B CHROMOSOME NONDISJUNCTION IN CORN: CONTROL BY FACTORS NEAR THE CENTROMERE

WAYNE R. CARLSON AND TAU-SAN CHOU

Botany Department, University of Iowa, Iowa City, Iowa 52242

ABSTRACT

B chromosomes of corn are stable at all mitotic and meiotic divisions of the plant except the second pollen mitosis. In the latter division, *B* chromosomes undego mitotic nondisjunction at rates as high as 98%. Studies by several workers on *B-A* translocation chromosomes have provided evidence for the existence of four factors on the *B* chromosome that control nondisjunction and are separable from the centromere. Two of these factors, referred to here as factors 3 and 4, flank the *B* chromosome centromere. Factor 3 is the centromere-adjacent heterochromatin in the long arm of the *B* chromosome; factor 4 is located in the minute short arm. Evidence is presented here supporting the existence of factors 3 and 4. Deficiencies that include each factor were identified following centromeric misdivision events, with breaks at or near the centromere of a *B*-translocation chromosome. *B* chromosomes lacking factors 3 or 4 show much less nondisjunction than do chromosomes containing them. The possible function of factor 4 in nondisjuntion is also discussed.

B chromosomes are supernumerary chromosomes that are not essential for the life of an organism and are considered by some to be parasitic (OSTERGREN 1945; RHOADES and DEMPSEY 1972). Contributing to the theory of parasitism is the observation that *B* chromosomes in a number of organisms possess systems for increasing their frequency above that expected in the gametes of an organism. The systems, known as accumulation mechanisms, differ widely between organisms, and the one in corn is unique. In corn, *B* chromosomes undergo mitotic nondisjunction at a very high frequency in one cell division, the second pollen mitosis. At this stage, nondisjunction in cells with a single *B* chromosome produces two sperm, one containing two and the other no *B* chromosomes. Subsequent preferential fertilization of the egg by sperm containing two *B* chromosomes increases chromosome frequency. (ROMAN 1947, 1948).

The precise timing of nondisjunction at the second pollen mitosis and its very high frequency (usually 50–95%) make studies of nondisjunction possible. The findings of several workers indicate that four separate factors on the *B* chromosome control nondisjunction. The approximate locations of the factors and references are given in Figure 1. The assignment of four nondisjunctional loci on the *B* chromosome is provided for convenience. However, the sites are not considered settled, as noted in the legend to Figure 1. In this report, misdivision of
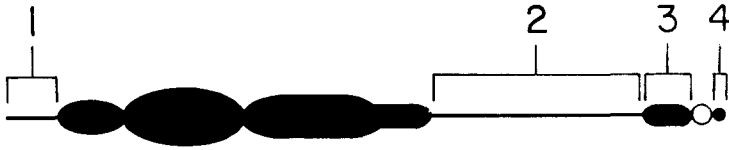
FIGURE 1.—The *B* chromosome of corn. (Darkened areas are heterochromatic regions. The open circle is the centromere.) Factors that control nondisjunction of the *B* are numbered 1–4, and approximate locations are shown. The existence of these factors was suggested in the following references: factor 1 (ROMAN 1949; WARD 1973); factor 2 (FIGURE 2 and Table 4, CARLSON 1973; translocation 1866, CARLSON 1978a; LIN 1978); factor 3 (RHOADES, DEMPSEY and GHIDONI 1967; RHOADES and DEMPSEY 1972); factor 4 (CARLSON 1978b; LIN 1979). The assignment of factor numbers required certain assumptions when comparing results of different workers. For example, the large deletion of proximal *B* chromatin in CARLSON's modified *B⁹* chromosome and the more accurately localized deletions constructed by LIN are believed to identify the same site, factor 2.

the *B* centromere is utilized to provide further evidence supporting the existence of factors 3 and 4. Experiments were carried out with the *B-A* translocation, *B-9b*. The *B* chromosome breakpoint of this translocation is within the distal *B* heterochromatin, as shown in Figure 2. Nondisjunction is restricted to the chromosome with the *B* centromere (*B⁹*) and nondisjunction rates refer to this chromosome. The translocation helps simplify analysis of the *B* chromosome because chromosome *9* genetic markers can be attached to it.
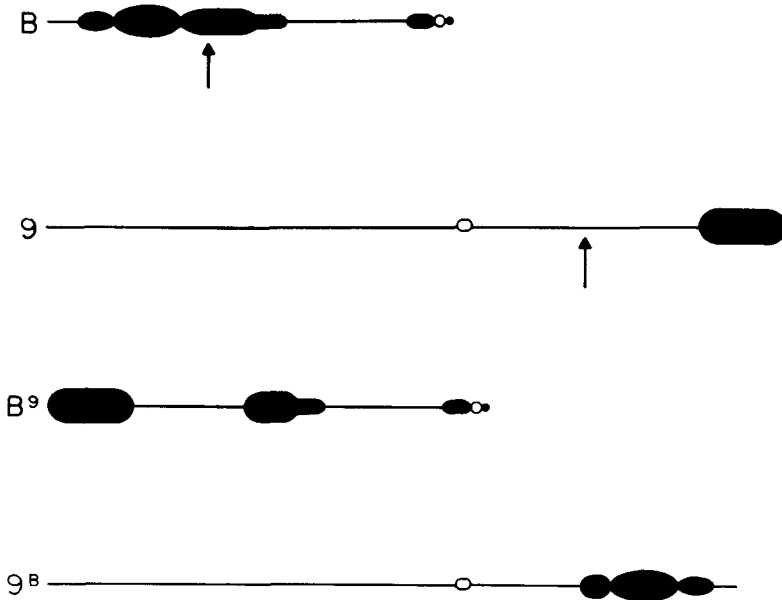

FIGURE 2.—Formation of the *B-9b* translocation. Exchange points on the *B* and chromosome *9* are shown with arrows. Note the unique arrangement of heterochromatin on the *B⁹*.

MATERIALS AND METHODS

The techniques used in analyzing *TB-9b* crosses are given elsewhere (CARLSON 1978a). Here, a brief review of the calculation of nondisjunction rates will be given. Testcrosses are carried out in which the female parent carries recessive markers for *9S*, and the male parent carries *TB-9b*, with dominant alleles on *B9*. Nondisjunction of the *B9* in the male parent produces pollen with sperm containing either 2 or no *B9*'s. During double fertilization, the deficient sperm may unite with the egg and produce a partially hemizygous plant. If the female parent carries the recessive *yg* marker, the result is a yellow-green plant. Alternatively, the deficient sperm may fertilize the polar nuclei and produce a recessive endosperm phenotype. Ordinarily, *c* (colorless endosperm) or *bz* (bronze aleurone) are the endosperm markers used in the tester. The rate of nondisjunction in the cross is the proportion of kernels with either a recessive plant or a recessive endosperm phenotype.

Kernels with the recessive plant phenotype are referred to as class I nondisjunctional phenotypes. The recessive endosperm group is the class II phenotype. The frequency of class II is considerably higher than that of class I, since sperm with extra *B*-type chromosomes have an advantage in fertilization of the egg (ROMAN 1948). The extent of preferential fertilization of the egg is reported here as the percent of class II kernels per total cases of nondisjunction.

In some testcrosses, *TB-9B* is homozygous (*9B 9B B9 B9*), and all progeny are included in the calculation of nondisjunctional rates. For *TB-9b* heterozygotes (*9 9B B9*), the *Wx* marker is used to distinguish presence of the translocation in progeny. (The *Wx* locus controls starch composition in the endosperm. Classification of dominant *vs.* recessive is made by an iodine-staining procedure.) The *Wx* gene is located on *9B* near the translocation breakpoint (ROBERTSON 1967). Heterozygotes are constructed with the recessive *wx* allele on chromosome *9* and *Wx* on *9B*. Only progeny with the *Wx* phenotype are classified for nondisjunction.

Chromosome preparations of meiotic chromosomes were made in a conventional manner with propiocarmine stain. Mitotic cells were arrested with 8-hydroxyquinoline, followed by fixation and Feulgen staining. Usually, Feulgen staining was followed by squashing in aceto-orcein stain.

RESULTS

Studies of occasional instability of the *B9* chromosome led to the recovery of a *B9* isochromosome. The isochromosome, now referred to as the original isochromosome, generally gives low rates of nondisjunction. In addition, it produces mosaic endosperm phenotypes at a high frequency in testcrosses. The mosaic phenotypes probably result from misdivision of the isochromosome and the production of unstable telocentrics (CARLSON 1973). An experiment was carried out to determine more accurately the properties of the original isochromosome. A controlled comparison with the standard *B9* was made. The initial cross was as follows: *9* (*c wx*) *9B* (*Wx*) iso-*B9* (*C C*) × *9* (*c wx*) *9B* (*Wx*) *B9* (*c*) *B9* (*c*).

An isochromosome class was selected from progeny kernels with the *C Wx* endosperm phenotype. In addition to endosperm classification, plants were selected with the homozygous *Wx Wx* constitution and with 50% pollen sterility. These should contain *9B* (*Wx*) *9B* (*Wx*) iso-*B9* (*C C*). A testcross, as female, to a *c c wx wx* stock was used to confirm the plant constitution. (The rationale for this selection procedure is given in CARLSON 1978a). A standard *B9* class of plants was selected from the same progeny, using kernels with a *c Wx* endosperm phenotype. *Wx wx* plants that displayed 25% pollen sterility were selected. They should contain *9* (*c wx*) *9B* (*Wx*) *B9* (*c*). This constitution was also confirmed by crossing the plants as female to the *c c wx wx* tester.

After selection of isochromosome and standard B^9 classes, the plants were crossed as male parents to a bz bz wx wx tester. Results are given in Table 1. The rate of class II nondisjunction (recessive endosperm) for the standard B^9 was 66%, while that for the isochromosome was 28%. The bz bz wx wx tester mentioned above was also heterozygous Yg yg. A sample of kernels from the testcross was grown and classified for the yellow-green (yg) phenotype. In this manner, an estimation of class I nondisjunction was obtained. Preferential fertilization rates were calculated and found to be very similar for the isochromosome and the standard B^9 (Table 2). The difference between the isochromosome group and the standard B^9 group in class II nondisjunction, therefore, reflects a true difference in nondisjunction rate.

TABLE 1

Endosperm classification

Family number of male parent	Bz	bz	Bz/bz: Multiple sectors	Bz/bz: Very large sector	Bz/bz: Large sector	Bz/bz: Small sector
Isochromosome group						
3331–4	1060	384	67	9	8	5
10	761	515	121	7	14	7
41	781	310	63	9	8	13
42	948	344	92	4	9	12
45	888	430	57	3	14	7
47	1191	451	66	7	9	11
51	890	408	79	6	13	5
	6519	2842	545	45	75	58
		% bz = 28%				
		% mult. = 5.4%				
Standard B^9 group (Wx progeny classified)						
3332–11	300	493	2	2	1	1
14	328	493	0	0	0	1
16	183	390	0	1	1	1
26	234	520	1	0	5	2
29	239	549	2	0	0	0
36	218	543	1	1	1	2
38	269	561	3	0	4	0
	1771	3549	9	4	12	7
		% bz = 66%				
		% mult. = 0.2%				

Endosperm classification of testcrosses involving the original B^9 isochromosome (family 3331) and the standard B^9 (family 3332). Seven plants were selected from each group and crossed as males to a bz bz wx wx tester. Chromosomal and genetic constitution of family 3331 was 9^B (Wx) 9^B (Wx) iso-B^9 (Bz Bz). The constitution of family 3332 plants was 9 (wx) 9^B (Wx)B^9 (Bz). Only progeny with the Wx phenotype were recorded for crosses of 3332, since only they contained the translocation.

Several categories of chimeric (Bz/bz) phenotypes are listed in the table. The multiple class is any kernel with more than one recessive sector on the endosperm. The very large sectorial kernel has a single recessive area that occupies more than 3/4 of the endosperm. The large sector class has a single recessive area that occupies 1/4 to 3/4 of the endosperm. Small events include all kernels with visible single sectors that cover less than 1/4 of the endosperm.

TABLE 2

Seedling classification

Chromosome tested	Endosperm phenotype of seeds planted	Number of seeds planted	Yg	yg	Yg/yg Multiple sectors	Yg/yg Very large sector	Yg/yg Large sector	Yg/yg Small sector
Isochromosome B^9	Bz	1723	1488	181	23	1	4	5
	bz	719	683	7	0	0	0	2
	Bz/bz multiple	110	98	1	6	0	1	3
	Bz/bz single sector (all types)	46	39	3	1	0	0	2

Preferential fertilization = 65%
Yg/yg multiples = 1.2% ($\times$2)

Chromosome tested	Endosperm phenotype of seeds planted	Number of seeds planted	Yg	yg	Yg/yg Multiple sectors	Yg/yg Very large sector	Yg/yg Large sector	Yg/yg Small sector
Standard B^9	Bz	555	304	225	0	0	0	3
	bz	996	858	0	0	0	0	0
	Bz/bz multiple	1	1	0	0	0	0	0
	Bz/bz single sector (all types)	4	4	0	0	0	0	0

Preferential fertilization = 66%
Yg/yg multiples = 0%

Partial classification of crosses listed in Table 1 for the plant character, yellow-green (yg). The female parent of the crosses was heterozygous Yg/yg, so that all yellow green and chimeric phenotypes must be multiplied by 2. For each cross, a single ear was planted in its entirety and classified in the seedling stage. Among sectored plants, the multiple phenotype contains more than one yellow stripe on the plant. The very large sector class has a single yellow sector that covers more than 3/4 of the plant. Large sectors cover 1/4 to 3/4 of the plant. Small sectors have a single yellow stripe smaller than 1/4 of the plant.

All kernels that contained a dominant phenotype in the endosperm and a recessive phenotype in the plant were class I nondisjunctional phenotypes. The frequency of class I phenotypes was multiplied by 2, because of the heterozygosity (Yg yg) of the tester. Kernels with the recessive endosperm phenotype and dominant plant phenotype were class II phenotypes (see MATERIALS AND METHODS). Preferential fertilization was calculated as the frequency of class II phenotypes per total nondisjunction. No correction for germination rates was used, since their effect on the results would be small.

The data reported in Tables 1 and 2 show that a high frequency of mosaic endosperm and plant phenotypes were produced in the isochromosome crosses. The variegated phenotypes of the plant and endosperm may have a common origin in misdivision of the B^9 isochromosome. Previous evidence suggested that unstable telocentric derivatives of the isochromosome produce the mosaic endosperm phenotype (CARLSON 1973). Similarly, analysis of root-tip mitoses of mosaic Yg-yg plants indicates the presence of telocentric B^9's. The 30 multiple sectored plants of Table 2 were classified as follows: 24 had a single B^9 telocentric, 1 had two telocentrics, 2 showed chimerism for one and two telocentrics, 1 was not classifiable, and 2 had one isochromosome. These findings indicate that unstable telocentrics are frequently produced by the isochromosome. However, it is not yet known whether instability is heritable or is corrected, by some mechanism, after one generation.

In previous work, six telocentric B^9's were recovered from the isochromosome. These telocentrics were not checked for instability in their original generation, but have subsequently proven to be somatically stable. With respect to nondis-

junction, the six stable telocentrics separate into two groups. One class, now referred to as type 1, is virtually incapable of nondisjunction. The second class (type 2) gives nondisjunction at variable rates (CARLSON 1973; Tables 2 and 3). The ability of the original isochromosome to produce two types of telocentrics was explained when isochromosome bivalents were examined in the pachytene stage of meiosis. Previously, only univalents that paired on themselves were seen in plants with two isochromosomes. Observations on the relatively rare bivalents reveal that the isochromosome is asymmetric, with little heterochromatin on one side of the centromere (Figure 3). Formation of the isochromosome occurred, therefore, in such a way as to delete most or all of the centromeric heterochromatin from one arm. The original isochromosome is, therefore, a pseudo-isochromosome. A comparison of the pachytene morphology of type 1 and type 2 telocentrics suggests that they arose from opposite arms of the isochromosome (Figures 4 and 5). The chromosome with little centromeric heterochromatin is the type 1 telecentric. In mitotic preparations, the type 1 telocentric appears to lack centromeric heterochromatin (Figure 6), while the type 2 telecentric clearly contains it (Figure 7).

A second type of isochromosome, referred to as the new isochromosome, was recovered following misdivision of the type 1 telocentric (CARLSON 1978b). Pachytene morphology of the new isochromosome shows that it is a symmetrical, true isochromosome (Figure 8). Although the type 1 telocentric rarely undergoes nondisjunction, its isochromosome derivative displays significant rates of nondisjunction. It also produces mosaic endosperm phenotypes at high frequencies. Data for three separately derived new isochromosomes are given in Table 3.

DISCUSSION

Previous studies with the *B-9b* translocation resulted in the discovery of a B^9 isochromosome, now referred to as the original isochromosome. The original isochromosome gave variable, but usually low, rates of nondisjunction and a
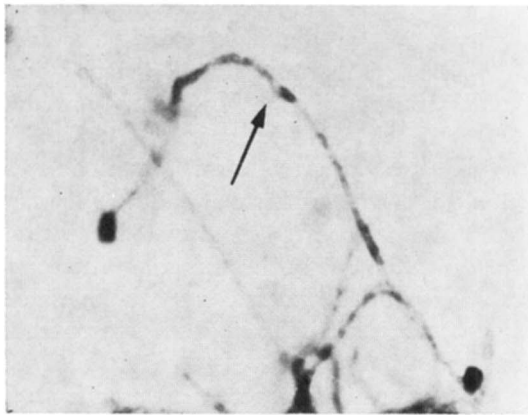

FIGURE 3.—A pachytene bivalent of the original isochromosome. Arrow indicates the centromere. Centric heterochromatin is found primarily on one side of the centromere.
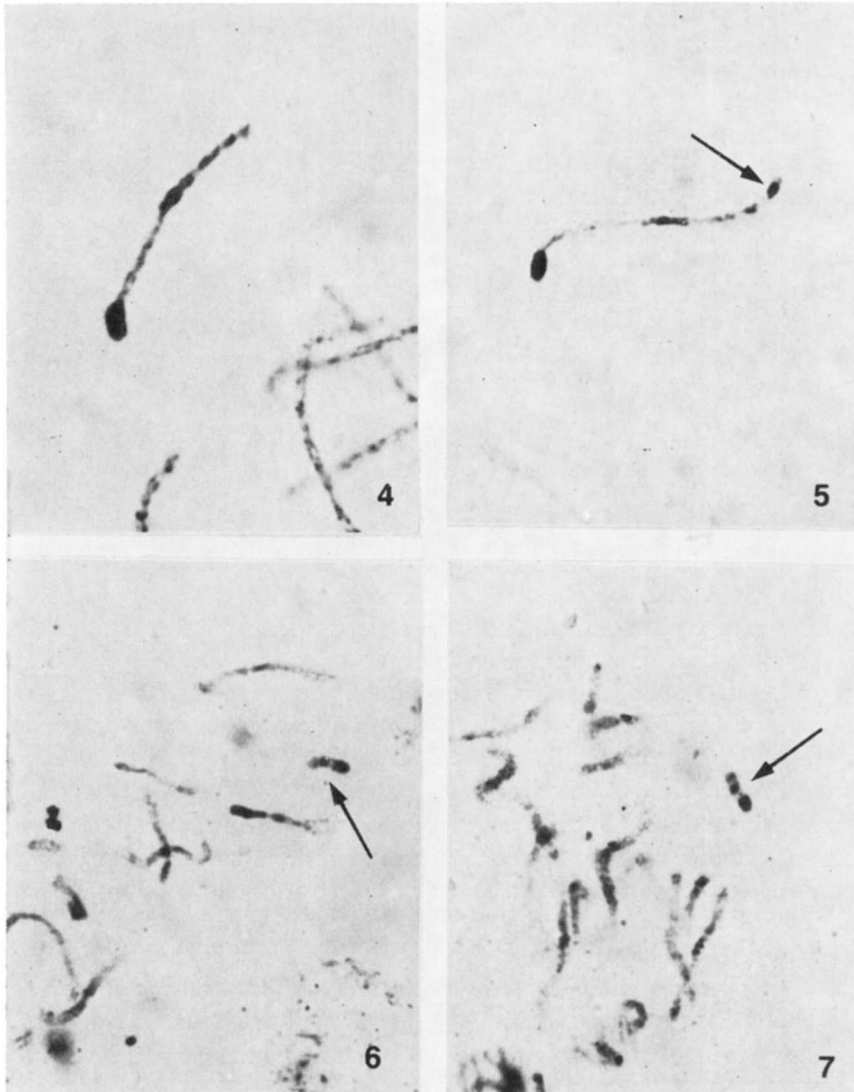
FIGURE 4.—A type 1 telocentric B^9 in pachytene. (Family number 2075).

FIGURE 5.—A type 2 telocentric B^9 in pachytene. (Family number 2003). Arrow indicates centric heterochromatin.

FIGURE 6.—A type 1 telocentric B^9 in mitotic prophase. (arrow)

FIGURE 7.—A type 2 telocentric B^9 in mitotic prophase (arrow).

high frequency of mosaic kernels in testcrosses. Mosaic phenotypes were attributed to unstable telocentric B^9's produced by misdivision of the isochromosome (CARLSON 1978b). Misdivision is believed to result from an incomplete nondisjunctional event at the second pollen mitosis (Brannen 1978).

Here, a comparison of testcrosses between the isochromosome and the standard B^9 is reported. The findings reinforce the view that the original isochromosome
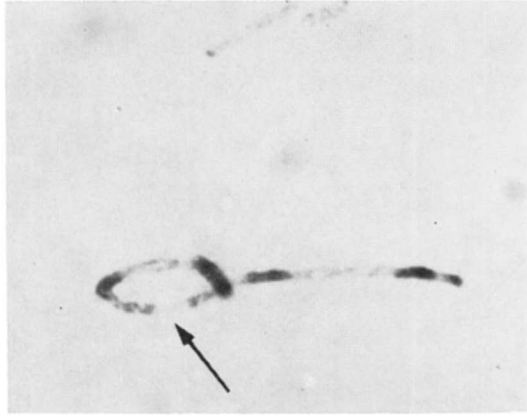
FIGURE 8.—Pachytene bivalent of the new isochromosome. Note the deficiency of centric heterochromatin. Arrow indicates the centromere.

is defective in the nondisjunctional process. As reported in Table 1, the rate of class II nondisjunction for the standard B^9 is 66%; whereas, the rate for the isochromosome is 28%. The data of Table 2 show that the class II nondisjunction rates need not be adjusted for different levels of preferential fertilization. In addition, the isochromosome displays a high level of endosperm (5.4%) and plant (2.35%) mosaicism not found in the standard B^9 crosses. Previous evidence that unstable telocentric B^9's produce endosperm mosaicism is supplemented by our finding that 27 of 30 mosaic Yg-yg plants examined from Table 2 contained B^9 telocentrics, rather than isochromosomes.

Although unstable B^9 telocentrics are produced in isochromosome crosses, it is not known whether their instability is heritable or is corrected in some way following the generation of origin. Progeny tests on the mosaic Yg-yg plants of Table 2 have not yet been carried out. It is known, however, that stable telocentric B^9's can be recovered from the isochromosome. Six telocentric B^9's isolated earlier (CARLSON 1973) appear stable in both plant and endosperm divisions. Whether they were stable in their generation of origin was not determined.

The six stable telocentric B^9's were found to differ among themselves in nondisjunctional properties. Four of the telocentrics (type 1) showed virtually no nondisjunction; whereas, two (type 2) gave significant levels of nondisjunction. The ability of the original isochromosome to produce two classes of telocentrics is explained by the finding of asymmetry in isochromosome morphology. One arm of the isochromosome has very little centromeric heterochromatin; whereas, the other arm appears to have as much as a normal B chromosome (Figure 3). The isochromosome presumably arose through breakage at different points on two chromatids and is, in fact, a pseudo-isochromosome. The type 1 telocentric derives from the isochromosome arm that is deficient in centromeric heterochromatin (Figures 4 and 6), while the type 2 telocentric comes from the other arm (Figures 5 and 7). A deletion of most centromeric heterochromatin can, therefore, explain the lack of nondisjunction by type 1 telocentrics. It should be noted,

TABLE 3

Endosperm classification

Family number of male parent	Bz	bz	Bz/bz Multiple sectors	Bz/bz Very large sector	Bz/bz Large sector	Bz/bz Small sector
New isochromosome 2820						
2820A	204	25	31	0	9	4
2820B	255	29	28	2	6	3
2820C	161	30	43	4	11	1
2820D	248	21	26	1	3	4
2820E	216	43	78	0	15	8
	1084	148	206	7	44	20
% bz = 9.8%						
% multiples = 13.7%						
New isochromosome 2821						
2821A	269	33	14	0	8	4
2821B	255	48	31	3	5	2
2821C	230	10	5	1	2	2
2821D	263	55	8	4	7	0
2821E	264	50	30	1	8	2
	1281	196	88	9	30	10
% bz = 12.1%						
% multiples = 5.5%						
New isochromosome 2822						
2822A	307	71	23	1	2	2
2822B	293	21	9	0	1	2
2822C	263	65	14	0	12	5
2822D	282	17	18	0	6	5
	1145	174	64	1	21	14
% bz = 12.3%						
% multiples = 4.5%						

Endosperm classification of testcrosses involving three new isochromosomes. Each isochromosome was derived independently from the type 1 telocentric. Four or five plants from each group were crossed as follows: bz bz × 9^B 9^B iso-B^9 (Bz). The method of classification of chimeric Bz/bz kernels was the same as in Table 1.

however, that the relationship between centromeric heterochromatin and nondisjunction depends on a correlation established for the six telocentric B^9's. Additional telocentrics are now being derived from the original isochromosome to determine whether the presence or absence of centromeric heterochromatin is always correlated with the ability or inability, respectively, to carry out nondisjunction.

Centromeric heterochromatin (factor 3) is assumed to be the "sticky" region that produces nondisjunction of the B chromosome (RHOADES and DEMPSEY 1972). Loss of the centromeric heterochromatin is expected to have a *cis*-dominant effect on chromosomal stickiness. However, stickiness of the type 1 telocentric can apparently be induced by the addition of extra B chromosomes. The type 1 telocentric gives nondisjunction at low rates and sometimes produces

mosaic kernels in the presence of extra B chromosomes (CARLSON 1978a). It seems likely, therefore, that a region of chromatin that is capable of stickiness remains on the type 1 telocentric. A small amount of centromeric heterochromatin is sometimes visible on the type 1 telocentric and may account for its residual stickiness.

The type 1 telocentric can produce isochromosome derivatives, and three new isochromosomes were recovered from it. Nondisjunctional properties of the new isochromosomes are given in Table 3. They give significant levels of nondisjunction, as well as a high rate of unstable endosperm phenotypes. The results appear comparable to those for type 1 telocentrics in the presence of extra B chromosomes. Apparently, the duplication of B chromatin during isochromosome formation increases chromosomal stickiness. The finding suggests again that a site of chromosomal stickiness is present on the type 1 telocentric.

The behavior of the new isochromosomes in testcrosses is somewhat similar to that of the original isochromosome. However, comparison of Tables 1 and 3 indicates a higher rate of nondisjunction for the original isochromosome. Although the data are from separate experiments and cannot provide a controlled test, the higher level of nondisjunction by the original isochromosome is probably due to its intact centromeric heterochromatin.

The reported findings of misdivision products of the original isochromosome suggest a role for centromeric heterochromatin in nondisjunction. However, they do not explain nondisjunctional properties of the original isochromosome. It contains a large, apparently intact region of centromeric heterochromatin, but is defective in nondisjunction, compared to the standard B^9 (Table 1). The isochromosome may, therefore, lack a factor (factor 4 in Figure 1) that assists in nondisjunction. Both the low nondisjunctional rate of the isochromosome and its tendency to undergo misdivision may result from a single defect in the nondisjunctional process. Factor 4 may assist in unipolar migration of the B centromere during nondisjunction. The isochromosome, lacking factor 4, may have a strong tendency toward bipolar orientation that conflicts with heterochromatic stickiness (factor 3) during the second pollen mitosis. The result is lowered nondisjunction and frequent centromeric misdivision. The factor proposed here and earlier (CARLSON 1978b) to explain the behavior of the original isochromosome may be equivalent to one reported by LIN (1979). LIN found that a factor in the B short arm assists nondisjunction, but is not required for its occurrence.

An alternate proposal to explain the data of Table 1 is that isochromosome formation itself may have an adverse effect on nondisjunction. Perhaps the attachment of a second long arm to the centromere has a cis-dominant effect on its behavior. This hypothesis can be tested by comparing the type 2 telocentric to the standard B^9 for nondisjunctional properties. The type 2 telocentric presumably lacks factor 4, and may show less nondisjunction than an intact B^9. Preliminary tests suggest that type 2 telocentrics give less nondisjunction than a standard B^9, but further data are needed.

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