Meiotic Transmission Rates Correlate With Physical Features of Rearranged Centromeres in Maize

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ABSTRACT

The centromere of the maize B chromosome was used as a model to study the physical features of a functional centromere. Pulsed-field gel electrophoresis was previously used to determine the organization of a repetitive sequence (referred to as the B-specific repeat) localized in the centromeric region of the maize B chromosome. The centromere is composed mostly of this repeat. In this report, a collection of 25 B chromosome derivatives that suffered from misdivision of the centromere was examined for the content and organization of the B repeat. Meiotic transmission of these derivatives was also determined and compared with rearrangements within the centromere. This analysis revealed that there is a strong correlation between the size of the centromere and meiotic transmission. In addition, the loss of a particular Pm1I fragment of 370 kb considerably reduced meiotic transmission. This sequence contains a 55-kb EcoRI fragment that is also present in all but four derivatives. Because the centromere of the maize B chromosome can be divided by successive misdivisions to derivatives with centromeres of <300 kb, it should be possible for artificial chromosomes to be produced in maize.

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Birchler 1996). This region was defined by breakpoints in a collection of misdivision derivatives. Misdivision of univalent chromosomes occurs at meiosis and results in breaks within the centromere. Pulsed-field gel electrophoretic analysis of a collection of such derivatives showed that after misdivision, only a fraction of the B-specific cluster remains, suggesting that the B-specific sequence is present in the functional centromere and is spread over its length. Furthermore, misdivision can reduce the size of the centromere by a considerable amount without any effect on chromosomal inheritance.

In this report, B chromosome derivatives with reduced meiotic transmission were examined for the content and organization of the B-specific repeat. This analysis reveals that there is a strong correlation between the amount without any effect on chromosomal inheritance.

Figure 1.—Genetic analysis of misdivision derivatives. The pedigree begins with the reciprocal translocation TB-95b.

**MATERIALS AND METHODS**

*Maize genetic strains:* Misdivision derivatives were successively produced from other chromosomes previously recovered from misdivision of the centromere of the reciprocal translocation TB-95b. Chromosomes used to generate new misdivision products were described in a previous study (Kaszás and Birchler 1996). TB-95b is the progenitor chromosome. It exchanges part of the B chromosome and the short arm of chromosome 9, the two components referred to as B-9 and 9-B. The first derivative recovered was a pseudoisochromosome (also referred to as pseudoiso) involving the B-9 chromosome of the translocation (Carlson and Chou 1981).

In one arm, this new chromosome has all the features of the normal B-9. In the other arm, there is a break in the euchromatic portion of the B long arm. Therefore, this new derivative will be referred to as iso4-10(–). It exchanges part of the chromosome that created the derivative, as well as the presence (+) or absence (–) of the centric knob (Carlson 1978a, 1986, 1988). Different isolates with the same chromosomal constitution and products of the same number of misdivisions are numbered.

**Cytological analysis:** The structure of the chromosomes was determined by cytological analysis of mitotic root tip cells. Roots collected from germinating seeds or 2-wk-old seedlings were fixed in a solution of 45% acetic acid containing 0.2% orcein for 5 days at 5°C. Roots were subsequently destained by boiling in 45% acetic acid for a few seconds. The cells were then squashed onto a glass slide; a coverslip was gently pressed onto the cells to allow maximum spreading. Chromosomes were visualized by phase contrast microscopy using a Zeiss Universal microscope. The centromeric region was identified as a constriction on metaphase chromosomes. Telocentrics could be recognized because they lack the constriction, whereas constrictions centered in the chromosome indicated an isochromosome. Ring chromosomes are circular and can be further verified by a high frequency of anaphase bridges that are indicative of sister-chromatid exchange that is not seen in sib stocks that do not carry the rings.

**Southern blot analysis:** High-molecular-weight DNA was prepared from protoplasts of inner leaves from one-to-four maize plants that were 4–5 wk old, as described by Kaszás and Birchler (1996). Briefly, leaves were cut into rectangles of ~2 × 5 mm and digested for 3 hr at room temperature in a solution of 0.5 m mannitol and 0.01 m CaCl2 containing 1% cellulase and 0.5% pectinase. Protoplasts were then filtered successively through a 95- and 20-μm opening nylon mesh and spun at 90 × g for 10 min in a clinical centrifuge. The pellet was resuspended in 10-15 ml of 0.5 m mannitol and spun again for 5 min. The resulting pellet was suspended in a small volume of mannitol, embedded in 1.2% low-melting-content agarose at a final concentration of 3–8 × 105 cells/ml, and treated with solutions containing 1% sodium N-lauroyl-
sarcosine, 1 mg/ml proteinase K, and 0.5x EDTA, pH 8.0, to release the DNA from the cells. The agarose-embedded DNA was subjected to electrophoresis (6 hr, 60 sec constant pulse, 200 V) to remove sheared fragments of <3 Mb. Digests with restriction enzymes were performed as follows: a portion of a plug (~1-4 μg of DNA) was rinsed twice with 1x restriction buffer, the enzyme was added on ice in 150-μl buffer and allowed to diffuse overnight at 5°C before incubation at the appropriate temperature for 4 hr. Approximately 100 units of the enzyme EcoRI was used per sample, or 50 units of PstI. Pulsed-field gel electrophoresis (PFGE) was conducted on a CHEF-DRII apparatus (Bio-Rad, Richmond, CA) in 0.5x TBE in 1% pulsed-field gel grade agarose (Sigma, St. Louis). Pulsed-field gels were stained with ethidium bromide and irradiated at 60 mJ/cm² for 2 min before transfer of the DNA to nylon filters. Dot-blot analysis was performed as follows: ~5 μg of undigested DNA was subjected to a short CHEF electrophoresis (5 hr, 1 sec constant pulse, 200 V) and then Southern blotted as described above. DNA from a line with no B chromosomes is also added as a control for nonspecific hybridization with the B-specific sequence.

Radioactive probes: Southern blots were probed with radio-labeled DNA from the appropriate DNA sequences subcloned into the Bluescript vector. DNA probes were synthesized by oligolabeling (Feinberg and Vogelstein 1983). For B-chromosomal-specific and chalcone synthase gene (c2) probes, hybridizations were performed under conditions of 42°C with 5x SSC and 50% formamide, and they were washed at 68°C with 0.2x SSC and 0.1% SDS. The dot-blot analysis was performed as described in Kaszas and Birchler (1996). In this study, DNA blots were first hybridized to the c2 gene used as a loading control and then with the B-chromosomal-specific sequence. A mean ±SD value was calculated from at least three replicates.

RESULTS

Genetic analysis of B-chromosome derivatives: Chromosomes were generated by misdivision of the centromere, a breakage of univalents at meiosis (Darlington 1939; Sears 1952; Carlson 1970, 1978a). In the normal segregation of bivalents at anaphase I, each homolog is pulled to one pole, whereas sister chromatids remain cohesive, especially in the centromeric region. A univalent usually behaves in the same fashion; i.e., it migrates to one pole or the other. On rare occasions, the univalent misdivides; i.e., one replicated arm migrates to one pole and the other migrates to the opposite pole. The centromere is pulled from both poles, and the tension eventually leads to rupture. If there is repair of the broken centromere, then new chromosomes are formed. They will be telocentrics, isochromosomes, or rings. In this study, translocations between the B chromosome and the short arm of chromosome 9 were used to generate new chromosomes that have a rearranged centromere as a consequence of misdivision. The behavior of the short arm of chromosome 9 (B9) was followed by two markers, the C1 and Sh1 genes. Dominant alleles give colored and plump kernels, respectively; the recessive phenotypes are colorless and shrunked. The 9-B chromosome is marked by the dominant allele of the waxy gene (Wx), which conditions the type of starch in the endosperm.

The B-9 translocations were crossed as males onto the recessive tester line. Misdivisions that break the centromere in meiosis can be recognized because a breakage-fusion-bridge cycle (McClintock 1939) is initiated, although breaks within a centromere might create an unusual situation involving only repeated breakage and fusion. Broken chromosomes do not repair in endosperm tissue and, thus, create a mosaic phenotype for C1 and Sh1. In the embryo tissue, the broken end “heals” and is stabilized (McClintock 1939). In the next generation, according to our experience, telocentric derivatives return to a nonmosaic phenotype (Figure 2A), whereas ring chromosomes, because of their inherent instability, will again exhibit a mosaic pattern (Figure 2B). Other mosaic kernels with one or a few colorless and shrunked sectors (referred to as fractional kernels)

Figure 2.—Phenotypic selection of misdivision derivatives. (A) Left, mosaic kernel exhibiting a breakage-fusion-bridge cycle in the endosperm, or repeated cycles of misdivision. The loss of the C1 and Sh1 markers (Sh1 loss is not observed on this kernel) creates a mosaic phenotype. Right, kernel showing a uniform colored phenotype that corresponds to a stabilized chromosome. (B) Left, mosaic kernel that gave rise to a ring chromosome. Right, mosaicism persists in the next generation because a ring chromosome is formed after misdivision. A ring is inherently unstable and exhibits a mosaic pattern.
also occur, but they do not represent a misdivision event (Carlson 1973; Alfenito and Birchler 1990).

Standard Southern analysis was performed (data not shown) on plants grown from those kernels showing cyclical mosaicism. Differences in the restriction pattern of the B-specific repeat between a derivative and its parental chromosome indicate that the centromere has been rearranged after a misdivision event. Such derivatives were then analyzed on CHEF gels and grown to establish their transmission rate. The succession of misdivisions is shown in Figure 1.

We examined 25 misdivision derivatives that were selected from a larger collection derived from chromosomes characterized previously (Kaszás and Birchler 1996). The meiotic transmission was calculated when the B-9 chromosome was carried as a heterozygote (in the constitution 9 9-B 8-9). As noted above, the B-9 chromosome carries the dominant markers C1 and Sh1. The 9-B chromosome carries the dominant Wx1, which is extremely closely linked to the translocation breakpoint and serves to identify the heterozygous translocations (Robertson 1967; Carlson 1978b) from other possible genotypes. The heterozygous translocations were crossed as female by a recessive c1 sh1 wx1 tester line. The transmission was averaged from 4 to 18 individuals, each producing 80–500 progeny kernels. The results from individual ears were given equal weight regardless of the number of progeny produced. Table 1 summarizes the transmission rates of the various chromosomes.

The derivatives were generated from the isochromosome iso3(−), the telocentric telo2-2(−), or the telocentric telo2(+) (see Materials and Methods for the designation of the derivatives). These chromosomes have a meiotic transmission rate of 43% [telo2-2(−)] to 53% [iso3(−)] as heterozygotes when crossed as female. In a female heterozygous for a B-9 translocation (9 9-B 8-9), three meiotic products are viable. These are 9, 9-B 8-9, and 9 B-9; the 9-B chromosome alone

<table>
<thead>
<tr>
<th>Chromosomal designation</th>
<th>Transmission</th>
<th>Sum of Pme restriction fragments (kb)</th>
<th>Ratio (B specific:c2)</th>
<th>Presence of specific restriction fragments</th>
</tr>
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<tbody>
<tr>
<td>Iso3(−)</td>
<td>10 ± 3</td>
<td>1170</td>
<td>53.0</td>
<td>■</td>
</tr>
<tr>
<td>Tel04-5(−)</td>
<td>44 ± 6</td>
<td>2180</td>
<td>42.7 ± 3.0</td>
<td>□</td>
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<tr>
<td>Iso4-7(−)</td>
<td>19 ± 4</td>
<td>790</td>
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<td>Ring4-8(−)</td>
<td>ND</td>
<td>500</td>
<td>ND</td>
<td>○</td>
</tr>
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<td>ND</td>
<td>○</td>
</tr>
<tr>
<td>Ring4-12(−)</td>
<td>ND</td>
<td>1240</td>
<td>ND</td>
<td>○</td>
</tr>
<tr>
<td>Tel04-11(−)</td>
<td>42 ± 5</td>
<td>2360</td>
<td>53.1 ± 3.5</td>
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<tr>
<td>Iso4-10(−)</td>
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<td>1360</td>
<td>72.0 ± 5.7</td>
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<td>Tel05-1(−)</td>
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<td>ND</td>
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<td>□</td>
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<tr>
<td>Iso5-2(−)</td>
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<td>4070</td>
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<tr>
<td>Tel06-4(−)</td>
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<td>2580</td>
<td>76.9 ± 7.2</td>
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<td>Iso6-6(−)</td>
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<td>2500</td>
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<tr>
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<td>4070</td>
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<td>Tel06-7(−)</td>
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<td>990</td>
<td>46.4 ± 3.6</td>
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<td>Tel06-8(−)</td>
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<td>■</td>
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<td>Iso3-2(−)</td>
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<td>Tel03-3(−)</td>
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<td>280</td>
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<td>Tel02(+)</td>
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<td>90.0</td>
<td>■</td>
</tr>
<tr>
<td>Tel03-4(+)</td>
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<td>2035</td>
<td>26.4 ± 2.0</td>
<td>■</td>
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<tr>
<td>Tel03-5(+)</td>
<td>16 ± 4</td>
<td>1665</td>
<td>17.3 ± 2.8</td>
<td>○</td>
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</table>

The B-9 chromosome is carried as a heterozygote in the constitution 9 9-B (Wx1) B-9 (C1 Sh1) and crossed as female by a recessive tester line (c1 sh1 wx1). The mean (± SD) transmission is indicated (calculated from 4 to 18 ears). The size of the chromosomes is estimated by the sum of Pme restriction fragments. The transmission rate of ring chromosomes is not shown (ND, not determined) because of their inherent instability in somatic tissue. Quantitation of the B-specific repeat is indicated as the B-specific repeat:c2 ratio (± SD). An arc sin transformation was applied for all hybridization values that were expressed initially as a fraction of iso3(−), telo2-2(−), or telo2(+).

The presence of a 370-kb Pme, a 55-kb EcoRI, or the absence of both sequences is indicated by a solid box, an open box, or an open circle, respectively.
aborts because it is missing a portion of chromosome 9 (Figure 3A). If the translocated chromosome arm 9 carries the dominant markers C1 and Sh1, then one would expect a 2 to 1 ratio of colored plump to colorless shrunken kernels (Figure 3B). In the translocation TB-9Sb, a value of 60% is observed, which is close to the expected 66% (Robertson 1967). The reduced transmission (43-53%) observed in the three chromosomes mentioned above has been suggested to result from the loss of noncentromeric regions of the B chromosome (Carlson 1978a; Carlson and Roseman 1992). Nevertheless, these chromosomes can be used as a reference to compare the rate of meiotic transmission to the new derivatives. To avoid problems of interpretation resulting from transmission distortions in the male parent because of reduced competition of duplicated gametes or nondisjunction at the second pollen mitosis (Roman 1947) for some chromosomes, only female transmission frequencies are considered.

Many of the chromosomes derived from iso3(–) have a reduced transmission rate. Patterns of inheritance vary from near normal transmission to 10% [telo4-4(–) and iso6-6(–)] (Table 1). Ring chromosome transmission is not reported because it is highly variable, but usually <30%. This behavior is caused by frequent sister chro-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Determination of meiotic transmission. (A) Four possible meiotic products are obtained from the heterozygous translocation 9, 9-B, B-9. The 9-B chromosome carries the dominant markers C1 and Sh1. The 9-B chromosome carries the dominant marker Wx1. The gamete carrying only the 9-B chromosome will abort, and it is not shown in B. The long arm of chromosome 9 is not drawn to scale. (B) Three possible combinations are found in the progeny after a cross with a recessive c1 sh1 wx1 tester line.}
\end{figure}

\textbf{Figure 3.}—Determination of meiotic transmission. (A) Four possible meiotic products are obtained from the heterozygous translocation 9, 9-B, B-9. The 9-B chromosome carries the dominant markers C1 and Sh1. The 9-B chromosome carries the dominant marker Wx1. The gamete carrying only the 9-B chromosome will abort, and it is not shown in B. The long arm of chromosome 9 is not drawn to scale. (B) Three possible combinations are found in the progeny after a cross with a recessive c1 sh1 wx1 tester line.
Derivatives with a further reduced transmission (<30%) have a centromere organization that is more simplified than in iso3(−). The centromere of iso4-10(−) has a size of ~1360 kb. Its organization is similar to iso3(−), with an additional 190-kb PmeI sequence. The significant reduction in transmission of iso4-10(−) [32 vs. 53% in iso3(−)] is difficult to attribute to any physical feature because several of the derivatives from iso4-10(−) with equivalent transmission are also very similar in organization [Figures 4 and 5, chromosome derivatives 5-3(−), 5-2(−), 6-4(−), 6-7(−), 6-8(−), and 6-9(−)]. Thus, correlations of transmission frequencies with specific restriction fragments cannot be definitively assigned using these derivatives.

However, other chromosomes studied here suggest that the reduced inheritance directly correlates with the complexity of the centromere. Only a few B-specific fragments remain in derivatives telo4-4(−) and iso4-7(−) derived from iso3(−), and the sizes of their centromere are 490 and 790 kb, respectively (Figures 5 and 6). The telocentric chromosome telo4-4(−) generates mosaic kernels at a high frequency. When examined closely, the fully colored kernels show a few colorless sectors that are indicative of late losses in the aleurone. This instability in the endosperm tissue suggests that the centromere of this chromosome often misdivides after meiosis, frequently nondisjoins, or is lost. Other chromosomes derived from iso4-10(−) also have less complexity in the centromeric region. A large portion of the centromere was deleted upon formation.
Figure 6.—Graphical representation of the centromere size of B chromosome derivatives that illustrates rearrangements after successive misdivisions. Data are taken from Table 1 (sum of Pmel restriction fragments). The filled boxes represent the centromeric region. The chromosome arms are shown as thin lines and are not drawn to scale.

1689

Centromere Transmission

of iso6-6(−) (10% transmission), indicated by the loss of several EcoRI fragments relative to iso5-2(−) (Figure 4). The centromere of telo5-1(-) is also smaller than its progenitor (800 vs. 1360 kb, Figure 6), and its size correlates with its low transmission of 17%.

The correlation between centromere size and transmission cannot solely be explained by size; for example, transmission of very small derivatives, such as telo3-3(−) or telo4-4(−), is almost equivalent to the transmission of iso6-6(−), which has a centromere of 2500 kb (Table 1 and Figure 7). This observation raised the possibility that some B-specific fragments are more critical than others for centromere function. Indeed, all the linear derivatives with a transmission of <30% are missing a 370-kb Pmel sequence (Figure 7 and Table 1). Three ring chromosomes are also missing the 370-kb Pmel sequence. A portion of this fragment remaining in these cases could provide some marginal centromere function or in the case of the rings, the circular nature of the chromosome might compensate in some manner.

The analysis of telo2(+) and telo2-2(−) derivatives addresses the association between centromere size and chromosome transmission. The centromere of telo3-5(+) is smaller than its progenitor, telo2(+) (1665 vs. 3235 kb, Figures 5 and 6). It is missing the 370-kb fragment and is poorly transmitted (16%) relative to telo2(+) (43%). In contrast, telo3-4(+) is reduced to 2035 kb, but retains the 370-kb fragment as well as a high transmission frequency (49%).

Derivatives of telo2-2(−) suggest that a critical sequence is required for normal transmission. Telo3-3(−) shows the most simplified B repeat organization of all derivatives (Figures 5 and 6). Only two Pmel fragments remain (Figure 5) that sum to <280 kb, which is seven times smaller than the size of its progenitor, telo2-2(−). The transmission of telo3-3(−) is 13%, which correlates well with the absence of a 370-kb Pmel sequence. This chromosome also transmits poorly at mitosis, as it is frequently lost during development of the plant (as indicated by a large proportion of fully colorless, shrunken ears on plants grown from colored plump kernels); it is also lost in the endosperm tissue because it generates a high frequency of mosaic kernels. On the other hand, iso3-2(−) was directly derived from telo2-3(−), and it shows several B-specific fragments, including the 370-kb B sequence after a Pmel digest (Figure 5). This derivative, which has one of the most simplified centromeres, retains the 370-kb fragment and has a nearly normal transmission (48%) relative to its progenitor.

Five ring chromosomes were generated, and they have some of the most simplified centromere structures. Three rings directly derived from iso3(−) all differ from it and from each other (Figures 4 and 5). The smallest centromere is found in ring4-9(−), with a size of 270 kb. Ring4-8(−) and ring4-12(−), directly derived from iso3(−), have centromeres of 500 and 1240 kb, respectively (Figure 6). It is difficult to evaluate the transmission behavior of rings because they often undergo sister chromatid exchange, and as a consequence, they often

Figure 7.—Plot of meiotic transmission of 20 linear derivatives against the size of their centromere. The presence of the 370-kb Pmel, the 55-kb EcoRI, or the absence of both sequences is shown as a solid square, an open square, or an open circle, respectively.
change in size. In addition, smaller rings are often lost by failure to congress to one or the other pole at anaphase (McClintock 1932, 1938). Other examples are found in rings 6-1(−) and 6-2(−), which contain a simplified B-specific region of 630 and 1875 kb, respectively (Figure 6). The significantly smaller centromere size found in rings may reflect a stabilizing effect of chromosomal regions that surround the centromere, compared to telocentrics.

An EcoRI 55-kb fragment remains in all derivatives but four (Figure 4). These derivatives are either rings [ring4-8(−), ring4-12(−)] or they have a severely reduced transmission rate [telo3-5(−), telo3-3(−)]. In a previous study of misdivision derivatives (Kaszás and Birchler 1996), a 55-kb EcoRI fragment remained in all derivatives. The fact that the loss of the 55-kb fragment in these four derivatives correlates with severely deficient transmission further suggests that it represents a core sequence that is necessary for normal transmission.

Relation between chromosome transmission rate and the copy number of the B centromeric repeat: The relative copy number of the B-specific repeat was estimated by dot-blot analysis for all the linear chromosome derivatives (Table 1). Equal amounts of DNA from each genotype were transferred to a nylon filter and then hybridized with a probe from the B-specific repeat or from the c2 gene (Wienand et al. 1986) as a loading control. The level of hybridization was measured by phosphorimaging. Misdivision leads to a reduction in the copy number of the B repeat, as indicated by changes in the B-specific c2 hybridization ratio (Table 1). Reduction in the amount of the B repeat does not significantly affect transmission in derivatives telo4-5(−), telo4-11(−), and iso5-2(−). In contrast, several derivatives that have a transmission of <20% [telo4-4(−), iso4-7(−), telo3-3(−), and telo3-5(−)] have lost a large portion of the B centromere.

Nevertheless, the results obtained from several derivatives contradict a strict correlation between chromosomal transmission and the copy number of the B repeat. A substantial fraction of the centromere remains in iso4-10(−) and telo6-4(−) has the same copy number of the B repeat as in iso3(−), but the transmission of both derivatives is reduced to ~30%. The reverse situation takes place in telo5-1(−) and iso6-6(−): half of the B repeat region persists, although the transmission is low (17 and 10%, respectively). Additionally, derivatives iso3-2(−) and telo3-4(−) [directly generated from telo2-2(−) and telo2(+)], respectively, have a nearly normal transmission, although the amount of the B repeat is decreased by one-third or more.

Chromosomal transmission rate is correlated with a 370-kb Pmel restriction fragment: Our observations can be reconciled by postulating that the presence of a 370-kb Pmel fragment is responsible for a high transmission rate. For example, iso6-6(−) is missing the 370-kb Pmel sequence, but it still retains several B-specific fragments. A 370-kb Pmel sequence remains in iso3-2(−), so it contains the information necessary for normal transmission. The only difference between telo3-4(−) and telo3-5(−) is the absence of the 370-kb sequence in the latter, which causes a drop of 33% in transmission (Table 1). This last result clearly demonstrates the requirement of a 370-kb fragment. This fragment contains a 55-kb EcoRI sequence that is found in most derivatives after a 2-D analysis (Kaszás and Birchler 1996). The 55-kb EcoRI sequence consists mostly of the B-specific repeat, and it is only missing in some ring chromosomes and in some low-transmission (<16%) derivatives (Figure 4). The 370-kb fragment contains one of the 55-kb fragments present in the full centromere, which (because of its position in the full original TB-9Sb centromere) is the one remaining after the misdivisions examined in this study. Consequently, the 370-kb fragment might be needed in linear chromosomes simply to have sufficient surrounding DNA for this particular 55-kb fragment. Four of five ring chromosomes are missing the 370-kb fragment, but because of their structure, they could provide stabilizing flanking DNA to a critical 55-kb unit by other means. We note, however, that isochromosomes missing the 370-kb fragment [iso4-7(−) and iso6-6(−)] have severely reduced transmission, so the nature of the flanking sequence or chromosome structure might also influence transmission. Because isochromosomes fold back on themselves for meiotic pairing, this configuration might affect the way that flanking sequences interact with the meiotic spindle relative to ring chromosomes.

DISCUSSION

In this study, the maize B chromosome misdivision system was used to generate partially functional centromeres. Twenty-five derivatives were analyzed by PFGE to determine the organization of their centromeres. We were then able to establish correlations between stability of the chromosomes and physical features of their centromeres. We find a correlation between size and transmission (see Figure 7), but there are some exceptions.

Comparison with functional studies of centromeres in other organisms: A common theme for centromere sequences in the multicellular eukaryotic genomes is emerging where information is available: a short repeat organized in tandem is present several thousands of times in mammals, maize, and Arabidopsis (Birchler 1997). The centromere sequences in Drosophila melanogaster (Le et al. 1995) are the exception, where very short satellite sequences are present. The centromere region in the Drosophila minichromosome Dp1187 is 420 kb, which is composed mostly of satellite sequences interspersed with single copies of retrotransposons (Murphy and Karpen 1995; Sun et al. 1997).

Although the composition of the Drosophila centro-
meme is different, its size requirement for function is similar to the minimum size of the maize B centromere. The transmission of Dp1187 derivatives drops dramatically when there is <600 kb in the centromeric region. In many maize derivatives, transmission significantly decreases when centromere size is <1000 kb (Figure 7), although there are exceptions [see telo3-5(−) in Table 1]. More precisely, a 370-kb Pmd4 fragment is correlated with full transmission of rod chromosomes. This sequence contains a 55-kb EcoRI fragment that is repeated several times in an intact centromere (Kaszaés and Birchler 1996). We propose that the presence of at least one of these 55-kb sequences is critical for full meiotic function, and that the surrounding DNA that leads to a larger fragment of 370 kb potentially provides additional stabilizing DNA. This 55-kb sequence is mostly composed of the B-specific repeat, as demonstrated in a previous study (Kaszaés and Birchler 1996).

**Concluding remarks:** Most of the derivatives with reduced meiotic transmission are apparently stable through mitosis, as evidenced by faithful recovery from one generation to the next. Notable exceptions are telo3-3(−) and telo4-4(−), which have a very reduced centromere (<500 kb). In these derivatives, mosaic kernels are recovered at a high frequency, which is indicative of either mitotic misdivision, nondisjunction, or loss. The faithful transmission through the life cycle would suggest that further centromere subdivisions might still function reasonably well in mitosis in accordance with the finding of Zinkowski et al. (1991) and that the critical sequences identified here might be necessary for proper meiotic transmission.

The maize B chromosome derivatives described here provide a manipulatable system to dissect components of the centromere. The finding of partially functional centromeres of <300 kb in size opens the possibility of creating artificial constructs in maize, which could be very useful for basic studies on chromosome inheritance and in genetic engineering.

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**LITERATURE CITED**


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