Genome Rearrangements in Maize Induced by Alternative Transposition of Reversed *Ac/Ds* Termini

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ABSTRACT

Alternative transposition can induce genome rearrangements, including deletions, inverted duplications, inversions, and translocations. To investigate the types and frequency of the rearrangements elicited by a pair of reversed *Ac/Ds* termini, we isolated and analyzed 100 new mutant alleles derived from two parental alleles that both contain an intact *Ac* and a fractured *Ac* (*fAc*) structure at the maize *p1* locus. Mutants were characterized by PCR and sequencing; the results show that nearly 90% (89/100) of the mutant alleles represent structural rearrangements including deletions, inversions, translocations, or rearrangement of the intertransposon sequence (ITS). Among 37 deletions obtained, 20 extend into the external flanking sequences, while 17 delete portions of the intertransposon sequence. Interestingly, one deletion allele that contains only a single nucleotide between the retained *Ac* and *fAc* termini is not competent for further alternative transposition events. We propose a new model for the formation of intertransposon deletions through insertion of reversed transposon termini into sister-chromatid sequences. These results document the types and frequencies of genome rearrangements induced by alternative transposition of reversed *Ac/Ds* termini in maize.

TRANSPOSABLE elements are the major component of many eukaryotic genomes, where they play important roles in plant gene evolution and genome reorganization (BENNETZEN *et al.* 2005; DOONER and WEIL 2007). In maize, nearly 85% of the genome is composed of mobile DNA and other repeated sequences (SCHNABLE *et al.* 2009). Transposable elements are broadly classified as either retroelements (class I) or DNA transposons (class II) (WICKER *et al.* 2007). Retroelement transposition occurs via a “copy and paste” mechanism, which can lead to massive amplification and is thought to be a major contributor to genome size differences. In contrast, most DNA elements move by a “cut and paste” mechanism. Although DNA elements are less abundant compared with retroelements, they can lead to a variety of genome structural changes (ROSS *et al.* 1979; GRAY 2000; WATANABE *et al.* 2007; HUANG and DOONER 2008) including large chromosomal rearrangements, which may contribute to genome evolution (McCLINTOCK 1951; McCLINTOCK 1978; LISTER *et al.* 1993; ZHANG *et al.* 2009).

The well-known *Ac/Ds* elements in maize were initially identified by Barbara McClintock through their ability to induce chromosome breakage. In addition, McClintock identified a number of major chromosome rearrange-

ments that were apparently induced by the *Ac/Ds* system (McCLINTOCK 1951; McCLINTOCK 1978). Subsequent genomic cloning and sequence analysis suggested that the original chromosome-breaking *Ds* element has a “double *Ds*” structure, *i.e.*, one *Ds* element inserted into a second identical *Ds*, in the opposite orientation (DORING *et al.* 1984; KLEIN *et al.* 1988; MARTINEZ-FEREZ and DOONER 1997). Chromosome breakage is thought to occur when the *Ac* transposase attempts to transpose a pair of directly oriented *Ds* 5′ and 3′ termini located on different sister chromatids (ENGLISH *et al.* 1993). This reaction, termed sister-chromatid transposition, not only breaks chromosomes but can also generate deficiencies and inverted duplications (ZHANG and PETERSON 1999). A second type of alternative transposition reaction involves reverse-oriented 5′ and 3′ *Ac/Ds* termini; for example, two directly oriented *Ac* or *Ds* elements will have their apposed 5′ and 3′ termini in reversed orientation. Previously our lab isolated and characterized the *P1-r11* allele, which contains a full-length *Ac* element and a terminally deleted *Ac* (*fAc*) inserted in the maize *p1* locus. The 5′ end of *Ac* and the 3′ end of *fAc* are in reversed orientation, separated by a ~13-kb intertransposon segment (ITS). In this configuration, reversed-ends transposition can induce a variety of chromosome rearrangements, including deletions, inversions, translocations, and ITS rearrangements (ZHANG and PETERSON 2004; ZHANG *et al.* 2009). In addition, this reversed-ends configuration can induce chromosome breakage (HUANG and DOONER 2008; YU *et al.* 2010).

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TABLE 1
PCR primers and their sequences used in this study

Primer ID	Primer sequence
P1-2415f	CGCCGAACCTTTCAGTCTGCTCTGCTA
9D9A5537f	CGCCACCTGATGATCGAAGC
P1-2915r	GACAGTTCGCAGTTGGGTTGGG
P1-7061r	GGTTTGTGTTGTGCTGCCTCC
P1-15588f	GCTATCAAACAGGACACGGGAGAGAAT
PP1'	GACCGTGACCTGTCCGCTC
P1-7869f	ATTCAACCAATAACTTTACGACTGTTCTCTTCTCA
P1-12046r	GATGATGTCTTCTTCTCCTTGG
9D9A33680r	GCCCTTATTTTCAGCCGTATTCAA
Ac4436f	GATTACCGTATTTATCCCCTTCGTTTTTC
Ac120r	CCCGTTTCCGTTCCGTTTTTCGT
Ac2680f	TGCCATCTTCCACTCCTCGGCTTTAG
PA-A11	GCGACGCCAAATCATCGATAG

In this article, we investigated the types and frequency of genome rearrangements produced by reversed-ends transposition at the *p1* locus. The results show a high relative frequency of deletions and inversions, in agreement with previous reports (ZHANG and PETERSON 2004; HUANG and DOONER 2008). In addition, we identified a substantial class (17%) of previously uncharacterized new mutant alleles, which contain deletions of various segments of the DNA between the reversed *Ac/Ds* termini. On the basis of structures of these deletions and the known propensity of *Ac/Ds* elements to transpose during DNA replication (GREENBLATT and BRINK 1962; GREENBLATT 1968, 1974, 1984), we propose a model for their formation. Furthermore, we investigated the frequency of chromosome breakage induced by the resulting alleles that contain various lengths of DNA separating the reversed *Ac/Ds* termini. Interestingly, the chromosome-breakage frequency (and hence the alternative transposition frequency) declines precipitously for alleles in which the reversed *Ac/Ds* termini are in very close proximity (91 and 1 bp).

MATERIALS AND METHODS

Maize stocks and crosses: The maize alleles *P1-rr11*, *P1-rr910*, and *P1-ovov454* were isolated and described previously (ZHANG and PETERSON 2004; ZHANG *et al.* 2009; YU *et al.* 2010). Plants in which the *P1-rr11*, *P1-rr910*, or *P1-ovov454* alleles were heterozygous with an allele for colorless kernel pericarp (*p1-wv* or *p1-wr*) were crossed by pollen from plants of genotype *p1, r1-m3::Ds*. The *r1-m3::Ds* allele contains a *Ds* element inserted in the *r1* gene required for kernel aleurone pigmentation; *Ac*-induced excision of *Ds* from *r1-m3::Ds* results in purple aleurone sectors (KERMICLE 1980). The mature ears were screened for kernels with colorless pericarp and spotted aleurone, as these were predicted to have undergone a loss of *p1* function, but to have retained *Ac* activity. Selected kernels were sown, and pollen from mature plants was checked using a hand microscope to estimate pollen abortion frequency. Plants were self-pollinated to make the new mutant alleles homozygous for molecular analyses.

DNA extraction and PCR analyses: Young leaves of individual plants were ground in liquid nitrogen, and genomic

DNA was extracted with CTAB (cetyltrimethylammonium bromide) reagent (SAGHA-MAROOF *et al.* 1984). HotMaster Taq polymerase from Eppendorf (Hamburg, Germany) was used in the PCR reaction. The PCR mix was heated at 94° for 3 min to denature the DNA template, followed by 35 cycles of 20 sec at 94°, 30 sec at 60°, and 1 kb/1 min at 65°, and one cycle of 8 min at 65°. Primers used for PCR analyses are listed in Table 1. Candidate ITS rearrangement alleles were analyzed using the following primer pairs: P1-2915r and P1-15588f (for alleles derived from *P1-rr11*) or P1-7061r and P1-15588f (for alleles derived from *P1-rr910*). Candidate internal deletions flanking the *Ac* 5' terminus were analyzed using the following primers: Ac120r plus one of Ac4436f, P1-12046r, or P1-7061r (for alleles derived from *P1-rr11*); and Ac120r plus one of Ac4436f or P1-12046r (for alleles derived from *P1-rr910*). Candidate internal deletions flanking the *fAc* 3' terminus were analyzed using the following primers: Ac4436f plus one of Ac120r, 9D9A5537f, P1-7869f, or 9D9A33680f (for alleles derived from *P1-rr11*); and Ac4436f plus one of Ac120r, P1-7869f, or 9D9A33680f (for alleles derived from *P1-rr910*). PCR products were excised from agarose gels, purified using the Pfect-prep gel cleanup system (Eppendorf HotMaster Taq, Westbury, NY), and sequenced by the DNA Synthesis and Sequencing Facility, Iowa State University. Sequence of *P1-ovov454* is available in GenBank (accession number GU595146).

Evaluation of chromosome-breakage frequency: The maize *Dek1* gene is required for differentiation of kernel aleurone cells; endosperm cells lacking *Dek1* function fail to differentiate into aleurone cells and thus cannot synthesize anthocyanin pigments (LID *et al.* 2002). Wild-type *Dek1 C1 R1* were crossed as ear parents by pollen from plants homozygous for the weak *dek1-Dooner* allele (BECRAFT *et al.* 2002) to generate a stock of heterozygous *dek1-Dooner/Dek1* tester plants. The *dek1/Dek1* plants were then crossed as ear parents by pollen from plants homozygous for the candidate *p1* alleles to be tested. Kernels of mature ears were examined and the numbers of colorless sectors were used as an index to the chromosome-breakage frequency. The chromosome-breakage frequency grading is evaluated according to Yu *et al.* (2010).

RESULTS

Inversion and deletion are the major products of reversed-ends transposition: The *P1-rr11* and *P1-rr910* alleles condition predominantly red kernel pericarp

pigmentation with white (colorless) sectors of variable size (ZHANG and PETERSON 2004; ZHANG *et al.* 2009). Both alleles contain an identical *fAc* element in the second intron of the *p1* gene and a full-length *Ac* element inserted upstream of the *p1* transcription start site. The distances between the *Ac* 5' terminus and *fAc* 3' terminus in *P1-rr11* and *P1-rr910* are approximately 13 and 9 kb, respectively. In both alleles, *p1* exons 1 and 2 are located between the *Ac/fAc* termini and are predicted to be deleted by reversed-ends transposition (ZHANG and PETERSON 2004; ZHANG *et al.* 2009). Therefore, we reasoned that new alleles arising from reversed-ends transposition could be selected on the basis of loss of *p1* function (colorless kernel pericarp) and retention of *Ac* activity (purple aleurone sectors) (MATERIALS AND METHODS). In this way we selected and characterized a total of 100 putative mutant alleles (42 and 58 derived from *P1-rr11* and *P1-rr910*, respectively).

To determine what types of structural changes had occurred in the mutant alleles, we performed PCR using four pairs of primers specific for the four junctions of *Ac/fAc* and the flanking genomic DNA: (1) the *Ac* 3' terminus; (2) the *Ac* 5' terminus; (3) the *fAc* 3' terminus; and (4) the *fAc* 5'-end junction (Figure 1A and Table 2). Representative gel analysis results are shown in Figure 1B, and a summary of the data are shown in Table 3.

The largest class (52%) was composed of alleles in which PCR primer pairs 1 and 4 were both positive while pairs 2 and 3 were negative (pattern + - - +). This pattern could be produced by one of several different types of rearrangements including translocations, inversions, and ITS rearrangements (classes a, b, and c).

- Translocations and large inversions (five cases). Chromosome translocations and very large inversions would be expected to exhibit a higher than normal percentage of pollen abortion (50% for translocations, and up to 50% for inversions depending on size). By screening for pollen abortion followed by further molecular characterization we identified four translocations (*p1-wwC15*, *p1-wwB47*, *p1-wwB33*, *p1-wwB1023*) and one pericentric inversion (*p1-wwC30*). These cases have been described elsewhere (ZHANG *et al.* 2009).
- Small inversions (46 cases). Alleles with a + - - + pattern and normal pollen abortion frequency were classified as putative inversions of less than 10 cM in size. Larger inversions would be expected to exhibit significant levels of pollen sterility resulting from crossovers within the inverted segment. Among these 46 alleles, the breakpoint sequences of 6 representative cases were cloned by the "Ac casting" method (SINGH *et al.* 2003; ZHANG *et al.* 2009). The *p1-wwB53* and *p1-wwB58* alleles contain inversions of less than 10 kb, with breakpoints located in repeat sequences

FIGURE 1.—PCR analyses of rearrangement alleles. (A) Schematic structure of the parental alleles, showing the locations of the four PCR primer pairs. (B) PCR results from 19 candidate rearrangement alleles (samples 1–19) and parental allele *P1-rr11* (sample 20). See Tables 1 and 2 for primer names and sequences.

flanking the *p1* 3' region. The *p1-wwB9*, *p1-wwC14*, and *p1-wwC4* alleles contain inversions with breakpoints located 138, 142, and 370 kb distal to *p1*, respectively. Finally, the *p1-wwC10* allele contains an ~1-Mb inversion proximal to *p1*.

- ITS rearrangements (1 case). ITS rearrangements are defined as those cases in which the ITS have been circularly permuted; *i.e.*, the sequences between the transposon termini have remained, but they have changed their relative position and, in some cases, their orientation. These permutations result from reversed-ends transposition followed by insertion into the ITS of the same chromatid (ZHANG and PETERSON 2004; HUANG and DOONER 2008). Candidate ITS rearrangement alleles were subject to one additional PCR test designed to detect the junction formed by excision of the *Ac/fAc* termini (MATERIALS AND METHODS). Only one case (derived from *P1-rr11*) was confirmed as an ITS rearrangement.
- External deletion (20 cases). Alleles in which only PCR primer pair 1 or pair 4 gave a positive result (pattern - - - + or + - - -) were classified as probable *p1* left- or right-side flanking deletions. For example, one allele (*p1-wwB4*) has a deletion which

TABLE 2
Combinations of PCR primers used to determine allele structures

Target sequence/PCR primers	<i>P1-rr11</i>	<i>P1-rr910</i>
3' <i>Ac</i> junction/pair 1	P1-2415f and Ac4436f	9D9A5537f and Ac4436f
5' <i>Ac</i> junction/pair 2	Ac120r and P1-2915r	Ac120r and P1-7061r
3' <i>fAc</i> junction/pair 3	P1-15588f and Ac4436f	P1-15588f and Ac4436f
5' <i>fAc</i> junction/pair 4	Ac2680f and PP1'	Ac2680f and PP1'
Fused <i>Ds</i> ends/pair 5	Ac120r and Ac4436f	Ac120r and Ac4436f
ITS rearrangement/pair 6	P1-2915r and P1-15588f	P1-7061r and P1-15588f

removed the ITS region, the 2.0-kb *fAc* element plus 105 bp of the flanking *p1* gene sequence.

- e. Internal deletions (17 cases). The PCR pattern of this class is + - + + or + + - +. These alleles are described in detail below.
- f. No *Ac/fAc* structure (7 cases). This class produced no positive reaction with any of the four primer pairs (- - - - pattern). These may represent segregation of the *p1-ww* or *p1-wr* allele together with a transposed *Ac*. They were not investigated further.
- g. *Ac* excision (two cases). Two alleles (one each from *P1-rr11* and *P1-rr910*) gave - - + + PCR patterns, which could be obtained by excision of *Ac* from the parental alleles. However, a simple transposition would not elicit a colorless pericarp phenotype; possibly, excision was accompanied by a deletion of essential *p1* sequences. These cases were not characterized further.
- h. Unknown (two cases). Two cases show PCR patterns (+ + - - and + + + -) that are not expected from any known single standard or alternative transposi-

tion event. The origin of these cases is unknown and is not considered further here.

Previous research has identified examples of “fused ends”; *i.e.*, cases in which the ITS is deleted and the 5' and 3' *Ac/Ds* termini are ligated together (GORBUNOVA and LEVY 1997; KRISHNASWAMY *et al.* 2008). The presence of fused ends among the mutant alleles tested here could be detected by PCR with *Ac* 5' end primer (Ac120r) and *Ac* 3' end primer (Ac4436f) (Table 2). Three alleles (*p1-wwB42*, *p1-wwB54*, *p1-wwB59*) from *P1-rr11* showed a single strong band, but subsequent sequence analysis showed that each of these cases was in reality an internal deletion (+ + - + pattern). In summary none of the alleles we identified here contains fused *Ac/fAc* ends.

Sequence analysis of the flanking *Ac* 5'-end or *fAc* 3'-end deletion lines: The breakpoints of 12 of the other 14 internal deletion alleles were isolated by PCR and sequencing (MATERIALS AND METHODS). DNA

TABLE 3
PCR patterns and inferred structures

PCR pattern	<i>P1-rr11</i>	<i>P1-rr910</i>	Inferred rearrangement	Frequency (%)
+ - - -	5	10	<i>Ac</i> 5' adjacent external deletion	15
- - - +	3	2	<i>fAc</i> 3' adjacent external deletion	5
+ - - +	3	2	Major rearrangement ^a	5
+ - - +	1	0	ITS rearrangement ^b	1
+ - - +	13	33	Inversion	46
+ + - +	6	2	<i>Ac</i> 5' adjacent small internal deletion ^c	8
+ - + +	4	5	<i>fAc</i> 3' adjacent small internal deletion	9
- - + +	1	1	<i>Ac</i> excision?	2
+ + - -	0	1	Unknown	1
+ + + -	0	1	Unknown	1
- - - -	6	1	Reference <i>p1-ww</i> or <i>p1-wr</i> allele	7
	42	58	Total	100

Results of diagnostic PCR using the four primer pairs shown in Figure 1; + and - represent positive or negative results from each primer pair. The number of cases derived from each progenitor allele (*P1-rr11* or *P1-rr910*) is shown. Deletions are classified as “adjacent external deletion” if they extend into the external flanking DNA. Deletions within the intertransposon segment (*i.e.*, between the *Ac* 5' end and the *fAc* 3' end) are termed “small internal deletion.”

^aAlleles containing major chromosome rearrangements (translocations or large inversions) were identified by high-frequency pollen abortion.

^bITS rearrangement alleles were identified using an additional primer pair (Table 2).

^cThree alleles (*p1-wwB42*, *p1-wwB54*, and *p1-wwB59*) produced + - - + patterns in initial PCR analysis. Subsequent sequence analysis placed *p1-wwB54* and *p1-wwB59* in the + + - + group; *p1-wwB42* could be in either + + - + or + - + + group; it is arbitrarily placed in the former group.

FIGURE 2.—Structures of progenitor and ITS deletion alleles. Schematic structures of *P1-rr11* (A), *P1-rr910* (B), and *P1-ovov454* (C) are shown. The solid black boxes indicate *p1* gene exons 1, 2, and 3 (left to right). The open and solid red triangles represent the 3' and 5' termini of *Ac/fAc*, respectively. Short horizontal arrows indicate PCR primers. Extent of ITS deletions (gapped lines) in alleles derived from *P1-rr11* and *P1-rr910* are shown in A and B, respectively.

sequence results show that in each case, the *Ac/fAc* structure is intact, and the deletions are confined to the intertransposon sequences flanking either *Ac* or *fAc* (Figure 2). Each deletion extends precisely from either the *Ac* 5' end or the *fAc* 3' end to a site in the intervening DNA. None of the alleles has a deletion of sequences flanking both the *Ac* and *fAc* termini. Because the deletions were selected on the basis of colorless kernel pericarp (*i.e.*, lack of *p1* function), it is expected that each deletion should remove some sequences essential for *p1* expression. Indeed, all deletions but one (*p1-wwB3*) have removed part or all of *p1* gene exons 1 and 2, which encode the Myb-homologous DNA binding domain of the *p1* transcriptional activator protein. The *p1-wwB3* allele removes a ~2.5-kb region of the distal *p1* promoter region. Approximately half of the deletions have endpoints within a 1-kb region including *p1* exons 1 and 2; this region is a hot spot for *Ac* insertion as previously reported (ATHMA *et al.* 1992). One allele (*p1-wwB42*) has only a single nucleotide (C) remaining between the *Ac* and *fAc* termini; it is not possible to determine from which side (*Ac* or *fAc*)

the deletion originated, because the same nucleotide is present flanking both the *Ac* and *fAc* termini in the progenitor *P1-rr11* allele.

Deletions flanking the external *Ac* 3' end occur at much lower frequency than intertransposon events: The results described above show that deletions flanking the *Ac* 5' end and *fAc* 3' end occur at a significant frequency. We wondered if deletions flanking the *Ac* 3' end can also occur at similar frequency (Figure 2). However, we could not readily detect deletions from this terminus in the *P1-rr11* and *P1-rr910* alleles because such deletions would not remove sequences essential for *p1* function. Therefore we screened for deletions using a third allele termed *P1-ovov454*, which conditions orange variegated pericarp and cob, *i.e.*, orange tissue with red and colorless sectors. In the *P1-ovov454* allele, both *Ac* and *fAc* are located in the *p1* gene intron 2 separated by a distance of approximately 0.8 kb (Figure 2) (ZHANG *et al.* 2009). Importantly, deletions extending from the *Ac* 3' end into the upstream flanking sequence could remove *p1* exons 1 and or 2, both of which are essential for *p1* function. To screen for such deletions, we

selected 133 kernels with colorless pericarp and spotted aleurone from *PI-ovov454* heterozygotes. These kernels should carry a nonfunctional *p1* gene and retain *Ac* activity. Plants grown from these kernels were screened by PCR using primers Ac4436f and Ac120r to identify those plants that retained the *Ac/fAc* structure of the progenitor allele. Only 3 of the 133 plants tested positive for this band. The other 130 plants most likely contain deletions of the ITS as a consequence of reversed-ends transposition involving the *Ac* 5' end and *fAc* 3' end. The three plants that retained the *Ac/fAc* structure were then tested using an *Ac* 3' end primer (Ac4436f) together with a flanking sequence primer (PA-A11) to detect possible one-sided deletions. Two of the plants were positive for this product; *i.e.*, they retained an intact *Ac* 3' junction and hence did not contain flanking deletions. One of the three tested plants was negative for the *Ac* 3' flanking sequence product, and therefore likely represents an *Ac* 3' flanking deletion. These results indicate that one-sided deletions flanking the *Ac* 3' end occur less frequently (1/133) than intertransposon deletions flanking the *fAc* 3' end (8/100).

Reversed *Ds* ends in very close proximity exhibit reduced alternative transposition in maize: Previously we reported that alleles containing pairs of reverse-oriented *Ac/Ds* ends can cause chromosome breakage, and chromosome-breakage frequency is inversely proportional to the distance between the element termini (YU *et al.* 2010). The previous study examined pairs of *Ac/Ds* elements separated by distances ranging from 0.8 to 13 kb. To examine the effects of shorter intertransposon distances on chromosome breakage, we tested the intertransposon deletion alleles described here for the frequency of loss of a distal visible marker gene (*Dek1*; MATERIALS AND METHODS). Most of the intertransposon deletion alleles derived from *PI-rr11* or *PI-rr910* show increased chromosome breakage as the distance between the reversed *Ac/Ds* ends decreases (Table 4 and Figure 3). For example, allele *p1-wwB54* has an *Ac/fAc* separation distance of 331 bp and exhibits a relatively high chromosome-breakage frequency (grade 2.9). However, two alleles with shorter element separation distances have drastically reduced chromosome breakage: *p1-wwB59* (91 bp between *Ac/fAc*) exhibits a relatively low chromosome-breakage frequency (grade 1.4). This frequency is similar to that of the progenitor allele *PI-rr11*, in which the *Ac/fAc* termini are separated by 13 kb. Even more striking, *p1-wwB42*, which has only one nucleotide separating the *Ac/fAc* termini, exhibits little or no detectable chromosome breakage (grade 0). To determine whether these alleles have mutation(s) in the *Ac/fAc* termini that would render them immobile, ~450 bp of the 5' *Ac* and 3' *fAc* termini and subterminal regions were sequenced, but no changes were found. Both *p1-wwB59* and *p1-wwB42* alleles show normal *Ac*-transposase gene activity by crossing with the *r1-m3::Ds* tester line. We then tested

TABLE 4
Chromosome-breakage frequency exhibited by different alleles

Allele	Distance between <i>Ac</i> and <i>fAc</i> (nt)	Ear BFB grade (total ear number)	Average BFB grades
<i>PI-rr11</i> and derived deletion alleles			
<i>PI-rr11</i>	13175	1, 1, 1, 1 (4)	1.0
<i>p1-wwB3</i>	9073	ND	ND
<i>p1-wwB1163</i>	4238	2, 2 (2)	2.0
<i>p1-wwB16</i>	4196	3, 2, 2 (3)	2.3
<i>p1-wwB8</i>	3962	2, 2 (2)	2.0
<i>p1-wwB56</i>	3748	2, 2, 2, 2 (4)	2.0
<i>p1-wwB34</i>	924	2, 3, 3 (3)	2.7
<i>p1-wwB54</i>	331	3, 3, 3, 3, 3, 3 (7)	2.9
<i>p1-wwB59</i>	91	1, 1, 1, 2, 2 (5)	1.4
<i>p1-wwB42</i>	1	0, 0, 0, 0 (4)	0.0
<i>PI-rr910</i> and derived deletion alleles			
<i>PI-rr910</i>	8919	2, 2, 2, 2, 2 (5)	2.0
<i>p1-wwC51</i>	4232	3, 3, 2, 2, 2 (5)	2.4
<i>p1-wwC32</i>	3943	3, 2, 2, 2, 2, 2 (6)	2.2
<i>p1-wwC28</i>	3920	3, 3, 2, 2, 2, 2 (7)	2.3
<i>p1-wwC107</i>	3493	ND	ND
<i>p1-wwC40</i>	4686	2, 2 (2)	2.0
<i>p1-wwC90</i>	2678	3 (1)	3.0

BFB, chromosome break-fusion-bridge; ND, not determined.

whether the *Ac* elements in the *p1-wwB59* and *p1-wwB42* alleles were still capable of standard transposition. PCR analysis using specific primers flanking the *Ac* insertions produced bands as expected for normal *Ac* excision (data not shown). We conclude that reversed *Ac/Ds* ends in very close proximity (<100 bp) have reduced frequencies of alternative transposition.

DISCUSSION

The aim of this study is to investigate the types and frequency of genome rearrangements induced by reversed-ends transposition at the maize *p1* locus. The results indicate that about half of rearrangements are chromosome inversions, translocations, or ITS rearrangements; 20% are chromosome external deletions; and 17% are internal deletions (ITS deletion). Our previous data indicate that the inversions, translocations, external deletions, and ITS rearrangements were generated via reversed *Ac* end transposition (ZHANG and PETERSON 2004; ZHANG *et al.* 2009), but it is not clear how the internal deletions were generated. Here, we propose a mechanism for the generation of small internal deletions. We also discuss the implications of our results for the development of transposon-based genome rearrangement tools.

Model for generation of transposon-induced flanking deletions: We have previously presented evidence that pairs of reversed *Ac/fAc* termini on the same chromatid are competent for transposition (ZHANG and PETERSON

the chromosome-breakage frequency (YU *et al.* 2010). The photographs show grade 0 to grade 3 representative ears produced by crossing with pollen from plants homozygous for the following alleles: *p1-wwB42* (grade 0); *p1-wwB59* (grade 1); *p1-wwB8* (grade 2); *p1-wwB34* (grade 3).

2004). To explain the high frequency of intertransposon segment deletions, we propose that, during reversed-ends transposition, the active *Ac/fAc* termini can insert into the region between *Ac* and *fAc* on the sister chromatid; this scenario would produce two daughter chromatids containing reciprocal deletions of the intertransposon segment. Each resulting chromatid would have a deletion flanking either the 5' *Ac* or 3' *fAc* ends (Figure 4). Insertion of the *Ac/fAc* termini in the opposite orientation would lead to formation of a chromatid bridge and chromosome breakage, as previously proposed (HUANG and DOONER 2008; YU *et al.* 2010). This model fits previous observations, including those that: (1) *Ac/Ds* elements exhibit a pronounced preference for local transposition; (2) the *Ac* element from the standard *p1-ww* allele often inserts into the sister chromatid (GREENBLATT and BRINK 1962); (3) the maize *p1* gene promoter and exon 1 sequences are preferred sites for *Ac* insertion (ATHMA *et al.* 1992), and this region is located within the *Ac/fAc* intertransposon segment in the *PI-rr11* and *PI-rr910* alleles.

There are at least three other existing models for the formation of transposon-flanking deletions. For the bacterial Tn5 transposon, flanking deletions were proposed to occur by abortive transposition of a single transposon end (JILK *et al.* 1993). If this model were correct for the maize *Ac/Ds* elements, then the frequency of “one-sided” deletions flanking the *Ac* 3' end should be equal to or greater than that of deletions flanking the *fAc* 3' end. This is based on the premise that transposition of the *Ac* 3' end should occur at least as frequently as that of the *fAc* 3' end, because (1) the *Ac* 3' end is present in the context of a complete *Ac* element, whereas the *fAc* 3' end is present on a smaller (2059 bp) *Ac* fragment, and (2) the *fAc* 3' end is located farther away from the *Ac* 5' end (9 and 13 kb in *PI-rr910* and *PI-rr11*, respectively) than is the 3' end of an intact *Ac* element (4565 bp). However, we detected significantly more “one-sided” deletions flanking the *fAc* 3' end (total 8/100 from *PI-rr910* and *PI-rr11*) than the *Ac* 3' end (1/133 from *PI-ovov454*) (corrected $P = 0.0056$; odds ratio = 11.38 with a 95% confidence interval [1.48, 511.80] by the Fisher exact test). These

FIGURE 3.—Representative ears for evaluating the frequency of chromosome breakage. Ears of *dek1/+* tester plants were crossed with pollen from plants homozygous for the indicated *p1* alleles. The functional *Dek1* gene conditions solid purple aleurone color; chromosome breakage at the proximal *p1* locus results in loss of *Dek1* as evidenced by colorless aleurone sectors. The ears were borne on heterozygous *dek1/+* tester plants, hence only half of the kernels can show *Dek1* loss. The number and size of colorless sectors indicate

results do not fit the predictions of the single-end abortive transposition model.

An alternative model (PAGE *et al.* 2004) proposed that deletions flanking *Ds* elements in *Arabidopsis* result from a hybrid transposition mechanism involving two sequential steps: First, a newly replicated *Ds* element inserts into a nearby unreplicated region and forms a pair of *Ds* elements in reversed orientation. Second, the 5' end of one *Ds* and the 3' end of the other *Ds* excise, leading to deletion of the internal sequence. The chromosome arm is repaired by ligation of the two broken ends. This model is not consistent with the deletions we isolated for at least three reasons: (1) The *fAc* in the *PI-rr11*

FIGURE 4.—Alternative transposition model for formation of ITS deletions. Symbols are the same as in previous figures. (1) The chromosome containing the *Ac/fAc* locus is partially replicated. *Ac* transposase (open, black circles) recognizes the reversely oriented *Ac/fAc* termini on the same chromatid; cleavage by transposase occurs at *Ac* and *fAc* termini. (2) Excised 5' and 3' termini of *Ac/fAc* insert into a site between *Ac/fAc* in the sister chromatid. (3–4) Reciprocal deletion alleles are generated following completion of DNA replication.

and *PI-rr910* alleles is immobile and hence cannot participate in the first transposition reaction of the Page *et al.* model (2) the deletion junctions we isolated are precise and exhibit no evidence of the involvement of NHEJ-type repair, and (3) this model does not explain the difference in frequency of deletions flanking the external *Ac* 3' side *vs.* the intertransposon *fAc* 3' end.

In *Drosophila*, the *P* element can undergo a type of alternative transposition involving the termini of *P* elements located on homologous chromosomes (hybrid element insertion model) (PRESTON *et al.* 1996; PARKS *et al.* 2004). If such a hybrid element inserted into a nearby site, a “one-sided” flanking deletion could be generated (Figure 5). However, no evidence for a similar hybrid element mechanism was detected in two different experiments in maize (YU *et al.* 2010). Previous work has shown that pairs of *Ac/Ds* elements on the same chromosome can induce chromosome breakage by an alternative transposition mechanism (WEIL and WESSLER 1993; HUANG and DOONER 2008; YU *et al.* 2010). When the distance between the two interacting *Ac/Ds/fAc* termini is increased to >200 kb, the chromosome-breakage frequency is highly reduced (HUANG and DOONER 2008). Therefore, even if alternative transposition reactions involving hybrid elements were to occur, the frequency is expected to be too low to explain the relatively high frequency of intertransposon segment deletions observed for the *PI-rr11* and *PI-rr910* alleles.

Generation of inversions *vs.* deletions by reversed-ends transposition: The *Ac/Ds* transposon system is well known for having a preference for transposition to closely linked sites (ATHMA *et al.* 1992; VOLLBRECHT *et al.* 2010). In contrast, the orientation of insertion of the *Ds* termini appears to be random in several large collections produced for gene tagging (KOLESNIK *et al.* 2004; NISHAL *et al.* 2005; PAN *et al.* 2005). If the insertion orientation of reversed-ends transposition is also random, then one would expect that inversions and deletions would be generated at a similar frequency. However, in the experiments described here, inversions were recovered at a twofold greater frequency than deletions. This difference may be due in part to the fact that deletions that extend from the 3' *fAc* upstream beyond the *Ac* element would not have been included in our analysis due to the initial selection for spotted kernels, *i.e.*, kernels that retain *Ac* activity. Moreover, very large deletions are known to have reduced transmission frequency (BIRCHLER and LEVIN 1991; LIN *et al.* 1997), presumably due to the loss of essential genes, whereas inversions have a normal gene balance and should transmit normally. Consistent with this point, a higher frequency of inversions than deletions was reported at the maize *Bz* locus (HUANG and DOONER 2008).

Reversed *Ds* ends distance and chromosome rearrangement tool development: This and earlier reports indicate that reversed *Ac/Ds* termini can produce chromosome rearrangements such as inversions, deletions,

FIGURE 5.—*P*-element-induced flanking deletion model (modified from PARKS *et al.* 2004). (1) Transposase (open, black circles) recognizes and binds the termini of two different *P* elements located on homologous chromosomes. (2) *P* element termini are cleaved and inserted into a flanking target site. (3) Structure of the deletion chromosome formed.

and translocations (ZHANG and PETERSON 2004; HUANG and DOONER 2008; ZHANG *et al.* 2009). The ability to generate chromosome rearrangements may be valuable for plant chromosome engineering and/or functional genomics analysis, and it will be important to determine the parameters affecting the frequency of alternative transposition events. Previous reports have shown that chromosome-breakage frequency is inversely related to the distance between the interacting *Ac/Ds* termini (DOONER and BELACHEW 1991; YU *et al.* 2010). Here, we have shown that alleles in which the *Ac/fAc* termini are separated by 0.3 kb to 3 kb segments have the highest chromosome-breakage frequency (Table 4). Strikingly, alleles in which the *Ac/fAc* termini are separated by less than 100 bp exhibit much reduced chromosome-breakage frequency (Table 4, alleles *p1-wwB59* and *p1-wwB42*). Chromosome breakage in these alleles can be fully explained as a consequence of reversed *Ac/Ds* ends transposition, and hence should be a good indicator of alternative transposition frequency. Interestingly, somatic extrachromosomal circular *Ds* molecules were previously reported in maize and transgenic tobacco tissues; these circular *Ds* elements appeared to be incapable of transposition and were considered likely to be abortive transposition products (GORBUNOVA and LEVY 1997; GORBUNOVA and LEVY 2000). Consistent with this idea is the fact that the *p1-wwB42* allele, which has only a single nucleotide separating the *Ac* and *fAc* termini, exhibits little or no detectable chromosome breakage. Together these observations suggest that structures in which the *Ac/Ds/fAc* termini are separated

by less than 0.1 kb are not competent for transposition. While other factors such as epigenetic modifications and local sequence context are known to affect transposition frequency, our results indicate that the spacing of *Ac/Ds* transposon termini is also important.

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