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Instability of the Maize *B* Chromosome[1]

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Summary. The B^9 chromosome of maize exhibits a very ordered type of instability at the second pollen mitosis, when nondisjunction may reach a level of 95%. Much less commonly the chromosome is unstable during early development of the kernel. Instability in the kernel produces recessive sectors in either the endosperm or the sporophyte, reflecting the absence of dominant markers carried by the B^9. The causes of B^9 loss in the endosperm and the sporophyte were investigated for the two observable classes of sectoring: fractional loss (single event) and multiple loss (mosaic pattern). The fractional class represents isochromosome formation by the B^9 (Carlson, 1970, 1971). Data presented here suggest that the isochromosome is a by-product of telocentric formation at the second pollen mitosis, and does not arise directly from the B^9 chromosome. The chromosomal basis for the mosaic pattern of B^9 loss is not completely known. However, one class of mosaic kernels displays a heritable instability of the B^9 chromosome which apparently results from ring chromosome formation by the B^9. The time of origin of the ring B^9 chromosome is prior to the second pollen mitosis, since the unstable chromosome generated in the male parent is transmitted to both the endosperm and the sporophyte. Finally, a genetic factor controlling B^9 stability in the developing endosperm has been found. A single plant (1818-1), crossed as a female parent to a B^9-containing stock, induced a mosaic pattern of B^9 loss in the endosperm at a very high rate. The characteristics of this plant are being investigated.

Introduction

The *B* chromosome of maize is a small, acrocentric, supernumerary chromosome with no essential function in the plant. Retention of the *B* chromosome in maize populations may depend upon its ability to undergo nondisjunction at the second pollen mitosis (Roman, 1947; 1948). While the *B* chromosome is relatively stable at all other divisions, the rate of nondisjunction at the second pollen mitosis can reach 95% (Roman, 1948; Carlson, 1969a). In plants with small numbers of *B*'s, nondisjunction is coupled with preferential fertilization of the egg by the sperm carrying extra *B*'s (Roman, 1948). The combination of nondisjunction and preferential fertilization acts to increase the number of *B* chromosomes in a population. However, preferential fertilization breaks down in plants with more than a few *B*'s, thus setting an upper limit to the accumulation of *B*'s in a population (Carlson, 1969a).

The above concept of *B* chromosome behavior was derived from experiments involving translocations between the *B* chromosome and members of the regular (*A*) complement. Additional work with the $A-B$ translocations has revealed that the centromeric region of the *B* (B^A chromosome) undergoes nondisjunction at the second pollen mitosis, while the distal region (A^B) does not. However, the distal region participates in nondisjunction, since the presence of the A^B chromosome is required for nondisjunction of the B^A (Roman, 1950; Longley, 1956; Ghidoni, 1968; Carlson, 1969b; Ward, 1972). A small amount of *B* chromosome instability can be detected at divisions other than the second pollen mitosis (Bianchi *et al.*, 1961). The instability occurs during early development of the kernel, and results in a loss of dominant genes (carried by B^A chromosomes) in sectors of the endosperm or sporophyte. In this paper and a previous one (Carlson, 1970) the chromosomal mechanisms of sector formation are discussed.

Materials and Methods

The translocation $B-9b$ was used for all experiments. Its properties are discussed in detail by Robertson (1967). The B^9 chromosome of $TB-9b$ carries the dominant alleles *C* and *Yg*, while the 9^B is marked by the *Wx* allele. The *Wx* marker allows identification of the 9^B+B^9 balanced pollen class (balanced with respect to the tube nucleus) in testcrosses of $9^{wx}\,9^{B^{Wx}}\,B^9\,(+B^9)$ plants. Crossing over between *Wx* and the translocation break point occurs less than 0.5% of the time (Robertson, 1967). Nondisjunction of the B^9 in the $9^B\,B^9$ pollen class is shown by the absence of *C* from the endosperm.

Genetic Markers

All genes referred to are located in the short arm of chromosome 9. Gene order from the distal to proximal ends of 9S is *Yg C Wx*. The recessive *wx* allele produces an endosperm deficient in amylose, while the *Wx* allele produces normal starch. The waxy (*w x*) vs normal phenotype is determined by the staining properties of the endosperm in an iodine-potassium iodide solution. The recessive *c* allele inhibits anthocyanin color production in the endosperm and *yg* produces a yellow-green plant.

Cytological Techniques

Root tips were pre-fixed in a saturated monobromonaphthalene solution for 3 hours at room temperature, and fixed overnight in 3:1 ethanol/glacial acetic acid. The roots were stained by the Feulgen method and further stained during slide preparation with aceto-orcein.

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[1] Dedicated with much appreciation and respect to Dr. M. M. Rhoades on the occasion of his 70th birthday.

Results and Discussion

Sector formation in both the endosperm and sporophyte was studied using the translocation $B - 9\,b$. Two general types of B^9 loss have been observed which produce either a fractional or a mosaic pattern of sectoring. (Fractional refers to the appearance of a single recessive sector, while mosaic indicates a pattern of repeated loss.) Among fractional losses, a distinction can be made between large sectors (covering $^1/_4$—$^1/_2$ of the endosperm or plant) and small sectors. The large sectors occur much more frequently than expected on the basis of a random distribution of events during kernel development (Bianchi *et al.*, 1961). However, the large sectors occur only when the 9^B chromosome accompanies the B^9, and only when the 9^B and B^9 are transmitted through the male parent. Small fractional sectors occur independently of the 9^B chromosome and are not influenced by male or female transmission. The large fractionals, therefore, appear to be a unique class of events possibly controlled by the system which regulates nondisjunction at the second pollen mitosis (Carlson, 1970). One might expect that large sectors result from nondisjunction of the B^9 in the endosperm or sporophyte. Surprisingly, cytological analysis of plants containing large sectors (yellow-green) reveals that the B^9 loss results from isochromosome formation rather than non-disjunction (Carlson, 1970; 1971). Two explanations for isochromosome formation can be proposed: 1. The nondisjunction system is activated at the second pollen mitosis and this activity is transmitted through the sperm to the egg or polar nuclei. The nondisjunction mechanism remains active, at a reduced rate, for the first few divisions of the developing endosperm and sporophyte. Separation of the B^9 centromere is occasionally prevented during early development, but the environment of the sporophyte (and perhaps the endosperm) prevents migration of both chromatids to one pole. Instead misdivision of the centromere and isochromosome formation occur. 2. Alternately, the isochromosome could be formed in a two-step process, with the initial event occurring at the second pollen mitosis. The B^9 chromosome might occasionally undergo misdivision at the second pollen mitosis, forming an unstable telocentric (Darlington, 1939, 1940; Rhoades, 1940). This telocentric could give rise to an isochromosome during early divisions of the sporophyte. A third possibility, that B^9 instability develops prior to the second pollen mitosis, can be ruled out. Such a hypothesis would allow for sector formation by the unstable B^9 in both the endosperm and sporophyte of a single kernel, a phenomenon which is not found. To decide between hypotheses 1. and 2., crosses were made in which a homogeneous F_1 line carrying the $B - 9\,b$ translocation was crossed as a female parent to two lines that are isogenic except for the presence or absence of B chromosomes. The experiment determines whether the transmission of normal B chromosomes to the progeny by the male parent has any effect on the stability of the B^9 chromosome from the female parent. If activation

Table 1. *Influence of B chromosomes on stability of the B^9 chromosome*

Type of cross	Male parents	Yg	Large fractional ($^1/_4 - {}^1/_2$ of plant)	Small fractional
$9^{yg\,wx}\,9^{B^{Wx}}\,B9^{Yg}\,B9^{Yg}$ ♀	No B chromosomes			
× $9^{Yg\,Wx}\,9^{yg\,wx}$ ♂	1805 A	5,290	0	12
	1805 B	4,995	1	13
	1805-2	4,380	0	15
	1805-4	5,299	0	8
		19,964	1	48
		Corrected percent of large fractionals = .01%		
$9^{yg\,wx}\,9^{B^{Wx}}\,B9^{Yg}\,B9^{Yg}$ ♀	Two to four			
× $9^{Yg\,Wx}\,9^{yg\,wx} + B$'s ♂	B chromosomes			
	1806-1 (2 B's)	5,698	0	13
	1806-4 (2 B's)	5,929	0	26
	1806-8 (4 B's)	6,089	0	12
	1840-3 (2 B's)	4,793	1	9
		22,509	1	60
		Corrected percent of large fractionals = .009%		

The experimental design places the $B9^{Yg}$ chromosome in a *yg* background so that sporophytic losses of the B^9 produce yellow-green sectors on the plant. The female parent in the crosses given in the table is a genetically uniform F_1 derived from a *yg wx* stock and an inbred *TB-9b* line. The male parent is also a genetically uniform F_1, obtained by crossing the *yg wx* stock to two inbred Black Mexican lines. The Black Mexican lines are isogenic except for the presence of B chromosomes in one line, and absence of B's in the other. Almost all the progeny of these crosses receive from the female parent either a $9^{yg\,wx}\,B9^{Yg}$ gamete or a $9^{B^{Wx}}\,B9^{Yg}$ gamete (Robertson, 1967). In both cases, only the B^9 chromosome carries the Yg allele and a test of B^9 stability is possible. However, the male parent contributes the *yg* allele to only $^1/_2$ the progeny, reducing the effective size of the test population. Data are therefore adjusted as a "corrected percent" value for the frequency of B^9 instability. Classification of the progeny was performed by growing the seeds on a sand bench and classifying the first two leaves of seedlings for large *yg* sectors (covering $^1/_2$—$^1/_4$ of the plant) and small sectors (less than $^1/_4$ of the plant, but extending the length if one leaf).

of genes controlling nondisjunction is all that is required for subsequent formation of fractionals, the activation of normal B's in the pollen is expected to induce instability in the B^9 from the female parent. However, if telocentric formation at the second pollen mitosis is a prerequisite for isochromosome formation, the B^9 should be stable in all crosses. Results are given in Table 1. The plant marker *yg* was used as an indicator of sectors and both large sectors (covering $^1/_2$—$^1/_4$ of the plant) and smaller sectors were classified. According to hypothesis 1., the number of large fractionals should increase in the crosses involving extra B chromosomes. However, large sectors are almost absent from both the experimental and the control groups, and no change in B^9 stability can be detected. In addition, the relative frequencies of large fractionals to small fractionals show that no preference for large sectors is occurring in either group. Such a preference is expected for instability caused by the nondisjunction system (Carlson, 1970). The two large fractionals found in the present study were probably not produced by the nondisjunction system, but by a random distribution among cell divisions of the event that usually produces small sectors. The B^9 chromosome appears to be considerably more stable in the present set of data than in a previous experiment (Carlson, 1970 table 2). The reason for the greater stability is not known, but it is probably not relevant to the present experiment, since it involves the formation of small fractionals. The data suggest that the B^9 isochromosome is produced by telocentric formation at the second pollen mitosis, followed by isochromosome formation. One can still argue that a gene product of the nondisjunction system is transmitted through the pollen and is active during kernel development, but that the activity is chromosome limited. However, the previously cited ability of the A^B chromosome to influence nondisjunction of the B^A demonstrates that at least some gene activity controlling nondisjunction is transmissable between chromosomes.

In addition to fractional loss of the B^9, multiple losses can occur, giving a mosaic appearance to the plant or endosperm. Contrary to the findings with fractional kernels, mosaic kernels can form in the absence of the 9^B chromosome. In fact, plants lacking the 9^B have been preferentially used in studying mosaic kernels, since the complexities of nondisjunction and fractional sector formation are absent. The B^9 chromosome can be maintained as a supernumerary chromosome in plants containing two normal chromosomes 9. Mosaic kernels were obtained from crosses of the following types:

$$cc\,♀ \times 9^c\,9^c\,B^{9^C}\,♂.$$

The 9 9 B^9 plants were used as male parents because identification of mosaics is easier when the unstable chromosome is present in one dose (from the male parent) rather than two doses (from the female parent) in the triploid endosperm. It is not known whether the frequency of mosaic kernels differs when the B^9 is transmitted through male or female gametes. In the above cross, the B^9 chromosome is transmitted in a hyperploid pollen grain containing 9 and B^9. The colored (C) phenotype identifies progeny receiving the B^9, and among these kernels about 1—2% are mosaic for C vs c. Thirty-one mosaic kernels were selected, grown, and the plants testcrossed to a $c\,c$ line. In seven of the plants an unstable B^9 chromosome was present, since in the testcrosses virtually all colored kernels were mosaic (Column 1, Table 2). Therefore, the unstable B^9 in the endosperm of the seven selected kernels was also found in the sporophyte, indicating an origin prior to the second pollen mitosis. (Parallel findings with the B^4 chromosome are given by A. Ghidoni in this volume.) One unstable B^9 chromosome was transferred into a *y g* background, and mosaicism for *Yg* — *yg* in the sporophyte was observed. The demonstration of B^9 mosaicism in both the endosperm and sporophyte, and the heritability of that mosaicism, suggest that the unstable B^9's are ring chromosomes (McClintock, 1938, 1941a). In fact, examination of mitotic root tip cells from these plants reveals small, rounded derivatives of the B^9 chromosome. They are considerably smaller than the normal B^9 and are probably ring chromosomes, but their small size prevents accurate classification. No adequate explanation for the origin of ring B^9 chromosomes is known. The two other classes of mosaics in Table 2 have not been analyzed. They could result from ring chromosome formation, a breakage-fusion-bridge cycle, or some other phenomenon (McClintock, 1941b).

Table 2. *Classification of plants derived from* 31 *kernels with a C/c mosaic endosperm phenotype*

Ear segregates colorless (c) and mosaic (C — c) endosperm phenotypes (Unstable B^9)	All kernels colorless (c) (B absent or lacking C locus)	Ear segregates colorless (c) and solid-colored (C) endosperm phenotypes (Stable B^9)
7	12	12

The mosaic kernels were found in crosses of the type cc♀ × $9^c\,9^c\,B^{9^C}$♂. Classification of plants was accomplished by testcrossing to a cc tester (male) and observing segregation on the ears.

The B^9 instability described thus far usually arises at low rates in crosses with a stable B^9. However, in one cross, multiple losses of the B^9 chromosome were found frequently. A *c wx* plant (1818-1) was crossed as female to a $T\,B-9\,b$ male (1819 J). The female parent occurred in the F_2 generation of a cross between two inbred *c wx* lines, while the male was a hyperploid $9^{c\,wx}\,9^{B^{Wx}}\,B^{9^C}\,B^{9^C}$ member of an inbred $T\,B-9\,b$ line. Phenotypic results of the cross are as follows:

$$\begin{aligned} C\,Wx &= 78 \\ C/c\,Wx &= 35 \\ C\,wx &= 27 \\ C/c\,wx &= 15 \\ c\,Wx &= 103 \\ c\,wx &= 5 \end{aligned}$$

The presence of the B^9 in the endosperm is indicated by the colored (C) or mosaic (C/c) phenotype. Of the total colored seeds, 50/155 are variegated. The appearance of both Wx and wx kernels with C/c variegation demonstrates that the 9^{BWx} chromosome is not required for variegation. Thus the nondisjunction system of the *B* chromosome is probably not involved (Carlson, 1969b). Plant 1818-1 and fifteen other members of family 1818 were crossed as females to plant 1819J, using one pollen shedding. Since only 1818-1 produced an ear with a high frequency of mosaics, the cause of B^9 instability can be attributed to an unusual genetic constitution of this parent. Either a rare combination of genes in the F_2 plant or a new mutation could account for the uniqueness of 1818-1. Tests of inheritance for the gene (s) controlling instability are underway. (Recent crosses, that have not been thoroughly analyzed, confirm the genetic basis of the trait and suggest a single gene mode of inheritance.) It seems likely that the factor (s) responsible for B^9 instability is not specific for *B* chromosomes. Variable amounts of necrotic tissue were found on the C/c mosaic kernels, suggesting the loss of other chromosomes. Perhaps a mutation that produces a general instability of chromosomes is involved.

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