. McClintock, B.: Maize Genetics. Carnegie Inst. Wash. Yearbook **42**, 148–152 (1943). — 22. McClintock, B.: Chromosome organization and genic expression. Cold Spring Harbor Symp. Quant. Biol. **16**, 13–47 (1951). — 23. Miles, J. H.: Influence of modified K10 chromosomes on preferential segregation and crossing over in *Zea mays*. Ph. D. thesis, Indiana University (1970). — 24. Phillips, R. L.: Recombination in *Zea mays* L. I. Location of genes and interchanges in chromosomes 5, 6 and 7. Genetics **61**, 107–116 (1969). — 25. Rhoades, M. M.: A cytogenetic study of a chromosome fragment in maize. Genetics **21**, 491–502 (1936). — 26. Rhoades, M. M.: Different rates of crossing over in male and female gametes of maize. Jour. Amer. Soc. Agron. **33**, 603–615 (1941). — 27. Rhoades, M. M.: Preferential segregation in maize. Genetics **27**, 395–407 (1942). — 28. Rhoades, M. M.: Preferential segregation in maize. In: Heterosis. Ed. by J. W. Gowen, pp. 66–80. Ames, Iowa: Iowa State College Press 1952. — 29. Rhoades, M. M.: Studies on the cytological basis of crossing over. In: Replication and recombination of genetic material. Ed. by W. J. Peacock and R. D. Brock, pp. 229–241. Canberra: Aust. Acad. Sci. 1968. — 30. Rhoades, M. M., Dempsey, E.: Further studies on preferential segregation. Maize Genet. Coop. News Letter **31**, 77–80 (1957). — 31. Rhoades, M. M., Dempsey, E.: The effect of abnormal chromosome 10 on preferential segregation and crossing over in maize. Genetics **53**, 989–1020 (1966). — 32. Rhoades, M. M., Dempsey, E.: Crossing over and preferential segregation in chromosome 10 induced by abnormal chromosome 10. Maize Genet. Coop. News Letter **44**, 57–58 (1970). — 33. Robertson, D. S.: Increased crossing over

in chromosome 5 in the presence of abnormal 10. Maize Genet. Coop. News Letter **42**, 89 (1968). — 34. Simchen, G., Zarchi, Y., Hillel, J. L.: Supernumerary chromosomes in the second outbreeding species of the wheat group. Chromosoma **33**, 63—69 (1971). — 35. Sprague, G. F.: The nature and extent of heterofertilization in maize. Genetics **17**, 358—368 (1932). — 36. Suzuki, D. T.: Interchromosomal effects on crossing over in *Drosophila melanogaster*. II. A reexamination of X chromosome inversion effects. Genetics **48**, 1605—1617 (1963). — 37. Swanson, C. P.: Cytology and Cytogenetics, Chapter 8. London: Macmillan and Co. 1957. — 38. Vosa, C. G., Barlow, P. W.: The effect of supernumerary chromosomes on meiosis in *Puschkinia libanotica* (*Liliaceae*). Chromosoma **30**, 344—355 (1970). — 39. Ward, E. J.: Localization of genetic factors in the B chromosome of maize. Ph. D. thesis, Indiana University (1972). — 40. Westerman, M.: Parallel polymorphism for supernumerary segments in *Chorthippus parallelus* (Zetterstedt). II. French populations. Chromosoma **26**, 7—21 (1969). — 41. Wolf, B. E.: Kontrolle des crossing over vom temperaturbedingten Allozykliegrad und vom α-Heterochromatin des X-Chromosoms bei *Phryne cincta*. Chromosoma **13**, 646—701 (1963). — 42. Wolf, B. E.: Adaptiver chromosomaler Polymorphismus und flexible Kontrolle der Rekombination bei *Phryne cincta* (Diptera, Nematocera). Zool. Beitr. (Berlin) **14**, 125—153 (1968). — 43. Zečević, L., Paunović, D.: The effect of B-chromosomes on chiasma frequency in wild populations of rye. Chromosoma **27**, 198—200 (1969).

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