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RESEARCH ARTICLES

Maize Genome Structure Variation: Interplay between Retrotransposon Polymorphisms and Genic Recombination [W]

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Although maize (*Zea mays*) retrotransposons are recombinationally inert, the highly polymorphic structure of maize haplotypes raises questions regarding the local effect of intergenic retrotransposons on recombination. To examine this effect, we compared recombination in the same genetic interval with and without a large retrotransposon cluster. We used three different *bz1* locus haplotypes, McC, B73, and W22, in the same genetic background. We analyzed recombination between the *bz1* and *stc1* markers in heterozygotes that differ by the presence and absence of a 26-kb intergenic retrotransposon cluster. To facilitate the genetic screen, we used *Ds* and *Ac* markers that allowed us to identify recombinants by their seed pigmentation. We sequenced 239 recombination junctions and assigned them to a single nucleotide polymorphism–delimited interval in the region. The genetic distance between the markers was twofold smaller in the presence of the retrotransposon cluster. The reduction was seen in *bz1* and *stc1*, but no recombination occurred in the highly polymorphic intergenic region of either heterozygote. Recombination within genes shuffled flanking retrotransposon clusters, creating new chimeric haplotypes and either contracting or expanding the physical distance between markers. Our findings imply that haplotype structure will profoundly affect the correlation between genetic and physical distance for the same interval in maize.

INTRODUCTION

Maize (*Zea mays*) has a highly polymorphic genome structure (Fu and Dooner, 2002; Song and Messing, 2003; Wang and Dooner, 2006). Retrotransposons, which constitute the bulk of the genome (SanMiguel and Bennetzen, 1998), differ among lines in their makeup and location relative to genes. Consequently, the pattern of interspersion of genes and retrotransposons varies from line to line, defining sharply distinct haplotypes. The extent of sequence variation in the *bz1* genomic region is remarkable. In a recent vertical comparison of eight *bz1* locus haplotypes, any two haplotypes shared between 25 and 84% of their sequences (Wang and Dooner, 2006). Haplotypic variation is common in the genome (Song and Messing, 2003; Brunner et al., 2005; Yao and Schnable, 2005) and could lead to huge differences in estimates of genetic distance in different backgrounds. This does not happen because the variable retrotransposon component of the genome is recombinationally inert (Fu et al., 2002; Yao et al., 2002). Yet, twofold to threefold variations in estimates of map distances for single genetic intervals have been reported in several maize mapping populations (Beavis and Grant, 1991; Fatmi et al., 1993; Williams et al., 1995). Much of this variation is probably due to *trans*-acting modifiers, such as the recently demonstrated quantitative trait loci that affect global recombination frequencies in recombinant inbred lines (Esch et al., 2007), but some of it may be due to *cis*-acting factors.

Cis-acting factors were demonstrated in a study that examined recombination rates across the *a1-sh2* genetic interval in three heterozygotes containing the same maize haplotype and different teosinte-derived haplotypes in a common maize background (Yao and Schnable, 2005). This region measures 130 kb in maize inbred UE85, and although most of the intervening teosinte DNA between *a1* and *sh2* was not sequenced, several large insertion/deletion (indel) polymorphisms relative to maize, including two LTR retrotransposons, one MITE transposon, and one *hAT* transposon, were uncovered in the sequenced region. The analysis identified up to threefold differences in recombination rates and statistical differences in the distribution of recombination junctions across subintervals among haplotypes. Although levels of sequence polymorphism correlated negatively with rates of recombination in the sequenced region, they did not fully account for the observed results. The authors proposed that other types of *cis* factors, such as region-specific chromatin structure, may affect the rate and distribution of recombination across the *a1-sh2* interval.

The polymorphic chromosomal organization of maize, due mainly to intergenic retrotransposon variation, prompted us to ask the specific question: to what extent does heterozygosity for large retrotransposon insertions, which occurs in every mapping population, affect recombination in the adjacent genes? The highly methylated retrotransposon clusters are probably heterochromatic, as are similar blocks in the knobs of maize (Ananiev et al., 1998) and *Arabidopsis thaliana* (Lippman et al., 2004), and most likely affect recombination. Genes next to retrotransposon clusters may be less recombinogenic, because the more

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condensed chromatin state of the retrotransposon cluster may interfere with the access of the recombination machinery to the adjacent euchromatic regions. In order to investigate this, we compared recombination in the same genetic interval in the presence and absence of a large retrotransposon cluster. Fu and Dooner (2002) found that the 1.5-kb *bz1-stc1* intergenic segment in the McC *bz1* locus haplotype (Fu et al., 2001) was replaced by a 26-kb retrotransposon block in the B73 haplotype (Figures 1A and 1B). We have identified a *bz1* haplotype, W22, that resembles McC in its retrotransposon–gene junctions but differs from McC in many single nucleotide polymorphisms (SNPs) and indel polymorphisms. The availability of these three distinct haplotypes has enabled us to examine the effect of retrotransposon heterozygosity on recombination in the adjacent *bz1* and *stc1* genes. Potential *trans* effects in such an experiment were eliminated by first introgressing all haplotypes into a common inbred background.

The confinement of recombination to the genic space in maize protects the genome from the massive disruptive rearrangements that would otherwise occur if the dispersed repetitive retrotransposons (SanMiguel and Bennetzen, 1998) recombined ectopically. However, the orderly exchange of different intergenic retrotransposon clusters by recombination between alleles should occur regularly in populations, leading to nondisruptive genomic changes that amplify the variability created by the explosive increase in retrotransposons in the recent maize ancestry (SanMiguel and Bennetzen, 1998; SanMiguel et al., 1998). We confirm here that recombination in genes shuffles heterozygous retrotransposons flanking them, creating new chimeric haplotypes and expanding or contracting chromosomal segments.

RESULTS

Structure of the W22 *bz1* Haplotype

Because of extensive polymorphisms in the content of retrotransposons, helitrons, and other transposons, the size of the *bz1* region can vary by more than threefold among maize lines (Wang and Dooner, 2006). *Not*I fragments containing the *bz1* region of W22 are not very different in size from those of McC (Fu and Dooner, 2002), yet the *Bz1-McC* and *Bz1-W22* alleles are known to differ in >1% of their sequences (Ralston et al., 1988), so W22 is an excellent candidate for a contrasting *bz1* locus haplotype lacking the retrotransposon cluster in the *bz1-stc1* intergenic region. In order to fully characterize the W22 *bz1* haplotype, the entire 238-kb *Bz1-W22* genomic region was cloned as two adjacent *Not*I fragments in the pNOBAC1 vector

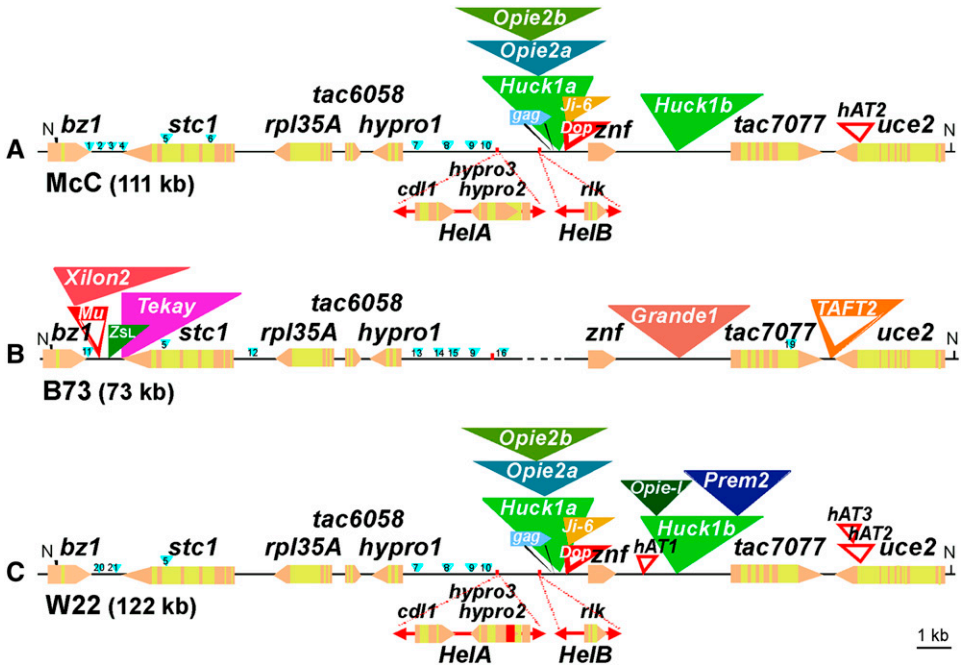

Figure 1. Organization of McC, B73, and W22 *bz1* Haplotypes.

Each haplotype is identified by the name of the line from which it was extracted, followed by the size of the cloned *Not*I (N) fragment in parentheses. The eight genes (*bz1*, *stc1*, *rpl35A*, *tac6058*, *hypro1*, *znf*, *tac7077*, and *uce2*) are shown as pentagons pointing in the direction of transcription, with exons in peach and introns in yellow. The same symbols are used for gene fragments carried by helitrons *HelA* and *HelB*, which are represented as bidirectional arrows below the line in McC and W22. The vacant site for *HelA* in B73 is marked with a short vertical stroke. Dashed lines represent deletions. Retrotransposons are indicated by closed triangles of different colors. DNA transposons are indicated by open triangles of red and orange. Small insertions are indicated in light blue and numbered as by Wang and Dooner (2006). Only the genes have been drawn to scale.

(Fu and Dooner, 2000). Sequencing confirmed that the *bz1-stc1* intergenic segment of *Bz1-W22* lacked retrotransposons. The structure of the distal 122-kb *Not*I BAC, which contains the genetic interval analyzed in this study, is presented in Figure 1C.

The overall structure of the W22 *bz1* haplotype is remarkably similar to that of McC (Figure 1). The two haplotypes share all of the boundaries between *Helitron* or retrotransposon insertions and intergenic regions, as well as several *hAT*, CACTA, and small DNA insertions in either intergenic regions or introns. Surprisingly, the lowest sequence variation between the two haplotypes occurs in the large intergenic region between *hypro1* and *znf*. The percentage divergence in that 67-kb stretch is 0.06%: the two haplotypes share the same four MITEs, the same two *Helitrons*, although *HelA* in W22 has experienced a 700-bp deletion of the *hypro3* gene fragment, the same *Doppia* DNA element, and the same 53-kb three-level retrotransposon nest distal to *HelB*. The 5′ and 3′ LTRs of each of the three retrotransposons differ in both haplotypes, allowing dating of the insertions (SanMiguel et al., 1998; Ma and Bennetzen, 2004) to a time period 0.4 to 1.2 million years ago, from youngest (top) to oldest (bottom) (see Supplemental Table 1 online). Yet, five of the six LTRs in the nest are identical in sequence between W22 and McC, suggesting that this chromosomal segment in the two haplotypes derives from a very recent common ancestor.

The *Huck1b* retroelement between the *znf* and *tac7077* genes has diverged in W22 relative to McC by the gain of a fractured 1.9-kb *Opie*-like element, consisting only of the 5′ LTR and the primer binding site, and a 9.5-kb *Prem2* element, estimated from its LTR sequence identity to have inserted only 60,000 years ago. These two insertions account for the 11-kb difference in size of the *Not*I fragment in the two lines. Using LTR sequence information from both haplotypes, *Huck1b* is estimated to have inserted between 1 and 1.2 million years ago, so it is about as old as *Huck1a* in the triple-level nest. A comparison of the *Huck1b* 5′ LTRs and 3′ LTRs in W22 versus McC indicates that they diverged from each other between 0.4 and 0.5 million years ago, pointing to an older common ancestry for this chromosomal segment of the two haplotypes. The subsequent acquisition of retroelement sequences by *Huck1b* in only one of the haplotypes supports this inference. As discussed below, retrotransposon clusters are shuffled by recombination between the genes that flank them: possibly, two nonconcurrent recombination events in *znf* and *hypro1* in the history of these haplotypes led to the replacement of the *hypro1-znf* intergenic region of one haplotype by that of the other. Lastly, the 1-kb *hAT* element in the last intron of *uce2*, which is present in both haplotypes, contains a second, unrelated 0.5-kb *hAT* element only in W22.

Remarkably, however, the sequences of most genes and nonrepetitive intergenic segments are just as polymorphic between W22 and McC as they are among other lines (Wang and Dooner, 2006). For example, the transcribed *bz1* segments differ by 1.6% in their coding sequences and by 3.2% plus one MITE indel in their noncoding sequences (intron and 3′ untranslated region). The *bz1-stc1* intergenic regions differ in six MITEs or other small insertions; excluding those insertions, they differ in 4.6% of their sequences. The transcribed *stc1* segments differ by 1.5% in their coding sequences and by 2.2% plus one MITE indel in their noncoding sequences (introns and 3′ untranslated region).

Recombination between *bz1* and *stc1*

In order to examine the effect of retrotransposon heterozygosity on recombination in the adjacent genes, we compared recombination between *bz1* and *stc1* markers in McC/B73 and McC/W22 heterozygotes. The *bz1-stc1* interval in these heterozygotes differs by the presence and absence, respectively, of a 26-kb retrotransposon cluster in the intergenic region. Each haplotype was first introduced into the common genetic background of the inbred W22 to minimize background differences (see Methods).

The experimental setup is diagrammed in Figure 2. The 26-kb retrotransposon cluster in B73 is represented in a much smaller scale than the adjacent *bz1* and *stc1* genes, which are drawn approximately to scale. The cluster is made up of a 9.4-kb *Xilon* retrotransposon inserted into a 1.7-kb *Mu1* element, a 0.7-kb *Zeon* solo LTR, and a 12.8-kb *Tekay* retrotransposon. The McC parental haplotype carries *bz1-m2(D1)*, a *bz1* allele containing a *Ds* element in the second exon, and *stc1-m1(Ac6087)*, an *stc1* allele with an *Ac* insertion in the first exon (Shen et al., 2000). The *bz1-m2(D1)* allele produces a spotted phenotype in the presence of *Ac* and a stable bronze phenotype in its absence. Flanking *bz-m2(D1)* and *stc1-m1(Ac6087)* are the endosperm mutations *sh1* and *wx1*, which serve as recombination markers in the

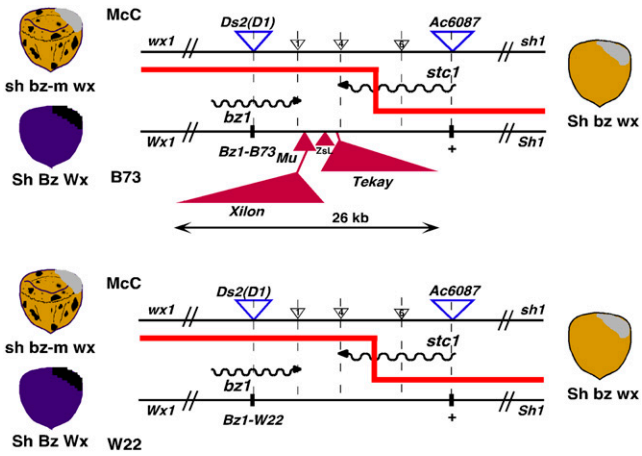

Figure 2. Genetic Scheme for Identifying Recombinants in McC/B73 (Top) and McC/W22 (Bottom) Heterozygous Haplotypes.

The cartoon depicts the spotted (bz-m) and solid purple (Bz) parental phenotypes at left and the solid bronze (bz) recombinant phenotype at right. The *sh1* flanking alleles condition either shrunken or plump seeds; the *wx1* flanking alleles condition either waxy or nonwaxy seeds (shown here as staining light or dark with iodine for diagrammatic purposes only). Recombination anywhere between the *Ds2(D1)* element in *bz1* and the *Ac6087* element in *stc1* gives rise to Sh bz recombinants, most of which will also carry wx. Recombination between *Ac6087* and *sh1* gives rise to Sh bz-m recombinants. The reciprocal recombinants of both classes are sh Bz. The *stc1* and *bz* transcripts are indicated by the wavy arrows. A 26-kb retrotransposon cluster containing *Tekay*, a *Zeon* solo LTR, and a *Mu1-Xilon* nest separates *stc1* from *bz* in the *Bz-B73*, but not the *Bz-W22*, haplotype. The small triangles represent indel polymorphisms (*Ins3*, *Ins5*, and *Ins6* [Wang and Dooner, 2006]) used to assign Sh bz recombinants to one of four intervals within the larger *Ds2(D1)-Ac6087* interval.

experiment. (Note that the marker order in Figure 2, with the *9S* centromere to the left, is the same as in Figure 1 and previous publications [Fu and Dooner, 2002; Wang and Dooner, 2006] but opposite to the more common way of presenting *9S* markers with the centromere to the right.) The other parental haplotype, either B73 or W22, carries normal *Bz1* and *Stc1* alleles and produces a purple phenotype. Flanking *Bz1* and *Stc1* are the contrasting markers *Sh1* and *Wx1*.

The *sh1 stc1-m1(Ac6087) bz-m2(D1) wx1/Sh1 Stc1-B73 Bz1-B73 Wx1* and *sh1 stc1-m1(Ac6087) bz-m2(D1) wx1/Sh1 Stc1-W22 Bz1-W22 Wx1* heterozygotes were pollinated with a *sh1-bz1-X2 wx1* stock, which carries a deletion of the *sh1-bz1* region, including the entire *bz1-uce2* interval depicted in Figure 1 (Mottinger, 1973; Shen et al., 2000). Use of this deletion allows the recovery of selections in a hemizygous condition, greatly simplifying their molecular analysis. The above test crosses will produce crossover haplotypes conditioning a plump bronze (Sh bz) seed phenotype if recombination occurs between *Ds* and *Ac*, as illustrated by the heavy lines in Figure 2. Most of these exceptions will carry a *Sh1 wx1* crossover arrangement of flanking markers.

Other exchanges between *sh1* and *bz1* are also identifiable. Crossovers between *Ac6087* and *sh1* will produce plump, spotted (Sh bz-m) seed, and the reciprocal crossover class of both Sh bz and Sh bz-m classes will be shrunken and purple (sh Bz). Thus, the sum of Sh bz and Sh bz-m crossovers should equal the number of sh Bz crossovers. Furthermore, the ratio of Sh bz to Sh bz-m kernels should not vary from family to family within a heterozygous haplotype genotype. This expectation provides an internal check that *Ac* did not transpose from *stc1* in any of the McC parent plants. A χ^2 analysis (data not shown) revealed that the data were homogeneous for different families of the same genotype and could be combined. The pooled data are shown in Table 1. As can be seen, the reciprocal crossover classes Sh (bz + bz-m) and sh Bz occur in approximately equal numbers in both heterozygotes. The length of the *sh1-bz1* interval, estimated from the sum of the last two columns, is significantly greater in the McC/W22 heterozygote than in the McC/B73 heterozygote (2.52 versus 1.89 centimorgan [cM]). In particular, the frequency of the Sh bz class, which provides a rough estimate of the distance between the *Ds* element in *bz1* and the *Ac* element in *stc1*, is about twice as high in McC/W22 (0.33%) than in McC/B73 (0.17%). This class includes crossovers of the type shown in Figure 2 as well as rare excisions of either *Ds* or *Ac* accompanied by coincidental exchanges in the *sh1-bz1* interval. All but 3 of the 283 Sh bz selections from both heterozygotes in Table 1 were also wx, which is not surprising, as interference in the *sh1-bz1-wx1* region is very high and double crossovers are rare (Dooner, 1986). The frequency of the Sh bz-m class, which provides an estimate of the distance between *Ac* and *sh1*, is not significantly different in the two heterozygotes, suggesting that potential local differences in recombination may average out over the longer *stc1-sh1* interval.

The Sh bz selections were first genotyped by PCR for a series of five key indel polymorphisms in the region (shown as open triangles in Figure 2), which allowed us to assign them to the *bz* gene, the intergenic region, or different parts of the *stc1* gene. Selections carrying markers exclusively from the McC haplotype were characterized for the presence of the *Ds-bz1* junction, in order to eliminate *Ds* excisions, and of *Ac* target site duplication footprints, in order to eliminate *Ac* excisions. Of the successfully tested Sh bz selections, ~10% arose from coincidental excisions of *Ac* or *Ds* and exchanges in the *sh1-bz1* interval (9 of 90 from McC/B73 and 17 of 175 from McC/W22). The vast majority arose from recombination between the *Ds* element in *bz1* and the *Ac* element in *stc1*.

Based on the indel marker analysis, the fragments bearing the recombination junctions were PCR-amplified and sequenced in order to precisely locate the recombination junction relative to the nearest flanking polymorphisms, most of which are SNPs. The results from sequencing 239 junctions are shown graphically in Figure 3. In this figure, gene exons are colored peach, introns are colored yellow, and SNPs are shown as vertical lines. The 26-kb retrotransposon cluster in the B73 haplotype is drawn in maroon and in a much smaller scale than the adjacent 5.8-kb genic region. MITEs in noncoding gene sequences or the *bz1-stc1* intergenic segment are represented as small blue triangles. As can be seen from the respective SNP densities, the *bz1* and *stc1* alleles of McC and B73 are somewhat more closely related than those of McC and W22. In both heterozygotes, most intervals are delimited by two SNPs. The smallest interval is a few base pairs in length; the largest one is a 1.8-kb stretch of uninterrupted homology at the 3′ end of *stc1* in the McC/B73 haplotype. The number of junctions falling within specific intervals is shown beneath the common McC haplotype in each heterozygote.

In order to assess the effect of the heterozygous retrotransposon cluster located between *bz1* and *stc1* on recombination in

Table 1. Recombinant Kernel Selections from Heterozygotes of McC *stc1-m1(Ac6087) bz-m2(D1)* with either B73 or W22 *Stc1 Bz1*

Heterozygous Haplotype	Kernel Population	Recombinant Kernel Class Phenotypes			
		Sh bz	Sh bz-m	Sh (bz + bz-m)	sh Bz
McC/B73	56670	96	473	569	507
Percent		0.17	0.83	1.00	0.89
95% C.L.		0.14 to 0.20	0.78 to 0.89	0.94 to 1.06	0.84 to 0.95
McC/W22	56460	187	524	711	710
Percent		0.33	0.93	1.26	1.26
95% C.L.		0.30 to 0.36	0.87 to 0.99	1.20 to 1.32	1.20 to 1.32

C.L., confidence limit.

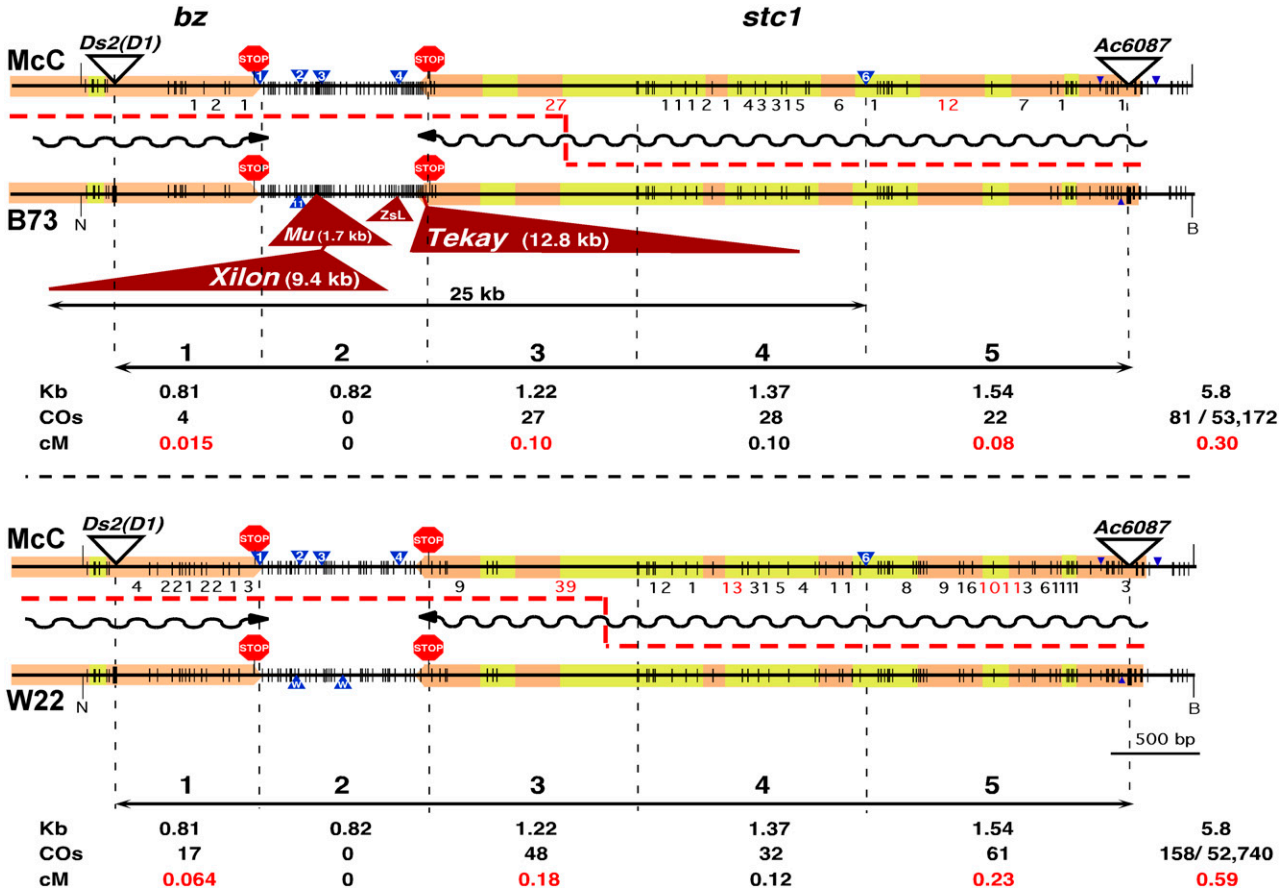

Figure 3. Distribution of Recombination Junctions within the *Ds2(D1)-Ac6087* Genetic Interval among Sh bz Recombinants from McC/B73 (Top) and McC/W22 (Bottom) Heterozygotes.

As in Figure 1, exons are in peach and introns are in yellow; the stop codon for each gene is indicated by a red octagon. The retrotransposon cluster in B73 is in maroon and drawn in a smaller scale than the rest of the interval. Polymorphisms are represented as vertical lines (SNPs) or blue triangles (indels), numbered as indicated by Wang and Dooner (2006). The number of recombination junctions in each subinterval defined by these polymorphisms is shown beneath the common McC haplotype in each heterozygote, single-digit numbers in black and double-digit numbers in red. The *Ds2(D1)-Ac6087* genetic interval was subdivided into five segments for analysis: *bz1*, intergenic region, and three roughly equally sized *stc1* segments. Significantly different estimates of genetic distances for a segment in the two heterozygotes are indicated in red. See text for additional details.

the region, we subdivided the *Ds2(D1) Ac6087* genetic interval into five segments and, for each segment, compared recombination between McC/B73, which had the retrotransposon cluster, and McC/W22, which did not. The segments, numbered 1 to 5 in the figure, are as follows: the 0.81-kb *bz1* interval (1), which corresponds to about half the length of the *bz1* gene because of the central location of the *Ds* insertion in the gene; the 0.82-kb common intergenic sequences between the termination codons of *bz1* and *stc1* (2); and three similarly sized segments of the *stc1* gene (3 to 5), which span practically the entire length of *stc1* and are defined by common polymorphisms in the two heterozygotes. The 1.22-kb distal segment 3 extends from the distalmost SNP in the fifth intron of *stc1* to a SNP just a few base pairs upstream of the *stc1* stop codon and is completely conserved between McC and B73; the 1.37-kb central segment 4 extends from the MITE indel *Ins6* in the third intron of *stc1-McC* to the last SNP in the fifth intron of *stc1*; and the 1.54-kb proximal segment 5 extends from the *Ac6087* marker in the first exon of *stc1-McC* to *Ins6* in the third intron.

Under each of the five segments are given the stretch of homology, in kilobases, the number of identified crossovers, and the genetic length, in centimorgan. The sum for all intervals is shown at right. The *Ds2(D1)-Ac6087* genetic distance for each heterozygote is slightly less than would have been calculated from the Sh bz recombinant class of Table 1, which is uncorrected for *Ac* or *Ds* excisions and simultaneous exchanges in the *sh1-bz1* region (0.30 versus 0.34 cM for McC/B73 and 0.59 versus 0.66 cM for McC/W22). The total length of the *Ds2(D1)-Ac6087* interval is significantly smaller in the B73 heterozygote than in the W22 heterozygote ($\chi^2 = 25$, 1 *df*, $P < 0.001$).

An examination of the distribution of junctions in Figure 3 reveals that exchanges occur only in the *bz1* and *stc1* genes. No recombination at all occurs in the intergenic region in either heterozygote. This is not surprising in the McC/B73

heterozygote, given that its intergenic region contains the large retrotransposon insertion, but the absence of crossovers in the McC/W22 intergenic region suggests that recombination is inhibited by the high density of SNP and indel heterologies in the interval, an effect previously documented for recombination within *bz1* (Dooner and Martinez-Ferez, 1997; Dooner, 2002) and *a1* (Yao et al., 2002; Yandeau-Nelson et al., 2006). Recombination was significantly higher in the *bz1* interval and two of the three *stc1* intervals of the McC/W22 heterozygote. The size of the *bz1* genetic interval 1 is about four times larger in W22 than in B73 ($\chi^2 = 8.15$, 1 *df*, $P < 0.01$). The size of the distal *stc1* segment is twice as large ($\chi^2 = 6.05$, 1 *df*, $P < 0.01$) and the size of the proximal *stc1* segment is three times larger ($\chi^2 = 18.6$, 1 *df*, $P < 0.001$). Only in the central *stc1* segment did recombination apparently not differ. Although both heterozygotes differ in multiple SNPs, which are known to have an inhibitory effect on recombination, SNP density in the *bz1* and *stc1* genes is actually lower in the McC/B73 heterozygote than in the McC/W22 heterozygote. Thus, the overall lower recombination observed in the McC/B73 heterozygote is most likely due to the presence of the large retrotransposon cluster in the B73 *bz1-stc1* intergenic region.

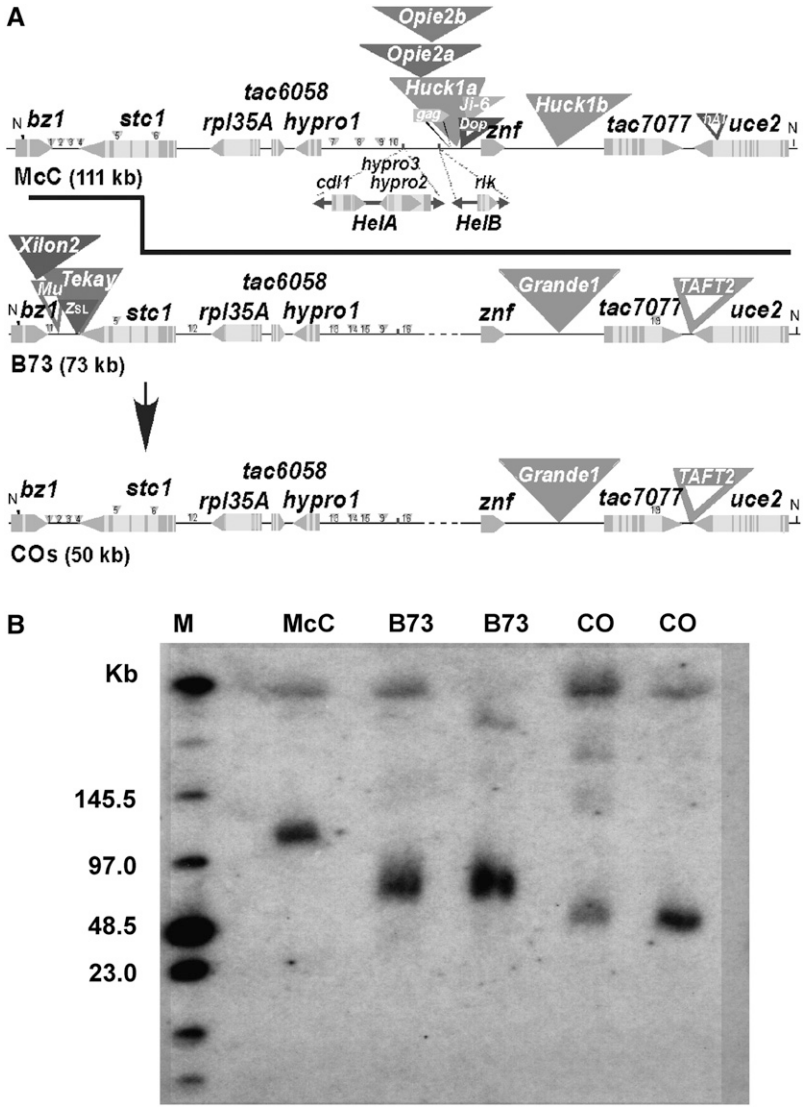

Figure 4. Generation of New Chimeric Haplotypes in McC/B73 Heterozygotes by Recombination within the *stc1* Gene.

(A) Diagram illustrating how an exchange event in *stc1* recombines the flanking retrotransposon clusters, producing a novel haplotype that lacks both clusters.

(B) CHEF gel DNA gel blot analysis of parental and recombinant haplotypes (*Not*I digest, *stc1* probe). M, size markers in kilobases; McC, *bz-m2(D1) stc1*-M1(*Ac6087*); B73 (left), B73 *Bz1-B73 stc1-B73* introgressed into W22; B73 (right), B73 *Bz1-B73 stc1-B73* from the original B73 inbred; CO (left) and CO (right), Sh bz wx crossovers resulting from exchanges in *stc1* gene intervals 3 and 4 (see Figure 3), respectively.

Recombination in the common gene space of heterozygous haplotypes has been proposed to shuffle retrotransposon blocks, creating new chimeric haplotypes (Wang and Dooner, 2006). In the McC/B73 heterozygote, recombination events within the *stc1* gene should produce a recombinant arrangement of retrotransposon blocks, as shown in Figure 4A. Like McC, they should lack the 26-kb retrotransposon cluster located between *bz1* and *stc1* in B73, and like B73, they should lack the 53-kb retrotransposon nest and the *Helitrons* of McC. To verify this, we characterized the size of the *stc1*-hybridizing *Not*I band in several *stc1* crossovers by CHEF gel DNA gel blot analysis. Representative data from two crossovers in segments 3 and 4 of *stc1* (Figure 3) are shown in Figure 4B. These crossovers were known from the initial analysis of the recombination junctions to lack the 26-kb retrocluster from B73. As expected, the parental McC haplotype gives a 111-kb *Not*I fragment and the parental B73 haplotype gives a 73-kb *Not*I fragment, both in the W22-introgressed line used in the recombination experiment and in the original B73 inbred. The two different *stc1* crossovers give a smaller, ~50-kb *Not*I fragment, the size expected from the recombinational loss of the large retroclusters of B73 and McC and the retention of the sole distal *Grande1* retrotransposon of B73.

DISCUSSION

In this study, we investigated whether retrotransposon polymorphisms, which are widespread in maize (Wang and Dooner, 2006), affect recombination in neighboring genes. We compared recombination between markers in the adjacent *bz1* and *stc1* genes in heterozygotes between haplotypes that differed by the presence or absence of a heterozygous retrotransposon cluster in the intergenic region and found that the genetic distance between the markers was twice as large in the absence of the cluster. To monitor recombination, we made use of *Ac* and *Ds* markers that affected seed pigmentation, allowing high-resolution analysis of the interval. In McC and W22, haplotypes that lack the retrotransposon cluster, the physical distance between the *Ds2(D1)* marker in *bz1* and the *Ac6087* marker in *stc1* is ~6 kb, and the measured size of the genetic interval in an McC/W22 heterozygote is 0.59 cM. In the B73 haplotype, the physical distance is ~32 kb because of the insertion in the *bz1-stc1* intergenic region of a retrotransposon cluster, consisting of *Tekay*, a *Zeon* solo LTR, and a *Xilon-Mu1* nest, and the measured size of the genetic interval in an McC/B73 heterozygote is 0.28 cM.

The heterozygous structure within the genetic interval is more than five times the size of the interval itself, so it could reduce recombination by interfering with the normal pairing of the adjacent genic sequences. Furthermore, the retrotransposon cluster is heavily methylated and probably more condensed than the adjacent euchromatin. Recombination is reduced by fivefold in the 0.81-kb segment of *bz1* located between *Ds2(D1)* and the cluster and by twofold overall in the ~4-kb segment of *stc1* located between *Ac6087* and the cluster. However, the pattern of reduction within *stc1* is somewhat unexpected. Recombination is reduced in the proximal (3′) one-third and distal (5′) one-third, but not in the middle. This is not the pattern one would expect if the retrotransposon effect diminished with distance from the cluster. It is unlikely that the observed differences result from a small experimental sample, as 77 and 141 *stc1* recombinants were characterized in McC/B73 and McC/W22 heterozygotes, respectively. Differences in the density of heterologies, which shows a negative correlation with intragenic recombination in maize (Dooner and Martinez-Ferez, 1997; Dooner, 2002; Yao et al., 2002; Yandeau-Nelson et al., 2006), could, in principle, account for some of the observed differences, but the *bz1* and *stc1* alleles of B73 are less polymorphic with McCs in intervals 3 and 5 than in those of W22. One possible explanation for the higher recombination in the 5′ *stc1* segment of McC/W22 heterozygotes would be that that segment is normally more recombinogenic in W22 than in B73, perhaps because of sequence differences in the two *Stc1* promoters, although the

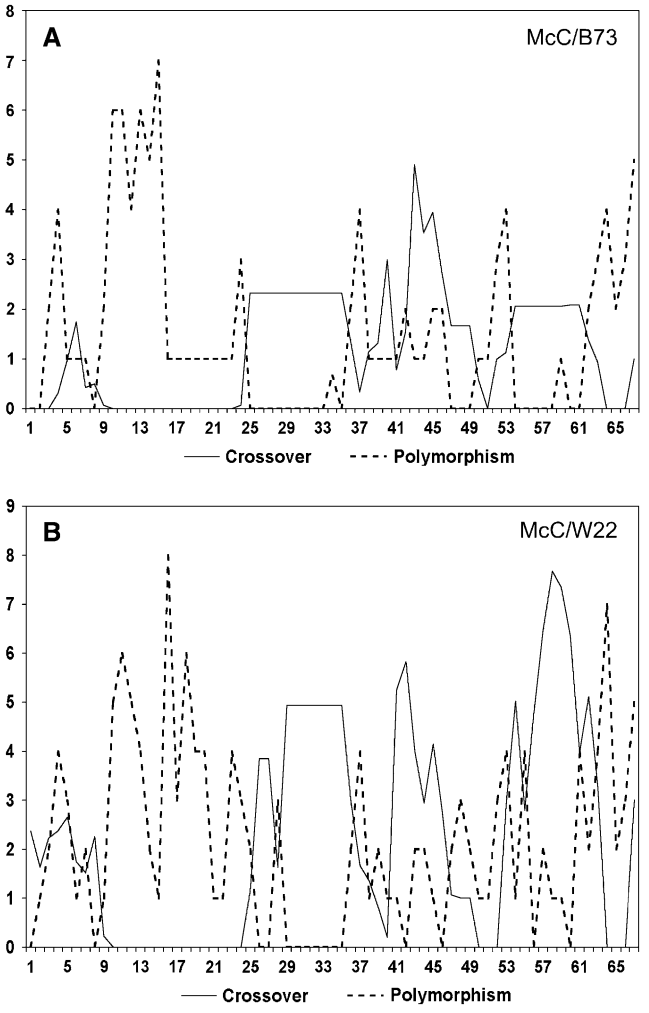

Figure 5. Frequencies of Recombinants (Solid Lines) and Polymorphisms (Dashed Lines) in the 6.7-kb Interval between *Ds2(D1)* and *Ac6087*.

bz1 is to the left and *stc1* is to the right. The 6.7-kb interval has been divided into 67 100-bp segments. Segments that make up fractions of an interval have been assigned a number of crossovers in proportion to the fraction of the interval constituted by that segment. The intergenic region lies between intervals 9 and 24.

enhancement of recombination by promoter-adjacent sequences, as in yeast and mammals (Petes, 2001), has not been demonstrated in plants.

As is evident from a comparison of the three haplotypes in Figure 1, B73 and W22 differ in other large insertion polymorphisms besides the 26-kb retrotransposon cluster in the *bz1-stc1* intergenic region, so the reduction in recombination observed between *bz1* and *stc1* could be partly the result of heterozygosity for other large insertions in the introgressed segment. However, recombination in the *bz1-stc1* interval, which contains the retrotransposon cluster and constitutes about one-fourth of the *bz1-sh1* distance, is reduced by twofold, whereas recombination in the *stc1-sh1* interval, which includes the polymorphic sequences to the right of *stc1* in Figure 1, is not reduced significantly in McC/B73 relative to McC/W22 heterozygotes. These observations argue that the reduction in recombination reported here arises principally from the heterozygosity of the large retrotransposon cluster in the *bz1-stc1* interval.

The distribution of recombination junctions in the *Ds2(D1)-Ac6087* interval correlates negatively with the density of polymorphisms in both heterozygotes. In Figure 5, frequencies of crossovers and polymorphisms in the 6.7-kb interval are plotted as a moving average every 100 bp from the proximal to the distal end. The highly polymorphic intergenic region lies between intervals 9 and 24 and contains no junctions in either heterozygote. Almost all of the polymorphisms outside of this region are SNPs. As can be seen, there is a general inverse relationship between the frequencies of crossovers and heterologies across the entire interval. These data confirm and extend the earlier observations at *bz1* and *a1* cited above.

All of the recombination junctions fell within introns or exons of *bz1* or *stc1* (Figure 3). The *bz1-stc1* intergenic region does not include the 5′ end of either gene, the end often associated with high conversion and the initiation of recombination in yeast (Petes, 2001). However, analysis of recombination junctions in a 100-kb stretch extending upstream (distal) of *stc1* that included several intergenic regions confirms that most crossovers fall within genes rather than in intergenic regions (L. He and H.K. Dooner, unpublished data). Similar observations have been made in the maize *a1-sh2* region (Yao et al., 2002). In *Arabidopsis*, a much less polymorphic species than maize, most crossover junctions fall in intergenic regions (Mezard, 2006), so the distribution of recombination appears to differ in the two plants.

The methylated retrotransposons that are interspersed with genes in the maize genome are probably heterochromatic, as they are in the knobs of maize and *Arabidopsis* (Ananiev et al., 1998; Lippman et al., 2004), and can affect recombination in neighboring genes. The general recombinational inertness of heterochromatin in many organisms (Harper and Cande, 2000) and the reduction of recombination in euchromatic regions adjacent to heterochromatin in *Drosophila* (Baker, 1958; Westphal and Reuter, 2002) are well-known phenomena. The distribution of LTR retrotransposons in the euchromatic portions of *Drosophila* chromosomes varies among wild-type strains (Franchini et al., 2004), but the effect of this variability on recombination has not been studied. More relevant to this study is a recent finding in rye (*Secale cereale*), showing that a polymorphic interstitial heterochromatic sequence in chromosome 2R significantly suppressed recombination in the arm (Kagawa et al., 2002). What sets maize apart from other species studied to date is the extensive variation in the distribution of retrotransposons, hence interspersed heterochromatin, from line to line. Our results suggest that this variability will affect the local distribution of recombination events across the genome.

The implication of the finding reported here is that, for very closely linked markers, local variation in haplotype structure will have a strong influence on estimates of genetic distance. Intergenic retrotransposons add physical length to the interval and reduce recombination in adjacent genes, thus doubly affecting the ratio of genetic to physical length in some mapping populations relative to others. Heterozygosity for intergenic retrotransposons is probably extensive in maize mapping populations. For example, the B73 and Mo17 *bz1* locus haplotypes differ from each other by the presence of the 26-kb *bz1-stc1* intergenic retrocluster (Fu and Dooner, 2002; Brunner et al., 2005), so the *bz1-stc1* distance in a B73×Mo17 map, such as the widely used IBM map (Lee et al., 2002; Sharopova et al., 2002), will be different from that in a W22/McC or W22/Mo17 map. When sequenced, the B73 genome will become the standard for map-based cloning efforts in maize. Using it as the reference for the physical map, one would compute a centimorgan:kilobase ratio of 0.0087 for the *Ds2(D1)-Ac6087* interval in an McC/B73 heterozygote (0.28 cM:32 kb). By contrast, the centimorgan:kilobase ratio for the same interval in an McC/W22 heterozygote would be 10-fold higher (0.59 cM:6 kb, or 0.098). Over longer genetic distances, variations in centimorgan:kilobase ratios between different mapping populations may average out.

Recombination within genes can lead to either the loss or gain of intergenic retrotransposons in the recombinants. We showed here that recombination within *stc1* in an McC/B73 heterozygote leads to the joint loss of flanking retrotransposons and to a calculated reduction of the distance between the *bz1* and *znf* genes from 82 and 44 kb in McC and B73, respectively, to 18 kb in the recombinant. The recombinant now carries a *bz1-znf* gene island uninterrupted by retrotransposons. In the reciprocal product, which should be present in the unanalyzed sh Bz recombinant class, both retroclusters would be retained and the distance between *bz1* and *znf* would expand to 126 kb. Again, taking B73 as the reference maize genome, the reciprocal products would have intraspecific expansion factors of 0.4 and 2.9, similar to those reported in an interspecific comparison of longer syntenic blocks from two maize (B73) homeologous regions with rice (*Oryza sativa*) as the standard (Bruggmann et al., 2006). Whether similar contraction:expansion ratios will occur when comparing megabase-sized distances within maize will have to await a fuller characterization of the genome from other maize lines.

METHODS

Plant Materials

All of the maize (*Zea mays*) stocks used in this study shared the common genetic background of the inbred W22. The *bronze1* alleles and the aleurone phenotypes of the various stocks are described below. Except for the W22 stock carrying a *Bz1-B73* allele, the derivation of the other stocks has been described previously.

Bz1-McC (purple): the normal allele of the McC haplotype (Ralston et al., 1988). *bz1-m2(DI)* (bronze in the absence of *Ac*, spotted in its presence) harbors a 3.3-kb *Ds* element at positions 755 to 762 in the second exon of *Bz1-McC* (McClintock, 1962; Dooner et al., 1986). *sh1-bz1-X2* (shrunken, bronze): an x-ray–induced deletion of a large chromosomal fragment that includes the *sh1* and *bz1* loci (Mottinger, 1973). *stc1-m1(Ac6087)*: an insertion mutation in the first exon of *stc1-McC* produced by the transposition of *Ac* from the nearby *bz1* locus in McC (Shen et al., 2000). *Bz1-W22* (purple): the normal allele of the color-converted version of the inbred W22 (Ralston et al., 1988). This stock traces back to the W22 *R1-r:standard* stock derived by Brink (1956) for his now classical studies of *R1* locus paramutation. *Bz1-B73* (purple): the normal allele of the inbred B73. It was introgressed as part of the *Sh1 Bz1* segment of that inbred by repeated backcrosses to a W22 stock carrying the *sh1-bz1-X2* deletion, thus precluding any recombination within the introgressed segment. The B73 parent was genotypically *c1 Sh1 Bz1*, and the recurrent W22 parent was *C1 sh1-bz1-X2*. Plump, purple kernels were selected in each generation, and after three backcrosses, a line carrying a *C1 Sh1 Bz1* recombinant chromosome was identified and selfed twice to establish homozygotes. The line was genotyped for *bnl1401* and *wx1* markers, located 13 and 25 cM proximal to *bz1*, respectively, and determined to carry W22 alleles at both of those loci. Thus, the *Sh1 Bz1* introgressed fragment from B73 is small. Its distal crossover junction lies in the 4-cM *c1-sh1* interval and its proximal junction lies in the 13-cM *bz1-bnl1401* interval, so the size of the fragment ranges from a minimum of 2 cM to a maximum of 17 cM.

Selection and Analysis of Crossovers

The mutations *sh1* (shrunken endosperm) and *wx1* (waxy endosperm) were used as markers flanking *bz1*. They map, respectively, ~2 cM distal and 25 cM proximal to *bz1* in *9S*. The *sh1-wx1* region exhibits high chiasma interference (Dooner, 1986), so double crossovers in the region are rare. The *sh1 stc1-m1(Ac6087) bz-m2(D1) wx1/Sh1 Stc1-B73 Bz1-B73 Wx1* and *sh1 stc1-m1(Ac6087) bz-m2(D1) wx1/Sh1 Stc1-W22 Bz1-W22 Wx1* heterozygotes were hand-pollinated with a *sh1-bz1-X2 wx1* stock. Sh bz recombinants were selected as single seeds with a plump, solid bronze phenotype in ears segregating spotted and purple seed and grown in the greenhouse for analysis. Leaf DNA was made from all selections by a urea extraction procedure (Greene et al., 1994) and used for subsequent PCR amplification. The selections were genotyped with a series of molecular markers, as described in Results.

PCR and Sequencing

PCR was performed using Qiagen Taq polymerase (Qiagen). The PCR products were run on 1% agarose gels or 8% polyacrylamide gels based on their size and the polymorphisms to be discriminated. For sequencing, PCR products were purified by isopropanol precipitation and 70% ethanol washing. The same PCR primers were used as sequencing primers to directly sequence purified PCR products using ABI BigDye Terminator V3.1 reagent (Applied Biosystems). DNA sequencing was performed in an ABI 3700 DNA analyzer.

BAC Isolation and Sequencing

*Not*I BAC clones of the *bz* genomic region of W22 were isolated as described previously (Fu and Dooner, 2000). The W22 BAC clones were sequenced by the shotgun sequencing strategy, assembled, analyzed, and annotated as described for other *bz* BAC clones from different maize inbreds and land races (Wang and Dooner, 2006).

DNA Gel Blot Analysis

DNA was prepared from isolated nuclei of 4-week-old shoots and leaves. Agarose plugs containing ~10 μg of high molecular weight DNA from different haplotypes were digested to completion with *Not*I. The digested genomic DNAs were resolved on 1% agarose gels by pulsed-field gel electrophoresis (CHEF-DR II system; Bio-Rad). The gels were blotted to a Hybond$^+$ nylon membrane (Amersham Pharmacia Biotech), and the membranes were hybridized with random primer–labeled ^{32}P probes from *stc1-McC*. Conditions for hybridization, high-stringency washing, and exposure to x-ray film were standard.

Accession Number

The GenBank accession number for the 238-kb W22 *bz* region sequence is EU338354.

Supplemental Data

The following material is available in the online version of this article.

Supplemental Table 1. Estimated Times of Insertion of LTR Retrotransposons in the McC and W22 Haplotypes.

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