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Molecular Evidence That Chromosome Breakage by *Ds* Elements Is Caused by Aberrant Transposition

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The transposable *Dissociation* (*Ds*) element of maize was first discovered as a site of high-frequency chromosome breakage. Because both *Ds*-mediated breakage and transposition require the presence of the *Activator* (*Ac*) element, it has been suggested that chromosome breakage may be the outcome of an aberrant transposition event. This idea is consistent with the finding that only complex structures containing multiple *Ds* or *Ac* and *Ds* elements have been correlated with chromosome breakage. In this report, we describe two chromosome-breaking maize alleles that contain pairs of closely linked but separate *Ds* elements inserted at the *Waxy* locus. A polymerase chain reaction assay was utilized to isolate intermediates in the breakage process. The DNA sequence of these intermediates reveals deletions and base pair changes consistent with transposon footprints that may represent the junctions between fused sister chromatids. These results provide direct molecular evidence that chromosome breakage is the result of aberrant transposition events.

INTRODUCTION

McClintock (1946) identified a site of high-frequency chromosome breakage in maize and called it *Dissociation (Ds).* Cytological and genetic analyses revealed that *Ds*-mediated breakage initiates the breakage-fusion-bridge cycle. Following breakage at *Ds*, sister chromatids fuse, forming a dicentric chromosome and an acentric fragment (McClintock, 1946, 1948). The dicentric chromosome forms an anaphase bridge and breaks again at random points between the centromeres. During subsequent mitosis, these broken ends fuse, forming another dicentric chromosome and acentric fragment, thus continuing the breakage-fusion-bridge cycle (McClintock, 1938).

Movement of the original site of high-frequency breakage to new chromosomal locations led to the proposal that *Ds* could transpose (McClintock, 1948). This observation that *Ds* could break the chromosome as well as transpose and the finding that both processes require the presence of an *Activator* (*Ac*) element (McClintock, 1947, 1948) suggested that breakage and transposition are related events. Additional information about the relationship between *Ds*-mediated breakage and transposition came from McClintock's comparison of several *Ds* alleles. She discovered that *Ds* elements differ in their frequencies of breakage and transposition and that these frequencies vary inversely (McClintock, 1949). These data provided the first indication that all *Ds* elements are not alike and might differ in structure.

Subsequent molecular studies have confirmed that *Ds* elements are structurally heterogeneous and that elements that predominantly transpose differ from those that predominantly break chromosomes. Whereas frequently transposing *Ds* elements are usually individual deletion derivatives of *Ac*, more complex types of structures, such as those in Figure 1, have been associated with frequent chromosome breakage. The chromosome-breaking *Ds* allele *shrunken* (*sh*)-*6233* contains a 4-kb so-called "double *Ds*" in which one 2-kb element has inserted into an identical copy of itself, with the two elements in opposite orientation relative to one another (Figure 1A; Weck et al., 1984; Döring et al., 1989). Similar structures have been found as part of other chromosome-breaking alleles such as *sh-5933* (Courage-Tebbe et al., 1983).

The *Ac* element itself can also be part of a complex structure that breaks chromosomes. Chromosome breakage associated with the *bronze* (*bz*)-*s:2094* allele (Figure 1B) has been attributed to the action of a terminally deleted, or "fractured," *Ac* inserted at *bz* and a tightly linked, intact *Ac* element (Ralston et al., 1989; Dooner and Belachew, 1991). Unlike double *Ds* elements, this *Ac* composite contains elements that are physically separated by at least 20 kb and are in the same relative orientation. Pairs of *Ac* elements and combinations of *Ac* and *Ds* elements separated by even greater distances have also been associated with chromosome breakage (Dooner and Belachew, 1991).

Although the physical arrangement of these elements provides some clues about the mechanism of chromosome breakage, the structure of breakage intermediates would be far more informative in deducing the relationship between

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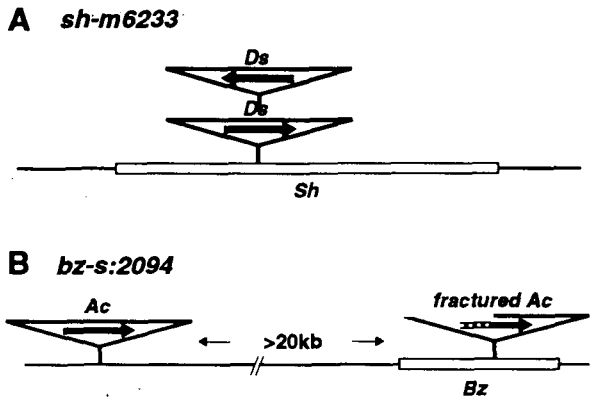

Figure 1. Structures Associated with Chromosome-Breaking *Ac/Ds* Alleles.

Arrows indicate element orientation, with the single BamHI site nearer the tail of the arrow. Elements and genes are not drawn to scale.
(A) The *sh-6233* allele contains a double *Ds* element (Weck et al., 1984) as does the *sh-5933* allele (Courage-Tebbe et al., 1983).
(B) The *bz-s:2094* allele contains a terminally deleted, or "fractured," *Ac* inserted at the *bz* locus and an intact *Ac* inserted 0.05 cM away (Ralston et al., 1989).

breakage and transposition. Transposition of *Ac/Ds* elements is characterized by the formation of transposon footprints at the excision site (reviewed in Wessler, 1988), and an example of this can be seen in Figure 2. These footprints are composed of all or part of an 8-bp duplication of target site sequences generated when the element inserts that subsequently flanks the element (Figures 2A and 2B). The footprint is usually not a perfect 8-bp duplication as internal bases can be deleted or changed (Figure 2C). If aberrant transposition events are responsible for *Ds*-mediated chromosome breakage and sister chromatid fusion, then transposon footprints may be evident at the site where the sister chromatids are fused.

In this report, we describe two examples of a new *Ds* structure that causes high-frequency chromosome breakage at the maize *Waxy* (*Wx*) locus. This structure contains two *Ds* elements that are inserted in the *Wx* gene in opposite relative orientations but are not in a double *Ds* configuration. The relative proximity of the two elements has allowed us to examine the sequences of intermediates in the breakage process. Detection of transposon footprints in these intermediates provides molecular evidence for a direct relationship between *Ds*-mediated chromosome breakage and transposition in maize.

RESULTS

Characterization of Chromosome-Breaking Derivatives of *Wx-m5*

In an earlier study, we reported that pairs of closely linked *Ds* elements can be readily isolated as unstable *wx* derivatives of the *Wx-m5* allele (Weil et al., 1992). *Wx-m5* contains a 2-kb *Ds* at position −470 bp relative to the start of *Wx* transcription ("−470") (Figure 2A). Frequent intragenic transpositions of this *Ds* into the *Wx* coding region produce unstable *wx* derivatives (Weil et al., 1992). Although most of these so-called "*wx-m*" derivatives arise from excision of the *Ds* at −470 and transposition into the *Wx* coding region, ~10% retain *Ds* at −470 and have a second, identical *Ds* element inserted in the *Wx* coding region. Such two-element alleles are believed to arise following *Ds* transposition from one chromatid to a nearby site on the sister chromatid (Greenblatt and Brink, 1962; Greenblatt, 1984).

Two derivatives of *Wx-m5*, each containing two *Ds* elements in opposite relative orientations, are the subject of this report. One of these alleles, *wx-m5:8313* ("*8313*"), has a *Ds* insertion at −470 and a second *Ds* inserted in the opposite orientation at +980 (Figure 2B) (Weil et al., 1992). A second allele, *wx-m5:8313∂14* ("*∂14*"), was isolated as a stable null derivative of *8313*

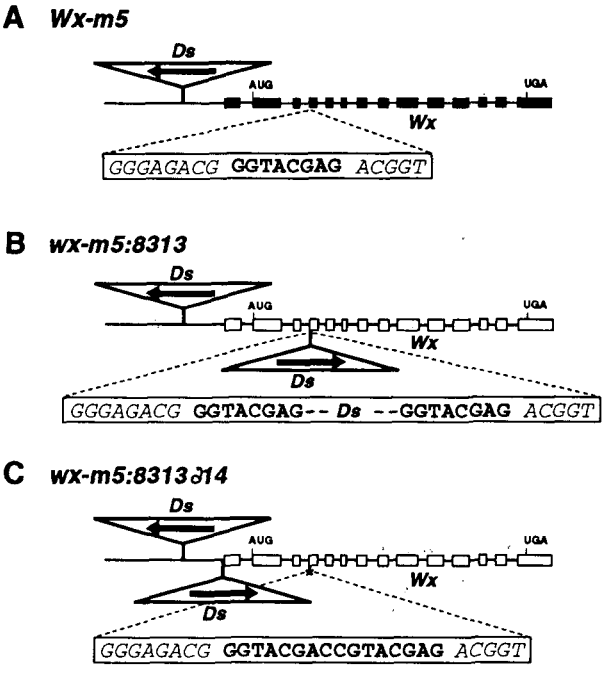

Figure 2. The Structure of *Wx-m5* and Two Derivatives.

Wx exons are shown as small boxes and introns as the intervening lines. Black boxes represent a functional *Wx* gene; white boxes indicate a null allele. The positions of the start and stop of translation are noted. Arrows indicate element orientation as in Figure 1.
(A) The *Wx-m5* allele (Weil et al., 1992) with the wild-type sequence at position +980 noted in the inset. The 8-bp target site prior to element insertion is in bold, and flanking sequences are italicized.
(B) The *wx-m5:8313* allele (Weil et al., 1992) with the 8-bp duplication of target sequences flanking the element shown in the inset.
(C) The *wx-m5:8313∂14* allele was derived from *wx-m5:8313* following intragenic transposition of the *Ds* at +980 (see text). The 16 bp in bold type at the site of *Ds* excision represent an 8-bp insertion (transposon footprint) relative to the wild-type sequence in **(A)**.

Table 1. Analysis of Chromosome Breakage by *Ds* Alleles of *Wx*

Allele	Proportion (p) of *Ac*-Containing Kernels Showing Chromosome Breakage[a]	n
Wx-m5	0.110 ± 0.012[b]	474
wx-m5:8313	0.973 ± 0.004	1437
wx-m5:8313∂14	0.997 ± 0.002	547

[a] Progeny kernels were examined individually at 40× magnification. Chromosome breakage was assayed by loss of the dominant *C1* allele producing colorless sectors of aleurone on the otherwise purple background (see Methods). Kernels with ≥five colorless sectors were scored as showing chromosome breakage (after Dooner and Belachew, 1991). Standard error was calculated as $\sqrt{p(1-p)/n}$.
[b] Chromosome breakage by *Wx-m5* is probably overestimated because somatic transposition of *Ds* into *Wx* may create element pairs that cause chromosome breakage.

(B. Burr, unpublished data). Two lines of evidence suggest that *∂14* was derived from *8313* in a single step when the *Ds* at position +980 transposed to position −12 (Figure 2C). First, *∂14* contains a footprint of 8 bp at +980, indicative of *Ds* transposition (Figure 2C). The footprint has created a frameshift in the *Wx* coding region and explains the null phenotype of this allele. Second, *∂14* contains two insertions of 2 kb each at the *Wx* locus. As *∂14* arose from *8313* in a single generation, the second *Ds* at −12 almost certainly reflects reinsertion of the *Ds* that excised from +980.

Even though *∂14* does not contain a double *Ds*, preliminary genetic analysis indicated that this allele caused frequent, *Ac*-dependent chromosome breakage (B. Burr, unpublished data). The presence of two *Ds* elements in opposite orientations in both *∂14* and *8313* led us to ask whether *8313* could also break chromosomes. The frequencies of chromosome breakage in *∂14*, *8313*, and the progenitor *Wx-m5* were quantified. As shown in Table 1, *8313* breaks chromosomes with a frequency nearly equal to that of *∂14*.

A Model for Chromosome Breakage at *∂14* and *8313*

The unusual structures of *8313* and *∂14* prompted us to consider how these alleles can break the chromosome, form dicentric chromosomes, and initiate the breakage-fusion-bridge cycle. A review of certain features of *Ac/Ds* transposition is useful in examining this question. Transposition of these elements has been associated with DNA replication (Greenblatt and Brink, 1962; Greenblatt, 1984; Chen et al., 1987). Furthermore, genetic experiments suggest that only one of the two replicated daughter elements transposes (Greenblatt and Brink, 1962; Greenblatt, 1984). Because only one daughter element is successfully recognized as a transposition substrate ("transposition competent"), it has been proposed that the daughter elements are differentially "marked" in some manner (Fedoroff, 1989). The mechanism by which *Ac/Ds* elements acquire transposition competence is currently under investigation but has not yet been determined (Coupland et al., 1988; Ott et al., 1992).

Segregation of competent and incompetent daughter elements following replication of two closely linked *Ds* elements, such as in *8313* and *∂14*, could lead to chromosome breakage and initiate the breakage-fusion-bridge cycle. A model for how this might occur is diagrammed in Figure 3 using *8313* as an example. If, following replication, the competent daughters of each element in the pair are on different sister chromatids (Figure 3B), then transposase could act on these elements in either of two ways. Each competent daughter element could be transposed individually, leaving a footprint behind where the element excised. Examples of individual transposition by the *Ds* elements in *8313* have been described previously (Weil et al., 1992) and also include the formation of the *∂14* allele. Alternatively, if two elements were close enough to one another, transposase might occasionally recognize both competent daughter elements simultaneously (Figure 3B). "Excision" in these aberrant transpositions would involve both elements and therefore both sister chromatids, producing an acentric fragment containing multiple copies of the 2-kb *Ds* as well as the entire chromosome arm distal to *Wx* (Figure 3C). The transposon footprint created following such an "excision" would join one sister chromatid to the other (Figure 3D). Just as described cytologically and genetically by McClintock (1946, 1948), the chromosome would be broken at the site containing the pair of *Ds* elements, sister chromatids fused, a dicentric chromosome formed, and the first round of a breakage-fusion-bridge cycle initiated.

Polymerase Chain Reaction Analysis of Breakage Intermediates

Our model of chromosome breakage by aberrant transposition could be tested directly because the *Ds* pairs in *8313* and *∂14* are each separated by well-characterized *Wx* sequences (Figure 2). This feature allowed us to monitor individual *Ds* insertion sites before and after transposition. A polymerase chain reaction (PCR) assay was devised to detect, isolate, and characterize transposition products that fuse sister chromatids. Two primers in the same orientation along the *Wx* sequence cannot successfully prime a PCR reaction (Figure 3A). For this same primer pair to form PCR products, the template would have to undergo an inversion that changes the orientation of one primer with respect to the other (Figures 3C and 3D). The sister chromatid fusion predicted by our model should create just such an inversion and, furthermore, this inversion should only occur in the presence of *Ac*. Finally, the DNA sequence of the PCR product is predicted to have the following 5′ to 3′ order (Figure 3D): (1) PCR primer 1, (2) the sequence between primer 1 and the most 5′ *Ds*, (3) an excision footprint formed from a copy of the insertion site of the most 5′ *Ds* and an inverted copy of the insertion site of the most 3′ *Ds*, (4) an inverted copy of the sequence between the most 3′ *Ds* and PCR primer 2, and (5) an inverted copy of primer 2.

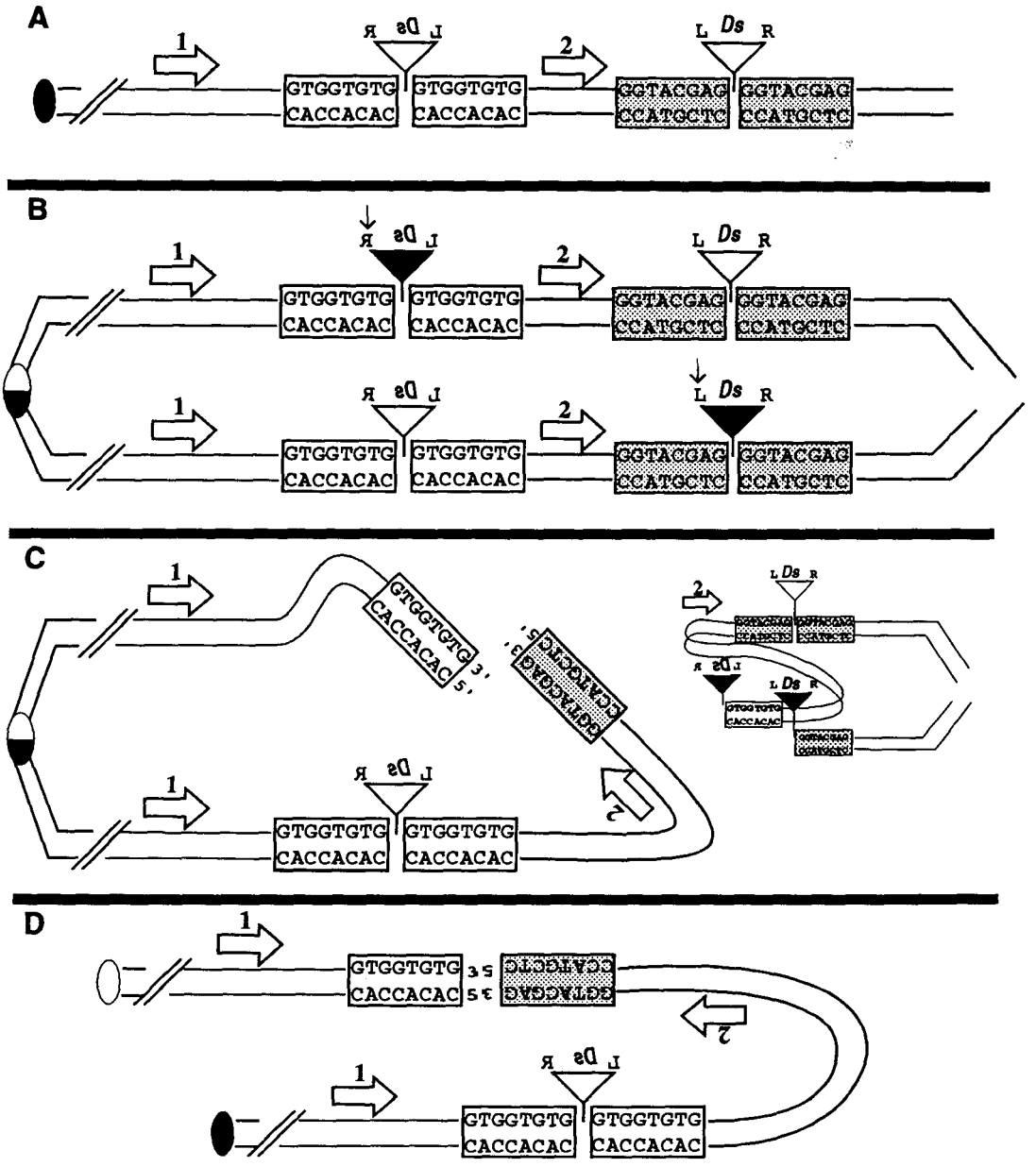

Figure 3. Model of Sister Chromatid Fusion by Aberrant Transposition in the *8313* Allele.

The 8-bp target site duplication for each element is shown in the boxes flanking the insertion. Open arrows indicate PCR primers. The centromere is represented by the oval at the left.

(A) Two *Ds* elements prior to replication.

(B) The same elements following DNA replication. The transposition-competent elements (solid triangles) occur on different sister chromatids for each insertion site. Cleavage by transposase occurs at the vertical arrows.

(C) Aberrant transposition event involves cleavage at the right end of one competent element and the left end of the other competent element. Cleavage results in the loss of the entire chromosome arm distal to the centromere from the excision points on each sister chromatid. Following this "excision," sequences immediately adjacent to the cleavage sites are brought together and religated in a transposase-mediated event.

(D) Structure of the dicentric chromosome following ligation and centromere division. Primers 1 and 2 are now positioned to amplify the intervening DNA.

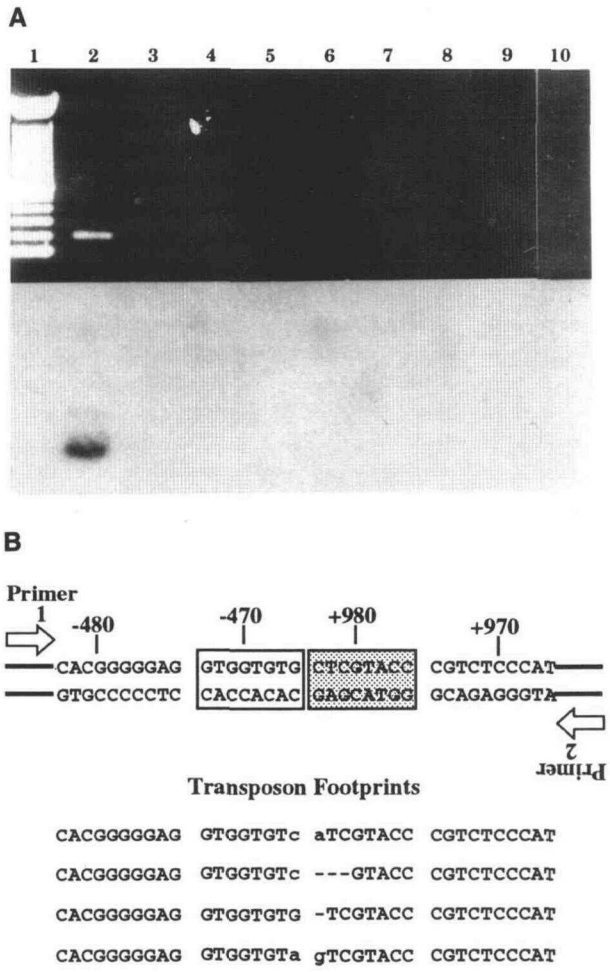

Figure 4. PCR Products of the Sites of Sister Chromatid Fusion in *8313*.

(A) Ethidium bromide–stained agarose gel of PCR products is above a DNA gel blot of the same gel probed with ^{32}P-labeled *Wx* sequences. Lane 1, size standard (100-bp ladder). The primers and genomic DNA used were as follows: lane 2, 1+2, *8313*(+*Ac*); lane 3, 1 only, *8313*(+*Ac*); lane 4, 2 only, *8313*(+*Ac*); lane 5, 1+2, *8313*(−*Ac*); lane 6, 1 only, *8313*(−*Ac*); lane 7, 2 only, *8313*(−*Ac*); lane 8, 1+2, *Wx-m5*(+*Ac*); lane 9, 1+2, *Wx-m5*(−*Ac*); lane 10, 1+2, no DNA template.

(B) Sequences of PCR products at the site of chromatid fusion. At the top is diagrammed the expected sequence if the insertion sites at −470 and +980 of *Wx* are joined as shown in Figure 3D. Sequences are, from left to right, the 10 bp immediately 5′ to the −470 insertion site, the 8-bp −470 target site, an inverted copy of the 8-bp +980 insertion site, and an inverted copy of the 10 bp immediately 5′ to the +980 site in a wild-type *Wx* gene. Aligned below are the sequence classes found among 16 clones of the PCR product pictured in **(A)**, lane 2. Dashes and lower case letters represent the positions of deletions and base changes, respectively.

Template DNA was prepared from stocks carrying *8313* or *∂14* heterozygous with a deletion of the entire *Wx* gene (*wx-C34*; Wessler and Varagona, 1985) to ensure that any inversions detected were the result of sister chromatid fusion and not exchange between homologous chromosomes (Figure 3). The results of PCR reactions with these templates are shown in Figure 4A. Pairs of primers in the same orientation did not yield PCR products using *8313*(−*Ac*), *∂14*(−*Ac*) (data not shown), the progenitor *Wx-m5*(−*Ac*), or *Wx-m5*(+*Ac*) genomic DNA as template (Figure 4A). However, the DNA from *8313* (+*Ac*) (Figure 4A) and from *∂14*(+Ac) (data not shown) could be amplified, demonstrating an *Ac*-dependent inversion requiring the presence of two linked elements as predicted by our model. Sixteen clones containing the PCR product from *8313* (Figure 4A, lane 2) were sequenced. Each clone contained the expected *Wx* sequence inversions and an excision footprint imprecisely joining half the *Ds* target site duplication at −470 to half the inverted target site duplication from +980 (Figure 4B). The sequences flanking the transposon footprints reflect an inversion of the *Wx* sequences from −470 to +980. These results are entirely consistent with the sister chromatid fusion predicted by the model in Figure 3.

DISCUSSION

We have characterized two *Ds* alleles of the maize *Wx* locus, *8313* and *∂14*, that break chromosome 9S at high frequency. In contrast to previously described chromosome-breaking structures, *8313* and *∂14* do not contain double *Ds* elements or pairs of elements separated by more than 20 kb. Instead, both alleles contain two *Ds* elements inserted in opposite orientations at *Wx* and separated by 1.5 kb (*8313*) and 0.45 kb (*∂14*). We have used a PCR assay to amplify *Ac*-dependent intermediates in chromosome breakage and have identified the sites of sister chromatid fusions. The DNA sequences of PCR products from the *8313* allele, with *Ds* insertions at positions −470 and +980, reveal that an inverted copy of the +980 insertion site has been joined to the −470 site. The deletions and base pair changes at the −470/+980 junction resemble transposon footprints associated with *Ac* and *Ds* excisions.

Element Interactions Lead to Chromosome Breakage

It seems likely that interaction between the *Ds* elements in both the *8313* and *∂14* alleles is responsible for causing chromosome breakage. All available evidence indicates that the individual *Ds* elements of *8313* and *∂14* transpose at high frequencies but are incapable of initiating high-frequency chromosome breakage. For example, the single copy of the 2-kb *Ds* in the progenitor allele, *Wx-m5*, transposes frequently and causes infrequent chromosome breakage (Table 1; Weil et al., 1992). *8313* arose in a single generation directly from *Wx-m5* and contains two identical copies of this same 2-kb element (Figures 2A and 2B). Both copies of the *Ds* elements in *8313* are capable of independent, high-frequency transposition (Weil et al., 1992). Similarly, *∂14* contains the same transposition-competent *Ds* elements as *8313* because it arose

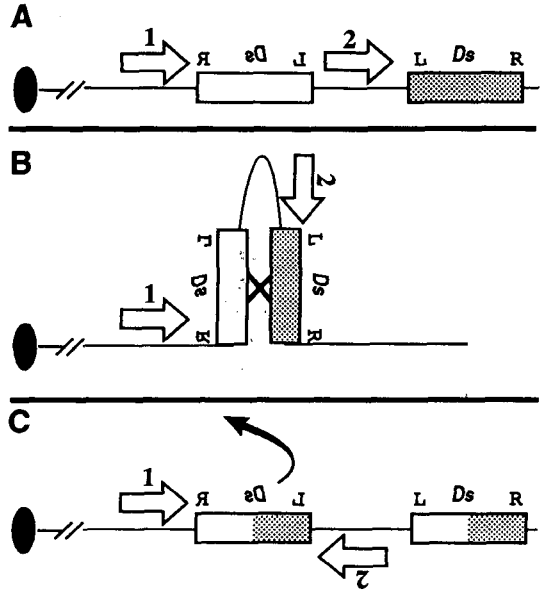

Figure 5. Intrachromosomal Recombination between *Ds* Elements.

Arrows signifying primers are as in Figure 3. Different *Ds* elements are indicated by shading, and left (L) and right (R) element ends are shown for each element. The centromere is indicated by the oval at the left.

(A) Two elements in opposite orientations.

(B) Recombination between elements produces an inversion.

(C) Excision of the recombinant *Ds* resulting in a transposon footprint similar to those shown in Figure 4B.

in a single generation from *8313* following intragenic transposition of the *Ds* at position +980 (Figures 2B and 2C). Thus, it appears that chromosome breakage and dicentric chromosome formation result from unusual events that are made possible when two otherwise unremarkable *Ds* elements are brought close to one another.

Interestingly, the *Ds* elements inserted at *Wx* in *Wx-m5* and its derivatives are nearly identical in sequence to the elements in the double *Ds* structures at the *sh* locus (Döring et al., 1984). A 28-bp deletion in the elements of the *wx* alleles includes the site where one *Ds* has inserted into the other in the *sh* double *Ds* structure. These sequence similarities may reflect a relationship between the double *Ds* and the *wx Ds* elements.

Alternative Models Do Not Explain Both the Observed Rearrangements and *Ds*-Mediated Chromosome Breakage

Other models, besides that of aberrant transposition (Figure 3), can be put forth to explain the joining of the −470 insertion site to the inverted +980 site and the generation of a transposon footprint. However, any successful model must account for four features of the data: (1) the excision footprints in the *8313* PCR products indicating *Ds* transposition, (2) the inversion of sequences between the *Ds* elements that precedes or is coincident with transposition and juxtaposes the −470 and inverted +980 sites, (3) McClintock's (1946) observation of *Ds*-mediated sister chromatid fusion and the formation of a dicentric chromosome that subsequently breaks, and (4) frequent chromosome breakage is *Ac* dependent.

Intrachromosomal pairing and recombination between the two *Ds* elements would invert the sequences between them, as shown in Figure 5. Subsequent excision of either *Ds* would leave a footprint that joined the +980 and −470 sites (Figure 5B) to give products like those in Figure 4B. However, this scenario of recombination followed by excision would not produce a dicentric chromosome. Alternatively, dicentric chromosomes could result from unequal crossing over between mispaired *Ds* elements on sister chromatids, as shown in Figure 6. Subsequent excision of the recombined *Ds* element would fuse the −470 insertion site to an inverted copy of the +980 insertion site in a transposon footprint like those in Figure 4B. This

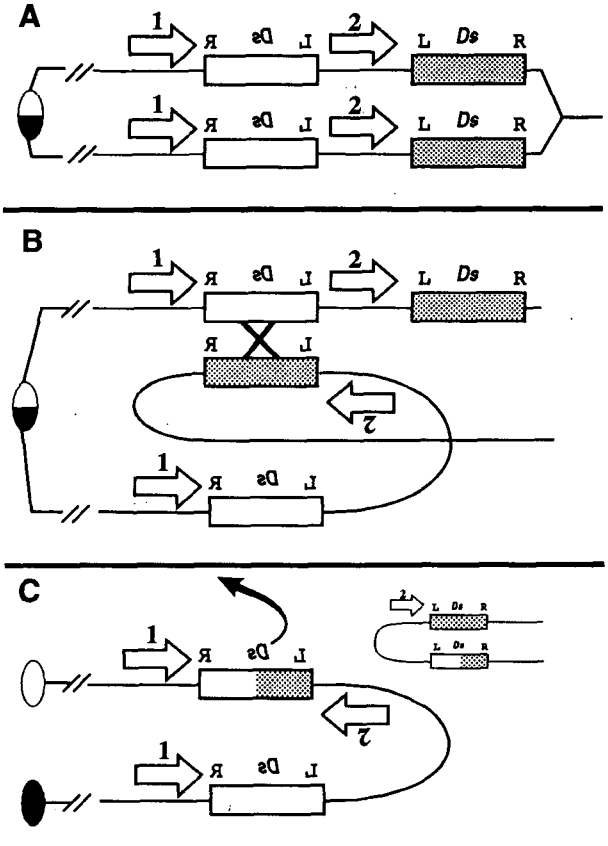

Figure 6. Sister Chromatid Exchange between Mispaired *Ds* Elements.

The centromere is indicated by the oval at the left.

(A) Element pairs following replication.

(B) Mispairing and recombination of elements in opposite orientations from different sister chromatids to create a dicentric chromosome.

(C) Excision of the recombinant *Ds* resulting in a transposon footprint similar to those shown in Figure 4B.

model accounts for both the formation of a dicentric chromosome and the observed PCR products. However, it is inconsistent with the observation that breakage is *Ac* dependent because sister chromatid exchange between mispaired elements should cause frequent chromosome breakage even in the absence of *Ac*.

In contrast, the aberrant transposition model (Figure 3) is consistent with all four features of the data. It can account for the creation of transposon footprints involving both *Ds* elements, as observed for *8313*. In addition, the model explains the inversion of sequences between the *Ds* elements observed in both *8313* and *∂14*, as well as the joining of sister chromatids to form a dicentric chromosome observed cytologically by McClintock (1946). Finally, because this mechanism is initiated by *Ds* transposition, it is dependent on the presence of *Ac*, also as observed (Figure 4A).

Our model suggests that the actual *Ds*-mediated chromosome breakage occurs when transposase cleaves both sister chromatids during an aberrant transposition event. This same mechanism also accounts for chromosome breakage by other structures in which two transposition-competent *Ds* elements are near one another but on opposite sister chromatids. For example, if following replication the competent daughter of each component element in a double *Ds* (Figure 1A) segregates onto different sister chromatids, similar aberrant transposition events as those described here could occur. Interestingly, double *Ds* elements and the *8313* and *∂14* alleles all have pairs of closely linked, 2-kb *Ds* elements in opposite orientations. This correlation may indicate that *Ds* elements in opposite orientations preferentially segregate competent daughter elements onto opposite sister chromatids. Further study of interacting elements may be useful in understanding how *Ac/Ds* elements acquire transposition competence.

Our data support McClintock's (1946) original assertion that *Ds*-mediated transposition and chromosome breakage occur by similar mechanisms. Both events require *Ac*-dependent breakage at element ends followed by element excision and ligation of the "empty site." Although chromosome breakage at *Ds* initiates the breakage-fusion-bridge cycle, it is certainly not responsible for the additional rounds of breakage and fusion that characterize the cycle. Rather, the dicentric chromosomes formed by aberrant transposition of complex *Ds* structures do not break at *Ds* but at random sites between the centromeres during anaphase. Due to the randomness of these secondary breakage and fusion sites, it will be challenging to design experiments that permit the isolation and characterization of the intermediates from subsequent rounds of the breakage-fusion-bridge cycle.

METHODS

Maize Stocks and Crosses

Strains carrying the *Waxy* (*Wx*)-*m5* and *wx-m5:8313* alleles (from B. McClintock, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), the *wx-m5:8313∂14* allele (from B. Burr, Brookhaven National Laboratory, Upton, NY), and the *wx-C34* (from the Maize Genetics Cooperative Stock Center, Urbana, IL) were obtained as indicated. The *wx-m5:CS22* ("*CS22*") *Dissociation* (*Ds*) allele was isolated as described previously (Weil et al., 1992).

Alleles to be tested for chromosome breakage were each placed proximal to a wild-type *C1* allele on the short arm of chromosome 9 in the absence of *Activator* (*Ac*). These stocks were then crossed onto homozygous *CS22* (*Ds*), *c1* females containing *Ac*. Chromosome breakage was assayed by somatic loss of the dominant *C1* marker producing a colorless sector. The presence of *Ac* in progeny was determined by filing away the aleurone of each kernel and staining the underlying endosperm with I_2/KI to assay genetic instability of the *Wx* locus.

DNA Isolation and Cloning

Genomic DNA was isolated from 14- to 21-day-old seedlings, as described previously (Shure et al., 1983). The *∂14 Ds* insertions were cloned as a single 7-kb SstI fragment by ligating size-selected SstI cut *∂14/wx-C34*(−*Ac*) genomic DNA into SstI cut λZAPII phage (Stratagene). The library was screened using a 1.8-kb SalI-SstI subfragment of *Wx* SalI fragment 1 (Wessler and Varagona, 1985) radioactively labeled by the random oligonucleotide method (Feinberg and Vogelstein, 1984). Positively hybridizing plaques were purified to homogeneity. After restriction mapping, the phage was used as a template for the polymerase chain reaction (PCR) (see below), the amplified subfragment cut with EcoRI and cloned into pUC119 (Vieira and Messing, 1987).

PCR products amplified from *8313*- and *∂14*-containing genomic DNA were gel purified to remove primers. After filling in the ends using the Klenow fragment of DNA polymerase I, these products were blunt-end cloned into SmaI-digested pUC119.

PCR Amplification and DNA Sequencing

Amplification reactions were performed in 100 μL containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.2 mM each dATP, dCTP, dGTP, and dTTP, and 0.5 U Taq polymerase (Perkin-Elmer-Cetus) final concentrations. The *Ds* insertion sites in *∂14* were determined after PCR amplification of 10 ng of λZAPII/*∂14* phage DNA using 70 ng of a single primer with an added EcoRI site near the 5′ end of the *Ds* element (5′-GGAATTCGAAATCGATCGGG-3′) (Weil et al., 1992). The cloned PCR product, containing the 5′ ends of both *Ds* elements and the *Wx* DNA between them, was sequenced using the dideoxy method (Sanger et al., 1977).

Amplification of genomic DNA utilized 200 ng of template DNA in each reaction. For *8313/wx-C34*(−*Ac*), 70 ng each of primer 1 (5′-GGACACGCAGCAGAGAGCGGAGA-3′ corresponding to positions −516 to −494 of the *Wx* sequence; Klosgen et al., 1986) and primer 2 (5′-CTGGGACACCAGCGTCGTG-3′, positions +841 to +860) were added (Figure 3). Amplification of *∂14/ws-C34*(−*Ac*) DNA used 70 ng each of primer 1 and a different primer 2 (5′-AGGATCCGGTCACGCAACGC-3′, an EcoRI site plus positions −79 to −66) chosen to reflect the different position of the more 3′ *Ds* in the *∂14* allele.

PCR was performed using a thermal cycler (model 480; Perkin-Elmer-Cetus). After a 2-min hold at 95°C, samples were cycled 40 times through 1 min at 95°C, 2 min at 65°C, and 3 min at 72°C. After a 10-min final extension at 72°C, samples were held at 4°C until analyzed.

PCR products were cloned as described above, and double-stranded DNA from mini-preps (Sambrook et al., 1989) of these clones was sequenced.

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