

# B-CHROMOSOME-STIMULATED CROSSING OVER IN MAIZE<sup>1</sup>

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ACCESSORY, supernumerary, or B-chromosomes are present in numerous species of plants and animals. Usually individuals possessing them exhibit little physiological evidence of their presence. If the B-chromosomes alter the normal phenotype, the effect is most commonly unfavorable to the host (BATTAGLIA 1964). These chromosomes, which are usually smaller than the normal "A" chromosomes often exhibit a numerical variability in different cells, tissues, individuals, or populations due to abnormal behavior at mitosis and/or meiosis. In many species of plants the number of B-chromosomes increases in the progeny (BATTAGLIA 1964).

RANDOLPH (1941), LONGLEY (1927), ROMAN (1947, 1948), and BLACKWOOD (1956) have described the behavior of the B-chromosomes in maize throughout the life cycle and noted that the progenies of individuals with B-chromosomes tend to have higher numbers of B's than the parents. ROMAN's studies showed that nondisjunction of the B-chromosomes at the second pollen grain mitosis could result in one sperm nucleus containing no B's while the other might have two or more B-chromosomes. This, followed by preferential fertilization of the egg by the hyperploid sperm nucleus would yield offspring with increased numbers of B-chromosomes as observed by ROMAN (1948a). The accumulation of B's in maize brought about by nondisjunction and preferential fertilization is compensated in nature by the decreased fertility and vigor of plants possessing large numbers of B's (RANDOLPH 1941). The two opposing mechanisms interact to maintain B-chromosomes at a relatively low level in the maize population. This study constitutes an investigation of the relationship between B-chromosomes and crossing over in the standard or A-chromosomes of maize and is an extension of preliminary reports (HANSON 1961, 1962).

## MATERIALS AND METHODS

Two sublines of maize were extracted by RHOADES (personal communication) from a highly inbred strain of Black Mexican sweet corn originally produced by E. W. LINDSTROM. The two sublines, one carrying accessory chromosomes and the other with no B's, have been maintained separately for many years and apparently differ only by the presence or absence of B-chromosomes. These two strains were used as male parents in crosses with chromosome tester stocks. Root tips from F<sub>1</sub> hybrids were prepared by the method of RANDOLPH (1940) and the number of B-chromosomes possessed by each plant ascertained. The hybrids were then backcrossed to their respective testers and the crossover frequencies studied. Two segments of the maize genome

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were tested: the  $\gamma g_2-c-sh_1-wx$  region on the short arm of chromosome 9 and the  $gl_6-lg_2-a_1-et$  region on the long arm of chromosome 3. The recessive alleles  $c, sh_1, wx, a_1,$  and  $et$  are endosperm markers while  $\gamma g_2, gl_6$  and  $lg_2$  yield seedling abnormalities.

Standard errors were calculated on the basis of the binomial distribution using the formula  $k_1-k_2 [k(1-k)(1/n_1+1/n_2)]^{-1/2}$  where:

$k_1, k_2,$  and  $k$  = observed crossover frequency of the B, non-B, and entire population, respectively;

$n_1$  and  $n_2$  = total number of individuals in the B and non-B sample, respectively.

## RESULTS

The data obtained from the testcrosses involving chromosome 9 are presented in Tables 1 and 2. Table 1 is concerned with the  $c-sh_1$  and  $sh_1-wx$  regions of chromosome 9 which could be tested by classifying kernels. Table 2 includes data from seedling classification of some of the material represented in Table 1. When all plants containing B-chromosomes are considered together and compared with those having no B's, certain crossover classes, particularly the double crossovers, are significantly higher in the B-chromosome plants. When the B-chromosome class is separated into two groups, the group with the higher amount of B's is significantly different from the non-B-chromosome class in recombination values for several regions. The group with 1-4 B's in general exhibits the same tendencies as the 6-9 B-chromosome class but the differences from the non-B-chromosome class are not significant. All crossover classes except single crossovers in the  $\gamma g_2-c$  interval (region 1) were increased in the presence of the accessory chromosomes; those in region 1 were decreased significantly.

Table 3 presents the data obtained in the chromosome 3 tests when the  $F_1$  hybrid was used as the female parent in backcrosses to the tester. Single crossovers in region 2 are reduced in the presence of B's, while those in region 3 are unchanged. The noncrossover class (0) is reduced in the presence of accessories. Region 1 and the multiple crossover classes show an increase with increasing numbers of accessories. It is interesting to note that, in both chromosome 3 and chromosome 9, the regions known in some stocks to possess or be near to knobs are the only ones which exhibit decreased crossing over in the presence of acces-

TABLE 1

*Crossover percentages on chromosome 9 as influenced by B-chromosomes*

		+ 2 + 3 + c sh <sub>1</sub> wx ♀ × c sh <sub>1</sub> wx ♂			Total recombination		Total progeny
		Crossover region			2	3	
	Noncrossovers	2	3	2,3			
with B chromosomes	75.8**	3.4	19.9**	0.25**	3.6	20.2**	14,060
without B chromosomes	78.6	3.2	18.2	0.13	3.3	18.3	17,436

\*\* Significantly different from population lacking B-chromosomes at 1% level.

TABLE 2  
Crossover percentages on chromosome 9 as influenced by B-chromosomes

	Crossover region										Total recombination			Total progeny
	♀ × $\gamma B_2$ c sh <sub>1</sub> wx δ										1	2	3	
	0	1	2	3	1,2	1,3	2,3	1,2,3	1	2				
with B's (1-9)	59.2	17.7	3.4	18.4*	0.31	0.86	0.21**	0.02	18.9	3.9**	19.5*	5225		
1-4 B's	59.6	18.3	3.3	17.8	0.24	0.60	0.10	0.02	19.2	3.6*	18.6	4156		
6-9 B's	57.4*	15.1**	3.7	20.7**	0.56	1.87**	0.66**	<0.09	17.5	5.0**	23.2**	1069		
without B's	60.8	18.6	2.9	16.9	0.26	0.40	0.06	<0.01	19.3	3.2	17.4	8565		
same family	61.4	19.0	2.5	16.4	0.15	0.53	<0.05	<0.05	19.7	2.7	17.0	2075		
different family	60.6	18.5	3.1	17.1	0.29	0.35	0.08	<0.01	19.1	3.4	17.5	6490		

\*, \*\* Significantly different from the 0 B-chromosome-bearing members of the same family at the 5% and 1% level, respectively.

TABLE 3  
Crossover percentages on chromosome 3 as influenced by B-chromosomes

Number of B's:	Crossover region										Total recombination			Total progeny
	♀ × $gl_6$ lg <sub>2</sub> a <sub>1</sub> et δ										1	2	3	
	0	1	2	3	1,2	1,3	2,3	1,2,3	1	2				
0	42.5	14.7	26.8	7.8	4.0	2.3	1.7	0.16	21.2	32.7	12.0	8221		
1-4	42.6	14.5	25.0*	7.7	4.9**	2.7	2.3**	0.36*	22.5	32.6	13.1	6191		
5-8	40.8*	16.4**	23.6**	7.7	5.4**	3.0**	2.5**	0.58*	25.4**	32.1	13.8	7415		
9-14	38.5**	18.2**	23.7*	7.6	5.9**	2.7	2.6*	0.75**	27.4**	33.0	13.7	1332		

\*, \*\* Significantly different from 0 B-chromosome class at 5% and 1% level, respectively.

TABLE 4

*Crossover percentages on chromosome 3 as influenced by B-chromosomes*

$$gl_6 \quad lg_2 \quad a_1 \quad \text{♀} \times \frac{+ \quad 1 \quad + \quad 2 \quad +}{gl_6 \quad lg_2 \quad a_1} \quad \text{♂}$$

Number of B's	Crossover region				Total recombination		Total progeny
	0	1	2	1,2	1	2	
0	51.2	19.4	24.5	4.8	24.2	29.3	4341
1-4	44.7**	21.2	25.1	9.0**	30.2**	34.1**	1617
5-8	46.9**	21.0	23.1	9.0**	30.0**	32.1**	56.39
9-14	44.3**	21.0	24.2	10.4**	31.4**	34.6**	3136

\*\* Significantly different from the 0 B-chromosome class at the 1% level.

sories. Further work with closely related tester stocks, one of which is knobbed and the other knobless, will be necessary to establish an interaction between knobs and B-chromosomes.

Table 4 presents the data from tests where the  $F_1$  hybrid was used as the male parent. The female tester parent did not carry the *et* allele so this locus was not considered in this experiment. The double crossover class increases with higher numbers of accessories while the single crossover classes exhibit no significant change. The total amount of recombination in each region was increased, however. As with the chromosome 9 data, there is a decided decrease in the frequency of the noncrossover class in the presence of B-chromosomes.

TABLE 5

*Coefficient of coincidence values from plants with varying numbers of B-chromosomes used as female parents in backcrosses to chromosome 9 testers*

Number of B's	Crossover regions			Multiple crossovers per single crossover
	1,2	1,3	2,3	
0	0.48	0.13	0.12	0.019
1-4	0.41	0.18	0.17	0.024
6-9	1.00	0.60	0.85	0.078
1-9	0.53	0.26	0.35	0.035

TABLE 6

*Coefficient of coincidence values from plants with varying numbers of B-chromosomes used as female parents in backcrosses to chromosome 3 testers*

Number of B's	Crossover region				Multiple crossovers per single crossover
	1,2	1,3	2,3	1,2,3	
0	0.57	0.91	0.45	0.20	0.17
1-4	0.67	0.92	0.53	0.38	0.22
5-8	0.66	0.85	0.56	0.52	0.24
9-14	0.65	0.71	0.58	0.60	0.24

TABLE 7

*Coefficient of coincidence values from plants with varying numbers of B-chromosomes used as male parents in backcrosses to chromosome 3 testers*

Number of B's	Crossover region 1,2	Double crossovers per single crossover
0	0.68	0.11
1-4	0.87	0.19
5-8	0.93	0.20
9-14	0.96	0.23

Columns 2,3, and 4 of Table 5, columns 2,3,4, and 5 of Table 6, and column 2 in Table 7 list the coefficient of coincidence values for these data. In chromosome 9, chromosome interference was reduced in all regions when accessory chromosomes were present. In chromosome 3, all regions showed a decrease in interference with the exception of the double crossover region, 1,3; this is not unexpected because of the relatively long length of the intervening region, 2. The last column in each of these tables lists the ratio of multiple to single crossovers for the three groups of data. This method of recording chromosome interference measures all of the concerned chromosomal regions simultaneously. If an increase in crossing over is not due to a decrease in chromosome interference, the ratio of multiple crossovers to singles in the B-chromosome plants should be the same as in the non-B-chromosome class; on the other hand, lowered interference should increase the multiples to singles ratio. Comparison of this ratio in plants with and without B-chromosomes shows that the presence of B-chromosomes reduced chromosome interference in the A-chromosomes in all instances.

Calculations of rank tetrad frequencies were made from the data with the assumption of no chromatid interference and the results are presented in Tables 8 and 9. Tetrad frequencies in microspore mother cells were not calculated. With each set of data, the frequency of single exchange tetrads was reduced and the calculated frequencies of double and triple exchange tetrads increased with increasing numbers of accessories.

From the tetrad calculations the total exchange frequencies were derived for each B-chromosome class as follows: the percentage of single exchange tetrads

TABLE 8

*Calculated frequencies of exchanges in megaspore mother cells heterozygous for loci on chromosome 9 in plants with varying numbers of B-chromosomes†*

Number of B's	Exchange tetrad class (percent)			Number of crossovers per tetrad
	None	single	double	
0	23.05	74.10	2.84	0.798
1-4	21.18	75.06	3.76	0.826
6-9	21.06	66.58	12.36	0.913

† Triple crossovers were ignored because of the small number of individuals involved.

TABLE 9

*Calculated frequencies of exchanges in megaspore mother cells heterozygous for loci on chromosome 3 in plants with varying numbers of B-chromosomes*

Number of B's	Exchange tetrad class (percent)				Crossovers per tetrad
	none	single	double	triple	
0	1.9	67.2	29.6	1.3	1.30
1-4	4.9	57.0	35.2	2.9	1.36
5-8	3.4	55.2	36.8	4.6	1.43
9-14	-0.8	59.2	35.6	6.0	1.48

plus 2 times the percentage of double exchange tetrads plus 3 times the percentage of triple exchange tetrads equals the average number of crossovers per tetrad. These values are presented in the last column of Tables 8 and 9. They indicate that the positive effect of accessory chromosomes on crossing over in the standard A-chromosomes is additive.

#### DISCUSSION

In the natural populations of most species a balance seems to exist between individuals with B's and those without. Furthermore, B-chromosome-bearing members of some species occupy a separate niche from individuals lacking B's (BATTAGLIA 1964). For example: In *Secale* the B's are found in the primitive strains. In *Festuca pratensis*, B-bearing plants exhibit a preference for soils with a high clay content. B's of *Centaurea scabiosa* are associated with plants growing in drier habitats. BARKER (1960) sampled populations of the grasshopper *Myrmeleotettix maculatus* and found that populations from cool, wet, high altitude (over 1000 feet) regions of northern England lacked B-chromosomes while populations from warm, dry, low altitude (under 400 feet) regions of northern and southern England did possess B's. He compared the chiasma frequencies of male grasshoppers bearing B's with those of individuals lacking B's and found a significantly higher frequency of chiasmata among the A-chromosomes when 1-2 B's were present in the cell. JOHN and HEWITT (1965 a and b) subsequently restudied these grasshopper populations and found B's in the high altitude populations thus refuting BARKER's geographical arrangement based upon B-chromosomes. However, they confirmed that individuals with B-chromosomes have significantly higher mean chiasma frequencies than those individuals, from the same population, lacking them.

Since chiasmata are believed to be the cytological equivalent of genetic crossovers, BARKER's data are in agreement with those presented in this paper. Recently AYONOADU and REES (1968) determined that the mean chiasma frequency in pollen mother cells of Black Mexican sweet corn increased with increasing numbers of B-chromosomes, thus providing cytological confirmation to the present linkage data. AYONOADU and REES calculated the increase in chiasma frequency (in percent) due to B's and obtained a regression coefficient of 0.60. Similar

regression coefficients calculated from data obtained in the present study of crossing over yielded values of 1.9 for the region between  $yg_2$  and  $wx$  on chromosome 9 (megaspore mother cells), 2.2 for the  $gl_6$  to  $et$  region of chromosome 3 (microspore mother cells) and 2.8 for the same region employing megaspore mother cell data. The values reported by NEL (1969) and by RHOADES (1968 a and b) would probably yield regression coefficients far in excess of those reported for this study. As AYONOADU and REES note, the effect of B-chromosomes on chiasma and cross-over frequencies is not consistent for all chromosomes and chromosome regions.

Numerous studies have been conducted to investigate the genic content of B-chromosomes. However, due to differences in morphology of B-chromosomes it is difficult to generalize concerning the apparent inertness of B-chromosomes. Heterochromatic B's may be the result of the genetic material becoming inactivated (BATTAGLIA 1964). ABRAHAM and SMITH (1966) have shown that maize B-chromosomes synthesize DNA during the last of the S period, a time when portions of the A-chromosomes also become labeled. However, they found no apparent DNA synthesis in B-chromosomes during the beginning half of the S period, a time when some A-chromosomes become labeled in all cells synthesizing DNA. HIMES (1967) prepared autoradiograms of maize root tips with and without B-chromosomes following administration of tritiated thymidine and cytidine. That study indicated that B-chromosomes are responsible for very little, if any, DNA synthesis and for only a small amount of nonhistone protein synthesis. B-chromosomes of maize, although primarily heterochromatic, do possess euchromatic segments. RANDOLPH (1941) found no instances in maize B-chromosomes of alleles for A-chromosome genes which exert a distinct qualitative effect. It may be however, as DARLINGTON (1963) suggests, that the heterochromatic B-chromosomes contain genes with small quantitative influences.

EHRENDORFER (1960) believes that accessories tend to increase the plasticity of populations. He found that accessory chromosomes in *Achillea* influence fertility and the frequency of spontaneous chromosome aberrations. MOSS (1966) provided evidence that B-chromosomes in rye increase the variability of the population and thus facilitate the spread of the species, RHOADES, DEMPSEY, and GHIDONI (1967) have recently provided evidence that B-chromosomes in maize cause the elimination of A-chromosomes with heterochromatic knobs at the second microspore mitosis. They also found evidence that the B-chromosomes caused breakage of A-chromosomes and subsequent loss of chromosomal segments which bore large heterochromatic knobs. Although this evidence shows an interaction between knobs and B-chromosomes in maize, further studies are necessary to ascertain whether this phenomenon is related to the failure of B-chromosomes to influence the rate of crossing over in regions of the A-chromosomes which are known to bear knobs in some strains.

RHOADES (1968a and b) worked with an altered maize chromosome 9 containing a transposed piece of chromosome 3 inserted between the two markers  $sh$ , and  $wx$ . In plants homozygous for the transposition but heterozygous for the markers  $c$  and  $wx$  he found that B-chromosomes present in the meiotic cell drastically

increased recombination in the *c-wx* region from 16.9% for the 0 B class to 30.8% in the presence of 1 B, 38.8% with 2 B's and 41.7 with 3 B's. His data also show that B-chromosomes additively increased the crossover percentages in the *c-wx* region and additively decreased the amount of crossing over in the adjacent *yg-c* region as detected in the present study.

NEL (1968-1969) studied the effect of B-chromosomes on crossing over in maize chromosome 5 with results similar to that reported here. The two regions studied (*A-Bt* and *Bt-Pr*) both showed an additive recombination increase with increased numbers of B's. He did not detect any shift in the distribution of crossovers, however. Thus B-chromosomes in maize have been shown to cause an increase in the recombination index and to alter the distribution of exchanges. Linkage relationships are thereby altered and the range of variation in the maize population is increased via the B's.

The increase in double crossovers produced by B-chromosomes reported in this investigation is evidence of action on their part which could be of selective advantage. B-chromosomes are known to produce adverse effects, especially when present in large numbers. The persistence of low numbers of B's indicates that they play some useful role. Crossing over gives rise to new combinations of genes, some with possible adaptive value, and it is conceivable that an agent producing a greater number of gene constellations would be selected for. Such may be the role and function of the B-chromosome.

#### SUMMARY

Accessory, or B-chromosomes in maize were tested for their effect upon crossing over in the A-chromosomes 3 and 9. In both instances crossing over was increased primarily in the double crossover classes. B-chromosomes were shown to increase the inferred frequency of double and triple exchange tetrads at the expense of the singles. The overall effect of an increase in B's was an additive increase in crossing over and a general decrease in chromosome interference. A possible evolutionary role of the B-chromosome in nature is discussed.

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