Effects of *trans*-acting Genetic Modifiers on Meiotic Recombination Across the *a1–sh2* Interval of Maize

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ABSTRACT

Meiotic recombination rates are potentially affected by *cis*- and *trans*-acting factors, *i.e.*, genotype-specific modifiers that do or do not reside in the recombining interval, respectively. Effects of *trans* modifiers on recombination across the ~140-kb maize *a1–sh2* interval of chromosome 3L were studied in the absence of polymorphic *cis* factors in three genetically diverse backgrounds into which a sequence-identical *a1–sh2* interval had been introgressed. Genetic distances across *a1–sh2* varied twofold among genetic backgrounds. Although the existence of regions exhibiting high and low rates of recombination (hot and cold spots, respectively) was conserved across backgrounds, the absolute rates of recombination in these sequence-identical regions differed significantly among backgrounds. In addition, an intergenic hot spot had a higher rate of recombination as compared to the genome average rate of recombination in one background and not in another. Recombination rates across two genetic intervals on chromosome 1 did not exhibit the same relationships among backgrounds as was observed in *a1–sh2*. This suggests that at least some detected *trans*-acting factors do not equally affect recombination across the genome. This study establishes that *trans* modifier(s) polymorphic among genetic backgrounds can increase and decrease recombination in both genic and intergenic regions over relatively small genetic and physical intervals.

MEIOTIC recombination mediates the proper disjunction of chromosomes, generates novel allelic combinations across chromosomes, and provides the genetic diversity upon which selection can act. According to the double-strand break repair (DSBR) model (Szostak *et al.* 1983; Sun *et al.* 1991), meiotic recombination initiates with a double-strand break (DSB). Fungal, animal, and plant chromosomes exhibit regions of recombination hyperactivity, *i.e.*, hot spots, and hypoactivity, *i.e.*, cold spots (reviewed in Lichten and Goldman 1995; Schnable *et al.* 1998; Petes 2001; Nachman 2002), as compared to the genomewide average rate of recombination in the organism being studied. Consistent with the DSBR model, recombination hot spots in *Saccharomyces cerevisiae* are clearly associated with DSBs (reviewed in Lichten and Goldman 1995) and these DSB hot spots are not distributed randomly across the yeast genome (Gerton *et al.* 2000).

Nonrandom patterns of recombination have been identified in all organisms studied to date. For example, in most species recombination rates per megabase are suppressed in and surrounding centromeres (Lambie and Roeder 1986; Werner *et al.* 1992; Mahtani and Willard 1998; Puechberty *et al.* 1999; Kagawa *et al.*

2002). Similarly, in most species, with the exception of *Caenorhabditis elegans*, for which recombination rates are negatively correlated with gene density (Barnes *et al.* 1995), recombination rates are higher in gene-rich than in gene-poor regions of the genome (reviewed in Lichten and Goldman 1995; Schnable *et al.* 1998). In wheat and barley, for example, analyses of integrated genetic and cytogenetically based physical maps (Gill *et al.* 1993, 1996a,b; Hohmann *et al.* 1994; Delaney *et al.* 1995a,b; Mickelson-Young *et al.* 1995; Kunzel *et al.* 2000) have shown that most recombination occurs in relatively small gene-rich regions. Similarly, in maize recombination nodules cluster in regions of high gene density (Anderson *et al.* 2006). In addition, it is estimated that recombination in a gene-dense portion of the *bz1* region on chromosome 9S in maize is two orders of magnitude greater than in a flanking region of high retrotransposon density (Fu *et al.* 2002).

Although it is well established that high frequencies of recombination are positively correlated with gene-rich regions in the grasses, recombination breakpoints within these hot spots might resolve equally in genic and intergenic sequences. A high-resolution recombination mapping study across the ~140-kb multigenic maize *a1–sh2* interval supports that genes *per se* are preferred recombination hot spots and intergenic regions are cold spots; even so, nongenic hot spots and genic cold spots do exist (Yao *et al.* 2002). These maize genic hot

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spots can have either nonrandom distributions of breakpoints as observed in *a1* (Xu *et al.* 1995) and other genes (Eggleston *et al.* 1995; Patterson *et al.* 1995) or uniform distributions across the gene, as in *bz1* (Dooner and Martinez-Ferez 1997).

Variations in meiotic recombination rates occur not only among various regions of the genome but also among genetic backgrounds (reviewed in Simchen and Stamberg 1969) as evidenced in both animals (Koehler *et al.* 2002) and plants. In Arabidopsis, recombination rates vary among accessions (Sanchez-Moran *et al.* 2002). In maize, heterogeneity in recombination frequencies has been well documented within corn belt (Beavis and Grant 1991; Tulsieram *et al.* 1992), corn belt × exotic germplasm (Tulsieram *et al.* 1992), synthetic (Fatmi *et al.* 1993), exotic, and teosinte-maize hybrid (Williams *et al.* 1995) mapping populations and has often confounded the generation of composite genetic maps. Recombination rates in intervals with heterogenous frequencies varied two- to threefold among diverse mapping populations (Williams *et al.* 1995). Within a mapping population, recombination frequencies both in adjacent (Tulsieram *et al.* 1992) and between genetically unlinked (Beavis and Grant 1991; Fatmi *et al.* 1993) regions were often not correlated, demonstrating that recombination can be differentially regulated across a chromosome. But in addition, several intervals on different chromosomes were shown to have either negatively or positively correlated recombination frequencies (Fatmi *et al.* 1993). Together, these studies suggest that the control of variation in recombination rates might be polygenic and that some factors might control specific genetic intervals.

The heterogeneity in recombination frequencies among genetic backgrounds can be attributed to two general classes of genetic factors: *cis* and *trans*. *Cis*-acting elements are genetic factors that affect recombination in the region in which the factor resides. A study of recombination across several teosinte *a1–sh2* intervals, each having large insertion/deletion polymorphisms (IDPs) as compared to maize, has revealed significant differences in recombination rates and distribution of recombination breakpoints across the interval caused by the action of *cis* factors (Yao and Schnable 2005). Similarly, a not yet molecularly characterized *cis* factor increases recombination in the large A188 *Sh1–Bz1* interval on maize chromosome 9S (Timmermans *et al.* 1997).

In contrast to *cis* elements, *trans*-acting factors are genetic modifiers that are not closely linked to the interval in which they affect recombination. *Trans* modifiers might include factors involved in chromatin remodeling, proteins involved in the recombination machinery, and autonomous transposons. For example, the rate of crossover (CO) increases near a *Mu* insertion in *a1* in the presence of *MuDR* transposase (Yandeau-Nelson *et al.* 2005). Also, recombination is affected on the maize chromosome 9 *c1–sh1* and *bz1–wx1* intervals

as well as chromosome 7 by a single unidentified *trans* factor (Timmermans *et al.* 1997). The maize mapping studies described earlier established recombination rate heterogeneity among genetic backgrounds and suggest that this variation is under polygenic control (*i.e.*, multiple *trans* factors). However, these studies (Tulsieram *et al.* 1992; Fatmi *et al.* 1993) were unable to distinguish between the effects of *cis*- and *trans*-acting genetic modifiers, both of which potentially influence recombination rates. Also, available data only suggest that rates of recombination differ among genetic backgrounds, but the manner in which factors can affect the distribution of recombination breakpoints across a small physical distance containing both genic and nongenic intervals is unknown.

To elucidate the effects of *trans*-acting modifiers specifically, these factors must be studied in the absence of polymorphic *cis*-acting effects. This can be achieved by studying recombination across an interval that is sequence identical in several different genetic backgrounds. The ~140-kb *a1–sh2* interval on maize chromosome 3L is an ideal system in which to study how *trans* factors modulate both recombination rates and the distribution of recombination breakpoints across an interval because (1) recombination events across the interval are easily identified by nonparental kernel phenotypes, (2) high-resolution mapping of recombination breakpoints is straightforward due to a significant degree of sequence polymorphism between the *A1 Sh2* haplotypes and the availability of previously developed IDP markers across much of the interval (Xu *et al.* 1995; Yao *et al.* 2002), and (3) the interval is multigenic (Yao *et al.* 2002), which allows for the study of how *trans*-acting factors differentially affect recombination across genic and nongenic regions.

A sequence-identical *a1::rdt sh2* interval that had been introgressed into three unique genetic backgrounds (maize inbreds A632, Oh43, and W64A) was used to assess the extent to which *trans*-acting genetic modifiers that were polymorphic among the backgrounds affect meiotic recombination in the ~0.1-cM *a1–sh2* interval. This study reveals that *trans*-acting factors can influence both rates of recombination and distribution of recombination breakpoints within conserved hot and cold spots and can convert an average spot to a hot spot. The modifier(s) affecting recombination across this small interval on chromosome 3L does not appear to differentially affect recombination among genetic backgrounds in the same way across an interval on chromosome 1S, suggesting that at least some *trans*-acting factors affect specific regions of the genome.

MATERIALS AND METHODS

Genetic stocks: The *shrunken-2* (*sh2*) gene is located on chromosome 3 ~0.1 cM centromere-distal to the *a1* locus (Civardi *et al.* 1994). Mutations at these loci condition

shrunken and colorless kernel phenotypes, respectively. The *a1::rdt sh2* haplotype (GenBank accession no. AF072704) was described previously (reviewed in Xu *et al.* 1995). The *A1-LC Sh2* haplotype (GenBank accession nos. AF434192, AF347696, AF363390, X05068, and AF363391) is derived from the inbred line C (LC), a color-converted version of W22.

The *a1::rdt sh2* (A632)[7], *a1::rdt sh2* (Oh43)[9], and *a1::rdt sh2* (W64A)[6] genetic stocks were gifts from David Glover (Purdue University). To generate these stocks, the inbreds A632, Oh43, and W64A were crossed by a line that was homozygous for the *a1::rdt sh2* haplotype. Using the *a1* and *sh2* mutants as markers, the *a1::rdt sh2* haplotype was backcrossed into the inbreds for seven, nine, and six generations to generate the *trans* stocks *a1::rdt sh2* (A632)[7], *a1::rdt sh2* (Oh43)[9], and *a1::rdt sh2* (W64A)[6], respectively. The *trans* stocks were self-pollinated to generate homozygous *a1::rdt sh2* sources in each of the three genetic backgrounds.

Isolation of genetic recombinants and estimations of genetic distances: *Recombinants across a1–sh2:* To identify recombinants that resolve between *a1* and *sh2*, each of the three inbred *trans* stocks was used as a male onto the inbred line C (cross 1). Recombination was measured in the three resulting *trans* stock/line C F₁'s, *i.e.*, A632/LC, Oh43/LC, and W64A/LC, via testcrosses (cross 2) as described by Yao *et al.* (2002) and Yao and Schnable (2005). To control environmental effects on recombination, these testcrosses were performed during a single season (summer, 1997) in a single field.

Cross 1: *A1-LC Sh2/A1-LC Sh2* (line C) × *a1::rdt sh2/a1::rdt sh2* (*trans* stock)
Cross 2: *A1-LC Sh2/a1::rdt sh2* (F₁ from cross 1) × *a1::rdt sh2/a1::rdt sh2*

Progeny from cross 2 segregated for parental colored round and colorless shrunken kernels. Rare kernels with nonparental phenotypes (*viz.*, colored shrunken and colorless round) putatively carry recombination events between *a1* and *sh2*. Because the original A632, Oh43, and W64A inbreds are recessive for *r1*, a gene that encodes a transcription factor that acts upstream of *a1* in anthocyanin biosynthesis, colorless round (*a1′ Sh2*) kernels could be generated due to the absence of *r1* and not because of resolution of a recombination breakpoint between *a1* and *sh2*. For this reason, only the colored shrunken (*A1′ sh2*) recombinant class was analyzed in this study. Putative *A1′ sh2* recombinant kernels from each inbred background were tested, confirmed, and made homozygous as described in Xu *et al.* (1995). The actual number of *A1′ sh2* recombinants isolated from each genetic background was estimated from the frequency of confirmed recombinants out of the total number analyzed.

Recombinants across chromosome 1S: To identify genetic intervals unlinked to the *a1–sh2* interval on chromosome 3L, ∼1000 IDP genetic markers developed by the Maize Genetic Mapping Project (http://maize-mapping.plantgenomics.iastate.edu/) and genetically mapped to chromosomes 1–2, and 4–10 were surveyed. The criteria for selecting genetic intervals were that: (1) they be defined by two IDP markers that were 1–20 cM apart, (2) each IDP marker was polymorphic between line C (the *A1 Sh2* haplotype used in cross 1) and the three *trans* stocks, and (3) the genetic interval could be assayed in each of the three *trans*-stock experiments. Only two intervals that met these criteria were identified; both were located on chromosome 1S. Interval 1S.1 is defined by genetic markers IDP194 and IDP254 and 1S.2 is defined by markers IDP112 and IDP643. The IDP194 and IDP254 markers were designed from MEST54-C06 (GenBank accession no. BM072826) and MEST139-B10 (GenBank accession no. BM334583), respectively, and map to positions 115.6 and 115.0 cM, respectively, on the maize IDP_body map version 4 (http://magi.plantgenomics.

iastate.edu/cgi-bin/cmap). Markers IDP112 and IDP643 were designed from MEST19-B03 (GenBank accession no. BG841229) and MEST129-G08 (GenBank accession no. BM333984) and map to positions 82.8 and 84.6 cM, respectively.

Mapping populations were generated by crossing a single F₁ plant from cross 1 for each of the *trans* stocks to the inbred B77 (cross 3).

Cross 3: B77 × *A1-LC Sh2/a1::rdt sh2* (F₁ from cross 1)

Consequently, the progeny of cross 3 potentially carry alleles from the specific *trans* stock, line C, and B77. B77 was selected as the female parent for cross 3 because when tested with a sample of IDP markers across the genome it showed a high frequency of polymorphisms relative to line C and each of the three *trans* stocks (data not shown). Recombination rates across 1S.1 and 1S.2 were assayed in populations of ∼700 seedlings derived from progeny of cross 3 in each genetic background. Recombinants across each interval were identified as those individuals having nonparental combinations of genotypes at the two linked loci in each interval. Genetic distances in each background were calculated using the total number of recombinants identified in each *trans* stock and the total number of seedlings analyzed.

Genotypes were determined using PCR primers IDP112F, 5′-CTGTGACATGTTTGATGCCC-3′, IDP112R, 5′-GGTGATG ACCACGTACAAGC-3′, and IDP112AW, 5′-CCC TGC TGA TAG TGA TAG AC-3′; IDP643F, 5′-ACCCTCATCTTCAGCAG TCG-3′ and IDP643R, 5′-GGTGAAACGGCAGTACAAGG-3′; IDP194F, 5′-GACAGATCCCTAACACTTGGG-3′ and IDP194R, 5′-AACAAGGCAACCTGTGAAGC-3′; and IDP254F, 5′-ATG TTGGTTGAGCCTCTTGG-3′ and IDP254R, 5′-GATTCAGA GAGAGTGCATGGC-3′.

Seed sterilization, germination, and DNA isolation: Colored shrunken kernels that were homozygous for recombinant *A1′ sh2* haplotypes originally isolated from cross 2 and ∼700 kernels from each genetic background derived from cross 3 were germinated in 96-well flats. PCR-ready DNA was isolated as described (Dietrich *et al.* 2002).

Due to fungal contamination, many shrunken kernels do not germinate in the soil. For these recombinants, a second aliquot of shrunken kernels was sterilized with pure bleach (Clorox) for 1 min and then rinsed repeatedly (10–15 times) with sterile water. Sterilized kernels were plated on moistened autoclaved germination paper (Anchor Paper, St. Paul) in sterile Petri dishes, treated with 0.5% Captan 50-W fungicide (Platte Chemical, Fremont, NE), and covered with activated charcoal. Seeds were germinated at 28° for 5–7 days. Seeds were transferred to new plates, watered, and covered with fresh charcoal every 1–2 days. Tissue was collected from coleoptiles and DNA isolation was as described above.

Physically mapping recombination breakpoints: Recombination breakpoints across the *a1–sh2* interval were mapped relative to eight IDP markers that had previously been identified between the *A1-LC Sh2* and *a1::rdt sh2* haplotypes (Yao *et al.* 2002). These markers separated the *a1–sh2* interval into eight informative subintervals (Figure 1A); intervals are numbered according to Yao *et al.* (2002). Allele-specific primers not described by Yao *et al.* (2002) are listed below. Primers that when paired recognize a size polymorphism between the two haplotypes are XL3, 5′-ATGAGCGGGAGCCTATG-3′, and XL4, 5′-TCAGCATCCATACCATTG-3′. Allele-specific and size polymorphism primers are shown in Figure 1A.

The breakpoints associated with >85% of the confirmed recombinants from the A632 (90%; 117/130), Oh43 (87%; 155/178), and W64A (85%; 185/219) genetic backgrounds were physically mapped. It was not possible to map the remainder due to difficulties in obtaining homozygous recombinant chromosomes or in germinating shrunken kernels.

TABLE 1

Genetic distances across a common *a1–sh2* interval in three genetic backgrounds

Genetic background	No. isolated[a]	No. recovered[b]	No. tested[c]	No. confirmed[d]	No. correct recombinants[e]	Population sizes	Genetic distances (cM)[f]
A632/LC	229	138	130	130	229	320,718	0.143 ± 0.000667
Oh43/LC	323	188	188	178	306	758,271	0.081 ± 0.00033
W64A/LC	442	219	219	219	442	704,077	0.126 ± 0.000442

[a] Number of putative colored shrunken recombinants isolated from cross 2 (MATERIALS AND METHODS).

[b] Number of putative recombinants successfully propagated.

[c] Number of putative recombinants whose validity was tested (MATERIALS AND METHODS).

[d] Number of putative recombinants confirmed.

[e] Number of correct recombinants = no. isolated × (no. confirmed/no. tested).

[f] Genetic distance = 100 × no. correct recombinants × 2/population size. Because colorless round recombinants were not isolated, genetic distances were calculated by multiplying the number of colored shrunken recombinants by two. Standard errors were calculated according to the formula $(pq/n)^{1/2}$. The genetic distances among genetic backgrounds varied significantly ($P < 0.03$).

Statistical methods: For each physical interval examined, genetic distances (in centimorgans) were compared among genetic backgrounds using χ^2 homogeneity tests. For the genetic distances between *a1* and *sh2*, the corrected number of *A1′ sh2* recombinants (Table 1) was doubled because the reciprocal class, *a1′ Sh2*, was not analyzed. This analysis makes the reasonable assumption that the rates at which the two recombinant classes arise are equal.

Statistical methods and population size adjustments were performed as described by YAO and SCHNABLE (2005). The actual numbers of mapped recombinants and the adjusted population sizes were used for subsequent χ^2 homogeneity tests. Pearson χ^2 tests were conducted as described by YAO and SCHNABLE (2005) to compare the actual recombinant breakpoint distribution in each genetic background across *a1–sh2* to (1) the expected distribution if recombination across the interval was equal to the genomewide average (2.4 cM/Mb) and (2) the expected distribution of recombinants if the rate of recombination across each subinterval was equal to the average recombination rate across the entire *a1–sh2* interval. In this study, rates of recombination were compared to a genomewide average recombination rate of 2.4 cM/Mb (Table 2). This is an updated rate compared to the 2.1 cM/Mb rate (YAO *et al.* 2002) based on a more recent maize genetic map that consists of 5917 cM (LEE *et al.* 2002).

The expected numbers of recombinants for each subinterval were calculated using the subinterval distances measured in the line C haplotype (Figure 1A). The sizes of each subinterval were previously determined via sequencing or DNA gel blot analyses (YAO *et al.* 2002). For the *a1::rdt sh2* haplotype, interval sizes were determined using the genomic sequence from *a1* to *yz1* of this haplotype (GenBank accession no. AF072704) for subintervals II (1.7 kb), III (1.34 kb), IV (0.4 kb), V (80 bp), VI (2.2 kb), VII (620 bp), and VIII (3.7 kb). To test if the use of subinterval sizes from line C affects comparisons of recombination rates to the genomewide and to the *a1–sh2* interval average rates of recombination, the expected numbers of recombinants and the corresponding statistical analyses were also conducted using subinterval sizes from the *a1::rdt sh2* haplotype. Because the significance or nonsignificance for each subinterval was not altered by which haplotype was used to determine sizes of each subinterval, only statistical analyses using subinterval sizes measured in line C are presented.

To investigate which subintervals contribute to the different breakpoint distributions among the backgrounds, an exploratory statistical analysis was conducted. Freeman–Halton tests

as described in YAO and SCHNABLE (2005) were conducted in which each subinterval was successively removed (and then replaced) from the analysis. If the recombination rates in a subinterval were contributing to differences in breakpoint distributions across the entire interval, then upon removal of this subinterval (or group of subintervals) the backgrounds should no longer differ significantly.

RESULTS

The genetic distance between *a1* and *sh2* and the distribution of recombination breakpoints varies among genetic backgrounds: A common *a1::rdt sh2* interval was introgressed into the inbred lines A632, Oh43, and W64A for multiple generations (MATERIALS AND METHODS). Each of the resulting *trans* stocks contains a sequence-identical *a1::rdt sh2* interval on chromosome 3L, while the bulk of its genome is expected to be derived from the recurrent parent: *i.e.*, A632, Oh43, and W64A. These stocks are ideal for studying the effects of *trans*-acting genetic modifiers on recombination across the *a1–sh2* interval because the presence of an identical *a1–sh2* interval in each of the three backgrounds ensures that the *cis* factors that affect recombination within the *a1–sh2* interval are identical in each of the inbred backgrounds. To analyze the effects of genetic background on recombination across the *a1–sh2* interval, each of the *trans* stocks was crossed to the inbred line C. Because each of the resulting F_1's is identically heterozygous at the *a1–sh2* interval (*A1-LC Sh2/a1::rdt sh2*), the only modifiers of recombination that are expected to differ among these three F_1's should be outside of the *a1–sh2* interval. Hence, any observed differences in meiotic recombination across the *a1–sh2* interval among the three genetic backgrounds can be attributed specifically to *trans* factors.

Meiotic recombination events that resolved between *a1* and *sh2* and yielded colored shrunken kernels (*A1′ sh2*) were identified from cross 2 (MATERIALS AND METHODS). Colorless round (*a1′ Sh2*) kernels were not

TABLE 2

Statistical analyses of recombination across *a1–sh2*

Informative subintervals	Genetic backgrounds	Comparisons to the average of *a1-sh2*[a]	Comparisons to the genome's average[a]	Recombination activities[b]
II	A632/LC	$2.8e^{-156}$↑ (22×)	$4.2e^{-63}$↑ (10×)	Hot spot (global, local)
	Oh43/LC	$8.8e^{-77}$↑ (14×)	$5.9e^{-13}$↑ (3.6×)	Hot spot (global, local)
	W64A/LC	$3.5e^{-100}$↑ (15×)	$3.0e^{-32}$↑ (5.8×)	Hot spot (global, local)
III	A632/LC	0.28	0.10	Average spot
	Oh43/LC	0.73	0.094	Average spot
	W64A/LC	0.41	0.41	Average spot
VI	A632/LC	$6.8e^{-104}$↑ (17×)	$2.1e^{-40}$↑ (7.5×)	Hot spot (global, local)
	Oh43/LC	$8.0e^{-49}$↑ (10×)	$6.3e^{-07}$↑ (2.6×)	Hot spot (global, local)
	W64A/LC	0↑ (23×)	$3.1e^{-110}$↑ (9.2×)	Hot spot (global, local)
VII	A632/LC	$7.1e^{-6}$↑ (6.9×)	0.018↑ (3.1×)	Hot spot (global, local)
	Oh43/LC	0.16	0.55	Average spot
	W64A/LC	0.030↑ (3.3×)	0.66	Hot spot (local)
VIII	A632/LC	$4.4e^{-137}$↑ (16×)	$3.3e^{-52}$↑ (7.1×)	Hot spot (global, local)
	Oh43/LC	0↑ (23×)	$9.7e^{-81}$↑ (5.8×)	Hot spot (global, local)
	W64A/LC	$4.9e^{-175}$↑ (14×)	$1.2e^{-54}$↑ (5.4×)	Hot spot (global, local)
IX	A632/LC	$3.1e^{-8}$↓ (NA)[c]	$1.1e^{-16}$↓ (NA)	Cold spot (global, local)
	Oh43/LC	$5.1e^{-10}$↓ (40×)	$1.5e^{-36}$↓ (160×)	Cold spot (global, local)
	W64A/LC	$3.1e^{-12}$↓ (NA)	$9.2e^{-29}$↓ (NA)	Cold spot (global, local)
X	A632/LC	0.74	0.23	Average spot
	Oh43/LC	0.40	0.11	Average spot
	W64A/LC	0.011 ↑(2.7×)	0.89	Hot spot (local)
XI–XIII	A632/LC	$4.1e^{-17}$↓ (NA)	$2.9e^{-38}$↓ (NA)	Cold spot (global, local)
	Oh43/LC	$2.8e^{-21}$↓ (51×)	$2.1e^{-86}$↓ (200×)	Cold spot (global, local)
	W64A/LC	$3.9e^{-25}$↓ (59×)	$1.6e^{-66}$↓ (150×)	Cold spot (global, local)

[a] *P*-values are shown for χ^2 goodness-of-fit tests used to compare the observed recombination rate in each subinterval with (a) the average rate of recombination (cM/Mb) across the *a1-sh2* interval in each genetic background and (b) the average rate of recombination in the genome (2.4 cM/Mb). This genome rate of 2.4 cM/Mb is an updated estimate based on a new maize genetic map that consists of 5917 cM (Lee *et al.* 2002) compared to the rate previously used (Yao *et al.* 2002). Arrows (↑ and ↓) denote a significantly higher or lower rate of recombination (*P*-value < 0.05), respectively, than the rate to which it was compared. For subintervals with significant *P*-values, the fold difference between rates is shown in parentheses.

[b] Hot and cold spots are intervals that have higher and lower rates of recombination, respectively, than the rates to which they are compared. Global and local spots significantly differ from the average genome and the *a1-sh2* interval rates of recombination, respectively. Average spots do not significantly differ from either the global or local rates.

[c] For genetic backgrounds that have no recombination in a given subinterval, the fold difference with the rate to which it is compared cannot be calculated. NA, not applicable.

analyzed for reasons described in MATERIALS AND METHODS. Each of the genetic backgrounds exhibited a statistically different rate of recombination across the *a1–sh2* interval and these rates varied almost twofold (Table 1).

Rates of recombination across the *a1–sh2* interval differ significantly due to the action of *trans*-acting modifier(s). To determine whether these *trans*-acting factors also affect the distributions of meiotic recombination breakpoints across genic and nongenic regions in this interval, recombination breakpoints isolated from each genetic background were mapped relative to sequence polymorphisms between the *A1-LC Sh2*

and *a1::rdt sh2* haplotypes (MATERIALS AND METHODS). Because the *a1–sh2* intervals present in each of the genetic backgrounds were identical, breakpoints could be mapped relative to the same sequence polymorphisms, thereby facilitating comparisons among genetic backgrounds.

In each background recombination breakpoints were not randomly distributed across the *a1–sh2* interval ($P < 4.0e^{-39}$). Indeed, >95% of the recombinants isolated from each background mapped within the *a1–yz1* interval (subintervals II–VIII; Figure 1, C and D) that composes only ~10% of the physical length of *a1–sh2*. The majority of the remaining recombinants resolved at
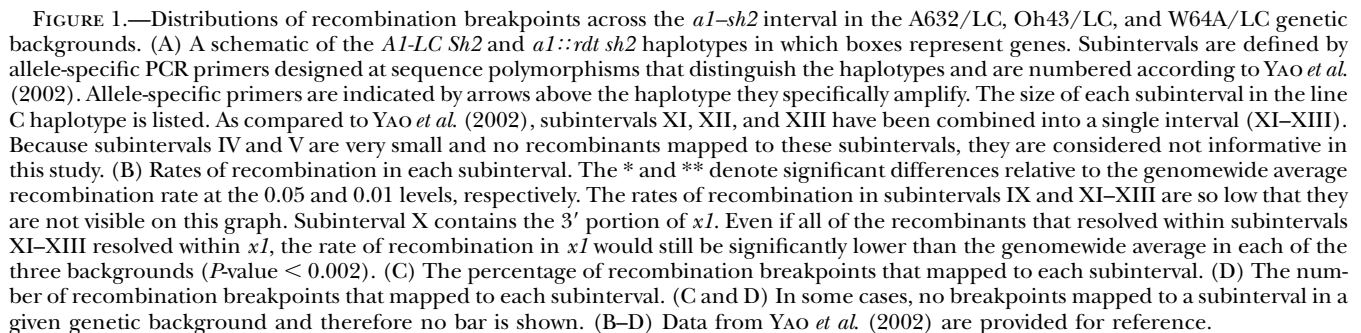
FIGURE 1.—Distributions of recombination breakpoints across the *a1–sh2* interval in the A632/LC, Oh43/LC, and W64A/LC genetic backgrounds. (A) A schematic of the *A1-LC Sh2* and *a1::rdt sh2* haplotypes in which boxes represent genes. Subintervals are defined by allele-specific PCR primers designed at sequence polymorphisms that distinguish the haplotypes and are numbered according to YAO *et al.* (2002). Allele-specific primers are indicated by arrows above the haplotype they specifically amplify. The size of each subinterval in the line C haplotype is listed. As compared to YAO *et al.* (2002), subintervals XI, XII, and XIII have been combined into a single interval (XI–XIII). Because subintervals IV and V are very small and no recombinants mapped to these subintervals, they are considered not informative in this study. (B) Rates of recombination in each subinterval. The * and ** denote significant differences relative to the genomewide average recombination rate at the 0.05 and 0.01 levels, respectively. The rates of recombination in subintervals IX and XI–XIII are so low that they are not visible on this graph. Subinterval X contains the 3′ portion of *x1*. Even if all of the recombinants that resolved within subintervals XI–XIII resolved within *x1*, the rate of recombination in *x1* would still be significantly lower than the genomewide average in each of the three backgrounds (*P*-value < 0.002). (C) The percentage of recombination breakpoints that mapped to each subinterval. (D) The number of recombination breakpoints that mapped to each subinterval. (C and D) In some cases, no breakpoints mapped to a subinterval in a given genetic background and therefore no bar is shown. (B–D) Data from YAO *et al.* (2002) are provided for reference.

Subintervals[b]	Comparisons among genetic backgrounds[a]		
	A632/LC *vs.* Oh43/LC	A632/LC *vs.* W64A/LC	Oh43/LC *vs.* W64A/LC
II	A > O ($1.8e^{-5}$)	A > W (0.019)	O $\simeq$ W (0.052)
III	A $\simeq$ O (0.36)	A $\simeq$ W (0.20)	O $\simeq$ W (0.45)
VI	A > O ($2.6e^{-5}$)	A $\simeq$ W (0.36)	W > O ($5.4e^{-9}$)
VII	A > O (0.049)	A $\simeq$ W (0.24)	O $\simeq$ W (0.45)
VIII	A $\simeq$ O (0.35)	A $\simeq$ W (0.24)	O $\simeq$ W (0.73)
IX	A $\simeq$ O (0.51)	A $\simeq$ W (ND)[c]	O $\simeq$ W (0.38)
X	A $\simeq$ O (0.83)	A $\simeq$ W (0.24)	O $\simeq$ W (0.16)
XI–XIII	A $\simeq$ O (0.36)	A $\simeq$ W (0.29)	O $\simeq$ W (0.79)

[a] For each subinterval, statistically significant relationships among rates of recombination in the A632/LC (A), Oh43/LC (O), and W64A/LC (W) genetic backgrounds are shown. The *P* values associated with χ^2 homogeneity tests are provided in parenthesis. In a given subinterval, rates of recombination are indicated as being not distinguishable ($\simeq$) if $P > 0.05$.

[b] The distribution of breakpoints across the entire *a1–sh2* interval in the Oh43/LC background differed significantly from the distributions in both the A632/LC ($P = 0.013$) and W64A/LC ($P = 0.0008$) backgrounds.

[c] Because no recombinant breakpoints mapped to this interval in either background, rates of recombination could not be compared. ND, not determined.

the 3′ end of *x1* (subinterval X; Figure 1, C and D). These observations are consistent with previously described distributions of recombination breakpoints across the *a1–sh2* interval (YAO *et al.* 2002; YAO and SCHNABLE 2005).

The distributions of recombination breakpoints across the eight *a1–sh2* subintervals defined by sequence polymorphisms between the *A1-LC Sh2* and *a1::rdt sh2* haplotypes (Figure 1) were compared among genetic backgrounds (MATERIALS AND METHODS). The distribution of breakpoints in the Oh43/LC background differed significantly from the distributions in both the A632/LC and W64A/LC backgrounds (Table 3). This finding indicates that *trans*-acting factors that are polymorphic in the Oh43/LC background as compared to the other two genetic backgrounds affect not only rates of recombination across the entire *a1–sh2* interval but also the distribution of recombination breakpoints within this interval. Using the exploratory statistical analysis described in MATERIALS AND METHODS it was possible to establish that in the Oh43/LC background the interloop (subinterval VI) and the *yz1* gene (subinterval VIII) were the most significant contributors to the differences in the distribution of breakpoints across the *a1–sh2* interval.

Recombination hot and cold spots are mostly conserved among genetic backgrounds: As defined by YAO and SCHNABLE (2005) "global" recombination hot and cold spots are regions that exhibit rates of recombination that are either higher or lower, respectively, than the genomewide average rate of recombination (2.4 cM/Mb). "Local" recombination hot and cold spots are regions that have rates of recombination that are higher and lower, respectively, than the average rate of recombination across the entire *a1–sh2* interval in the genetic background being examined. In each of the genetic backgrounds examined in this study the average recombination rate across the *a1–sh2* interval (*i.e.*, the local average) is lower than the genomewide average rate of recombination. "Average" spots exhibit rates of recombination that do not differ significantly from the genomewide average.

All three recombination hot spots previously identified by YAO *et al.* (2002) in the same *A1-LC Sh2/a1::rdt sh2* heterozygote used in this study but in another genetic background [the *a1* gene (subinterval II), the *yz1* gene (subinterval VIII), and the apparently nongenic interloop (subinterval VI)] are both global and local recombination hot spots in all three genetic backgrounds (Figure 1B; Table 2). Similarly, two intergenic regions and one gene (*x1*) identified by YAO *et al.* (2002) as recombination cold spots (subintervals IX, X, and XI–XIII, Figure 1B; Table 2), are also cold spots in all three genetic backgrounds analyzed in this study.

Trans-acting factors affect rates of recombination within hot spots: As discussed above, the *a1* (subinterval II) and *yz1* (subinterval VIII) genes and the nongenic interloop (subinterval VI) are conserved global recombination hot spots in each of the three genetic backgrounds (Table 2). If *trans*-acting modifiers affect each subinterval equally, the rates of recombination in each subinterval should be highest and lowest in the genetic backgrounds that have the largest (*i.e.*, A632/LC) and smallest genetic distances (*i.e.*, Oh43/LC), respectively, across the entire *a1–sh2* interval (Table 1). Because this is often not true (Figure 1; Table 3), we conclude that *trans*-acting factor(s) that are polymorphic among the genetic backgrounds are differentially affecting rates of recombination within the conserved hot spots.

The most notable example of differential control of recombination among genetic backgrounds occurs in

TABLE 4

Genetic distances across two genetic intervals unlinked to *a1–sh2* in three genetic backgrounds

Genetic background	1S.1 interval			1S.2 interval		
	No. recombinants[a]	Population sizes	Genetic distances (cM)	No. recombinants[a]	Population sizes	Genetic distances (cM)
A632/LC	15	704	2.13 ± 0.06[b]	5	729	0.69 ± 0.03[c]
Oh43/LC	15	717	2.09 ± 0.05	1	705	0.14 ± 0.01
W64A/LC	6	678	0.89 ± 0.04[b]	0	678	—[c]

[a] Total number from the two expected classes of recombinants.
[b] The 2.4-fold larger genetic distance in A632 as compared to W64A is weakly supported (P-value = 0.058).
[c] The genetic distance in A632 is higher than in W64A (P-value = 0.03).

yz1 (subinterval VIII; Figure 1A). Among the three backgrounds, the rate of recombination across *a1–sh2* is lowest in Oh43/LC (0.607 cM/Mb) and consistent with this low rate most subintervals show the lowest rate of recombination in this background (Figure 1B). However, more than half the recombination events isolated in the Oh43/LC background resolve in *yz1* (Figure 1, C and D) and the corresponding rate of recombination is 23-fold higher than the rate across *a1–sh2* (P-value ∼0; Table 2) in this background, making *yz1* the most recombinationally active region in Oh43/LC. In contrast, only ∼40% of the breakpoints associated with recombinants isolated in the A632/LC and W64A/LC genetic backgrounds resolve within *yz1* (Figure 1C). The recombination rate in *yz1* is approximately seven-, approximately six-, and approximately fivefold hotter than the genomewide rate (Table 2) in the A632/LC, Oh43/LC, and W64A/LC genetic backgrounds, respectively, with no significant differences in recombination rates among the backgrounds (Figure 1B; Table 3). In each of the backgrounds, the rate of recombination in each of the subintervals of *yz1* did not differ significantly from the rate of recombination for the entire gene (P-value > 0.05) and, further, the recombination rates in each of the subintervals of *yz1* did not differ significantly among the backgrounds (data not shown).

***Trans*-acting modifiers can convert a region with an average recombination rate into a hot spot:** Although their levels of recombination activity vary, recombination hot and cold spots are generally conserved among the three genetic backgrounds. The exception, however, is subinterval VII, a nongenic region proximal to *yz1*. While no recombination was observed in this interval in another genetic background (YAO *et al.* 2002), in the A632/LC genetic background subinterval VII is approximately threefold more recombinationally active than the genomewide average (7.4 cM/Mb; P-value = 0.018; Table 2; Figure 1B) and approximately sevenfold hotter than the average rate across *a1–sh2* in the A632/LC background (P-value = 7.1e^{-06}; Table 2). In the W64A/LC background, however, subinterval VII is an average spot when compared to the genomewide

average, but locally it is approximately three times more recombinationally active than the average rate across *a1–sh2* (P-value = 0.03; Table 2). In Oh43/LC, subinterval VII is an average spot both globally and locally (Figure 1B; Table 2). In summary, this interval is more recombinationally active in each of the genetic backgrounds in this study compared to the sequence-identical interval in the line C background (YAO *et al.* 2002).

Genetic distances of chromosomal intervals on chromosome 1: The genetic distances between *a1* and *sh2* differ significantly among the three genetic backgrounds tested in this study. To determine whether *trans*-acting modifiers that affect recombination across the *a1–sh2* interval of chromosome 3 are global or region-specific modifiers of recombination, recombination was assayed in ∼700 progeny of cross 3 in two intervals on chromosome 1 (intervals 1S.1 and 1S.2) in the three genetic backgrounds. Intervals 1S.1 and 1S.2 are separated by 30.4 cM on chromosome 1S in the maize IBM mapping population (IBM_IDP+MMP_ body map version 4.0; http://magi.plantgenomics.iastate. edu/cgi-bin/cmap). In both intervals, the genetic distances were highest in the A632/LC background consistent with results in *a1–sh2* and, unlike in the *a1–sh2* interval, lowest in W64A/LC (Table 4). Therefore, the relationships between genetic distances among the genetic backgrounds differ between the intervals examined on chromosome 1S and the *a1–sh2* interval on 3L.

DISCUSSION

***Trans*-acting genetic modifiers affect rates of recombination in the *a1–sh2* interval:** The heterogeneity in rates of recombination observed in previous studies among maize genomes (BEAVIS and GRANT 1991; TULSIERAM *et al.* 1992; FATMI *et al.* 1993; WILLIAMS *et al.* 1995) could be due to *cis*- and/or *trans*-acting genetic modifiers. In contrast, the genetic stocks used in this study ensured that elements that affect recombination in *cis* are the same in each background. This is, therefore, the first molecular analysis in plants, animals, or

yeast for which differences in recombination across a multigenic interval can be directly attributed to *trans*-acting modifiers that differ among genetic backgrounds.

To enhance our ability to identify inbred backgrounds that exhibit polymorphic *trans*-acting factors, recombination was assayed in three genetically distinct maize inbreds. A632 is a Stiff Stalk (SS) Reid Yellow Dent inbred, while the Non-Stiff Stalk (NSS) inbreds Oh43 and W64A (http://www.maizegenetics.net/index.php?page=germplasm/lines.html) are members of the Lancaster Sure Crop and the Hy:T8:Wf9 subgrouping (LIU *et al.* 2003), respectively. A632 and Oh43 are among the six inbreds that contribute to ~70% of all hybrids in the United States (NASS and PATERNIANI 2000).

Rates of recombination between *a1* and *sh2* varied up to approximately twofold among these three genetic backgrounds (Table 1). This establishes that *trans*-acting modifiers can affect rates of recombination and, not surprisingly, the effects of these factors vary among the inbreds within different heterotic groups.

***Trans* effects on a multigenic interval:** Unlike previous studies of *trans*-acting effects that used large genetic (and physical) intervals with uncharacterized molecular structures (TIMMERMANS *et al.* 1997) or studies that compare genetic sizes among different genetic maps (BEAVIS and GRANT 1991; TULSIERAM *et al.* 1992; FATMI *et al.* 1993; WILLIAMS *et al.* 1995), analysis of the well-characterized multigenic *a1–sh2* interval (Figure 1A) allowed for observation of the effects of *trans*-acting modifiers in defined genic and intergenic regions.

Although recombination hot and cold spots are largely conserved among the genetic backgrounds (Figure 1B; Table 2), the rates within recombination hot spots are affected by *trans*-acting factors that are polymorphic among the backgrounds (Table 3). For example, in the Oh43/LC genetic background *trans*-acting factor(s) have seemingly redirected recombination events that might normally resolve within the nongenic interloop (subinterval VI) to *yz1* (subinterval VIII) such that the resulting rate of recombination in *yz1* does not differ significantly from the rates in the A632/LC and W64A/LC genetic backgrounds. In each of the conserved recombination hot spots, the relationships between recombination rates among the backgrounds vary (Table 3). This demonstrates that the *trans*-acting modifier(s) act by increasing or decreasing the rates of recombination within particular hot spots.

Trans-acting modifier(s) affecting recombination in this study are affecting not only the overall rate of recombination between *a1* and *sh2* but also the distributions of recombinants across this interval. This observation is in direct contrast to the *trans*-acting autonomous transposon *MuDR* that increased the rate of recombination at an *a1* allele containing a *Mu1* insertion but did not change the distribution of recombinant breakpoints compared to patterns observed in other *a1* alleles (YANDEAU-NELSON *et al.* 2005). This suggests, not surprisingly, that different types of *trans*-acting modifiers will affect recombination in different ways.

***Trans*-acting modifier(s) can act as hot and cold switches in a region of lower sequence similarity:** Although there are exceptions (YAO *et al.* 2002), in general plant genes are recombination hot spots and nongenic regions are cold spots (reviewed in PUCHTA and HOHN 1996; SCHNABLE *et al.* 1998). Indeed, nongenic subintervals IX and XI–XIII are both local and global cold spots (Figure 1B; Table 2) in each of the three genetic backgrounds. In striking contrast, the nongenic subinterval VII is both a global and local hot spot in A632/LC, providing a second example of an apparently nongenic recombination hot spot. Because subinterval VII is only a local hot spot in the W64A/LC and an average spot in the Oh43/LC genetic backgrounds (Table 2), *trans*-acting modifiers not only are able to alter rates of recombination within hot spots but also can switch hot spots on and off.

Recombination hot spots have been shown to be "dialed down" by sequence heterology (*cis*-acting elements) in many organisms, including within the maize *a1–sh2* interval (YAO and SCHNABLE 2005), by a mechanism involving mismatch repair proteins (reviewed in MODRICH and LAHUE 1996; BORTS *et al.* 2000; EVANS and ALANI 2000; SCHOFIELD and HSIEH 2003). At the maize *bz1* locus, as little as 1.5% sequence divergence reduces rates of recombination twofold (DOONER 2002). In subinterval VII, the level of sequence heterology between the line C haplotype and the *a1::rdt-sh2* haplotype present in each genetic background is 3.1%. Even with this relatively high degree of heterology, the rate of recombination in A632 is approximately threefold higher than the average rate of recombination in the genome (7.4 *vs.* 2.4 cM/Mb; Table 2). This demonstrates that *trans*-acting modifiers in the A632/LC and W64A/LC (where recombination in this subinterval is also increased but to a lesser extent) backgrounds can overcome the suppression of recombination that often occurs between heterologous sequences. Further, this establishes that high sequence identity, a *cis*-acting factor, is not the only criterion that dictates whether a region is a recombination hot or cold spot.

Comparison of *cis*- and *trans*-acting effects on recombination: Both *cis*- and *trans*-acting factors can potentially affect the rate of recombination in a given interval. Only by studying each type of factor in the absence of the other (*e.g.*, *trans* in the absence of polymorphic *cis* effects) is it possible to assess how these two categories similarly and differently affect recombination. To that end, the *a1–sh2* interval in maize is the first region for which the effects of *trans*-acting modifiers on recombination (this study) can be compared to the effects of *cis*-acting elements on recombination that were characterized among different *a1–sh2* teosinte intervals introgressed into maize (YAO and SCHNABLE 2005).

Both the *cis*- and *trans*-acting modifiers in the two studies affect recombination similarly in that in both studies most of the recombination breakpoints resolve in the proximal ~10% of the *a1–sh2* interval. Together these studies suggest that although *cis* and *trans* effects on recombination in the *a1–sh2* interval are, in general, similar, the *cis* elements represented in the teosinte *a1–sh2* intervals affect recombination to a greater extent than do the *trans*-acting modifiers that are polymorphic in the A632/LC, Oh43/LC, and W64A/LC genetic backgrounds. For example, in a subinterval containing the interloop (subinterval VI in this study), which is a recombination hot spot in each of the *trans* stocks, *cis* elements act to make this region an average, cold, and hot spot in the three different intervals studied. Such comparisons are limited, however, because the *trans*-acting modifiers in the *cis* studies most likely differ from those in the inbred backgrounds in this study.

Do the *trans*-acting factors act globally or on specific genetic intervals? *Trans*-acting modifiers of meiotic recombination (reviewed in Wahls 1998) have been divided into two categories: those that affect recombination across the entire genome (*i.e.*, global modifiers) and those that act only on specific (*i.e.*, region-specific modifiers) genetic intervals (Catcheside 1977). Global *trans*-acting modifiers are most likely proteins intimately involved in the mechanisms of recombination (reviewed in van den Bosch *et al.* 2002). For example, in yeast rates of meiotic CO are globally reduced in mutants of DNA polymerase δ (Maloisel *et al.* 2004). In maize, *desynaptic* (Ji *et al.* 1999), which is involved in crossover control, affects recombination across the genome (Bass *et al.* 2003).

To test whether or not the *trans*-acting factors that affect the *a1–sh2* interval are global modifiers, in each genetic background recombination was assayed in two intervals genetically unlinked to *a1–sh2*. In both intervals on chromosome 1, the relationships among genetic distances were the same. However, these relationships differed from that seen in the *a1–sh2* interval (compare Tables 1 and 4). This could be due to the same *trans*-acting modifier(s) within a genetic background differentially affecting recombination in chromosomes 1S and 3L. Alternatively, different region-specific modifiers could be acting on at least portions of each of the two chromosomes. In addition, because the 1S.1 and 1S.2 intervals are most likely not sequence-identical among the backgrounds we cannot rule out the possibility that a combination of both *trans*- and *cis*-acting elements is affecting recombination in these intervals. Hence, these results suggest, but do not prove, that the *trans*-acting modifiers detected in this study do not have global effects and are instead region specific.

Region-specific *trans*-acting modifiers have been characterized in *Schizosaccharomyces pombe* (De Veaux *et al.* 1992; De Veaux and Smith 1994; Li *et al.* 1997; Krawchuk *et al.* 1999; Pryce *et al.* 2005) and *Saccharo-myces cerevisiae* (Rockmill and Roeder 1990). The effects of chromatin organization on DSB formation and subsequent meiotic recombination repair have been extensively demonstrated in many organisms (reviewed in Lichten 2001; Petes 2001), including plant species (reviewed in Schuermann *et al.* 2005). In *S. pombe, rec10*, a protein involved in the formation of lateral elements, is required for the activation of some but not all *M26*-containing recombination hot spots (Pryce *et al.* 2005). In this case, higher-order chromatin structure affects recombination at a known hot spot. *Trans*-acting modifiers involved in chromatin organization or remodeling might be responsible for the transformation of the nongenic subinterval VII from an average spot in Oh43/LC to a hot spot in the A632/LC genetic background by opening the chromatin and allowing access by the recombination machinery. If so, *trans*-acting modifiers of chromatin most likely act in context of local structures (*e.g.*, sequence motifs). Alternatively, genotype-specific patterns of crossover interference (reviewed in van Veen and Hawley 2003; Hillers 2004; Copenhaver 2005) could differently affect the distributions of recombination hot spots across a chromosome among genetic backgrounds.

Region-specific *trans*-acting modifiers could also include proteins (*e.g.*, transcription factors) for which binding to specific *cis*-acting elements [*e.g.*, unique sequence motifs (Wahls and Smith 1994; Kon *et al.* 1997; Pryce *et al.* 2005) or promoters] is necessary for activation of recombination hot spots as seen in α-hot spots in yeast (reviewed in Lichten and Goldman 1995; Petes 2001). Such interactions between *cis*-acting elements and *trans*-acting modifiers, if they occur in *a1–sh2*, must be due to differences among the *trans*-acting modifiers (*e.g.*, binding specificity or affinity for the *cis* element) because the *a1–sh2* intervals are sequence-identical among the genetic backgrounds.

LITERATURE CITED

Anderson, L., A. Lai, S. M. Stack, C. Rizzon and B. S. Grant, 2006 Uneven distribution of expressed sequence tag loci on maize pachytene chromosomes. Genome Res. **16:** 115–122.

Barnes, T. M., Y. Kohara, A. Coulson and S. Hekimi, 1995 Meiotic recombination, noncoding DNA and genomic organization in *Caenorhabditis elegans.* Genetics **141:** 159–179.

Bass, H. W., S. J. Bordoli and E. M. Foss, 2003 The desynaptic (dy) and desynaptic1 (dsy1) mutations in maize (Zea mays L) cause distinct telomere-misplacement phenotypes during meiotic prophase. J. Exp. Bot. **54:** 39–46.

Beavis, W. D., and D. Grant, 1991 A linkage map based on information from four F$_2$ populations of maize (*Zea mays* L.). Theor. Appl. Genet. **82:** 636–644.

Borts, R. H., S. R. Chambers and M. F. Abdullah, 2000 The many faces of mismatch repair in meiosis. Mutat. Res. **451:** 129–150.

Catcheside, D. G., 1977 *The Genetics of Recombination.* Edward Arnold Limited, London.

Civardi, L., Y. Xia, K. J. Edwards, P. S. Schnable and B. J. Nikolau, 1994 The relationship between genetic and physical distances in the cloned *a1-sh2* interval of the *Zea mays* L. genome. Proc. Natl. Acad. Sci. USA **91:** 8268–8272.

Copenhaver, G. P., 2005 Plant genetics: when not to interfere. Curr. Biol. **15:** R290–R291.

Delaney, D., S. Nasuda, T. R. Endo, B. S. Gill and S. H. Hulbert, 1995a Cytogenetically based physical maps of the group-2 chromosomes of wheat. Theor. Appl. Genet. **91:** 568–573.

Delaney, D., S. Nasuda, T. R. Endo, B. S. Gill and S. H. Hulbert, 1995b Cytogenetically based physical maps of the group-3 chromosomes of wheat. Theor. Appl. Genet. **91:** 780–782.

DeVeaux, L. C., and G. R. Smith, 1994 Region-specific activators of meiotic recombination in Schizosaccharomyces pombe. Genes Dev. **8:** 203–210.

DeVeaux, L. C., N. A. Hoagland and G. R. Smith, 1992 Seventeen complementation groups of mutations decreasing meiotic recombination in *Schizosaccharomyces pombe*. Genetics **130:** 251–262.

Dietrich, C. R., F. Cui, M. L. Packila, J. Li, D. A. Ashlock et al., 2002 Maize *Mu* transposons are targeted to the 5′ untranslated region of the *gl8* gene and sequences flanking *Mu* target-site duplications exhibit nonrandom nucleotide composition throughout the genome. Genetics **160:** 697–716.

Dooner, H. K., 2002 Extensive interallelic polymorphisms drive meiotic recombination into a crossover pathway. Plant Cell **14:** 1173–1183.

Dooner, H. K., and I. M. Martinez-Ferez, 1997 Recombination occurs uniformly within the *bronze* gene, a meiotic recombination hotspot in the maize genome. Plant Cell **9:** 1633–1646.

Eggleston, W. B., M. Alleman and J. L. Kermicle, 1995 Molecular organization and germinal instability of *R-stippled* maize. Genetics **141:** 347–360.

Evans, E., and E. Alani, 2000 Roles for mismatch repair factors in regulating genetic recombination. Mol. Cell. Biol. **20:** 7839–7844.

Fatmi, A., C. G. Poneleit and T. W. Pfeiffer, 1993 Variability of recombination frequencies in the Iowa Stiff Stalk Synthetic (*Zea mays* L.). Theor. Appl. Genet. **86:** 859–866.

Fu, H., Z. Zheng and H. K. Dooner, 2002 Recombination rates between adjacent genic and retrotransposon regions in maize vary by 2 orders of magnitude. Proc. Natl. Acad. Sci. USA **99:** 1082–1087.

Gerton, J. L., J. DeRisi, R. Shroff, M. Lichten, P. O. Brown et al., 2000 Inaugural article: global mapping of meiotic recombination hotspots and coldspots in the yeast Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **97:** 11383–11390.

Gill, K. S., B. S. Gill and T. R. Endo, 1993 A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. Chromosoma **102:** 374–381.

Gill, K. S., B. S. Gill, T. R. Endo and E. V. Boyko, 1996a Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics **143:** 1001–1012.

Gill, K. S., B. S. Gill, T. R. Endo and T. Taylor, 1996b Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics **144:** 1883–1891.

Hillers, K. J., 2004 Crossover interference. Curr. Biol. **14:** R1036–R1037.

Hohmann, U., T. R. Endo, K. S. Gill and B. S. Gill, 1994 Comparison of genetic and physical maps of group 7 chromosomes from Triticum aestivum L. Mol. Gen. Genet. **245:** 644–653.

Ji, Y., D. M. Stelly, M. De Donato, M. M. Goodman and C. G. Williams, 1999 A candidate recombination modifier gene for *Zea mays* L. Genetics **151:** 821–830.

Kagawa, N., K. Nagaki and H. Tsujimoto, 2002 Tetrad-FISH analysis reveals recombination suppression by interstitial heterochromatin sequences in rye (Secale cereale). Mol. Genet. Genomics **267:** 10–15.

Koehler, K. E., J. P. Cherry, A. Lynn, P. A. Hunt and T. J. Hassold, 2002 Genetic control of mammalian meiotic recombination. I. Variation in exchange frequencies among males from inbred mouse strains. Genetics **162:** 297–306.

Kon, N., M. D. Krawchuk, B. G. Warren, G. R. Smith and W. P. Wahls, 1997 Transcription factor Mts1/Mts2 (Atf1/Pcr1, Gad7/Pcr1) activates the M26 meiotic recombination hotspot in Schizosaccharomyces pombe. Proc. Natl. Acad. Sci. USA **94:** 13765–13770.

Krawchuk, M. D., L. C. DeVeaux and W. P. Wahls, 1999 Meiotic chromosome dynamics dependent upon the *rec8*$^+$, *rec10*$^+$ and *rec11*$^+$ genes of the fission yeast *Schizosaccharomyces pombe*. Genetics **153:** 57–68.

Kunzel, G., L. Korzun and A. Meister, 2000 Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics **154:** 397–412.

Lambie, E. J., and G. S. Roeder, 1986 Repression of meiotic crossing over by a centromere (CEN3) in *Saccharomyces cerevisiae*. Genetics **114:** 769–789.

Lee, M., N. Sharopova, W. D. Beavis, D. Grant, M. Katt et al., 2002 Expanding the genetic map of maize with the intermated B73 × Mo17 (IBM) population. Plant Mol. Biol. **48:** 453–461.

Li, Y. F., M. Numata, W. P. Wahls and G. R. Smith, 1997 Region-specific meiotic recombination in Schizosaccharomyces pombe: the rec11 gene. Mol. Microbiol. **23:** 869–878.

Lichten, M., 2001 Meiotic recombination: breaking the genome to save it. Curr Biol **11:** R253–R256.

Lichten, M., and A. S. Goldman, 1995 Meiotic recombination hotspots. Annu. Rev. Genet. **29:** 423–444.

Liu, K., M. Goodman, S. Muse, J. S. Smith, E. Buckler et al., 2003 Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics **165:** 2117–2128.

Mahtani, M. M., and H. F. Willard, 1998 Physical and genetic mapping of the human X chromosome centromere: repression of recombination. Genome Res. **8:** 100–110.

Maloisel, L., J. Bhargava and G. S. Roeder, 2004 A role for DNA polymerase δ in gene conversion and crossing over during meiosis in *Saccharomyces cerevisiae*. Genetics **167:** 1133–1142.

Mickelson-Young, L., T. R. Endo and B. S. Gill, 1995 A cytogenetic ladder-map of wheat homoeologous group-4 chromosomes. Theor. Appl. Genet. **90:** 1007–1011.

Modrich, P., and R. Lahue, 1996 Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. **65:** 101–133.

Nachman, M. W., 2002 Variation in recombination rate across the genome: evidence and implications. Curr. Opin. Genet. Dev. **12:** 657–663.

Nass, L. L., and E. Paterniani, 2000 Pre-breeding: A link between genetic resources and maize breeding. Scientia Agricola **57:** 581–587.

Patterson, G. I., K. M. Kubo, T. Shroyer and V. L. Chandler, 1995 Sequences required for paramutation of the maize *b* gene map to a region containing the promoter and upstream sequences. Genetics **140:** 1389–1406.

Petes, T. D., 2001 Meiotic recombination hot spots and cold spots. Nat. Rev. Genet. **2:** 360–369.

Pryce, D. W., A. Lorenz, J. B. Smirnova, J. Loidl and R. J. McFarlane, 2005 Differential activation of M26-containing meiotic recombination hot spots in *Schizosaccharomyces pombe*. Genetics **170:** 95–106.

Puchta, H., and B. Hohn, 1996 From centiMorgans to base pairs: homologous recombination in plants. Trends Genet. **1:** 340–348.

Puechberty, J., A. M. Laurent, S. Gimenez, A. Billault, M. E. Brun-Laurent et al., 1999 Genetic and physical analyses of the centromeric and pericentromeric regions of human chromosome 5: recombination across 5cen. Genomics **56:** 274–287.

Rockmill, B., and G. S. Roeder, 1990 Meiosis in asynaptic yeast. Genetics **126:** 563–574.

Sanchez-Moran, E., S. J. Armstrong, J. L. Santos, F. C. Franklin and G. H. Jones, 2002 Variation in chiasma frequency among eight accessions of *Arabidopsis thaliana*. Genetics **162:** 1415–1422.

Schnable, P. S., A. P. Hsia and B. J. Nikolau, 1998   Genetic recombination in plants. Curr. Opin. Plant Biol. **1:** 123–129.

Schofield, M. J., and P. Hsieh, 2003   DNA mismatch repair: molecular mechanisms and biological function. Annu. Rev. Microbiol. **57:** 579–608.

Schuermann, D., J. Molinier, O. Fritsch and B. Hohn, 2005   The dual nature of homologous recombination in plants. Trends Genet. **21:** 172–181.

Simchen, G., and J. Stamberg, 1969   Fine and coarse controls of genetic recombination. Nature **222:** 329–332.

Sun, H., D. Treco and J. W. Szostak, 1991   Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the ARG4 recombination initiation site. Cell **64:** 1155–1161.

Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein and F. W. Stahl, 1983   The double-strand-break repair model for recombination. Cell **33:** 25–35.

Timmermans, M. C., O. P. Das, J. M. Bradeen and J. Messing, 1997   Region-specific *cis*- and *trans*-acting factors contribute to genetic variability in meiotic recombination in maize. Genetics **146:** 1101–1113.

Tulsieram, L., W. A. Compton, R. Morris, M. Thomas-Compton and K. Eskridge, 1992   Analysis of genetic recombination in maize populations using molecular markers. Theor Appl Genet **84:** 65–72.

van den Bosch, M., P. H. Lohman and A. Pastink, 2002   DNA double-strand break repair by homologous recombination. Biol. Chem. **383:** 873–892.

van Veen, J.E., and R.S. Hawley, 2003   Meiosis: when even two is a crowd. Curr. Biol. **13:** R831–R833.

Wahls, W. P., 1998   Meiotic recombination hotspots: shaping the genome and insights into hypervariable minisatellite DNA change. Curr. Top. Dev. Biol. **37:** 37–75.

Wahls, W. P., and G. R. Smith, 1994   A heteromeric protein that binds to a meiotic homologous recombination hot spot: correlation of binding and hot spot activity. Genes Dev. **8:** 1693–1702.

Werner, J. E., T. R. Endo and B. S. Gill, 1992   Toward a cytogenetically based physical map of the wheat genome. Proc. Natl. Acad. Sci. USA **89:** 11307–11311.

Williams, C. G., M. M. Goodman and C. W. Stuber, 1995   Comparative recombination distances among *Zea mays* L. inbreds, wide crosses and interspecific hybrids. Genetics **141:** 1573–1581.

Xu, X., A. P. Hsia, L. Zhang, B. J. Nikolau and P. S. Schnable, 1995   Meiotic recombination break points resolve at high rates at the 5′ end of a maize coding sequence. Plant Cell **7:** 2151–2161.

Yandeau-Nelson, M. D., Q. Zhou, H. Yao, X. Xu, B. J. Nikolau *et al.*, 2005   *MuDR* transposase increases the frequency of meiotic crossovers in the vicinity of a *Mu* insertion in the maize *a1* gene. Genetics **169:** 917–929.

Yao, H., and P. S. Schnable, 2005   *Cis*-effects on meiotic recombination across distinct *a1-sh2* intervals in a common Zea genetic background. Genetics **170:** 1929–1944.

Yao, H., Q. Zhou, J. Li, H. Smith, M. Yandeau *et al.*, 2002   Molecular characterization of meiotic recombination across the 140-kb multigenic a1-sh2 interval of maize. Proc. Natl. Acad. Sci. USA **99:** 6157–6162.