CROSSING OVER AND CHROMOSOMAL SEGREGATION INVOLVING THE B^9 ELEMENT OF THE A-B TRANSLOCATION *B-9b* IN MAIZE[1]

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A-B translocations involve reciprocal exchanges between a member of the regular (A) complement of the maize genome and a genetically inert B chromosome. ROMAN (1947, 1948), ROMAN and ULLSTRUP (1951), BELLINI, BIANCHI and OTTAVIANO (1961), and BIANCHI, BELLINI, CONTIN and OTTAVIANO (1961) have described the behavior of such translocations when they are transmitted through the pollen. In the mitotic division of the generative nucleus to form the two sperm nuclei, the B^A member of these translocations frequently undergoes nondisjunction resulting in one sperm with two B^A elements (duplicate or hyperploid) and one sperm with no B^A (deficient or hypoploid). Fertilizations involving pollen grains in which non-disjunction has occurred will produce two classes of seed. One class will result from the fertilization of the polar fusion nucleus by the hyperploid sperm and the egg nucleus by the hypoploid sperm. Such a seed will have two B^A elements in the endosperm and none in the embryo. The second class will result from the hypoploid sperm nucleus fertilizing the polar fusion nucleus and the hyperploid sperm fertilizing the egg nucleus. The resulting seed will have no B^A chromosomes in the endosperm and two B^A chromosomes in the embryo. Both of the above classes will receive the A^B element of the translocation.

This study is primarily concerned with an analysis of crossing over involving the B^A element and its transmission through the female gametophyte.

MATERIALS AND METHODS

The translocation which was used, *TB-9b*, has the distal 60% of the short arm of chromosome 9 transferred to a B chromosome (ROMAN and ULLSTRUP 1951). This segment includes yg_2 (yellow-green plant), *c* (colorless aleurone), sh_1 (shrunken seed), and other loci. The *wx* (waxy endosperm) locus is proximal to the break point. Figure 1a shows the probable break point in chromosome 9.

This study began with hyperploid plants of the constitution 9 $9^B B^9 B^9$ in which the normal chromosome 9 carried the recessive genes *c*, sh_1, and *wx*, the B^9 chromosome carried dominant genes *C* (colored aleurone) and Sh_1 (plump seed), and the 9^B chromosome carried *Wx* (starchy seed). The genotype was thus *C C c*, Sh_1 Sh_1 sh_1, *Wx wx* (Figure 1b). These hyperploid plants were obtained originally by selecting colorless shrunken seeds from ears of a *c c* sh_1 sh_1 *wx wx* stock pollinated by a *TB-9b* stock homozygous for the dominant alleles. The hyperploid plants were pollinated by a "c tester stock" (a *c c* line that is homozygous for all of the other genes necessary for colored aleurone) of the constitution *c c* sh_1 sh_1 *wx wx*. To determine the chromo-

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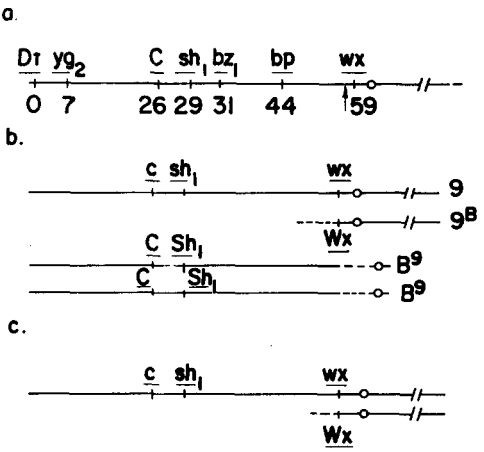

FIGURE 1.—(a) The genetic map of the short arm of chromosome 9 (RHOADES 1954). The arrow indicates the approximate break point of translocation *TB-9b*. The circle indicates the approximate position of the centromere, and the double slant lines indicate that the long arm has been foreshortened. Symbols: *Dt*—dotted, yg_2—yellow green, *C*—colored aleurone, sh_1—shrunken seed, bz_1—bronze, *bp*—brown pericarp, and *wx*—waxy. (b) The genetic and cytological constitution of the hyperploid plants in the original testcross. The dash segments represent the segments of the B chromosome involved in the translocation. (c) The genetic and cytological constitution of hypoploid plants used to determine the *wx*–break point distance.

TABLE 1

Testcross data of plants hyperploid for TB-9b (c sh_1 wx/C Sh_1–/C Sh_1–/– –Wx × c c sh_1 sh_1 wx wx)

Parents	Testcross phenotypes and frequencies							
	C Sh_1 Wx	*c sh_1 wx*	*C sh_1 wx*	*c Sh_1 Wx*	*C Sh_1 wx*	*c sh_1 Wx*	*C sh_1 Wx*	*c Sh_1 wx*
5561–4 / 4558–4	112	5	.	.	137	3	.	.
5561–5 / 4558–4	221	15	.	1	237	3	.	.
5561–6 / 4558–4	160	8	.	.	152	1	.	.
5561–16 / 4558–4	243	16	.	1	242	.	.	.
4564–2 / 4558–3	284	31	.	1	271	.	.	.
4564–16 / 4558–3	290	27	.	1	276	.	.	1
Totals	1310	102	.	4	1315	7	.	1
Percent	47.83	3.72	.	0.15	48.01	0.26	.	0.04

somal and genetic constitutions of the various genotypes resulting from this original testcross, further crosses were made with the same "c tester stock" or with one carrying yg_2.

The distance between *wx* and the translocation break point was determined by crossing hypoploid plants with the waxy "c tester stock". Seeds with hypoploid embryos were taken from the same ears as the hyperploid plants. The genetic and cytological constitution of such deficient plants is given in Figure 1c. The seeds that give rise to hypoploid plants have purple aleurone, and the plants are semisterile because the pollen grains and embryo sacs receiving the deficient 9^B chromosome abort. All functional gametes will thus carry the normal chromosome 9 and will usually transmit the *wx* allele. However, an occasional crossover between *wx* and the translocation break point will transfer the *Wx* allele from 9^B to the normal chromosome 9. The frequency of *Wx* seeds from the testcross of a hyperploid plant thus gives the crossover distance between this locus and the break point.

RESULTS AND DISCUSSION

Testcross of hyperploid female plants: Table 1 gives the classes of seeds that result from this testcross and Figure 2 gives the suggested chromosomal constitution and genotype for each testcross phenotype observed. Since most of the colorless classes occurred in low frequency their percentage values were probably not too reliable. To determine more reliable data for these classes, a larger number of testcross ears was scored exclusively for the frequency of the colorless classes. These results are given in Table 2.

TABLE 2

Summary of colorless classes in testcrosses of plants hyperploid for TB-9b (c sh_1wx/C Sh_1–/C Sh_1–/– –Wx × c sh_1 wx/c sh_1 wx)

Phenotypes	Frequency	Percentages
c sh_1 wx	446	2.72
c Sh_1 Wx	34	0.21
c sh_1 Wx	63	0.38
c Sh_1 wx	21	0.13
Total covered	15,805	96.55
	16,369	

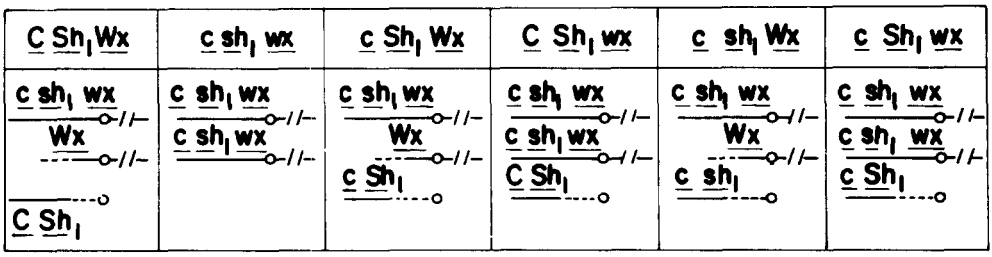

FIGURE 2.—Suggested chromosome constitution and genotype for each phenotype observed in the testcross results recorded in Table 1.

Classes C Sh_1 Wx *and* C Sh_1 wx: From Table 1 it can be seen that these are the most frequent classes observed and that they occur in approximately equal frequency. These results would be expected if most of the time the normal chromosome 9 paired with 9^B, the two B^9 chromosomes paired, and if these two pairs then assorted independently at the first division of meiosis.

Class c sh_1 wx: This is the next most frequent class in Table 1, and also the most frequent class in Table 2. These seeds would be expected if a gamete received only a normal chromosome 9, and could be the result of nondisjunction of the B^9 centromere either during meiosis or during development of the female gametophyte. The latter possibility is not very likely since nondisjunction would have to occur simultaneously in the two cell lineages giving rise to the polar nuclei. If the *c* sh_1 *wx* seeds result from loss of the B^9 chromosome, plants from these seeds should carry only normal 9th chromosomes and upon self-pollination would yield ears that were homozygous for *c*, sh_1 and *wx*. From the 102 seeds recorded in Table 1, 71 self-pollinated ears were obtained and all were homozygous for *c*, sh_1 and *wx*.

Classes c Sh_1 Wx *and* c Sh_1 wx: The simplest explanation of the *c* Sh_1 *Wx* class involves a crossover in the *c*-sh_1 region between chromosome 9 and the B^9 element followed by subsequent segregation, in which the crossover B^9 chromatid and a 9^B chromatid were included in the same megaspore. The presence of 21 *c* Sh_1 *wx* (Table 2) seeds suggests that crossing over between the B^9 and the normal 9 chromosomes in the *c*-sh_1 region does not affect the independent assortment of the two pairs of chromosomes (i.e., the 9 and 9^B pair and the pair of B^9 chromosomes). If such independent assortment occurs, half of the time the B^9 chromosome with the crossover chromatid will end up in the same nucleus as 9^B chromosome at the end of the first meiotic division. When this happens, one of the second meiotic division products of the nucleus receiving the B^9 chromosome with the crossover chromatid will have the *C* Sh_1 *Wx* phenotype (noncrossover class) and the other will be *c* Sh_1 *Wx* (a crossover). The *c* Sh_1 *wx* class would be expected if the B^9 chromosome with the crossover chromatid went to the same first telophase pole as the chromosome 9 with a crossover chromatid. However, the *c* Sh_1 *wx* class would be expected in only half the frequency of the *c* Sh_1 *Wx* class, since half the time the crossover B^9 chromatid and the crossover 9 chromatid will go to the same second telophase pole, resulting in a phenotype that cannot be distinguished from a noncrossover *C* Sh_1 *wx* phenotype. The other 50% of the time, when the chromosome alignment on the second metaphase spindle is such that the crossover B^9 chromatid and the noncrossover 9 chromatid go to the same pole, half of the second-division products will be *c* Sh_1 *wx*. In summary, a crossover in the *c*-sh_1 region followed by independent assortment of the chromosomes involved will result in two alignments of first metaphase chromosomes. The first, when the two crossover chromosomes go to opposite poles, will result in ¼ of the products being *c* Sh_1 *Wx*. The other alignment in which the two crossover chromosomes go to the same pole will result in ⅛ of the products being *c* Sh_1 *wx*. Thus, the *c* Sh_1 *Wx* class is expected twice as frequently as the *c* Sh_1 *wx* class; the data indicate that this is found.

If the foregoing explanation of the origin of the *c Sh₁ Wx* class is correct, plants from these seeds when used as pollen parents should show the typical nondisjunction of plants carrying an A-B translocation. When plants from *c Sh₁ Wx* seeds were crossed as pollen parents to plants carrying yg_2, which is located near the end of the short arm of chromosome 9, 13 of the 14 F_1 ears segregated for yg_2. These results are in agreement with the chromosome constitution suggested in Figure 2. The one exceptional plant, which did not produce ears segregating for yg_2, might have been the result of nondisjunction of the B^9 chromosomes in one of the divisions of the female gametophyte nuclei, resulting in an embryo sac with a deficient egg nucleus and a polar fusion nucleus that received the crossover B^9 chromosome. Plants from such a caryopsis would not give yg_2 seedlings on outcrossing. The same 14 plants when used as females in crosses involving yg_2 pollen did not segregate any yg_2 seedlings. As would be expected, reciprocal crosses of 12 plants of the *c Sh₁ wx* to plants carrying yg_2 did not segregate for this mutant.

Class C sh_1 wx: This class, which is the reciprocal of *c Sh₁ Wx*, is rare (from the material summarized in Table 2, only one *C sh₁ wx* seed was observed). This class is expected to be infrequent because it probably requires the simultaneous occurrence of two rare events, (1) a crossover in the *c-sh₁* region followed by (2) the nondisjunction of the B^9 chromosomes.

Class c sh_1 Wx: Three events could give rise to this class: (1) A crossover between *wx* and the translocation point, putting *Wx* on the normal chromosome 9 along with *c sh₁*, followed by nondisjunction of the B centromeres; (2) A crossover between the *sh₁* locus and the translocation point, putting *c* and *sh₁* on the B^9 chromosome, which, as the data from the *c Sh₁ Wx* class indicate, would result in the crossover B^9 chromatid occasionally being included in a spore with the 9^B chromosome carrying *Wx*; (3) Nondisjunction of the 9 centromeres and B centromeres, in which the normal 9 and 9^B chromosomes go to one pole and the two B^9 chromosomes to the other.

Table 3 gives the results of testcrosses of deficient (hypoploid) plants. From these data, the amount of crossing over between waxy and the break point has been determined to be .48%. This information permits the elimination of the first explanation for the *c sh₁ Wx* class. If explanation 1 were responsible for this class, the frequency with which these seeds are expected can be predicted by the following formula: *[(Probability of a crossover between* wx *and break point) × (Probability of* c sh_1 wx*)]/2* = (.0048 × .037)/2 = .00009. Since this predicted value is considerably less than the observed frequency of .004 for the *c sh₁ Wx* class, it seems likely that explanation 1 is responsible for very few of the seeds of this phenotype.

Of the remaining two explanations, (2) is the most likely, since (3) requires the occurrence of two rare nondisjunctions, one of which has not been demonstrated (i.e., nondisjunction of 9 centromeres). If the events incorporated in explanation (2) are correct, these plants will be heterozygous for *TB-9b* and should show the typical nondisjunction when used as pollen parents. Such plants were reciprocally crossed to plants carrying yg_2. No yg_2 plants were found in the

TABLE 3

Testcross data of plants hypoploid for TB-9b (c sh wx/– –Wx)*

Parents	*c sh*$_1$ *Wx*	*c sh*$_1$ *wx*
4558–8 / 4893–9	1	174
4558–13 / 4893–9	0	97
4893–2 / 4894–3	0	130
4893–2T / 4894–3	1	160
4893–3 / 4894–4	0	189
4893–9 / 4558–13	0	183
4894–2 / 4893–3	0	207
4894–5 / 4893–3	0	217
4894–8 / 4893–9	1	216
4895–1 / 4893–9	4	160
4895–3 / 4893–3	3	326
Total	10	2059
Percent crossovers	.48%	. . .

* These data were first presented by ROBERTSON (1963). BIANCHI and BORGHI (1966) repeated the testcross and obtained crossover values between .21% and .23%. The different results may be due to background differences in the two stocks or to the chance occurrence of more than the expected number of crossovers in the last two crosses in this table.

F_1 progeny when 26 *c sh*$_1$ *Wx* plants were used as females. However, when these same plants were outcrossed as males to plants carrying *yg*$_2$, 18 of the ears segregated for *yg*$_2$ seedlings while two did not. Six of the outcrosses failed to set seed. The two crosses that failed to segregate for *yg*$_2$ may be the result of the failure of B centromere to undergo nondisjunction when the crossover plants were used as males, or they could have been produced by the events incorporated in explanation 1, which would result in seeds of the *c sh*$_1$ *Wx* phenotype that have only normal ninth chromosomes, and thus would give only normal seedlings when crossed reciprocally with plants carrying *yg*$_2$.

The preceding interpretations for the genotypes observed in testcrossing hyperploid *TB-9b* plants as females are those that seem most likely. In most instances genetic tests substantiated the suggested genotypes (e.g., the tests of the *c* Sh_1 *Wx*, *c* sh_1 *Wx* and *c* Sh_1 *wx* as pollen parents with yg_2). However, in some instances other, less likely genotypes are possible. One such genotype was suggested to explain the exceptional behavior of the two *c* sh_1 *Wx* that did not give yg_2 seedlings when outcrossed to yg_2 female plants. Also, some of the genotypic classes undoubtedly contain chromosomal arrangements in addition to those suggested, since some crossover events followed by certain patterns of chromosomal assortment result in genotypes that are indistinguishable from noncrossover classes. One example of such a situation was discussed in connection with the production

TABLE 4

First metaphase counts on the frequency of cells with trivalents and univalents from plants of the genotype C Sh_1 wx *with a predicted 99B*[9] *chromosome constitution*

Plant No.	Spikelet	Anther	Metaphase I trivalents		Number of unclassified cells
			Frequency*	%	
1300–3	1	1	33/89	37	2
		2	51/131	39	0
		3	15/36	42	1
	2	1	55/151	36	1
		2	19/61	31	2
		Total:	173/468	37	
2300–9	1	1	42/122	34	7
	2	1	48/124	39	1
		2	41/103	40	2
		3	46/118	39	1
		Total:	177/467	38	
2300–10	1	1	33/104	32	2
		2	43/109	39	3
	2	1	43/105	41	1
	3	1	45/104	43	2
		Total:	164/422	39	
2299–1	1	1	34/104	33	0
		2	74/214	35	0
		3	35/125	28	1
		Total:	143/443	32	
1299–2	1	1	21/151	14	2
	2	1	25/137	18	5
		2	25/156	16	5
		Total:	71/444	16	
2299–8	1	1	34/107	32	9
	1	1	44/149	30	10
		2	43/142	30	3
		Total:	121/398	30	
Grand totals			849/2642	32.1	
Total omitting plant 1299–2			778/2198	35.4	

* Frequency = (*number of cells containing a trivalent*)/(*number of cells with a trivalent* + *number of cells with a univalent*).

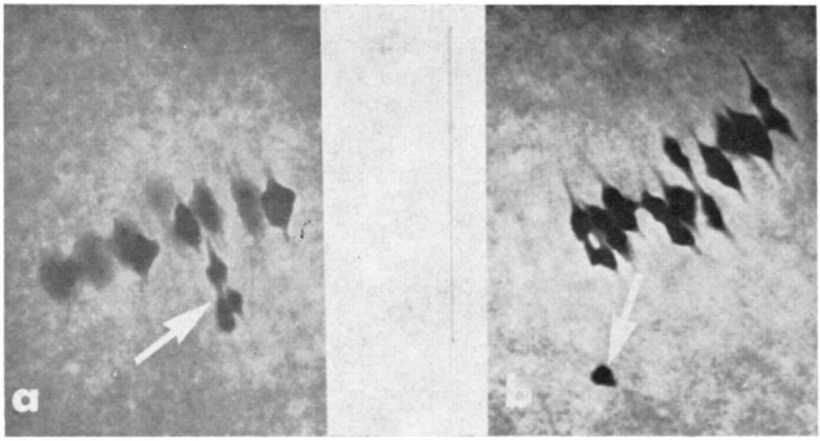

FIGURE 3.—First metaphase cells of "trisomic" *C Sh*$_1$ *wx* (9, 9, B^9) plants. The arrows indicate a trivalent (in a) and a univalent (in b).

of the *c Sh*$_1$ *Wx* class. In this instance, segregation after crossing over could produce a spore that received a crossover B^9 chromosome (*c Sh*$_1$) and a crossover chromosome 9 *C sh*$_1$ *wx* resulting in a noncrossover *C Sh*$_1$ *wx* phenotype. Other examples of minor importance could be cited, but they would not alter the main interpretation.

The two predominant noncrossover classes were tested further because they consisted of chromosomal configurations that have not been analyzed genetically before. The first test involved the "trisomic" *C Sh*$_1$ *wx* class which was suggested to result from the presences of a normal chromosome 9 (*c sh*$_1$ *wx*) and a B^9 chromosome (*C Sh*$_1$) in the same megaspore (see Figure 2). ("Trisomic" is used in this report to indicate that the trisomic condition of this genotype involves only the short arm of chromosome 9 rather than the whole chromosome.)

Genetic analysis of "trisomic" C Sh$_1$ wx *plants:* Cytological examination of 2642 first metaphase cells from six plants (Table 4) confirmed the predicted cytological configuration (see Figure 2); the cells possessed either the trivalent (Figure 3a) or univalent (Figure 3b) chromosomal associations expected of a plant trisomic for the short arm of chromosome nine. The frequency of trivalents and univalents in these cells is given in Table 4. The total frequency of trivalents is about 35.4% if counts from plant 1299–2 are omitted. (The values for this plant were consistently about half those of the others, for unknown reasons.)

Table 5 is a summary of testcross data in which *C Sh*$_1$ *wx* plants were used as male and female parents in crosses with *c c sh*$_1$ *sh*$_1$ *wx wx* stocks. Crosses in both directions give the crossover types *C sh*$_1$ and *c Sh*$_1$, indicating that trisomic pairing is strong enough to permit crossing over.

Using a trivalent frequency of .35, a crossover value of .33 for the distance from the break point to *c* (a value a little high since this is the total *c-wx* distance and the aberration undoubtedly reduces crossing over in this region), and an estimated map length for B^9 of 59 (again, probably an overestimated value),

TABLE 5

Summary of testcrosses of C Sh_1 wx *plants used as males and females*

	Male parent				Female parent			
	C Sh_1	*C* sh_1	*c* Sh_1	*c* sh_1	*C* Sh_1	*C* sh_1	*c* Sh_1	*c* sh_1
No.	509	21	5	2962	1719	5	12	4420
%	14.56	00.60	00.14	84.70	27.92	00.08	00.19	71.80

the frequency of *C* seeds on the ear of the "trisomic" female can be predicted for the situations where the B^9 chromosome moves at random after crossing over (16.28%), or where it segregates from its normal chromosome 9 crossover partner (17.5%). (See Appendix I for calculations.) These calculations are made on the assumption that the univalent B^9 is lost and not incorporated in functional megaspores, and represent maximal values since map distances have all been overestimated. That the observed value of 28.00 is considerably higher than predicted would indicate that the univalent B^9 chromosomes frequently are not lost and are transmitted as a duplication. This is not surprising because RANDOLPH (1941) and others have demonstrated that single B chromosomes frequently are transmitted both through the male and female gametes.

Calculations for the "trisomic" plants used as male parents give a value of 2.97% *C* if the crossover B^9 moves at random and 5.94% *C* if it regularly segregates from its crossover partner (see Appendix II for calculations). Neither value is as high as the observed 15.16%, which again suggests that the univalent B^9 chromosome is not always lost and that it is occasionally transmitted as a duplication through the pollen.

Since the transmission of noncrossover B^9 chromosomes when present as univalents makes it virtually impossible to estimate how much crossing over is taking place in the *C*–break point region, analysis of the crossovers in the *c-sh*$_1$ region is more revealing. In the case of transmission through the female gamete, using 3% as the expected crossover percent in this region, 0.22% *C sh*$_1$ and 0.33% *c Sh*$_1$ seeds are expected if the crossover B^9 assorts at random with the crossover chromosome 9. If the crossover B^9 regularly disjoins from the crossover 9, 0.44% *C sh*$_1$ and 0.44% *c Sh*$_1$ seeds are expected (see Appendix III for calculations). The observed frequencies of 0.08% and 0.19%, respectively, are far below those expected if crossing over took place at the normal frequency every time a trivalent was formed. These lower values could result from less frequent trivalent pairing in female than in male, or from pairing insufficiently strong to permit much crossing over. Also, random assortment of the B^9 crossover chromosome with respect to its crossover partner is indicated, since if assortment was not random the two crossover classes would be expected in equal frequency. This is not observed, and the class that is most frequent, *c Sh*$_1$, is one expected on the basis of the calculated data for random assortment. The frequency of the *c Sh*$_1$ crossover class in this test is 0.19%, while the frequency of the same class in the testcross of the original hyperploid plants was 0.34% (Table 2). This suggests

that in hyperploid plants, in which distal 9s has no pairing partner other than the B^9 chromosome, more crossing over with B^9 will take place than in the "trisomic" plants that have two normal ninth chromosomes.

The predicted frequency of the two crossover classes in the *c-sh₁* region when transmitted as a male (assuming that duplications are not transmitted) is 0.27% for the *C sh₁* class if random and 0.53% if directed assortment of the crossover B^9 chromosome took place (see Appendix IV for calculations). No *c Sh₁* classes are expected in either assortment. The observed 0.60% is higher than either calculated value but is closer to that expected for the regular disjunction of crossover chromosomes. In contrast to the female data, it would appear that crossing over in the male approximates that predicted from the observed frequency of trivalent figures at first metaphase and also that the male crossover products may be expected to regularly disjoin. Since such directed segregation of crossover products is not observed for the female, these results suggest that males and females might differ in the segregation of B^9 after crossing over. Further studies of the transmission of crossover B^9 chromosomes through the male are needed to establish if such a difference does exist.

The unexpected *c Sh₁* crossover class (0.14%) is observed in the male data which demonstrates conclusively that the B^9 element can be transmitted as a duplication through the pollen.

Genetic analysis of C Sh_1 Wx *plants:* Plants of this phenotype were suggested to be heterozygous for *TB-9b* and to have the genotype *c sh₁ wx/C Sh₁ Wx* with the dominant alleles carried on the translocated chromosomes (see Figure 2). A summary of this testcross is given in Table 6. The striking thing about these data is the high frequency of the *C Sh₁ wx* class (29.28%), which approximates those for the classes *C Sh₁ Wx* and *c sh₁ wx*. This distribution is expected when no crossing over takes place and the B^9 chromosome in meiosis I moves at random with respect to chromosomes 9 and 9^B, producing the following distributions in equal frequency: $9{:}9^B B^9{:}9\ B^9{:}9^B$. Since 9^B spores do not function, ⅓ of the noncrossovers would be expected to be *C Sh₁ Wx*, ⅓ *c sh₁ wx*, and ⅓ *C Sh₁ wx*. Although the data suggest the random assortment of the noncrossover B^9 chromosome, they also suggest that the B^9 chromosome is sometimes lost from the spindle and not included in a nucleus. If it were never lost and moved completely at random, the *c sh₁ wx* class should more closely approximate the frequencies of the *C Sh₁ Wx* and *C Sh₁ wx* classes. As it is, the frequency of the *c sh₁ wx* class is significantly higher than the values for the *C Sh₁ Wx* and *C Sh₁ wx* classes at the 1% level as determined by a chi-square test. An alternative possibility, that B^9 does not move completely at random but tends to go with the normal 9 chromosome, should elevate the *C Sh₁ wx* class to a level that would compensate for the deficiency in the *C Sh₁ Wx*. Such an increase is not observed. This is particularly significant since the *C Sh₁ wx* genotype also includes some crossover classes as well as those resulting from the random movement of the B^9 chromosome. (The *c sh₁ wx* class also includes some crossover classes; but if these are subtracted from the *c sh₁ wx* total, this class is still significantly in excess of the *C Sh₁ Wx* and *C Sh₁ wx* classes.)

The high frequency of the *c Sh$_1$ wx* as opposed to the reciprocal *C sh$_1$ Wx* class, suggests again that crossover B^9 chromosome sometimes moves at random with respect to its crossover partner. The *C sh$_1$ Wx* class is expected to occur in a very low frequency since it involves a crossover in the *c-sh$_1$* region and a second in the *sh$_1$-wx* region, probably in the *Sh$_1$* to break point region of the B^9 chromosome, since the *wx* to break point distance is extremely short. The most reasonable explanation for the unexpected high frequency of the *C sh$_1$ Wx* class would seem to be that most are not the result of double crossovers, but rather, the result of a single crossover in the *c-sh$_1$* region putting *c* on the B^9 chromosome. If this crossover B^9 chromosome moves to the same pole at anaphase I as the crossover chromosome 9, half the time the crossover B^9 chromatid would be included in the same microspore as the noncrossover 9 chromatid, resulting in a *c Sh$_1$ wx* phenotype. If random assortment after crossing over took place, then *C sh$_1$ wx*, *c Sh$_1$ Wx* and *c Sh$_1$ wx* would be expected in equal frequency, which is observed. The same could be said for the crossover producst involving the *Sh$_1$* to break point region. The three classes resulting from random assortment would be *C Sh$_1$ wx*, *c sh$_1$ wx*, and *c sh$_1$ Wx*. The frequency of the first two classes cannot be determined because they are phenotypically indistinguishable from noncrossover classes. The crossover class putting *C Sh$_1$* on normal nine is classified with the noncrossover class that receive normal nine and B^9 chromosomes, while the crossover that places *c sh$_1$* on one of the B^9 chromatids results in the noncrossover *c sh$_1$ wx* phenotype when the B^9 crossover chromatid is included in the same microspore as the noncrossover chromosome 9 chromatid. If the frequency of the *c sh$_1$ Wx* class is taken as the frequency of this crossover and random assortment of B^9 is assumed, then the *C Sh$_1$ wx* and the *c sh$_1$ wx* classes would be lowered by 66, or 2.38%. Table 7 gives the expected frequency of the crossover classes if these corrections for random assortment of B^9 are made. The frequency of the *c sh$_1$ wx* class is still significantly higher than the values for the *C Sh$_1$ Wx* and *C Sh$_1$ wx* classes, indicating the occasional loss of the B^9 chromosome. The crossover data calculated with and without the incorporation of the random assortment of the B^9 chromosome are in close agreement. The crossover value of about 2% for the *c-sh$_1$* region is only 1% less than the 3% given by RHOADES (1954) for this distance, which indicates that little interference with crossing over has occurred in this region. However, the 7% crossing over estimated for region two is far below the 30% expecetd, indicating that the heterozygous aberration markedly reduces crossing over in the region of the break points.

General conclusions: Two main conclusions emerge from this work: (1) To incorporate genes into A-B translocations, heterozygous A-B plants should be used rather than hyperploid plants, and (2) B^9 chromosomes involved in crossing over during megasporogenesis do not regularly disjoin from their crossover partner at metaphase I.

The first conclusion is limited in scope but is of practical importance to anyone using A-B translocation in genetic studies. The results of the original testcross of the hyperploid plant (Table 1) indicate that about 96% of the time the two

TABLE 6

Summary of testcross data for plants of the constitution C Sh_1 Wx

	C Sh_1 Wx	*c sh_1 wx*	*C sh_1 wx*	*c Sh_1 Wx*	*C Sh_1 wx*	*c sh_1 Wx*	*C sh_1 Wx*	*c Sh_1 wx*
No.	784	1054	18	19	812	66	1	19
%	28.27	38.01	0.65	0.69	29.28	2.38	0.04	0.69

TABLE 7

Testcross data of plants of the constitution C Sh_1 Wx *corrected for the effect of the random assortment of the* B^9 *chromosome*

	Parental		Crossovers Region 1			Crossovers Region 2			Crossovers Region 1 & 2	
	C Sh_1 Wx	*c sh_1 wx*	*C Sh_1 wx*	*C sh_1 wx*	*c Sh_1 Wx*	*c Sh_1 wx*	*C Sh_1 wx*	*c sh_1 Wx*	*c sh_1 wx*	*C sh_1 Wx*	*c Sh_1 wx*
Corrected totals with the random assortment of B^9											
	784	988	746	18	19	18	66	66	66	1	1
Totals		2518			55			198		2	
%		90.80			1.98			7.14		0.07	
Totals excluding the classes that result from the random assortment of B^9											
Totals		1772			37			132		2	
%		91.20			1.90			6.79		0.10	

B^9 chromosomes will pair, hence, greatly reducing the chances of obtaining a crossover between the B^9 chromosome and the normal chromosome 9. This means that the incorporation of genes into the B^A element of an A-B translocation will be extremely difficult to accomplish using hyperploid plants. However, use of hyperploid plants as females will result in the ready production of plants heterozygous for the translocation and in such plants crossing over between the B^A element and its A homologue is expected in an appreciably higher frequency (Table 6).

With regard to the second conclusion, RHOADES (1933) in an analysis of crossing over in a chromosome 5 trisomic demonstrated that when intact A chromosomes are involved in crossing over the orientation of the trivalent at metaphase I may be either adjacent with the two chromosomes involved in the crossover passing to the same pole or alternate, with crossover chromosomes moving to opposite poles. The same pattern of chromosome distribution has been demonstrated for *TB-9b* in the hyperploid (Table 1) and "trisomic" plants (Table 5). However, since only a B^9 element and not a whole chromosome is involved this similarity to the behavior of a trisomic may be just fortuitous. The hyperploid and "trisomic" plants of this study more closely resemble the aberrations studied by RHOADES (1940) and MAGUIRE (1964). RHOADES studied plants with two normal chromosomes 5 plus a telocentric chromosome for the short arm of the same chromosome, and found in one instance that one out of 5605 female gametes possessed the genotype expected from nondisjunction of crossover products; in another cross involving the testing of 2037 female gametes, only two were the result of nondisjunction of the crossover chromosomes following crossing over. MAGUIRE (1964) found that in a maize "trisomic" stock carrying reciprocal Zea-Tripsacum interchange chromosomes and a normal Zea homologue, distribution following crossing over was usually (although not always) disjunctive. In both of these instances partial "trisomics" were involved and the data indicate that nondisjunction following crossing over can take place, but it is not nearly as frequent as in *TB-9b*.

BURNHAM (1950) found that in reciprocal translocations of maize involving chromosome six and other members of the A genome, crossing over in the interstitial segment would result in the crossover partners moving to opposite poles. Whether the movements of chromosomes following crossing over in the *TB-9b* experiments should be compared with those of BURNHAM's (1950) is certainly open to question, since the nature of the rearrangements is so different and regions of crossing over are not comparable. He was concerned with crossing over in the interstitial region, but the crossovers in these tests were, for the most part, in the arms distal to the break point and involved aneuploidy for B^9. However, in the testcross of heterozygous *TB-9b* plants only one 9^B element was present, thus providing the opportunity to determine chromosome segregation free from the complications of aneuploidy. The data from Table 6 indicate that the B^9 chromosome moves at random with respect to chromosome 9 whether or not a crossover has occurred.

The independent behavior of the B^9 chromosome may result from some peculiar

characteristic of the B centromere (or some region of the B chromosome closely associated with it). These chromosomes are well known for their erratic behavior, which does not in any way seem to influence the segregation of A chromosomes (RANDOLPH 1941). Evidently neither the attachment of a piece of an A chromosome nor crossing over alters the independent behavior of the B chromosome (or its centromere).

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SUMMARY

The transmission of the B^9 chromosome through the female gametophyte has been studied for hyperploid ($B^9B^9B^9B^9$), heterozygous (B^9B^9), and "trisomic" (B^9 9) plants. Male transmission was also studied for the latter class. In all cases, B^9 chromosomes exhibited occasional pairing and crossing over with the normal chromosome 9. The amount of crossing over was greatest in the heterozygous plants although there was a marked decrease in crossovers in the region of the break points. In all cases where the B^9 chromosomes was transmitted through the female it exhibited a strong tendency to assort independently of the other chromosomes involved in the aberration. This independent assortment was observed whether or not pairing and crossing over occurred between the B^9 chromosome and the normal chromosome 9. In contrast, male transmission of crossover B^9 chromosomes, in the one instance studied ("trisomic" $B^9$9 9 plants), suggested regular disjunction of crossover products. Evidence was also found for the occasional transmission of the B^9 chromosome as a duplication through male and female gametes.

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APPENDIX I: Calculation of expected frequency of *C* on ears of "trisomic" plants of the constitution *C Sh*$_1$ *wx* (9, 9, B^9) when pollinated by *c c sh*$_1$ *sh*$_1$ *wx wx* plants. (Assuming 35% trivalent formation, 33% crossing over between the break point and *C*, a B^9 map length of 59 and loss of univalent B^9 chromosomes.)

.35 (Trivalent frequency)
× .56 (Proportion of trivalents with crossing over in *C*–break point region = *[33 — map length C–break point]/[59 — map length B^9]*)
.1960 (Frequency of trivalents with crossover in *C*–break point region)

Random assortment of B^9			Disjunction of crossover chromosomes	
Genotypes of functional gametes expected, and frequencies			Genotypes of functional gametes expected, and frequencies	
c	.04900		. .	. . . . .
c c	.01225		. .	. . . . .
C c	.02450*		. .	. . . . .
C C	.01225*		. .	. . . . .
or				
c	.02450		*c*	.049
C	.02450*	.35†	*C*	.049*
C c	.02450*	— .1960‡	*C c*	.049*
c c	.02450		*c c*	.049
	.1960	.1540§		.1960
		× .5		
*C (Total)	= .08575		*C (Total)	= .098
	+ .07700	.07700¶		+ .077
	.16275			.175
(Freq. of *C* expected if B^9 assorts at random)			(Freq. of *C* if crossover chromosomes regularly disjoin)	

† Trivalent frequency.
‡ Trivalent cells expected with crossing over in *C*–break point region.
§ Frequency of trivalent with crossing over distal to *C*.
¶ Frequency of *C* gametes from trivalents with crossovers distal to *C*.

APPENDIX II: Calculation of expected frequency of *C* when "trisomic" plants of the constitution *C* Sh_1 *wx* (9, 9, B^9) are used as pollen parents in crosses to *c c* sh_1 sh_1 *wx wx* plants. (Assuming 35% trivalent formation, 33% crossing over between the break point and *C*, a B^9 map length of 59, and that pollen grains carrying duplications do not function in fertilization.)

.35 (Trivalent frequency)
× .56 (Proportion of trivalents with crossing over in *C*–break point region = *[33 — map length* C–*break point]/[59 — map length* B^9*]*)

.1960 (Frequency of trivalents with a crossover in *C*–break point region)

Random assortment of B^9		Disjunction of crossover chromosomes	
Genotypes of functional gametes expected, and frequencies		Genotypes of functional gametes expected, and frequencies	
c	.0490		
or			
C	.0245*	*C*	.049*
c	.0245	*c*	.049
	.098		.098
*C (Total) = .0245/.825†	= .0297	*C (Total) = .049/.825	= .0594

† 100% minus percent of duplicate gametes from trivalents that do not function = ½ × .35.

APPENDIX III: Calculation of expected frequency of crossovers in the *c*-sh_1 region on ears of "trisomic" plants of the constitution *C* Sh_1 *wx* (9, 9, B^9) when pollinated by *c c* sh_1 sh_1 *wx wx* plants. (Assuming 35% trivalent formation, 3% crossing over between *c* and sh_1, a B^9 map length of 59, and that univalent B^9 chromosomes are lost.)

.35 (Trivalent frequency)
× .05 (Proportion of trivalents with crossing over in *c*-sh_1 region = *[3 —* c-sh_1 *map length]/[59 — map length of* B^9*]*)

.0175 (Prophase I cells expected with crossing over)

Random assortment of B^9		Disjunction of crossover chromosomes	
Genotypes of functional gametes expected, and frequencies		Genotypes of functional gametes expected, and frequencies	
c sh_1	.004375	. . . . .	. . . . .
c c Sh_1 sh_1	.00109375†	. . . . .	. . . . .
C c Sh_1 sh_1	.00218750	. . . . .	. . . . .
C C Sh_1 sh_1	.00109375	. . . . .	. . . . .
or			
c sh_1	.0021875	*c* sh_1	.004375
C sh_1	.0021875*	*C* sh_1	.004375*
c c Sh_1 sh_1	.0021875†	*c c* Sh_1 sh_1	.004375†
C c Sh_1 sh_1	.0021875	*C c* Sh_1 sh_1	.004375
	.0175		.0175
*C sh_1 (Total)	†c Sh_1 (Total)	*C sh_1 (Total)	†c Sh_1 (Total)
.0021875	.00328125	.004375	.004375

APPENDIX IV: Calculations of expected frequency of crossovers in the *c-sh*$_1$ region when "trisomic" plants of the constitution *C Sh*$_1$ *wx* (9, 9, B^9) are used as pollen parents in crosses to *c c sh*$_1$ *sh*$_1$ *wx wx* plants. (Assuming 35% trivalent formation, 3% crossing over between the *c* and *sh*$_1$ loci, a B^9 map length of 59, and that pollen grains carrying duplications do not function in fertilization.)

.35 (Trivalent frequency)
× .05 (Proportion of trivalents with crossing over in *c-sh*$_1$ region = *[3 — c-sh*$_1$ *map length]/[59 — map length of B*^{9}*]*)

.0175 (Prophase I cells expected with crossing over)

Random assortment of B^9		Disjunction of crossover chromosomes	
Genotypes of functional gametes expected, and frequencies		Genotypes of functional gametes expected, and frequencies	
c sh$_1$	.004375		
or			
c sh$_1$	.00218750	*c sh*$_1$	.004375
C sh$_1$	.00218750*	*C sh*$_1$	.004375*
	.00875		.00875
**C sh*$_1$ (Total)	*c Sh*$_1$ (Total)	**C sh*$_1$ (Total)	*c sh*$_1$ (Total)
.0021875/.825† = .002652	0	.004375/.825 = .005303	0

† 100% minus percent of duplicate gametes from trivalents that do not function = ½ × .35.