Changes in State of the *Wx-m5* Allele of Maize Are Due to Intragenic Transposition of *Ds*

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ABSTRACT

The molecular basis for the unusual phenotype conditioned by the *waxy(Wx)-m5 Ds* allele has been elucidated. Unlike most *Ds* alleles, *Wx-m5* is phenotypically wild-type in the absence of *Ac*. We find that the *Wx-m5* gene contains a 2-kb *Ds* element at −470 relative to the start of *Wx* transcription, representing the most 5′ insertion of any transposable element allele characterized to date in plants. Despite its wild type phenotype, *Wx-m5* has reduced levels of *Wx* enzymatic activity indicating that *Ds* insertion influences *Wx* gene expression. In the presence of *Ac*, *Wx-m5* kernels have sectors of null expression on a wild-type background and give rise to stable *wx* and unstable *wx-m* germinal derivatives. Seventeen of 20 derivatives examined are *wx-m* alleles and at least 15 of these appear to result from intragenic transposition of *Ds* from −470 to new sites within the *Wx* gene. Three *wx-m* alleles contain two *Ds* elements, one at −470 and a second in *Wx* coding sequences. Surprisingly, only 3 out of 20 derivatives are stable *wx* mutants and these have sustained gross rearrangements of *Wx* and flanking sequences. For most other maize transposable element alleles somatic sectors and germinal derivatives usually arise following element excision or deletions of element sequences. In contrast, element insertion following intragenic transposition is apparently responsible for most of the somatic sectors and germinal derivatives of *Wx-m5*.

THE insertion into and excision from maize genes of transposable elements result in diverse patterns of phenotypic expression. Most unstable alleles display somatic sectors of revertant and/or intermediate gene expression on a contrasting background of mutant expression. The quality, frequency and timing of somatic sectors can serve to distinguish a particular transposable element allele. The position of element insertion within a gene with respect to exons, introns and 5′ flanking sequences has a major influence on the sectoring pattern because element excision usually leaves nucleotides behind at the insertion site. Thus, alleles that preferentially give rise to large and frequent somatic sectors often contain transposable elements in regions where additional nucleotides have little effect on target gene function (*e.g.*, untranslated exons, introns or promoters) (SUTTON *et al.* 1983; BONAS *et al.* 1984; COEN, CARPENTER and MARTIN 1986; PEREIRA *et al.* 1986; SULLIVAN, SCHIEFELBEIN and NELSON 1989). Conversely, alleles that preferentially display small, infrequent revertant sectors are often the result of insertions into sequences important for gene function; in these cases most excision events result in null expression (VARAGONA and WESSLER 1990).

Transposable element alleles can also give rise to stable germinal derivatives (reviewed in WESSLER 1988). The phenotypes of these derivatives and the frequency of their isolation is usually a reflection of the somatic sectoring pattern of the unstable progenitor. For example, unstable alleles that condition many large revertant sectors usually have a higher germinal reversion frequency than alleles that display few small sectors.

Transposable element alleles also give rise to unstable germinal derivatives that have a heritable change in the timing and frequency of somatic sectoring. These "change in state" derivatives were discovered by MCCLINTOCK (1949) and have been studied most extensively in association with the *Spm/dSpm* and *Ac/Ds* transposable element systems. The *anthocyanin-less(a)-m1* and *bronze(bz)-m13* alleles contain nonautonomous *dSpm* elements that, in the presence of *Spm*, condition kernels with large and frequent sectors of purple aleurone against a colorless or bronze background (MCCLINTOCK 1951; NELSON and KLEIN 1984). Change in state derivatives of these alleles can be readily identified as kernels with smaller, less frequent purple sectors in the presence of *Spm* (MCCLINTOCK 1953a; PETERSON 1961; SCHIEFELBEIN *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985; RABOY *et al.* 1989). Many change in state derivatives of *a-m1* and *bz-m13* are attributable to changes in the *composition* of the resident *dSpm* element. For example, intraelement deletions that remove sequences essential for transposition lead to a reduction in the frequency of element excision (SCHIEFELBEIN *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985; RABOY *et al.* 1989). Deletions of

element sequences are also responsible for turning *Ac* alleles into *Ds* derivatives. The *Ds* alleles *wx-m9* and *bz-m2(DI)* were derived from *Ac*-containing progenitors following intraelement deletions of 0.18 and 1.2 kb, respectively (FEDOROFF, WESSLER and SHURE 1983; POHLMAN, FEDOROFF and MESSING 1984; DOONER *et al.* 1985).

Changes in state can also occur when an element transposes to a new *position* within the target gene (PETERSON 1976). The *P-ovov(Ac)* allele conditions a different pattern of pericarp color than its progenitor, *P-vv(Ac)* (PETERSON 1990). Comparison of the progenitor and derivative genes indicate that the *Ac* element has transposed to a new site 161 bp away but still within the *P* gene. Intragenic transposition is also responsible for a change in state of the *wx-m7(Ac)* allele (P. CHOMET, personal communication). To our knowledge, there are no reported instances of intragenic transposition by a *Spm* or *dSpm* element.

Change in state derivatives of *Ds* alleles have only been characterized in association with heritable changes in the frequency of *Ds*-mediated chromosome breakage (MCCLINTOCK 1948, 1949). Derivatives of the *Ds* alleles *shrunken(sh)-m5933* that display a lower frequency of chromosome breakage were found to differ from the progenitor allele in the composition of the complex *Ds* element at the *Sh* locus (COURAGE-TEBBE *et al.* 1983; DORING, TILLMAN and STARLINGER 1984; DORING *et al.* 1989). There are no published reports on the molecular basis of change in state derivatives of *Ds* alleles associated with new patterns of element excision. The *Wx-m5* allele (MCCLINTOCK 1953b) provides a unique opportunity to isolate many change in state derivatives from a *Ds* allele. The endosperm of kernels deficient for the *Wx*-encoded ADP-glucose glucosyltransferase lack amylose and are not stained with I/KI (NELSON and RINES 1962). Most *Ds*-induced *wx* mutations are stable nulls in the absence of *Ac*; in the presence of *Ac*, they give rise to clonal sectors of revertant tissue (a "*wx-m*" phenotype) (MCCLINTOCK 1952, 1963; WESSLER *et al.* 1986). In contrast, *Wx-m5(−Ac)* kernels are phenotypically indistinguishable from wild-type *Wx* kernels when *Ac* is not in the genome. However, in the presence of *Ac*, *Wx-m5* kernels display clonal sectors of null and intermediate *wx* expression (a "*Wx-m*" phenotype; see Figure 1a).

In this report, we show that the *Wx-m5* allele contains a 2-kb *Ds* element ~470 bp upstream of the *Wx* transcription start. We also present the genetic and molecular characterization of 20 germinal derivatives of *Wx-m5*: 17 are changes in state from *Wx-m* to *wx-m*, while three are stable null alleles. Whereas 15 of the changes in state appear to result from intragenic transposition, the stable alleles are associated with rearrangements of the *Wx* gene and flanking DNA.

MATERIALS AND METHODS

Maize stocks: The *Wx-m5* allele and two *wx-m* change in state derivatives, 8311B and 8313, were obtained from B. MCCLINTOCK. Additional change in state derivatives from *Wx-m5* were isolated from the following cross:

$$\frac{c\ wx}{c\ wx} \times \frac{c\text{-}m2(Ds)\ [Wx\text{-}m5\ or\ wx]}{Wx}\ (+Ac)$$

[*wx-C34*, a deletion of the entire gene, was used as the *wx* tester throughout these experiments (WESSLER and VARAGONA 1985)]. Ears segregating 1:1 for *Wx*:*wx* kernels had a *Wx/wx* parent and were discarded. Ears segregating for *Wx* and *Wx-m* kernels were screened for rare *wx* or *wx-m* kernels that were tested for heritability by selfing. Plants derived from mutant *wx* kernels were also crossed to *Ac* testers to distinguish *wx* from *wx-m* derivatives.

Wx and *Wx-m* derivatives of the *wx-m* alleles described above were obtained by crossing heterozygous *wx-m/wx-(+Ac)* plants onto homozygous *wx* testers, screening the resulting ears for kernels with *Wx-m*, *Wx* or intermediate phenotypes and testing for heritability and response to *Ac* as described above. We designate these *Wx* and *Wx-m* derivatives *Wx'* and *Wx-m'*, respectively, to acknowledge the possibility that the derivatives may differ slightly from wild type and the *Wx-m5* progenitor. Similarly, *wx-m"* denotes derivatives of *Wx-m'* alleles identified in crosses of *Wx-m'/Wx(+Ac)* stocks to homozygous *wx* testers and screened for rare *wx* kernels as described above.

Genomic DNA isolation and Southern blot analysis: DNA prepared from seedlings by the method of SHURE, WESSLER and FEDOROFF (1983) was digested according to the manufacturers' specifications, and analyzed on Southern blots as previously described (WESSLER and VARAGONA 1985). Hybridization probes of subcloned *Wx* gene fragments (see Figure 1c) isolated from low melting point agarose (SeaPlaque) gels were radiolabeled with [^{32}P]dATP and [^{32}]dCTP, either by nick translation (RIGBY *et al.* 1977) or random primer extension (FEINBERG and VOGELSTEIN 1983).

Genomic cloning of *Wx-m5* and *wx-m* derivatives: Samples of 100–200 μg of *Sal*I-digested *Wx-m5/Wx-m5(−Ac)* DNA were size fractionated in a 0.7% low melting point agarose gel and 2.7–6.8-kb fragments extracted from the agarose. This DNA was ligated into the *Xho*I site of LambdaZAP II phage (Stratagene), packaged into phage particles (Gigapack Plus, Stratagene) and the resulting phage used to infect *Escherichia coli* SURE cells (Stratagene). Screening approximately 120,000 plaques with a *Wx* fragment 1 probe (see Figure 1c; WESSLER and VARAGONA 1985) yielded 98 positives, 8 of which were purified to homogeneity and found to contain identical 5.4-kb inserts. A pBluescript plasmid containing this insert was obtained by coinfecting *E. coli* SURE cells with one of the LambdaZAP clones and R408 helper phage according to the manufacturer's instructions.

The *Ds* insertions from the derivative alleles *Wx-m5CS9* and *Wx-m5CS22* (hereafter all change in state alleles are abbreviated CS9, CS22, etc.) (see Table 1), and both *Ds* insertions from the 8313 allele were cloned from partial genomic libraries made in the same manner as for *Wx-m5*. Approximately 120,000 plaques were screened from each library. The CS9 library yielded 12 phage containing 2.7-kb inserts when probed with fragment 3. The CS22 library probed with fragment 2 resulted in the identification of five phage that contained 4.0-kb inserts. Finally, in a screen of ~120,000 plaques for the two *Ds* inserts in 8313, a fragment 1 probe revealed seven phage plaques that contained a 5.4-

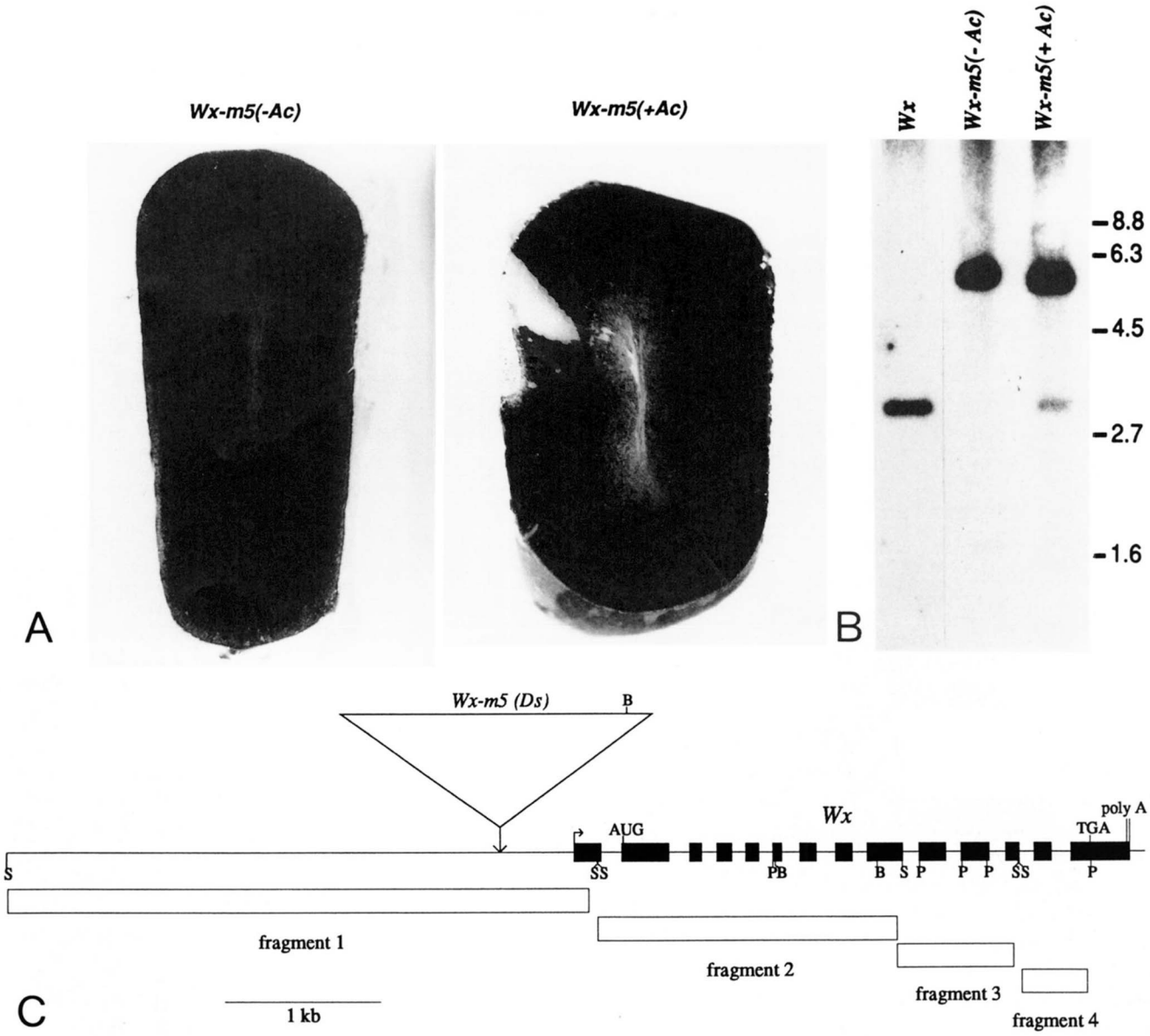

FIGURE 1.—Characterization of the *Wx-m5* allele. (A) Cross-sections of mature kernels stained for amylose with I/KI solution. (B) *Sal*I digest/fragment 1 probe. Southern blot of maize genomic DNA. Size standards are in kilobase pairs. (C) A diagram of the *Wx-m5* gene showing the position of the *Ds* element and the identity of the restriction fragments used as probes. Exons of the *Wx* gene are shown (black boxes) and the *Wx* transcription start is indicated by the arrow. B = *Bam*HI, P = *Pst*I and S = *Sal*I.

TABLE 1

Assays of Wx enzymatic activity

Allele	Wx enzymatic activity[a]	Percent wild-type activity
Wx	789	100
Wx-m5[b]	371	47.0
	198	25.1

[a] Activity given as cpm [^{14}C]UDP-glucose incorporated × mg starch granule^{-1} × 30 min^{-1}. Background activity (measured in endosperm homozygous for the deletion allele *wx-C34*) was subtracted from each measurement.

[b] Two different stocks carrying one dose of *Wx-m5* were assayed.

kb insert and a fragment 2 probe hybridized to 20 phage plaques containing a 4.0-kb insert. Plasmids carrying the CS9 insertion and each insertion from 8313 were evicted from LambdaZAP as described above. For CS22, the cloned LambdaZAP insert was subcloned as two *Bam*HI fragments of 2.2 kb and 1.2 kb in pUC119 (VIEIRA and MESSING 1987).

Sequence analysis: Unidirectional deletion of pBluescript and pUC119 subclones were carried out using the Exo/Mung system (Stratagene). Following transformation into *E. coli* strain DH5 (HANAHAN 1983), plasmids containing overlapping deletions were used as double-stranded templates for dideoxy sequencing (SANGER, NICKLEN and COULSON 1977) with either Sequenase or Taquenase enzyme (U.S. Biochemical).

Starch granule preparation and enzymatic assays: Starch granules were purified from the triploid endosperm

of immature (18 days after pollination) *Wx-m5/wx/wx*, *Wx/wx/wx*, or *wx/wx/wx* kernels by the method of SHURE, WESSLER and FEDOROFF (1983). Quantitation of Wx protein activity based on the incorporation of ^{14}C-labeled UDP-glucose into starch granules (NELSON, CHOUREY and CHANG 1978) used 10^5 cpm [^{14}C]UDP-glucose per assay and 5 mM unlabeled UDP-glucose. Eight samples of 2–10 mg starch granules per genotype were assayed, ^{14}C incorporation plotted against mg starch granules for each sample, and a line fitted to the points by regression ($r^2 > 0.95$). The slope of this line is a measure of Wx activity in terms of [^{14}C]UDP-glucose incorporated per mg starch granule per 30 min.

RESULTS

Molecular analysis of *Wx-m5*: Unlike other *Ds* alleles of the *Wx* gene, *Wx-m5* conditions a *Wx-m* phenotype: kernels appear wild type in the absence of *Ac* and display somatic sectors of mutant *wx* endosperm in the presence of *Ac* (Figure 1a). Southern blot analysis of genomic DNA from *Wx-m5(−Ac)* plants revealed that the mutation is caused by a 2.0-kb insertion in the 3.4-kb *Wx* fragment 1 (Figures 1, b and c). Fragments 2, 3 and 4 of *Wx-m5* are indistinguishable from wild type (data not shown). The insertion in fragment 1 represents a *Ds* element as evidenced by Southern blots of DNA from *Wx-m5(+Ac)* plants: in addition to a 5.4-kb band containing the insertion, labeled fragment 1 also hybridizes to a wild-type size band, demonstrating that somatic excision of the element occurs when *Ac* is present (Figure 1b).

Cloning and sequence analysis of the *Ds* element and flanking DNA indicated an 8-bp insertion site at positions −467 to −474 (hereafter referred to as "−470") with respect to the start of *Wx* transcription (KLOSGEN *et al.* 1986; Figure 1c). This *Ds* element is 2013 bp in length and has sustained two deletions relative to the published *Ac* sequence (MULLER-NEUMANN, YODER and STARLINGER 1984). The largest deletion includes positions 995–3515 of *Ac* and the other extends from 3607 to 3634. In addition, the *Wx-m5 Ds* has several nucleotide substitutions when compared to *Ac* (Figure 2).

Enzymatic activity of *Wx-m5*: Mutant alleles with reduced levels of enzymatic activity can still condition a nonmutant phenotype (KLEIN and NELSON 1983). To ascertain whether the 2-kb *Ds* element at −470 affects the level of *Wx* expression, *Wx* enzymatic activity was quantified. To this end, starch granules were isolated from immature *Wx-m5(−Ac)* and *Wx* endosperm tissue and assayed for glucosyltransferase activity. *Wx-m5(−Ac)* starch granules have only 25–50% of wild-type Wx activity (Table 1) suggesting that the site of *Ds* insertion and/or the presence of *Ds* sequences has a small influence on the level of *Wx* expression.

Changes in state of *Wx-m5*: The somatic sectors of mutant endosperm present on *Wx-m* kernels suggest that mutant *wx* alleles can be derived from *Wx-m5*. Germinal derivatives of *Wx-m5* were identified by crossing *Wx-m5/Wx* stocks containing 1 copy of *Ac* onto *wx/wx* testers and screening for mutant *wx* kernels (see MATERIALS AND METHODS). Of approximately 49,330 kernels screened, 44 *wx* derivatives were identified and 16/44 were found to be heritable (Table 2). Of these 16 derivatives, 13 were mutable in the presence of *Ac* (changes in state from *Wx-m* to *wx-m*) and three had a stable *wx* phenotype. All except one of the *wx-m* derivatives appear null in the absence of *Ac*; CS14(−*Ac*) displays a low level of *Wx* expression (Figure 3). In addition, each *wx-m* derivative has a distinctive phenotype with respect to the frequency, timing and quality of somatic sectors resulting from *Ds* excision (Figure 3). A similar broad spectrum of phenotypes has been found among 40 *Ds* insertions at the *R* locus (KERMICLE, ALLEMAN and DELLAPORTA 1989). The frequency of *wx-m* and *wx* derivatives obtained from *Wx-m5* in this experiment was 16/23,510 = 6.81×10^{-4} per chromosome. Two additional *wx-m* derivatives (CS7 and CS8) were isolated in a separate experiment. These alleles, in addition to the *wx-m* derivatives 8311B and 8313 isolated by MCCLINTOCK (see MATERIALS AND METHODS) bring the total number of derivative alleles to 20.

Genomic organization of *wx-m* derivatives: To understand how distinctive *wx-m* alleles arose from a *Wx-m* progenitor, the genomic DNA from 15 of the *wx-m* derivatives was analyzed on Southern blots following hybridization with labeled *Wx* fragment 1, 2, 3 or 4. Examples of this analysis are shown in Figure 4 and the genomic organization of all the *wx-m* derivatives examined is summarized in Figure 5. Each *wx-m* derivative of *Wx-m5* contains either one or two *Ds* elements. The vast majority of the *wx-m* alleles derived from *Wx-m5* (12 of 15) have a wild-type sized fragment 1 and a 2-kb insert in either fragment 2, 3 or 4. As shown below, these derivatives are apparently the result of *Ds* excision from fragment 1 and transposition into new sites within the *Wx* gene. To denote the presence of one *Ds* element at the *Wx* locus, these alleles are henceforth referred to as "class 1." In contrast, three *wx-m* alleles (8313, CS13 and CS14) appear to retain the *Ds* element in fragment 1 and also contain a second 2-kb insertion in fragment 2 (8313 and CS13) or fragment 3 (CS14; Figure 5); these are "class 2" derivatives.

Characterization of the *Ds* elements in the *wx-m* derivatives: All of the *wx-m* derivatives contain *Ds* insertions of approximately 2 kb in length. To determine the relationship between these elements and the *Ds* in the *Wx-m5* progenitor, the *Ds* insertions from CS9 and CS22 (chosen on the basis of their differing *wx-m* phenotypes) and both *Ds* insertions from the class 2 allele 8313 were cloned and partially sequenced. These four elements are identical to the *Ds*

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TAGGGATGAA AACGGTCGGg AACGGTCGGT AAAATACCTC TACCGTTTTC 1-50
ATTTTCATAT TTAACTTGCG GGACGGAAAC GAAAACGGGA TATACCGGTA 51-100
ACGAAAACGA ACGGGATAAA TACGGTAATC GAAAACCGAT ACGATCCGGT 101-150
CGGGTTAAAG TCGAAATCGG ACGGGAACCG GTATTTTTGT TCGGTAAAAT 151-200
CACACATGAA AACATATATT CAAAACTTAA AAACAAATAT AAAAAATTGT 201-250
AAACACAAGT CTTAATTAAA CATAGATAAA ATCCATATAA ATCTGGAGCA 251-300
CACATAGTTT AATGTAGCAC ATAAGTGATA AGTCTTGGGC TCTTGGCTAA 301-350
CATAAGAAGC CATATAAGTC TACTAGCACA CATGACACAA TATAAAGTTT 351-400
AAAACACATA TTCATAATCA CTTGCTCACA TCTGGATCAC TTAGCATGCA 401-450
TAAACTATTA CAACCAAGGC TCATCcGTCA ACAAACATAA GACACATTGC 451-500
TCATGGAGAG GAGCCACTTG CTACATCTTC ATTATTCTTA GAAAATTCTA 501-550
TTGCGTCTTC ATCCTGTTAA TACACAAAAA TAAGTCAGTT TTGGATAAAT 551-600
AAATACATAT AGAAGAACAT GAATTGATAT GCAGGGAGTA TAAATAAATA 601-650
CATATAGGAG AACATGAATC TGTGAACTAA CACGGCTGGG AGCTAGGCAG 651-700
CTAGCAGCTA GCGCCTAACA GCTGGGAGCC TAACAGCTAG CAGCTAGCAG 701-750
CCAATCAAAA CAAGGCGACA AGGCGCATGC AGTGAGATCA AAAATCTGTT 751-800
AATGCCAGCC ATGCAGGGAG TATAACACGG CTGGGCAGCA AGGCGCATGC 801-850
ATCAAAACAA GGCGACAGCA AACAGCCCAT GCATCAAAAC AGTAGTGAAT 851-900
AATAGCAAAT TAATAGCCCA TGCACGAAGT AAATAATAAT CTTTAAATAC 901-951
CTCATCCATA TGATTCTCAT GATTTGTTGC AGCAGCAATA ACAG------ 951-994
---------- 2521 bp deleted from Ac sequence ----------
---------- -----CATCA ACTTGGCCAA TCTTATGGCT GAGC-TGAGG 3515-3550
GAGGATTATT TCCAACCGGA GGCGTCATCT GAGGAATGGA GTCGTAGCCG 3551-3600
GCTAGC---- ---------- ---------- ----AGGTGT TCAGCAATCA 3601-3650
GCTTGGTGCT GTACTGCTGT GACTTGTGAG CACCTGGACG GCTGGACAGC 3651-3700
AATCAGCAGG TGTTGCAGAG CCCCTGGACA GCACACAAAT GACACAACAG 3701-3750
CTTGGTGCAA TGGTGCTGAC GTGCTGTACT GCTAAGTGCT GTGAGCCTGT 3751-3800
GAGCAGCCGT GGAGACAGGG AGACCGCGGA TGGCCGGATG GGCGAGCGCC 3801-3850
GAGCAGTGGA GGTCTGGAGG ACCGCTGACC GCAGATGGCG -ATGGCGGAT 3851-3900
GGGCGGACCC GGGATGGGCG AGCAGTGGAG TGGAGGTCTG GGCGGATGGG 3901-3950
CGGACCGCGG CGCGGATGGG CGAGTCGCGA GCAGTGGAGT GGAGGGCGGA 3951-4000
CCGTGGATGG CGGCGTCTGC GTCCGGCGTG CCGCGTCACG GCCGTCACCG 4001-4050
CGTGTGGTGC CTGGTGCAGC CCAGCGGCCG GCCGGCTGGG AGACAGGGAG 4051-4100
AGTCGGAGAG AGCAGGCGAG AGCGAGACGC GCGCCGGCGT CGGCGTGCGG 4101-4150
CTGCCGGCGT CCGGACTCCG GCGTGGGCGG GTGGCGGCGT GTGAATGTGT 4151-4200
GATGCTGTTA CTCGTGTGGT GCCTGCCGCC TGGGAGAGAG GCAGAGCAcg 4201-4250
GTTCGCTAGG TATTTCTTAC ATGGGCTGGG CCTCAGTGGT TATGGATGGG 4251-4300
AGTTGGAGCT GGCCATATTG CAGTCATCCC GAATTAGAAA ATACGGTAAC 4301-4350
GAAACGGGAT CATtCCGATT AAAAACGGGA TCCCGGTGAA ACGGTCGGGA 4351-4400
AACTAGCTCT ACCGTTTCCG TTTCCGTTTA CCGTTTTGTA TATCCCGTTT 4401-4450
CCGTTCCGTT TTCGTTTTTT ACCTCGGGTT CGAAATCGAT CGGGATAAAA 4451-4500
CTAACAAAAT CGGTTATACG ATAACGGTCG GTACGGGATT TTCCCATCCT 4501-4550
ACTTTCATCC CTa                                        4551-4563
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FIGURE 2.—DNA sequence of the *Wx-m5 Ds* element. Numbering is according to the *Ac* sequence of MULLER-NEUMANN, YODER and STARLINGER (1984). Nucleotide differences with the *Ac* sequence are indicated in lower case and deletions of *Ac* sequences are represented by dashes.

element in *Wx-m5* for all regions examined (data not shown). Specifically, all have the same deletion of *Ac* nucleotides 995–3515 and the same 28-bp deletion at positions 3607–3634 (see Figure 2) as well as identical base substitutions when compared to the *Ac* sequence. The *Ds* elements of CS9 and CS22 are inserted into 8-bp target sites at positions 2387–2394 and 965–972, respectively, within the *Wx* transcription unit. One of the two *Ds* elements of 8313 is inserted at positions 976–983. The second *Ds* element is inserted at positions −467 to −474, identical to the *Wx-m5* progenitor. Taken together, these data strongly suggest that the *wx-m* derivatives arise following intragenic transposition of the *Ds* element inserted at −470 in the *Wx-m5* progenitor.

If these derivatives have in fact resulted from intragenic transposition into the *Wx* coding region, the site of *Ds* excision at −470 might be expected to contain a transposon "footprint." These footprints often include partial duplications and inversions of the target sequence (SACHS *et al.* 1983; MARTIN *et al.* 1985; SOMMER *et al.* 1985; WESSLER *et al.* 1986). Analysis of the −470 region from CS22 indicates that a 7-bp transposon footprint is retained at the *Ds* insertion site (Figure 6). Interestingly, the sequence in the −470 region of a second allele (CS9) is identical to wild type (data not shown); the implications of this observation are considered in the DISCUSSION.

Reversion of *wx-m* derivative alleles: To further characterize the *wx-m* derivatives of *Wx-m5*, germinal revertants were isolated by crossing stocks containing the *wx-m* alleles and one copy of *Ac* onto homozygous *wx* testers and screening the resulting ears for *Wx′* or *Wx-m′* kernels (see MATERIALS AND METHODS). Frequencies of germinal reversion ranged from 5×10^{-2} to $<5 \times 10^{-4}$ (Table 3). The reversion frequencies of three *wx-m* alleles have not been characterized precisely; CS7, CS9 and CS13 yielded no germinal revertants out of 2560, 1600 and 1575 chromosomes screened, respectively. In addition, CS14 and CS24 were not analyzed. For the most part the reversion frequency of each *wx-m* derivative was consistent with

TABLE 2

Derivatives of *Wx-m5*

Wx-m parent	No. of chromosomes screened	Derivative	Phenotype
1439-10	1,995	*CS9*	*wx-m*
1440-2	1,705	*CS10*	*wx-m*
1452-4	680	*wx-11*	Stable *wx*
1452-8	1,225	*wx-12*	Stable *wx*
1452-9	1,710	*CS13*	*wx-m*
1452-12	935	*CS14*	Intermediate *wx-m*
		CS15	*wx-m*
1453-1	2,620	*CS16*	*wx-m*
		CS17	*wx-m*
		CS18	*wx-m*
1453-2	2,455	*CS19*	*wx-m*
		CS20	*wx-m*
		wx-21	Stable *wx*
1453-4	2,490	*CS22*	*wx-m*
		CS23	*wx-m*
		CS24	*wx-m*
Others	7,695		
Totals	23,510	16	
		4	Did not germinate
		3	Sterile
		21	*wx-m* phenotype not heritable
		44	

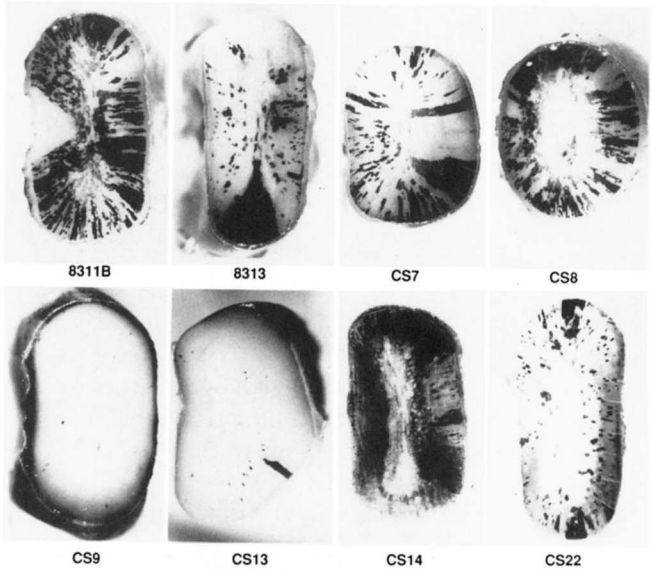

FIGURE 3.—Sectoring patterns of *wx-m* derivatives of *Wx-m5*. Cross-sections of mature kernels were stained with I/KI. In each case the genotype of the endosperm is *wx-m/wx/wx* plus one copy of *Ac*.

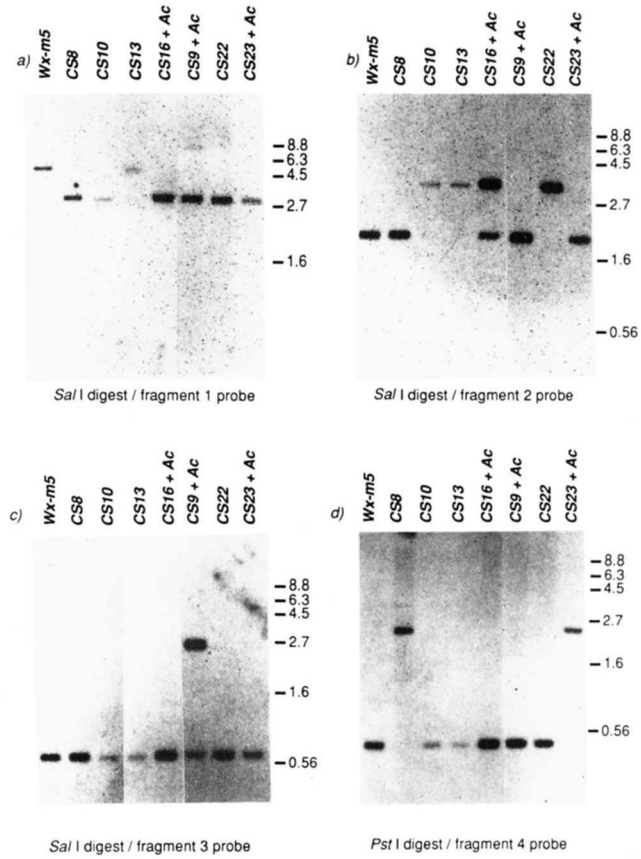

FIGURE 4.—Representative Southern blot analysis of *wx-m* derivative alleles. Restriction enzymes and probes used are indicated under each panel. Size standards are in kilobase pairs.

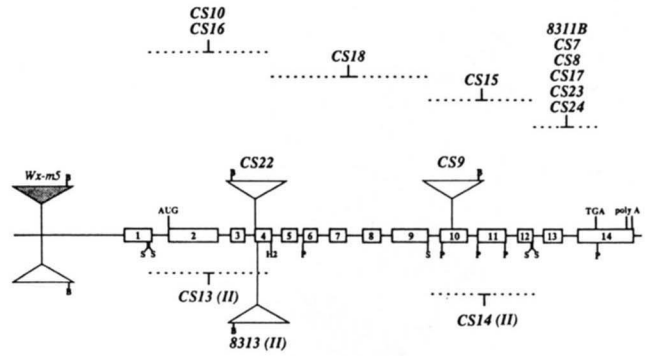

FIGURE 5.—Summary of the positions of *Ds* insertions in the *wx-m* derivatives of *Wx-m5*. Exons of the *Wx* gene are indicated by open boxes. *Ds* elements whose positions are precisely mapped are indicated as open triangles. The orientation of these *Ds* insertions is indicated by the position of the assymetric *Bam*HI site. The remaining insertion sites have been localized to one of the restriction fragments that comprise the *Wx* gene, and are indicated by the dashed lines. Class 1 derivatives are shown above the gene and class 2 derivatives below the gene. H2 = *Hin*CII, P = *Pst*I and S = *Sal*I.

its somatic phenotype. Alleles which preferentially show large, frequent somatic sectors (*e.g.*, 8311B) produced a higher frequency of germinal revertants than alleles that had late, rare somatic sectors (*e.g.*, CS9). One allele, CS7, did not yield germinal revertants even though it conditions relatively large and frequent revertant sectors (see Figure 3); this inconsistency is under investigation. All but one of the *wx-m* alleles that gave rise to germinal revertants produced stable derivatives. In contrast, most derivatives of 8313 were *Wx-m′* and indistinguishable from the *Wx-m5* progenitor.

The physical descriptions of the *wx-m* derivatives provide a simple explanation for their reversion be-

Wx	GTGCACGGGGGAG	*GTGGTGTG*	GAAGTG
Wx-m5	GTGCACGGGGGAG	*GTGGTGTG*-----Ds-----*GTGGTGTG*	GAAGTG
CS22	GTGCACGGGGGAG	*GTGGTCACTGGTGTG*	GAAGTG

FIGURE 6.—DNA sequence comparison of the region containing the *Wx-m5* insertion site in *Wx*, *Wx-m5* and the *wx-m* derivative CS22. The sequence duplicated following *Ds* insertion is shown in italics.

TABLE 3

Reversion of *Wx-m5* derivative alleles

wx-m derivative[a]	No. of chromosomes screened	No. of *Wx* kernels	Frequency
8311B	3300	71	2.15×10^{-2}
8313	2215	127[b]	5.73×10^{-2}
CS7	2560	0	$<3.91 \times 10^{-4}$
CS8	1980	18	9.09×10^{-3}
CS9	1600	0	$<6.24 \times 10^{-4}$
CS10	2430	5	2.06×10^{-3}
CS13	1575	0	$<6.34 \times 10^{-4}$
CS15	4340	113	2.60×10^{-2}
CS16	3595	13	3.62×10^{-3}
CS17	1380	2	1.45×10^{-3}
CS18	4780	18	3.76×10^{-3}
CS22	3520	5	1.42×10^{-3}
CS23	2110	2	9.48×10^{-4}

[a] *CS14, CS19, CS20* and *CS24* were not analyzed.
[b] Includes *Wx-m'* and *Wx'* kernels.

havior. Most of the *wx-m* alleles have one *Ds* insertion in the *Wx* gene and excision of that *Ds* results in stable revertants. The class 2 *wx-m* allele 8313, which gives rise to *Wx-m'* revertants, contains two *Ds* insertions (see Figure 5). If the *Ds* element within the *Wx* coding region excises and the insertion in fragment 1 does not, the *Wx-m5* allele is restored (except for any transposon footprint resulting from *Ds* excision). Southern blots comparing genomic DNA from 8313 and a *Wx-m'* derivative of 8313 confirm that the *Ds* inserted in *Wx* fragment 2 has excised to produce the *Wx-m'* derivative (Figure 7). A *Wx-m'* derivative of 8313 has been used to generate a set of *wx-m''* changes in state (data not shown; see MATERIALS AND METHODS). Based on our isolation of change in state derivatives of *Wx-m5*, approximately 2/13 of this new round of *wx-m''* derivatives may be expected to carry two *Ds* elements at the *Wx* locus. If these new class 2 alleles produce *Wx-m'''* revertants then the cycling between *Wx-m* and *wx-m* phenotypes would continue (Figure 8). Another *wx-m* allele containing two *Ds* elements, CS13, has not yielded germinal revertants (see Table 3). Our inability to recover revertants of CS13 is consistent with its infrequent and late somatic sectoring, however our prediction is that revertants of CS13 should be *Wx-m'*.

Genomic organization of stable *wx* derivatives: Only three of 20 derivatives of *Wx-m5* (*wx-11*, *wx-12* and *wx-21*) have a stable *wx* mutant phenotype in the

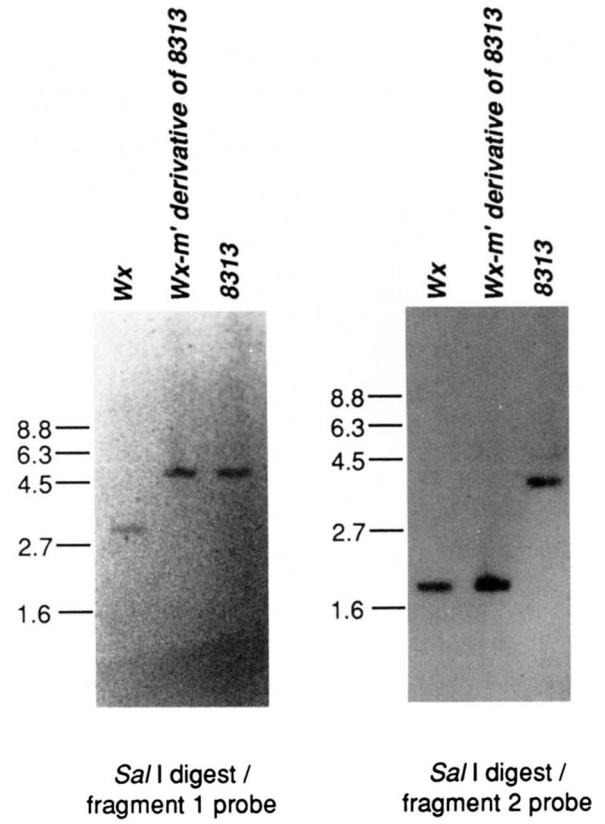

FIGURE 7.—Southern blot analysis of *Wx*, 8313 and a *Wx-m'* derivative of 8313. Restriction enzymes and probes used are indicated under each panel. Size standards are in kilobase pairs.

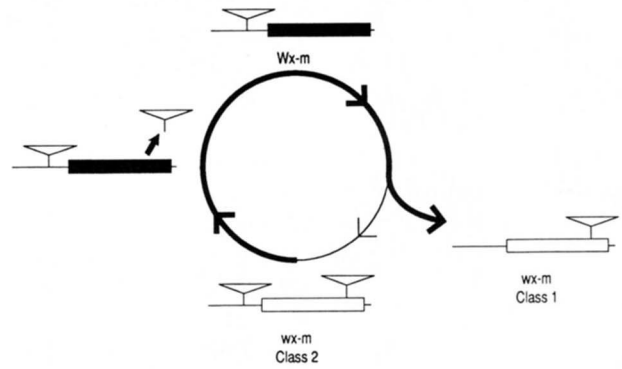

FIGURE 8.—Diagram summarizing the formation of *wx-m*, *Wx-m'* and *wx-m''* derivatives of *Wx-m5* (see text for details).

presence of *Ac*. Southern blot analysis of *wx-11* and *wx-12* shows an increase in the size of *Wx* fragment 1 to greater than 10 kb and no change in the size of fragments 2, 3 or 4 (Figure 9). The increase in size of only fragment 1 suggests that a large scale rearrangement of the sequences upstream of *Wx* has occurred in these two alleles. Genomic DNA from the third stable *wx* derivative, *wx-21*, shows no hybridization when probed with fragments 2, 3 or 4 (Figure 9), indicating that this allele has sustained a deletion of the *Wx* gene.

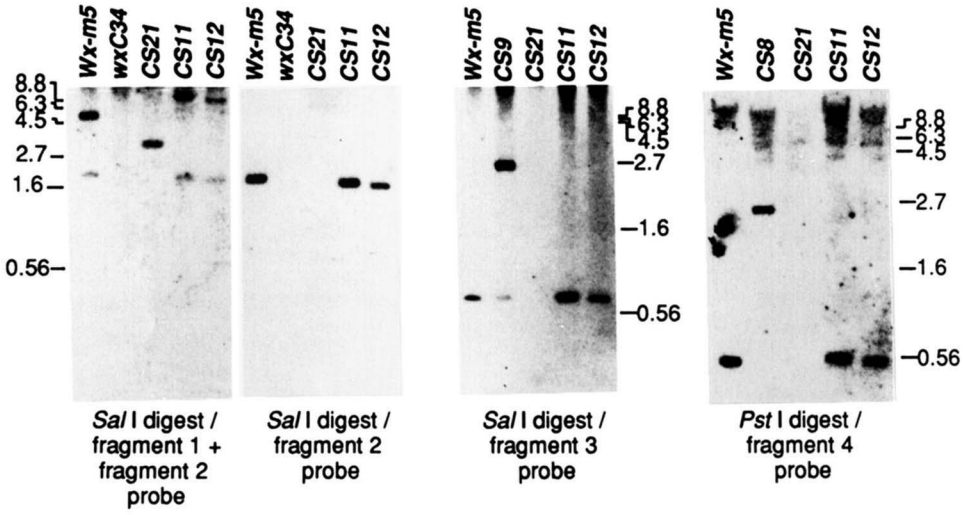

FIGURE 9.—Southern blot analysis of the stable *wx* derivatives of *Wx-m5*. Restriction enzymes and probes used are indicated under each panel. CS9 (+*Ac*) is included as a positive control on the filter hybridized to *Wx* fragment 3 and CS8 is included as a positive control on the filter hybridized to *Wx* fragment 4. Size standards are in kilobase pairs.

DISCUSSION

The *Wx-m5* allele has been cloned and found to contain a 2-kb *Ds* element at −470 relative to the *Wx* transcription start. Although phenotypically wild-type, *Wx-m5(−Ac)* conditions 2–4-fold lower levels of enzymatic activity. Since the *Ds* insertion influences *Wx* expression the insertion site at −470 is formally a part of the *Wx* gene. In the presence of *Ac*, *Wx-m5* displays mutant *wx* sectors and gives rise to *wx-m* and *wx* derivatives. The majority of these derivatives are *wx-m* change in state alleles that can be divided into two classes based on their gene structure. Class 1 contains one *Ds* element in the *Wx* gene whereas class 2 contains two *Ds* elements. The three remaining derivative alleles are stably mutant and are associated with large-scale rearrangements of *Wx* sequences.

Several lines of evidence indicate that class 1 *wx-m* alleles arise following intragenic transposition of *Ds* from −470 to a new site in the *Wx* gene. First, *Wx-m5* and all the class 1 derivatives contain 2-kb *Ds* elements. This finding in itself is not convincing proof of intragenic transposition since other transposable element alleles also harbor 2-kb *Ds* elements (FEDOROFF, WESSLER and SHURE 1983; DORING, TILLMAN and STARLINGER 1984; WECK *et al.* 1984; CONE, BURR and BURR 1986; KERMICLE, ALLEMAN and DELLAPORTA 1989). A second and stronger line of evidence is that the partial sequences of two class 1 *Ds* insertions (from CS9 and CS22) are identical to the *Wx-m5 Ds* over all regions compared. Specifically, the *Ds* elements in *Wx-m5* and its *wx-m* derivatives are characterized by a short deletion and eight base substitutions that have not been reported in other 2-kb *Ds* elements (see Figure 2; DORING, TILLMAN and STARLINGER 1984). Finally, in addition to a *Ds* insertion, some class 1 alleles should contain a transposon footprint at −470, the site of *Ds* excision. We find that CS22 does indeed contain a typical transposon footprint at this position.

A transposon footprint was not detected at −470 of the CS9 allele; the sequence in this region is wild-type. Although perfect *Ds* excision or pollen contamination could explain this inconsistency we believe these reasons unlikely. Perfect excisions of *Ac/Ds* elements are unusual (BARAN *et al.* 1991) and CS9 is probably not a contaminant because it contains a *Ds* that is indistinguishable from the *Wx-m5 Ds*. Rather, this allele may have arisen by intragenic transposition of *Ds* followed by gene conversion or recombination with the *Wx* homolog in the *Wx-m5/Wx(+Ac)* parent. Alternatively, *Ds* may have transposed into the *Wx* gene on the homologous chromosome.

Intragenic transposition of *Ds* can also explain the origin of the class 2 *wx-m* derivatives containing two *Ds* elements (8313, CS13 and CS14). Partial sequences of both *Ds* insertions in the 8313 allele indicate they are identical to each other and to the *Wx-m5* element over all regions compared. Furthermore, the *Ds* at −470 in 8313 is in the same position and orientation as the *Ds* in *Wx-m5*. Previous transposition models provide an explanation for how an allele containing one element can generate a derivative containing two identical elements (GREENBLATT and BRINK 1962; GREENBLATT 1984; CHEN, GREENBLATT and DELLAPORTA 1987). Class 2 *wx-m* derivatives probably result from intrachromosomal transposition of *Ds*, after its replication, into either an unreplicated target site or a replicated site on the sister chromatid. As described above for CS9, *Ds* transposition into the homologous chromosome followed by gene conversion or recombination could also explain the origins of 8313, CS13 and CS14. The small number of class 2 *wx-m* derivatives relative to class 1 derivatives suggests that events leading to the formation of class 2 alleles are relatively infrequent. It is also possible that our use of a heterozygous *Wx-m5/Wx(+Ac)* progenitor restricted the types of derivatives we were able to detect. Use of a *Wx-m5/Wx-m5* progenitor might be expected to yield a higher frequency of class 2 derivatives since, with

such a homozygous progenitor, both interchromatid and interchromosomal transpositions could result in derivatives containing two elements.

Alleles of the *bz* locus containing tightly linked *Ac/Ds* elements have been described previously (DOWE, ROMAN and KLEIN 1990; RALSTON, ENGLISH and DOONER 1990). These alleles are associated with "macrotransposition" events in which two elements and the sequences between them excise simultaneously. Macrotransposition may also result in chromosome breakage (RALSTON, ENGLISH and DOONER 1990). We are currently examining the class 2 derivatives of *Wx-m5* to determine whether they also lead to macrotransposition and chromosome breakage.

Although studies have clearly demonstrated that *Ac/Ds* elements have a propensity to transpose to genetically linked sites (ORTON and BRINK 1966; BRINK and WILLIAMS 1973; GREENBLATT 1984; CHEN, GREENBLATT and DELLAPORTA 1987; DOONER and BELACHEW 1989; KERMICLE, ALLEMAN and DELLAPORTA 1989), the behavior of these elements over very short distances has not been examined in depth. If short-range transposition is not sequence specific then the frequency of insertions into a region should be directly proportional to the size of that region. Among the *wx-m* derivatives of *Wx-m5*, the number of insertions into *Wx* fragments 2 and 3 are within expectations for random insertion into sequences of 2026 and 728 bp, respectively (YATES' corrected chi-squared values = 0.38 and 0.02, d.f. = 1; $0.5 < P < 0.9$). However, a higher number of *Ds* insertions occur in fragment 4 than would be expected if insertion were random. Of 15 insertions into the 3,933-bp *Wx* gene examined in this study, 1–2 insertions are expected in the 484 bp of fragment 4 [$(484/3933) \times 15 = 1.846$]; instead, 6/15 derivatives have a *Ds* element in fragment 4 (YATES' corrected chi-squared = 7.233, d.f. = 1; $0.001 < P < 0.01$). The high number of insertions in fragment 4 could indicate a preferred target site for *Ds* insertion. Such "hotspots" have been seen for the Drosophila *P* element and the *E. coli* Tn*7* and Tn*10* elements (KLECKNER *et al.* 1979; LICHTENSTEIN and BRENNER 1981; O'HARE and RUBIN 1983). There are two reasons why this is probably not the explanation for the clustering of *Ds* insertion sites among the *wx-m* derivatives. First, preliminary restriction mapping suggests that the insertions in fragment 4 have different target sites (C. WEIL, unpublished results). Second, only one of seven previously described and independent *Ac/Ds* alleles of *Wx* has an insertion in fragment 4 (VARAGONA and WESSLER 1990). Rather than postulating an insertional hotspot, we believe that the clustering of *Ds* elements indicates that *Ds* may have a preference for insertion sites at least 3 kb from the donor site.

In this study derivatives of *Wx-m5* were recovered at a frequency of only 6.81×10^{-4} per chromosome (Table 2). In contrast, the intensity of the excision fragment in Southern blots of *Wx-m5(+Ac)* genomic DNA probed with fragment 1 suggest a higher frequency of *Ds* excision (Figure 1b). What is the reason for this discrepancy? One possibility is that *Ds* excision from −470 is usually accompanied by the restoration of a stable non-mutant phenotype followed by *Ds* transposition away from the *Wx* gene. Although our genetic screen was not designed to identify these stable *Wx* derivatives, two lines of evidence presented in this study support the notion that *Ds* excision from −470 usually conditions a non-mutant phenotype. First, the *wx-m* derivative CS22 has a transposon footprint at −470 (Figure 6) and a *Ds* insertion in fragment 2 (Figure 5). Despite the footprint, excision of *Ds* from fragment 2 can restore the *Wx* phenotype both somatically (Figure 3) and germinally (Table 3). Second, our results demonstrate that the three derivatives with a stable, mutant phenotype are not attributable to transposon footprints at −470; rather they contain large-scale rearrangements of the *Wx* gene and flanking DNA (Figure 9).

If most *Ds* excisions from −470 do not have a mutant phenotype, what is the mechanism producing the mutant *wx* sectors associated with *Wx-m5* in the presence of *Ac* (Figure 1a)? The *wx* sectors on *Wx-m* kernels could reflect a chromosome-breaking *Ds* element located at or proximal to the *Wx* gene on chromosome *9* (MCCLINTOCK 1949). Two lines of evidence argue against this explanation. First, kernels can be found containing *Wx* subsectors within *wx* sectors, indicating that the *Wx* gene was not lost from these subsectors via chromosome breakage (data not shown). Second, using appropriately marked chromosomes, we find that markers distal to the *Wx* gene are not lost in *wx* sectors (data not shown). Based on our analysis of *Wx-m5* and its derivatives we believe that the *wx* sectors reflect intragenic transposition of *Ds* for the following reasons. Germinal derivatives of transposable element alleles are usually a reflection of somatic mutational events. For *Wx-m5*, most of the mutant germinal derivatives are *wx-m* alleles that apparently result from intragenic transposition of *Ds* from −470 into *Wx* coding sequences. In these derivatives, *Ds* excision alone does not appear to result in a mutant phenotype. Only the mutant phenotypes of the three stable *wx* derivatives may be directly related to *Ds* excision. Taken together, these data suggest that the molecular basis for the unstable phenotype of *Wx-m5(+Ac)* may differ from all previously described transposable element alleles. Unlike these other alleles, where clonal sectors arise from the *excision* of the transposable element and the restoration of gene expression, the clonal sectors of *Wx-m5* appar-

ently represent transposable element *insertion* and the concommitant loss of gene expression.

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