TWO NEW *B-10* TRANSLOCATIONS INVOLVED IN THE CONTROL OF NONDISJUNCTION OF THE *B* CHROMOSOME IN MAIZE[1]

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ABSTRACT

A *B-A* translocation, *TB-10(18)*, has been established involving breakpoints in the proximal region of the long arm of chromosome *10* and the minute short arm of the maize *B* chromosome. *TB-10(18)* differs in its nondisjunctional behavior at the second microspore division from *TB-10(19)*, which has a breakpoint in the same region of *10* but in the heterochromatic region of the long arm of *B*, in the following ways: (1) Nondisjunction of the B^{10} chromosome of the *TB-10(18)* translocation occurs in the absence of the reciprocal element (10^B), albeit at low frequency. (2) Presence of 10^B increases the frequency of B^{10} nondisjunction but not to the level found for *TB-10(19)* and certain other translocations. (3) The frequency of B^{10} nondisjunction varies among closely related sublines both when 10^B is present and when it is absent. It is inferred that the B^{10} of *TB-10(18)* carries all the components of *B* necessary for nondisjunction but that expression is weak in the absence of 10^B, suggesting the existence in the *B* chromosome short arm of a factor influencing efficient nondisjunction.

THE *B* chromosome of maize is interesting cytogenetically in two respects: (1) it is mainly heterochromatic and has no apparent function in the plant (McCLINTOCK 1933; RANDOLPH 1941) except that it interacts with knobs in producing chromatin loss at the second pollen mitosis (RHOADES and DEMPSEY 1973) and it enhances crossing over in the normal chromosome complements (RHOADES 1968; HANSON 1969 and WARD 1973b), and (2) it frequently undergoes nondisjunction during the second pollen mitosis. The results of nondisjunction can be detected genetically when a *B* chromosome is involved in a translocation with an *A* chromosome (ROMAN 1947). One derived chromosome, termed B^A and carrying the *B* centromere, can fail to disjoin during the second pollen mitosis. This failure results in one sperm that is deficient for the portion of the *A* chromosome borne on B^A and a second sperm that duplicates the same segment. Following double fertilization, a pollen grain with dissimilar sperm produces a kernel whose endosperm and embryo phenotypes do not correspond with respect to genes located in the B^A chromosome.

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ROMAN (1950) reported that although the A^B chromosome divides normally, it must be present in the same microspore in order for B^A to fail to disjoin. The necessity of A^B for the nondisjunction of B^A suggests that there are at least two separable components controlling nondisjunction, which, for convenience, may be said to occupy receptor and regulator sites. The receptor site is probably associated with the *B* centromere or the adjacent heterochromatic segment, and the regulator is associated with either or both of the two arms. WARD (1973a) mapped one regulator site in the distal euchromatic tip of the long arm. LIN (1978) discovered the second regulator site in the proximal region of the long arm of the *B* chromosome. He created deletions of different regions in the *B* long arm by combining 10^B and B^{10} chromosomes from two different *B-10* translocations. Deletion of the heterochromatic portion did not have any effect on nondisjunction. Some deletions of the proximal euchromatic portion resulted in the disappearance of this activity.

CARLSON (1973) isolated telocentric derivatives from the iso-B^9 chromosomes of the *TB-9b* translocation and found that the telocentric B^9 cannot nondisjoin at the second pollen mitosis. Since the telocentrics lack only the short arm and probably some centromere material, he concluded that a factor essential for nondisjunction is located in one of these regions. He advanced the hypothesis that two sticky sites flanking the centromere may be required for nondisjunction. The telocentrics have only one site, which is not sufficient to cause nondisjunction (CARLSON 1978a). In other words, they contain a defective receptor site. CARLSON (1978b) also recovered isochromosomes from the telocentric B^9 and observed that, like the original iso-B^9, the reconstituted iso-B^9 can undergo nondisjunction, as would be expected from the hypothesis.

This report describes the structure and behavior of a new *B-10* translocation, termed *TB-10(18)*, which has in the *B* short arm a minor regulator factor conditioning nondisjunctional behavior of the *B* chromosome. The behavior of this translocation will be compared with a second new translocation, termed *TB-10(19)*, which behaves as does another translocation, *TB-10a*, established earlier.

MATERIALS AND METHODS

The translocations *TB-10(18)* and *TB-10(19)* were isolated following X-irradiation of plants carrying *B* chromosomes (LIN 1972). The positions of the breaks in the *B* and *10* chromosomes are described below in the RESULTS. Both of the B^{10} translocated chromosomes carried the *R* and *EF* genes of chromosome *10* (LIN 1975).

Gene markers: The *R* genes for anthocyanin pigmentation used in this study have been previously described (BRINK 1964; KERMICLE 1970). Their expression in endosperms and seedlings can be summarized as follows:

r-g: kernels with a colorless embryo and aleurone; green seedlings.

R-r: kernels with a colorless embryo and a self-colored aleurone when the gene is inherited through the pistillate parent, but mottled aleurone when it is transmitted *via* the male parent; colored seedlings except for the coleoptile tip.

R-st: stippled kernels with a colorless embryo and a finely spotted aleurone; green seedlings.

R-scm: kernels with a uniformly colored embryo and a self-colored aleurone; colored seedlings with a deeply colored coleoptile tip.

R-nj: *R*-Navajo kernels with a colored embryo (the central region of the scutellum). Only the crown portion of the aleurone is self-colored; colored seedlings.

Six genes in addition to *R* were used to map the breakage positions of the two translocations. Five of the seven genes have been mapped on chromosome *10* (NEUFFER, JONES and ZUBER 1968). The map position on chromosome *10* are as follows: lineate (*li*) is located at position 28, dull endosperm (*du*) at 33, zebra necrotic (*zn*) at 35, golden (*g*) at 43, and *R* at 57. Blue fluorescent-2(*bf-2*) is situated 17.7 map units proximal to *g* (ROBERTSON 1975b). Male-sterile-*10* (*ms-10*) is known to be located in chromosome *10*, but its position has not been determined. The *li* gene is the only one assigned to the short arm of *10*.

Marker-uncovering analysis: The procedure followed was essentially that outlined by ROMAN and ULLSTRUP (1951). Stocks bearing the recessive genes were crossed as pistillate parents with the translocations bearing the wild-type alleles. The progenies were then scored for the occurrence of the recessive endosperm or embryo phenotypes.

Three-point linkage analysis: The translocation breakpoints were mapped in relation to *g* and *R* following the cross of $10(g,r\text{-}g)\ 10(g,r\text{-}g) \times 10(g,R\text{-}r)10^{B}\ B^{10(G,R\_scm)}$. Kernels with colored aleurone have various chromosome constitutions. Kernels with colorless aleurone, however, result only from nondisjunction in $10^{B}\ B^{10}$ microspores. This latter class was used for scoring the *g* and *r* phenotypes.

Cytological techniques: For pachytene analysis, microsporocytes were fixed in 3:1 ethanol/glacial acetic acid and stained with propionic carmine. The procedure outlined by LIN (1977) was adopted for root tip preparations.

Estimation of nondisjunction frequency: To estimate the frequency of B^{10} nondisjunction, plants with $10\ 10\ B^{10}$, $10^{B}\ 10^{B}\ B^{10}\ B^{10}$, and $10\ 10^{B}\ B^{10}\ B^{10}$ chromosome constitutions were crossed as pollen parents with a structurally normal *r-g* tester. The $10\ 10\ B^{10}$ male plant measures nondisjunction of B^{10} in the absence of 10^{B}. The $10^{B}\ 10^{B}\ B^{10}\ B^{10}$ plant gives the frequency of nondisjunction of B^{10} when 10^{B} is present in the same pollen grain. Finally, the $10\ 10^{B}\ B^{10}\ B^{10}$ plant crossed as the pollen plant measures the rate of B^{10} nondisjunction in microspores with and without 10^{B} produced by the same plant. The last cross assays the background effect on the rate of nondisjunction in the two different classes of microspores.

In the cross of $10(r\text{-}g)\ 10(r\text{-}g) \times 10(r\text{-}g)\ 10(R\text{-}st)\ B^{10(R-nj)}$, four microspores classes function with unequal frequencies. The genotypes and phenotypes of the derived kernels are as follows with normal disjunction: (1) $10(r\text{-}g)$, colorless aleurone and embryo; (2) $10(R\text{-}st)$, stippled aleurone and colorless embryo; (3) $10(r\text{-}g)\ B^{10(R-nj)}$, Navajo aleurone and embryo; (4) $10(R\text{-}st)\ B^{10(R-nj)}$, stippled plus Navajo aleurone and Navajo embryo. Nondisjunction of the B^{10} chromosomes in class (3) microspores during the second pollen mitosis gives two additional kernel classes that, for convenience of description, are termed nondisjunction classes. One of the two classes has a Navajo endosperm and a colorless embryo, and the other has the reverse phenotype. The last pollen grain class also contains chromosome *10* and B^{10}, but unlike the third class, these two chromosomes are separately marked with codominent alleles of *R*, namely, *R-st* and *R-nj*. When nondisjunction of B^{10} occurs, the pollen grain will yield two nondisjunction kernel classes that are distinguishable from those derived from the third pollen class. One possesses a stippled plus Navajo endosperm with a colorless embryo, and the other bears a stippled endosperm associated with a Navajo embryo. Furthermore, an estimate of the frequency of hyperploid $10\ B^{10}$ microspores can be obtained from the class (4) pollen, since they produce kernels with a phenotype diagnostic of their chromosome constitution.

In the cross of $10(r\text{-}g)\ 10(r\text{-}g) \times 10^{B}\ 10^{B}\ B^{10(R-scm)}\ B^{10(R-scm)}$, the male parent produces with normal disjunction only $10^{B}\ B^{10}$ sperm, which yield kernels with self-colored endosperms and embryos. Nondisjunction of B^{10} would likewise result in two nondisjunction kernel classes, either the endosperm or embryo being self-colored. In addition to the difference in anthocyanin phenotype, these two classes can also be distinguished by kernel size. Since one of the two nondisjunction kernel classes has a hypoploid endosperm lacking the paternal endosperm factor (*EF*), there is a reduction in kernel size of about 50%. The complementary class has normal kernel size due to the presence of two doses of paternal *EF* in the endosperm (LIN 1975).

In the cross of $10(r\text{-}g)\ 10(r\text{-}g) \times 10(R\text{-}st)\ 10^{B}\ B^{10(R-nj)}\ B^{10(R-nj)}$, the pollen parent produces

three major microspore classes whose genotypes and the phenotypes of the derived kernels are as follows in the absence of mitotic nondisjunction: (1) *10(R-st)* $B^{10(R-nj)}$, stippled plus Navajo aleurone and Navajo embryo; (2) 10^B $B^{10(R-nj)}$, Navajo aleurone and embryo; and (3) *10(R-st)*, stippled aleurone and colorless embryo. The first two classes are the products of normal meiotic disjunction, whereas the last results from the meiotic nondisjunction of B^{10}. Nondisjunction of B^{10} in *10* B^{10} and 10^B B^{10} microspores will result in two nondisjunction kernel classes: only the endosperm or the embryo will show the Navajo phenotype. Furthermore, those nondisjunction kernels derived from *10* B^{10} microspores are distinguishable from those derived from 10^B B^{10} microspores in two respects. First, kernels from the former have stippled endosperm, whereas those from the latter do not. Second, half of the kernels resulting from nondisjunction in 10^B B^{10} microspores possess hypoploid endosperms that are deficient for the paternal *EF* factor carried by B^{10}. These kernels will show a 50% reduction in kernel size, as mentioned above.

An infrequent class of kernels is also expected from this cross. Phenotypically, they would have a colorless embryo and endosperm and would come from 10^B B^{10} microspores in which the B^{10} chromosome carries the *R-st* allele by virtue of a crossover. The nondisjunction of B^{10} and the preferential fertilization of the egg with the hyperploid sperm would lead to the formation of kernels with a colorless embryo and endosperm.

RESULTS

The *B* chromosome of maize consists of two unequal arms (RANDOLPH 1941). The long arm comprises a proximal knob-like region followed by a euchromatic region and a distal heterochromatic segment associated with a euchromatic tip (Figure 1).

One break of *TB-10(18)* is in the short arm of the *B*, and the other is in the long arm of chromosome *10(10L)*. One of the two derived chromosomes, 10^B, consists of the short arm of *10(10S)*, the centromere of *10* and a portion of the *B* short arm (Figure 4). The reciprocal chromosome, B^{10}, has the *B* centromere, all of the long arm of the *B* and most of *10L* since the break in *10L* is close to the centromere. This conclusion is based on the fact that the proximal part of *10L* was not discernible in the 10^B chromosome (Figure 4). B^{10} is essentially metacentric, consisting of all of the long arm of the *B* chromosome and most of the long arm of chromosome *10* (Figures 3, 4 and 5).

As in *TB-10(18)*, the break in *TB-10(19)* in chromosome *10* is close to the centromere. The second break, however, is located in the distal, heterochromatic segment of the long arm of the *B* chromosome. One of the resulting chromosomes, 10^B, carries *10S*, the centromere of *10*, and the distal half of the *B* long arm including the distal heterochromatin and euchromatic tip. The other chromosome, B^{10}, contains the *B* short arm, the *B* centromere and the proximal half of the *B* long arm joined with all of *10L* (Figure 2).

The break positions of the two translocations in chromosome *10* were confirmed by a genetic study that involved marker-uncovering tests and three-point linkage analyses. As would be expected from the cytological studies, crossing *zn, du, g* and *r-g* plants as females with *TB-10(18)* and *TB-10(19)* carrying the dominant alleles, all the recessive phenotypes were uncovered, indicating that the breaks of both translocations are in *10L* proximal to the genes tested. Since *du* is the most proximal gene known in the long arm, the breaks are likely very close to the centromere. Also uncovered were *bf-2* and *ms-10*. Unexpectedly, *li*

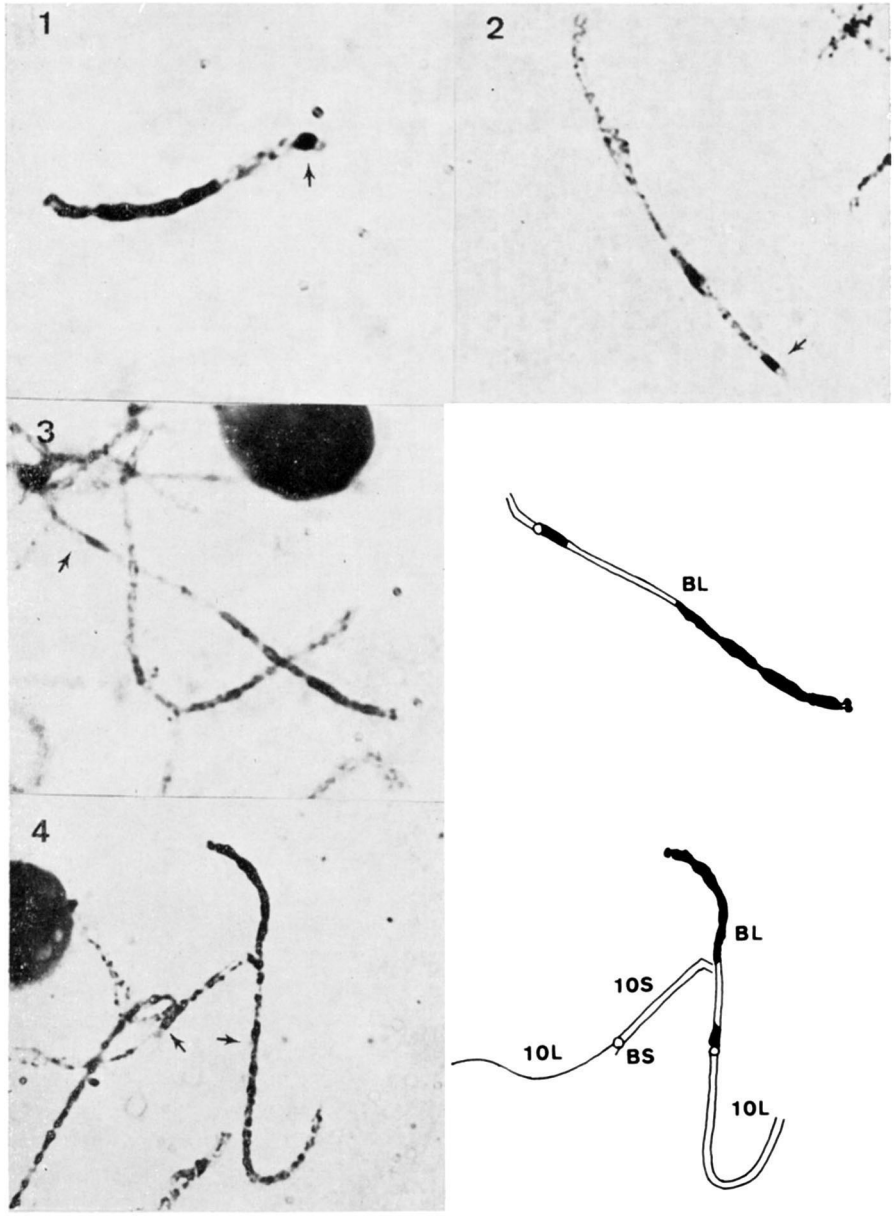

FIGURE 1.—*B* chromosome of maize consisting of the short arm, knob (centromere) and the long arm. The centromere region is marked with an arrow. FIGURE 2.—The B^{10} of *TB-10(19)* consisting of the short arm, knob (centromere), proximal euchromatic region and the proximal heterochromatic portion of the *B* chromosome and the long arm of chromosome *10*. The centromere is marked with an arrow. FIGURE 3.—The *B* chromosome portion of B^{10} of *TB-10(18)*. The centromere region is marked with an arrow. FIGURE 4.—B^{10} chromosome of *TB-10(18)* consisting of the whole long arm, knob and the centromere of the *B* chromosome and the long arm of chromosome *10*. The centromeres of 10^{B} and B^{10} are marked with arrows.

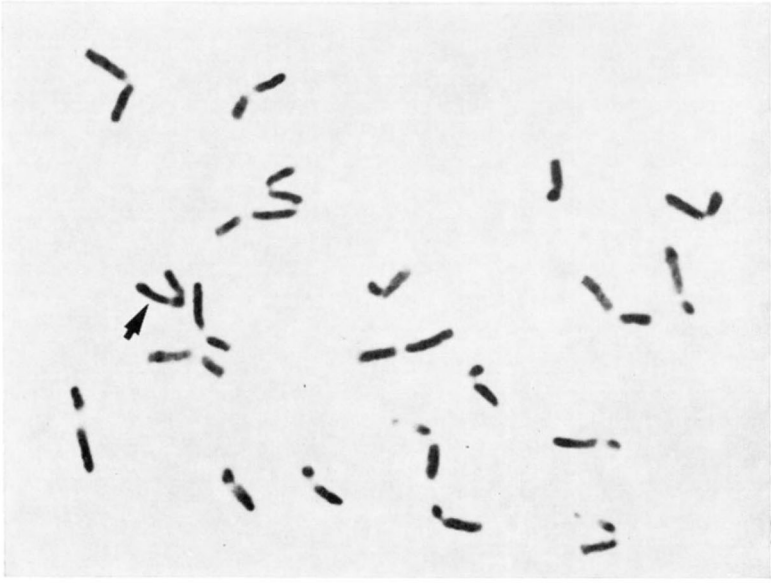

FIGURE 5.—Root tip metaphase of a *10 10 B^{10}* plant, where *B^{10}* is the component of *TB-10(18)*. Note that no telocentric is included and the metacentric *B^{10}* (arrow) is associated with the proximal *B* knob in the centromeric region.

likewise was uncovered by the two translocations, although its map position had earlier been assigned to *10S* (NEUFFER, JONES and ZUBER 1968). These results, therefore, serve to place *bf-2*, *ms-10* and *li* in *10L*. This assignment confirms BECKETT's (1973) report in which he placed the three genes in *10L* since they were uncovered by *TB-10b*, which also possesses a break in *10L*.

Table 1 presents the results of the cross of *10(g, r-g) 10(g, r-g)* × *10(g, R-r) 10^{B} $B^{10(G, R-scm)}$*. The kernels with colorless endosperm were selected for classification with respect to *g* and *R*. The reason for using only the colorless kernels is that they result from the nondisjunction of *B^{10}* followed by preferential fertilization of the egg with hyperploid sperm, and were the only progeny that serve to identify the breakpoints of the translocations. The results suggest the linkage positions of *TB-10(18)* and *TB-10(19)* as 34.3 and 28.0 map units from *g*, respectively. The percent of recombination in the *g-R* region is 25.4 and 21.6 in the

TABLE 1

Summary of the testcross data of 10(g,R-r) 10^{B} $B^{10(G,R-scm)}$ *male parents*

Translocation	Parental type *G R-scm*	Recombinant type: Single crossovers *g R-r*	Single crossovers *G R-r*	Double crossovers *g R-scm*	Total	Crossover (%): *T-g* region	Crossover (%): *g-R* region
TB-10(18)	150	100	70	15	335	34.32	25.37
TB-10(19)	227	100	73	17	417	28.05	21.58

Only the seeds with a colorless aleurone were germinated and classified for *g* and *R* phenotypes.

two tests. This is higher than the standard value of 14 and may be due in part to the *B* chromosome, which increases recombination. But 81% and 54%, respectively, of recombination enhancement is much more than that reported previously for the heterozygous *A-B* chromosome translocations, suggesting that the translocations per se may also be the basis. The exact nature of the latter is not yet certain. ROBERTSON (1967) found that the heterozygous *TB-9b* gives 2% crossing over in the *c-sh* region, which is 1% less than the standard value of 3%.

Since B^{10} of *TB-10(18)* contains essentially the whole *B* chromosome except for all or part of the short arm, it provides a mean of determining whether the short arm is necessary for nondisjunction. The simplest test is to cross plants carrying B^{10}, but not 10^{B}, of this translocation (*i.e.*, *10 10* B^{10}) as male parents with a standard tester. Nondisjunction of B^{10} in *10* B^{10} pollen grains would then result in seeds whose endosperm and embryo phenotypes do not correspond. *TB-10(19)* was tested at the same time as *TB-10(18)* in order to estimate the amount of heterofertilization, which can result in kernels with noncorresponding embryos and endosperms. *TB-10(19)* was chosen for three reasons: (1) its break position on chromosome *10* is in the same region as that of *TB-10(18)* so that the transmission behavior of the former should be similar to that of the latter; (2) since it does not carry the distal tip of the *B* chromosome long arm, B^{10} of *TB-10(19)* is not expected to undergo nondisjunction (WARD 1973a). Kernels with discordant phenotypes between endosperm and embryo would arise only from heterofertilization, and they can thus be used as a control for evaluating the amount of nondisjunction associated with *TB-10(18)*. (3) *TB-10(19)* and *TB-10(18)* were induced in the W22 inbred strain and were maintained by the same crossing system. This minimizes the effect of genetic backgrounds on the nondisjunction of the two translocations.

Table 2 gives the results of testcrosses of *10(r-g) 10(R-st)* $B^{10(R-nj)}$ plants. Among the eight kernel classes produced, five (I, II, III, IV and V) are the descendants of *10* B^{10} microspores whose chromosome constitutions can be determined from the kernel phenotypes. With respect to *TB-10(19)*, discordant kernels (classes I, II, III and IV) constituted 0.725% of the total offspring (entry 5, column 10). In the case of *TB-10(18)*, the same eight kernel classes were produced, but the frequency of discordant kernels was 4.173%, which is higher than that for *TB-10(19)*. Using the averaged measurements for *TB-10(19)* as an estimate of heterofertilization, nondisjunction of the B^{10} chromosome from *TB-10(18)* was calculated to occur in 20.72% of the hyperploid microspores. The results thus suggest that, in the absence of 10^{B}, the B^{10} chromosome of *TB-10(18)* is capable of undergoing some degree of nondisjunction. The necessity of the short arm for nondisjunction, therefore, seems unlikely.

Table 3 presents the testcross data from 10^{B} 10^{B} $B^{10(R-scm)}$ $B^{10(R-scm)}$ plants used as males. Of the three seed classes produced, two (classes I and II) are the discordant types, which averaged 68.90% and 93.31% for *TB-10(18)* and *TB-10(19)*, respectively. Since pollen parents bore only a single gamete class, the possible role of heterofertilization is excluded. All the discordant kernels originated from nondisjunction. It appears, therefore, that although B^{10} of

TABLE 2

Inheritance of seed color in 10(r-g) 10(r-g) × 10(r-g) 10(R-st) $B^{10(R-nj)}$ *crosses, relative to* B^{10} *nondisjunction in the absence of* 10^B

Progeny identification	Endosperm: Embryo : Class	r* Nj I	Nj r II	St Nj III	Number of kernels Nj + St r IV	Nj + St Nj V	Nj Nj VI	r r VII	St St VIII	Percentage of *R-nj* discordance†	Rate of nondisjunction (corrected)‡
Translocation *B-10(19)*											
1		2	0	2	1	21	79	217	188		
2		0	1	0	1	51	91	122	121		
3		0	3	0	1	36	87	155	186		
4		2	1	0	0	15	76	173	163		
5	(Total)	4	4	2	3	123	333	667	658	0.725	
Translocation B-10(18)											
6		9	1	9	0	21	105	174	190		26.71
7		7	7	12	2	51	99	196	197		18.96
8		5	5	10	4	32	77	141	151		24.63
9		3	2	5	0	24	73	160	169		12.47
10	(Total)	24	15	36	6	128	354	671	707	4.173	20.72

* r designates absence of anthocyanin (endosperm or embryo); Nj designates anthocyanin pigmentation governed by *R-nj* (endosperm or embryo); St designates anthocyanin pigmentation due to *R-st* (endosperm only).

† (Classes I through IV) × 100/Total.

‡ $M \times 100/(M + 2 \times$ class V), where $M =$ (classes I through IV) — Total × 0.725%.

TB-10(18) alone can undergo some nondisjunction in microspores lacking the 10^B chromosome, the addition of 10^B increases nondisjunction from 20.72% to 68.90%.

Table 4 gives the results of the cross of an *r-g* tester by $10^{(R-st)}$ 10^B $B^{10(R-nj)}$ $B^{10(R-nj)}$ male parents. This cross was conducted to measure the effect of the

TABLE 3

Analysis of seed classes following the cross 10(r-g) 10(r-g) × 10^B $10^{B(R-scm)}$ $B^{10(R-scm)}$, *testing the occurrence of* B^{10} *nondisjunction in the presence of* 10^B

Progeny identification	Endosperm: Embryo : Class	r* Scm I	Number of kernels Scm r II	Scm Scm III	*R-scm* discordance (%)
Translocation *B-10(19)*					
1		232	120	19	94.87
2		120	100	22	90.90
3	(Total)	352	220	41	93.31
Translocation *B-10(18)*					
4		59	52	56	66.46
5		63	50	48	70.18
6	(Total)	122	102	102	68.90

* r designates absence of anthocyanin (endosperm or embryo); Scm designates anthocyanin pigmentation due to *R-scm* (endosperm or embryo).

TABLE 4

Analysis of seed classes derived from 10(r-g) 10(r-g) × 10(R-st) 10^{B} $B^{10(R-nj)}$ $B^{10(R-nj)}$ *crosses in tests of the* B^{10} *nondisjunction in* 10 B^{10} *and* 10^{B} B^{10} *pollen grains from individual plants*

Progeny identification	Endosperm: Embryo Class	Number of kernels: r Nj I	Nj r II	Nj Nj III	St Nj IV	Nj + St r V	Nj + St Nj VI	r r VII	St r VIII	Total rate of nondisjunction in $10^{B}B^{10}$ pollen*	Corrected rate of nondisjunction in $10B^{10}$ pollen†
Translocation *B-10(19)*											
1		123‡	74	57	2	0	42	12	46	77.55	
2		175	95	59	2	0	34	17	27	82.06	
3		166	87	52	0	1	33	9	38	82.95	
4		172	120	39	2	1	8	21	39	82.21	
5		211	146	74	2	0	6	28	91	82.83	
6	(Total)	847	522	281	8	2	123	87	241	82.96§	
Translocation *B-10(18)*—subline 1											
7		130	61	102	1	0	51	13	46	65.18	— 1.82
8		131	74	175	0	0	21	20	52	53.94	—11.94
9		125	52	114	3	0	13	18	42	60.82	8.85
10		196	76	126	1	0	33	24	45	68.34	— 4.34
11		188	66	88	1	1	12	14	42	74.26	0.40
12		130	85	105	2	1	5	18	63	67.18	17.97
13	(Total)	900	414	710	8	2	135	107	290	64.92	— 1.62§
Translocation *B-10(18)*—subline 2											
14		82	75	109	2	1	26	16	49	44.10	3.23
15		98	87	309	3	2	23	9	47	37.67	9.00
16		122	78	224	3	1	15	14	44	47.16	9.78
17		60	42	305	5	0	39	12	76	20.06	5.93
18	(Total)	362	282	1034	13	4	103	51	216	38.37	6.56
Translocation *B-10(18)*—subline 3											
19		98	59	247	8	1	19	10	30	38.86	26.25
20		58	38	222	5	0	29	12	49	30.18	9.50
21		82	37	173	9	6	19	11	41	40.75	41.01
22		80	59	127	7	5	21	11	40	52.25	33.30
23		120	80	131	9	13	18	12	42	60.42	55.00
24	(Total)	438	273	900	38	25	106	56	202	44.13	33.48
Translocation *B-10(18)*—subline 4											
25		52	27	167	2	1	42	9	28	32.11	3.33
26		119	69	315	3	4	19	21	55	37.37	17.87
27		24	21	182	1	0	32	10	34	19.82	— 1.39
28		78	49	337	6	0	36	16	45	27.37	8.92
29		48	32	231	5	3	46	12	34	25.72	11.62
30	(Total)	321	198	1232	17	8	175	68	196	29.64	7.76

* (Classes I and II) × 100/classes I, II and III.
† $M \times 100/(M +$ class VI), where $M =$ classes IV and V — Total × 0.0047 (or 10/2111).
‡ Kernels on the same row were derived from the same ear.
§ Percentage calculated from the total for the family.

different genetic backgrounds on nondisjunction of the B^{10} chromosome in microspores with and without a 10^B chromosome. Two major classes of microspores were produced from the cross, namely, *10(R-st)* $B^{10(R-nj)}$ and 10^B $B^{10(R-nj)}$. The former provides an opportunity to evaluate B^{10} nondisjunction in the absence, and the latter in the presence, of 10^B. Among the eight kernel classes produced, six possess phenotypes that correspond to their chromosome constitution. Three of these classes (I, II and III) were derived from euploid 10^B B^{10} microspores and three others (IV, V and VI) from hyperploid *10* B^{10} microspores. Classes VII and VIII result from meiotic nondisjunction and crossing over in the T-*R* region, as explained in the MATERIALS AND METHODS section.

In the case of *TB-10(19)*, the frequency of nondisjunction in 10^B B^{10} microspores is 82.96%, which is less than the value in Table 3 (93.31%). The decrease may stem from an increase in class III, the disjunctional class, as the result of exchanges between *R* and the breakpoint of *TB-10(19)*, following pairing between chromosome *10* and B^{10}. The effect of different genetic background may also be a factor. The discordant kernels in IV and V arise from heterofertilization. Their frequency relative to the total progeny is 0.47% (ten out of 2111, entry 6).

Of the four sublines of *TB-10(18)*, subline 1 behaved similarly to *TB-10(19)*. The rate of nondisjunction in 10^B B^{10} pollen averages 64.92% (entry 13), which is very close to the corresponding value in Table 2 (68.90%). The averaged percentage of nondisjunction involving *10* B^{10} microspores (classes IV and V) is zero. (The calculated value is −1.62%. The negative sign means that the percentage of noncorresponding kernel classes is less than that of the control, *TB-10(19)*, resulting from heterofertilization, and thus indicates no nondisjunctional activity.) There is a considerable variation among crosses, ranging from zero to 17.97%. (The calculated range is −11.94% to 17.97%.) Within a given cross, an inverse relation between the values in the last column and class VI column is evident. For example, there are 51 seeds in the class VI column and −1.82% in the last column in the first cross (entry 7); whereas, the two corresponding values in the last cross (entry 12), are 5% and 17.97%. It appears that, in *10* B^{10} microspores, B^{10} does not fail to disjoin in this subline. Whenever such suspected activity occurs, it does not result from nondisjunction but from a reduction in class VI due to the inability of hyperploid *10* B^{10} pollen to compete with euploid grains when abundant pollen is applied. This phenomenon is not unique in this subline. It also occurs in the last two crosses of *TB-10(19)* (entry 4 and 5).

The effect of 10^B on the amount of nondisjunction of B^{10} is less in the three other *TB-10(18)* sublines. This is due in part to reduction in the nondisjunction kernel classes associated with 10^B B^{10} pollen (classes I and II), which varies from 44.13% to only 29.64% (entries 24 and 30, column 10). The same type of kernels resulting from *10* B^{10} pollen, on the other hand, increase, averaging from 6.56% to 33.48% (entries 18 and 24, last column). Despite such variations, the rate of nondisjunction in 10^B B^{10} microspores is always greater than that in *10* B^{10} microspores. The difference between the two rates is obvious in sublines 2 and 4, where the value for the former is more than four times that of the latter. In

the case of subline 3, the difference is less striking. As shown in Table 4, the difference persists not only at the subline level, but also at the level of individual crosses. It is apparent in these three sublines that B^{10}, when present alone, is capable of some nondisjunctional activity and that 10^B strongly enhances this activity. This effect of 10^B is particularly apparent in some crosses. For example, B^{10} in the first and third crosses in subline 4 (entries 25 and 27, last column) does not fail to disjoin in *10* B^{10} pollen, but it nondisjoins up to 32.1% and 19.8%, respectively, in 10^B B^{10} pollen (entries 25 and 27, column 10). It should be noted that the rate of nondisjunction calculated from the data in the last column was overestimated in some cases because the transmissional effect of class VI, the hyperploid class, was not taken into account. As a consequence, the difference between columns 10 and 11 for each individual cross gives a minimal estimate of the degree to which nondisjunction is enhanced by 10^B.

DISCUSSION

Unlike the previously established translocation *TB-10a* (LONGLEY 1956) and *TB-10(19)*, one break of *TB-10(18)* is in the short arm of the *B* chromosome, the other break in the proximal region of *10L*. Thus, the derived B^{10} chromosome carries the *B* centromere and the entire long arms of both the *B* chromosome and chromosome *10*. Since the lengths of the long arms of the *B* chromosome and of chromosome *10* are about equal in pachytene, B^{10} is essentially metacentric (Figures 4 and 5). The corresponding 10^B is acrocentric, bearing the short arms of both the *B* chromosome and chromosome *10*, in addition to the centromere of *10* (Figure 4).

Since B^{10} lacks the short arm of the chromosome *B*, it provides an opportunity to study the possibility of whether this arm carries a gene controlling nondisjunction of the *B* chromosome at the second pollen mitosis. Results of the study showed that in plants devoid of 10^B, B^{10} undergoes nondisjunction at a rate of 20.72%. Accordingly, it is concluded that B^{10} contains all of the genes necessary for nondisjunction. This conclusion is consistent with that of CARLSON (1970). He isolated isochromosomes of the long arm of B^9 from translocation *TB-9b* and observed that the isochromosomes, like B^{10} of *TB-10(18)*, could nondisjoin at variable rates in the presence of chromosome 9^B. Since neither iso-B^9 nor 9^B possessed the short arm, he concluded that this arm is dispensable for the nondisjunction of the maize *B* chromosome.

Yet, the effect of the short arm of chromosome *B* on nondisjunction is not entirely neutral. This is evident when the above rate of nondisjunction (20.72%) is compared with the rate measured when 10^B was also present in the same plant, 68.90%. The difference of 47.18% between the two rates is highly significant. Since the observed difference in this case might be due to unlike genetic backgrounds, an experiment was designed to measure the two rates on the same testcross ear. After crossing *10(R-st)* 10^B $B^{10(R-nj)}$ $B^{10(R-nj)}$ as the male parent with an *r-g* tester, two principal classes of microspores are produced, *10(R-st)* $B^{10(R-nj)}$ and 10^B $B^{10(R-nj)}$. The former provides a basis for estimating B^{10} nondisjunction

in the absence of *10^B*, and the latter, as a control, in its presence. Without exception, *B^{10}* in *10^B B^{10}* spores nondisjoin at a rate much higher than that in *10 B^{10}* spores. The average difference between the two rates varied widely, ranging from 10% to 64%. Variation occurred not only between sublines, but also within a given subline. The data suggest that although *B^{10}* displays some nondisjunctional activity when present alone, this activity is not fully expressed unless *10^B* also is present in the same cell. The importance of *10^B* is clearly evident in the extreme case, subline 1, where no nondisjunctional activity was detected in *10 B^{10}* spores, but up to 64% was observed in *10^B B^{10}* spores. The results thus show that the short arm of the *B* chromosome contains a minor regulator factor influencing efficient nondisjunction. Absence of the factor will lead to the reduction, but not the elimination of nondisjunction. This conclusion is in agreement with CARLSON's data (1970, 1978b) in which iso-*B^9* failed to disjoin at a rate lower than the normal *B^9*. This can be explained as a result of the absence of the short arm of the *B* chromosome in the former, as suggested by CARLSON (1978a). Since a proper control was not available in his experiment, applicability of this explanation in CARLSON's case is subject to reservation. CARLSON proposed, however, that the iso-*B^9* chromosome has a strong *B* centromere since it lacks the *B* short arm, which can invoke weakness at the *B* centromere. The effect is to separate the chromosome and result in reduction of the nondisjunctional rate.

The *B* chromosome break in *TB-10(18)* seems to impair the function of the components controlling nondisjunction. In *10^B 10^B B^{10} B^{10}* plants, the nondisjunction rate of *10^B B^{10}* spores averaged 68.90% (Table 3), the highest value ever measured for this translocation. The corresponding rate for *TB-10(19)* is 93.31%. The difference between the two translocations is 24.41%. The reduction in nondisjunction rate is also observed in *10 10^B B^{10} B^{10}* plants. The average rate of four *TB-10(18)* sublines combined is 52.57%, giving a difference of 30.39% from the corresponding rate of *TB-10(19)*, which was 82.96%. The two translocations possessed similar breaks in chromosome *10*, close to the centromere in the long arm. The breaks in the *B* chromosome are different; *TB-10(18)* involves the short arm and *TB-10(19)* the distal heterochromatic region of the long arm (Figures 2, 3 and 4). The former leads to reduction in the nondisjunction rate, whereas the latter gives a normal rate. The reduction in nondisjunction may be due to a damaged *B* short arm on the *10^B* of *TB-10(18)* where DNA was lost or rearranged before fusion. The alternative explanation is that separation of the *B* short arm by *TB-10(18)* breaks up *cis*-acting components essential for normal nondisjunction. The same conclusion was reached by CARLSON (1978c), based on the results of his studies on telocentric derivatives of iso-*B^9*. The telocentric *B^9* does not undergo nondisjunction at any significant rate, but the addition of extra *B* chromosomes did not result in restoration of full nondisjunctional activity. Thus, the presence in the cell of the chromatin comprising the short arm of the *B* chromosome on another chromosome is not sufficient for typical *B* chromosome nondisjunction.

The *B* short arm of *TB-10(18)* is very unstable with respect to its ability to increase nondisjunction. In each family in Table 4, *10^B* has a different potential

for enhancement of the nondisjunctional activity of B^{10}, ranging from 10% in subline 3 to 64% in subline 1. In part this is due to the nature of the B chromosome in maize. As mentioned by ROBERTSON (1975a), the frequency of nondisjunction in maize is variable, with variation not only between different *A-B* translocations, but also for a given translocation measured by different workers. The typical example is *TB-9b*, which nondisjoins at rates ranging from 67.6% (BIANCHI *et al.* 1961) to 98.2% (ROMAN 1947). But such variation is not as large as that shown in Table 4, suggesting the existence of additional variation associated with *TB-10(18)*. Since these four sublines were descendents of a common progenitor, a hybrid between inbred W22 and inbred W23 after three generations of backcrossing to W23, it is possible that the additional variation is attributable to the effect of genetic background. But the fact that *TB-10(19)* when tested in parallel with *TB-10(18)* did not exhibit variation of this magnitude seems to disagree with this explanation. Furthermore, the following observation also renders the effect of genetic background unlikely. When propagated as male parents (*10* 10^B B^{10} B^{10}) in inbred W22 background, occasional tester plants of *TB-10(18)* gave ears with very low rates of nondisjunction, 30% or less. Ears with a nondisjunction rate around 40% were frequently obtained, while the equivalent tester ears from *TB-10(19)* consistently exhibited a high rate of nondisjunction, 80% or more (LIN unpublished).

The alternative explanation for the variation is the breakpoint of *TB-10(18)*, which separates the short arm of the *B* chromosome from the centromere and the long arm. This separation somehow destabilizes the action of the controlling components of nondisjunction. The behavior of B^{10} is consistent with this explanation. The rates of nondisjunction in *10* B^{10} spores varied from zero up to 55% (column 11, Table 4), which is comparable to the variation of the 10^B B^{10} spores, from 19.82% to 74.26% (column 10). Presence or absence of a 10^B chromosome has no effect on the variation of nondisjunction. It follows that B^{10} is unstable in its nondisjunctional behavior, and this cannot be corrected by the presence of the short arm carried on 10^B. The simplest explanation is that the short arm, when in *cis* relationship to the long arm, functions to stabilize the controlling components. The disruption of the *cis* relationship by a translocation results in irregular action of the components in question, leading to variable nondisjunction rates. The damaged *B* chromatin on the B^{10} due to DNA loss or rearrangement before fusion can also explain the unstable B^{10} behavior.

The pachytene figure of *TB-10(18)* can also be interpreted as having a different structure. It is possible that one break is in the *B* chromosome's long arm between the centromere and the proximal knob, and the second is in the short arm of chromosome *10*, close to the centromere. This gives a B^{10} having a structure similar to the 10^B of the previous interpretation, except for the presence of the *B* centromere, and a 10^B that is similar to the corresponding B^{10}, but with the centromere of *10*. The quality of the pachytene preparation does not permit an unequivocal discrimination between these interpretations. Accordingly, in this case, it is 10^B, not B^{10}, that is capable of undergoing nondisjunction. Since this 10^B possesses the *B* proximal knob, as does the B^{10} of *TB-10a* and *TB-10(19)*,

this scheme will lead to the conclusion that this knob, not the centromere, is the receptor site of the nondisjunction. This conclusion is in accord with the hypothesis of RHOADES and DEMPSEY (1973). They advanced the proposal that faulty replication of heterochromatic knob leads to *B* chromosome nondisjunction.

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