

test-crosses of 31 plants derived from mosaic kernels.

Segregate solid-colored kernels	Segregate kernels with C-c mosaicism	All kernels colorless
12 ears	7 ears	12 ears

Seven independent cases of heritable mosaicism were found, among 31 individuals tested. One B^9 derivative, discovered before the seven cases above, has been tested for C-c mosaicism and Yg-yg mosaicism and found to exhibit both.

A number of explanations can be given for the unstable B^9 derivatives. They could simply be ring chromosomes. On the other hand, a mutation of the timing mechanism, which normally restricts nondisjunction of the B^9 to the second pollen mitosis, could account for their behavior. The fact that nondisjunction of the B^9 requires the presence of 9^B (Carlson), which is absent in these crosses, makes this idea less likely. A third explanation for the derivative B^9 's is loss of the minute short arm of the chromosomes (Randolph) and formation of an unstable telocentric. A cytological analysis should help decide between the alternatives.

Wayne Carlson

2. Regulation of nondisjunction of the B chromosome.

The B chromosome of maize undergoes nondisjunction at the second pollen mitosis, and rarely at other divisions (Roman, 1947). It has been shown, with TB-4a, that nondisjunction of the B^4 chromosome requires the presence of 4^B , even though the 4^B chromosome itself divides properly at the second pollen mitosis (Roman, 1949). The analogous situation was found with TB-10a (Longley, 1956) and TB-9b (Carlson, 1968). One can infer, therefore, that a gene(s) in the distal heterochromatin of the intact B (present on 4^B , 10^B , and 9^B) causes a "stickiness" or incomplete replication in the proximal part of the B, resulting in nondisjunction. The restriction of nondisjunction to the second pollen mitosis might result from repression of this gene(s) during all divisions except the second pollen mitosis. However, Battaglia proposed (1964) a different explanation for the localization of B chromosome nondisjunction to the second pollen mitosis. According to Battaglia, the B chromosomes of

maize and of other species may undergo nondisjunction only during especially rapid divisions. The rapidity of the second pollen mitosis would account for nondisjunction, rather than differential activity of a gene(s) in the distal heterochromatin.

The findings of Bianchi et al. (1961), together with the evidence presented here, indicate that a differential activity of the gene(s) controlling nondisjunction does occur. Bianchi showed, with A-B translocations, that losses of BA chromosomes occur at a low but significant rate during early development of the endosperm and embryo. This was reflected in endosperms and embryos with tissue sectors lacking dominant genes on the B^A. The sectors very frequently covered one-half of the endosperm or embryo. Small sectors were found much less frequently than would be expected if the B^A was equally susceptible to loss at all somatic divisions. Bianchi considered the losses he observed cases of somatic nondisjunction, although other phenomena could also explain the sectors. If somatic nondisjunction did occur, Bianchi's data suggest that the gene(s) regulating nondisjunction is differentially active in early divisions of the endosperm and embryo and relatively inactive at later divisions. The activity during early development can be explained if the gene(s) regulating nondisjunction is active primarily during the second pollen mitosis and inactive at all other divisions. A carry-over of residual gene activity or gene product to the egg and polar nuclei may occur, via the sperm, and account for nondisjunction in early somatic divisions.

Several tests have been made to determine whether early somatic sectoring is controlled by the distal region of the B, and whether a carry-over of gene activity from the second pollen mitosis does occur. Sected losses at early divisions of the endosperm and embryo were studied with TB-9b, using the markers C and Yg. The influence of the 9^B chromosome on somatic loss of the B⁹ was studied in the endosperm. Since the 9^B chromosome carries a gene(s) that is required for nondisjunction, absence of the chromosome should prevent the appearance of large colorless sectors in the endosperm. Crosses of the following type were made:

$$\underline{c} \ \underline{sh} \ \underline{wx} \quad X \quad 9^c \ sh \ wx \ 9^B \ wx \ B^9 \ C \ Sh \ B^9 \ C \ Sh$$

The major products of meiosis in the male parent are spores with 9^c sh wx B^{9C} Sh or 9^{Bwx} B^{9C} Sh. Both spore types function, although the unbalanced $9 B^9$ type does so at a reduced rate. Among the progeny, C Sh kernels were selected, divided into wx and Wx classes, and examined for colorless (c) sectors. Selection of wx progeny allows study of the $9 B^9$ spore type, and selection of Wx progeny insures that the $9^B B^9$ spore type is being examined. (Crossing over between Wx and the translocation breakpoint occurs less than 0.5% of the time--Robertson). Since all the kernels examined were C Sh in phenotype, they each contained at least one B^{9C} Sh in the endosperm. Nondisjunction does not occur in $9 B^9$ pollen, and all C Sh wx kernels carried one B^9 in the endosperm. However, nondisjunction occurs at a high rate in $9^B B^9$ pollen, and the endosperms of many C Sh Wx kernels contained two B^9 's. Consequently, somatic nondisjunction of the B^9 in C Sh Wx kernels can be hidden by the presence of a second B^9 . For this reason, a comparison of the absolute rates of sectoring between C Sh wx and C Sh Wx kernels cannot be accurately made. One can, however, compare sector sizes for the wx and Wx classes and determine whether early somatic loss is characteristic of the Wx kernels. Classification of progeny from three male parents was made. Colorless sectors which occupied 1/16 or more of the endosperm were designated "large" and lesser sectors "small". The results were as follows:

Male parent	Kernel type examined	Solid colored	Large colorless sector	Small colorless sector
748-2	C Sh Wx	451	6	1
	C Sh wx	188	1	3
748-10	C Sh Wx	530	7	3
	C Sh wx	100	0	4
713-3	C Sh Wx	968	9	11
	C Sh wx	<u>114</u>	<u>1</u>	<u>3</u>
TOTALS				
	C Sh Wx	1949	22	15
	C Sh wx	402	2	10

The distribution of sectors as to large vs. small is biased toward large sectors in the Wx class and toward small sectors in the wx class. The experiment was repeated in a different genetic background. Since more

data were available in the second experiment, the classification of large vs. small sectors was changed. Losses smaller than 1/16 were not recorded. Sectors covering 1/4 to 1/2 of the endosperm were designated large, and those 1/16 to 1/4 in size small. Only one male parent was used in this cross. The data are given below:

Male parent	Kernel type examined	Solid colored	Large colorless sector	Small colorless sector
1295-34	C Sh Wx	3100	22	3
	C Sh wx	873	2	12

Again, large sectors predominate among the C Sh Wx kernels, but not in the C Sh wx group. Kernels with sectors covering more than one-half of the endosperm were also found in the above experiments. These were not included in the tables. Such kernels probably result from nondisjunction of the B^9 at the first division, followed by irregular development of the endosperm (Stadler). As expected, sectors larger than 1/2 were found exclusively among Wx kernels. The data indicate that the $9^{B^{Wx}}$ chromosome is responsible for early somatic loss of the B^9 .

A second test was made comparing losses of the B^9 chromosome in the absence of 9^B with losses of the regular chromosome 9. Crosses of the following types were made:

1. c sh wx X 9^c sh wx 9^c sh wx B^{9^C} Sh
2. c sh wx X 9^c sh wx 9^C Sh wx

The male parents were sibs, and the 9^C Sh wx chromosome was derived from 9^c sh wx and B^{9^C} Sh by crossing over. The female parent was an inbred line. C Sh wx kernels were selected from each cross and examined for large vs. small sectors. (Large sectors were 1/16-1/2 in size, while small sectors were less than 1/16). The pooled data from several crosses of each type are given:

Male parent	Solid colored	Large colorless sector	Small colorless sector
9^c sh wx 9^c sh wx $B9^C$ Sh	2060	15	88
9^C Sh wx 9^c sh wx	1626	2	13

While loss of the B^9 chromosome seems to occur at a higher rate than loss of the normal 9, the distribution of losses, as to large or small sector sizes, is similar. Therefore, the prevalence of early losses of the B^9 chromosome in the presence of 9^B may be considered unusual, with regard to the normal pattern of chromosome loss.

A further test was made to determine whether somatic nondisjunction results from the carry-over of gene product (or gene activity) from the second pollen mitosis. Since B^A 's undergo nondisjunction in the pollen but not in the female inflorescences, somatic nondisjunction should only occur when the B^A is introduced through the male parent. Reciprocal crosses were, therefore, made between a chromosome 9 tester stock and plants containing TB-9b. To avoid differences in chromosome constitution of the endosperm in these crosses, a seedling trait, Yg , was used to identify B^9 losses. The crosses are given below:

1. yg bz wx X 9^{Yg} Bz wx $9^{B^{Wx}}$ $B9^{Yg}$ Bz $B9^{Yg}$ Bz
2. 9^{Yg} Bz wx $9^{B^{Wx}}$ $B9^{Yg}$ Bz $B9^{Yg}$ Bz X yg bz wx

The yg bz wx stock is an inbred line and the TB-9b line was backcrossed for three generations to an inbred line. Crosses (1) and (2) were not precisely reciprocal, but they were made at the same time and between the same families. The TB-9b parents were all from the same family and appeared uniform. The $9^{B^{Wx}}$ B^9 spore type was selected in the progeny of both crosses, using the wx marker. In the first cross, bz wx as well as Bz wx kernels were found, as a result of nondisjunction. Only the Bz wx kernels were selected for germination, and the seedlings were expected to be either yg (0 B^9) or Yg (1 B^9). The Yg plants that germinated were examined for yg sectors. In the second cross only Bz wx kernels were

found, since nondisjunction of the B^9 did not occur, and all the seedlings were Yg ($1 B^9$). The chromosomal constitution of seedlings examined from both crosses was the same: $9^{Yg} bz wx 9^{B^{Wx}} B^9 Yg Bz$. Only the source of the 9^B and B^9 chromosomes differed as to the male or female parent. Sectors for yg were classified as large if they occupied $1/4$ or more of the first two leaves of the seedling. Sectors smaller than this were also classified, regardless of size, if they extended the length of one leaf. The latter were designated small sectors. Results are given below:

Cross	Totally Yg	Large yg sector	Small yg sector
$Yg bz wx$ X TB-9b	738	10 (1.3%)	8 (1.1%)
TB-9b X $yg bz wx$	1152	0 (0.0%)	12 (1.0%)

Since the chromosome constitutions of the individuals classified in both crosses were identical, the absolute rates of sector formation can be compared. As expected, large sectors appeared only when the 9^B was transmitted through the male parent, while small sectors formed at equal rates in the two crosses.

Another experiment suggests that the distal heterochromatin of the B may be relatively inactive at the first division of meiosis. It was shown (Carlson, thesis) that nondisjunction of the B^9 chromosome occurs at a high rate in pollen carrying 9^B and B^9 , but probably never in $9 B^9$ pollen. This agrees with the results of Roman on TB-4a and Longley on TB-10a. In addition, the presence of the 9^B chromosome in the meiotic cells that give rise to $9 B^9$ pollen does not induce nondisjunction of the B^9 . There is no carry-over of activity of the 9^B from meiosis to the second pollen mitosis. The results are, therefore, consistent with a genetic inactivity of the distal heterochromatin of the B during the first meiotic division. (Alternative explanations are, of course, quite possible, and the sensitivity of the experiment to low levels of nondisjunction is questionable).

In conclusion, the data support the idea that a gene(s) controlling nondisjunction is differentially active in the second pollen mitosis, and relatively inactive at other divisions. The restricted activity of the gene(s) acts as a timing device for nondisjunction. A similar method for regulating nondisjunction is probably also present in the B's of rye

and Festuca, since genes required for nondisjunction have been found in these plants. However, it remains to be determined whether somatic losses observed in the present experiments resulted from nondisjunction. This requires cytological examination of fractional seedlings, which is currently underway. For preliminary results, see the following article.

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3. Isochromosome formation and the process of nondisjunction.

In the preceding article, sectorial loss of the B^9 chromosome in endosperm and sporophyte tissue is reported and attributed to somatic nondisjunction. Cytological examination of root tips of the fractional seedlings has been made for three plants. Surprisingly, the losses of the B^9 in these plants may be attributed to isochromosome formation, rather than nondisjunction. In one plant, two root tips gave chromosome counts of 20 and 21. The root tip with 21 chromosomes contained no telocentrics (as seen in metaphase) but did contain one chromosome with a large heterochromatic knob at each end (observable in prophase). In addition, two smaller knobs were seen halfway between the center of the chromosome and the distal knobs. The normal B^9 chromosome carries a large terminal knob ($K9^L$) and a second smaller knob (derived from the heterochromatic region of the B) in a median position. The extra chromosome found, therefore, was probably a B^9 isochromosome. In a second plant, only one root tip has been examined, but again metaphase and prophase cells indicate the presence of a B^9 isochromosome. A third plant was examined which showed multiple sectoring for loss of Yg, rather than single sector formation. Of four root tips that were examined, two contained 21 chromosomes, with one telocentric. One B^9 was apparently present in these roots. The other two root tips carried 22 chromosomes, only one of which was telocentric. Prophase cells showed one chromosome with the isochromosome knob pattern and another with one-half this pattern. One B^9 and one isochromosome B^9 were likely present. Somatic nondisjunction of the B^9 , followed by isochromosome formation could account for the findings in this plant.

In the previous article, evidence suggested that somatic sectoring is controlled by the same gene(s) that induces nondisjunction at the