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# *Cis*-effects on Meiotic Recombination Across Distinct *a1-sh2* Intervals in a Common Zea Genetic Background

**Hong Yao*,†,[1] and Patrick S. Schnable*,†,‡,§,[2]**

*Interdepartmental Genetics Program, †Department of Genetics, Development and Cell Biology, ‡Department of Agronomy and §Center for Plant Genomics, Iowa State University, Ames, Iowa 50011-3650*

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ABSTRACT

Genetic distances across the *a1-sh2* interval varied threefold in three near-isogenic stocks that carry structurally distinct teosinte *A1 Sh2* haplotypes (from *Z. mays* spp. *mexicana* Chalco, *Z. mays* spp. *parviglumis*, and *Z. luxurians*) and a common maize *a1::rdt sh2* haplotype. In each haplotype >85% of recombination events resolved in the proximal 10% of the ~130-kb *a1-sh2* interval. Even so, significant differences in the distributions of recombination breakpoints were observed across subintervals among haplotypes. Each of the three previously detected recombination hot spots was detected in at least one of the three teosinte haplotypes and two of these hot spots were not detected in at least one teosinte haplotype. Moreover, novel hot spots were detected in two teosinte haplotypes. Due to the near-isogenic nature of the three stocks, the observed variation in the distribution of recombination events is the consequence of *cis*-modifications. Although generally negatively correlated with rates of recombination per megabase, levels of sequence polymorphisms do not fully account for the nonrandom distribution of recombination breakpoints. This study also suggests that estimates of linkage disequilibrium must be interpreted with caution when considering whether a gene has been under selection.

HOMOLOGOUS recombination provides physical connections between pairs of homologous chromosomes during meiosis and thereby helps to prevent nondisjunction. In addition, meiotic recombination generates novel haplotypes upon which natural selection can act. Two types of recombination events result from meiotic recombination: reciprocal crossovers (CO) and unidirectional noncrossovers (NCO). Although evidence from yeast has shown that both events are initiated by double-strand breaks (DSB) (reviewed by Paques and Haber 1999), these two types of events probably arise via different pathways (Allers and Lichten 2001; Hunter and Kleckner 2001; Clyne *et al.* 2003). COs are thought to arise via the DSB repair pathway (Szostak *et al.* 1983; Cao *et al.* 1990; Sun *et al.* 1991), which involves the formation of double Holliday junctions (DHJs) following strand invasion; resolution of these DHJs can result in COs. Although NCOs could also arise via this pathway (following an alternative resolution of DHJs), several pieces of evidence suggest that NCO events may instead arise from the synthesis-dependent strand-annealing pathway that does not involve the formation of DHJs (reviewed by Paques and Haber 1999; Allers and Lichten 2001; Hunter and Kleckner 2001).

Meiotic recombination does not occur randomly in a genome or across a chromosome. Eukaryotic genomes contain recombination hot and cold spots where the rates of recombination per megabase are much higher and lower, respectively, than average (reviewed by Lichten and Goldman 1995; Puchta and Hohn 1996; Schnable *et al.* 1998; Petes 2001). Surprisingly, although the DNA sequences of the human and chimp genomes are highly similar, some human hot spots (*e.g.*, TAP2) are not conserved in chimps (Pennisi 2004; Ptak *et al.* 2004). This is consistent with the finding that within a species, *cis*- and *trans*-genetic modifiers can affect the nonrandom occurrence of meiotic recombination in a genome. *Cis*-regulation of recombination has been demonstrated in studies of fungi, mammals, and plants. In fungi, hot spots are classified as α, β, and γ according to the natures of the sequences that cause the hyperrecombination activity (reviewed by Petes 2001). α-hot spots are caused by sequences that are transcription factor binding sites and that require the binding of transcription factors to activate the hot spot. β-hot spots are caused by sequences that are thought to cause the exclusion of nucleosomes, resulting in higher accessibility of a region to the recombination machinery. γ-hot spots are associated with sequences with high G + C content. In addition to the natures of sequences within or in the vicinity of a hot spot that can regulate recombi-

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[1]*Present address:* Department of Biological Sciences, University of Missouri, Columbia, MO 65211.

[2]*Corresponding author:* Department of Agronomy, 2035B Roy J. Carver Co-Laboratory, Iowa State University, Ames, IA 50011-3650. E-mail: schnable@iastate.edu

nation in *cis*, sequence polymorphisms between DNA segments residing on a pair of homologs can affect both recombination rates per megabase and the distribution of recombination events. Both large insertion/deletion polymorphisms (InDeLs) and a high density of small sequence polymorphisms, including single nucleotide polymorphisms (SNPs) and small InDeLs, reduce recombination rates per megabase in fungi, mammals, and plants (reviewed by Modrich and Lahue 1996; Schnable *et al.* 1998; Borts *et al.* 2000). In *Saccharomyces cerevisiae*, two small sequence polymorphisms are sufficient to significantly decrease rates of meiotic recombination (Borts *et al.* 1990).

In maize, characterized *cis*-modifiers of meiotic recombination include heterochromatic centromeres that reduce frequency of COs in nearby regions; heterozygous knobs that are heterochromatic have similar effects (Carlson 1977; Rhoades 1978). Polymorphisms due to chromosome rearrangements caused by large deletions, inversions, and translocations also reduce recombination rates per megabase (Robertson 1967, 1984; Phillips 1969; Carlson 1977). Timmermans *et al.* (1997) identified a *cis*-factor in the *Sh1-Bz1* interval from the inbred line A188 that increases recombination rates per megabase locally, but the nature of this factor has not been defined. Higher-resolution analyses of *cis*-modifiers of meiotic recombination have been performed in genic recombination hot spots of maize. As is true in other species, sequence polymorphisms in maize genes can influence recombination in *cis*, although the impact seems to be significantly less than that in other species. Recombination rates per megabase in the *a1* (Xu *et al.* 1995) and *bz1* (Dooner and Martinez-Ferez 1997) loci are suppressed by nonautonomous transposon insertions. Sequence polymorphisms at the *bz1* locus also affect recombination resolution sites and the ratio of NCO/CO events (Dooner and Martinez-Ferez 1997; Dooner 2002). The insertion of a *Mu1* transposon at the 5′-end of the *a1* gene, however, does not change the pattern of recombination resolution (Xu *et al.* 1995). These studies of intragenic recombination have revealed *cis*-modifiers that influence meiotic recombination in maize genes. Nevertheless, absent an analysis of the *cis*-effects on the rates per megabase and distribution of recombination across a multigenic interval, it is not possible to answer questions such as why genes are more likely than intergenic regions to be recombination hot spots and whether intragenic and intergenic recombination are similarly regulated by *cis*-modifiers.

To answer these questions, the *a1-sh2* interval was used as a model. This region was selected because: (1) the multigenic nature of the *a1-sh2* interval (Yao *et al.* 2002) allows us to compare *cis*-effects on intragenic as well as intergenic recombination; (2) previous characterization of the distribution of recombination events across the *a1-sh2* interval identified an apparently nongenic hot spot and a genic non-hot spot (Yao *et al.* 2002), the analysis of which is informative; (3) the two genic markers defining this interval, *a1* and *sh2*, give kernel phenotypes that facilitate the isolation of meiotic recombinants.

In the current study, ~500 recombination events were isolated from near-isogenic plants that carried *A1 Sh2* haplotypes extracted from a maize inbred line and three maize relatives (*Z. mays* ssp. *mexicana* Chalco, *Z. mays* ssp. *parviglumis* and *Z. luxurians*) in combination with a common maize *a1 sh2* haplotype. Phylogenic studies suggest that maize arose from *Z. mays* ssp. *parviglumis* ~9000 years ago (Matsuoka *et al.* 2002) and diverged from ssp. *mexicana* ~75,000 years ago and that *Z. mays* diverged from *Z. luxurians* ~135,000 years ago (Hanson *et al.* 1996). As predicted by these evolutionary relationships, the *A1 Sh2* haplotypes used in this study are structurally diverse. This allowed us to observe the effects of varying levels of sequence divergence on recombination and to identify putative specific *cis*-modifiers that cosegregate with the *a1-sh2* intervals. Rates of recombination per megabase across the *a1-sh2* interval vary among the *A1 Sh2* haplotypes. Similarly, the distributions of recombination breakpoints within the *a1-sh2* interval also differ significantly among haplotypes. Each of three hot spots detected in a prior study was detected in at least one of the teosinte haplotypes and two of these hot spots were not detected in at least one teosinte haplotype. In addition, novel hot spots were detected in two of the teosinte haplotypes. These variations in recombination activity can be attributed to the *cis*-effects related to the divergent sequences of the *A1 Sh2* haplotypes.

## MATERIALS AND METHODS

**Maize genetic stocks:** The stocks used to produce progenies carrying recombinant *a1 sh2* haplotypes were derived from genetic crosses between the near-inbred maize *a1::rdt sh2* stock and three teosinte lines: *Z. mays* ssp. *mexicana* Chalco (Schnable lab accession no. 294; Iltis 28620), *Z. mays* ssp. *parviglumis* (Schnable lab accession no. 1322-292; Doebley 1993–1994 292), and *Z. luxurians* (Schnable lab accession no. 291; Beadle VII.A.4) as well as the maize inbred line C (a color-converted version of W22). Like the *A1-LC* allele from line C, the *A1* alleles derived from teosinte condition colored kernel phenotypes and in this report are designated *A1-mex*, *A1-par*, and *A1-lux*. The *a1::rdt* allele conditions a recessive colorless kernel phenotype because the function of the *a1* gene is disrupted by the *rdt* transposon insertion (Brown *et al.* 1989). The functional *Sh2* alleles derived from teosinte and line C condition a round kernel phenotype. Kernels homozygous for the mutant *sh2* alleles are shrunken (Mains 1949; Laughnan 1953; Hannah and Nelson 1976).

Stocks used to isolate meiotic recombinants were developed by introgressing the *A1 Sh2* haplotypes from the three teosinte lines and maize inbred line C into the maize *a1::rdt_ sh2* stock. First, $F_1$ plants were generated from crosses between the maize *a1::rdt sh2* stock and the three teosinte lines as well as line C. Then a single $F_1$ plant carrying the *A1 Sh2* haplotype from each teosinte and line C was selected to backcross to the *a1::rdt sh2* stock for 4–5 generations (teosinte) or 10 generations (line C). In each generation, colored round kernels carrying

the *A1 Sh2* haplotypes were selected for the next generation of backcrosses. The resulting stocks carry distinct *A1 Sh2* haplotypes in a common genetic background that is derived from the near-inbred *a1::rdt sh2* stock and have the genotype of *A1 Sh2/a1::rdt sh2*. In this article these heterozygous stocks are also referred to as the mex, par, lux, and LC2 stocks and the corresponding *A1 Sh2* haplotypes as the mex, par, lux, and LC haplotypes.

**Isolation and confirmation of meiotic recombinants and calculation of genetic distance:** The mex, par, lux, and LC2 stocks were used as female parents (listed first) and the near-inbred *a1::rdt sh2* stock as the male parent in the genetic crosses *A1 Sh2/a1::rdt sh2* × *a1::rdt sh2/a1::rdt sh2*, to generate meiotic recombinants (Table 2) following procedures similar to those described previously (Civardi *et al.* 1994; Xu *et al.* 1995). Kernels from these crosses that exhibit nonparental phenotypes (colored shrunken and colorless round *vs.* parental colored round and colorless shrunken) presumably carry recombinant chromosomes (designated *A1* sh2* and *a1* Sh2*) resulting from meiotic recombination that could be COs between the *a1* and *sh2* loci or NCOs (*e.g.*, gene conversions) at the *a1* or *sh2* loci.

Samples of the putative recombinants from each source were tested via genetic crosses and molecular marker analysis as described previously (Xu *et al.* 1995; Yao *et al.* 2002). On the basis of the frequency of putative recombinants confirmed within each sample, the number of the true recombinants isolated from each cross could be estimated and used to calculate the genetic distance between the *a1* and *sh2* loci in the corresponding female parent. Stocks homozygous for the recombinant haplotypes (*A1* sh2* and *a1* Sh2*) were generated as described previously (Civardi *et al.* 1994; Xu *et al.* 1995) and used to map the recombination breakpoints.

Breakpoints associated with the confirmed recombinants from the LC2 stock were not physically mapped because a detailed analysis of the distribution of recombination breakpoints associated with the LC haplotype had been conducted previously using a different stock (referred to as the LC1 stock in this article) that carries the same *A1 Sh2* and *a1::rdt sh2* haplotypes as the LC2 stock (Yao *et al.* 2002).

Because no significant differences (*P*-values $> 0.05$) were observed between the distributions of breakpoints associated with the two classes of recombinants (*A1* sh2 vs. a1* Sh2*), these two classes of recombinants were combined for subsequent analyses.

**Sequences of the *A1 Sh2* haplotypes from the three teosinte lines:** Portions of the *A1 Sh2* haplotype from line C (the "*A1-LC Sh2*" haplotype; Yao *et al.* 2002) (GenBank accession nos. AF434192, AF347696, AF363390, X05068, and AF363391) and the *a1::rdt sh2* haplotype (GenBank accession no. AF072704) have been sequenced previously. To sequence the corresponding regions of the three teosinte *A1 Sh2* haplotypes used in this study, plants with the genotype *A1 Sh2* (teosinte)/*a1::rdt sh2* were self-pollinated. Colored and round kernels were planted. DNA samples isolated from plants that are homozygous for the teosinte *A1 Sh2* haplotypes were PCR amplified using primers from the *a1*, *yz1* loci and the interloop region (IR) (Yao *et al.* 2002) between the two loci (Figure 2A). Purified PCR products were then sequenced directly.

The 11-kb *a1-yz1* interval from *Z. mays* ssp. *mexicana* Chalco (GenBank accession no. AY662984) was assembled from sequences of eight overlapping PCR fragments that ranged in size from ~1 to 3.5 kb. Results obtained from RFLP analyses using probes derived from the *a1* and *yz1* loci and partial sequencing of the amplified product from long-range PCR conducted using primers that anneal to the *a1* and *yz1* loci confirmed the organization of the assembled sequence of the 11-kb *a1-yz1* interval (data not shown). The 6.4-kb *a1-yz1* interval from *Z. luxurians* (GenBank accession no. AY662985) was assembled from sequences of five overlapping PCR fragments that ranged in size from ~0.5 to 2.5 kb, one of which includes the entire intergenic region and overlaps both the *a1* and the *yz1* locus. The entire *a1-yz1* interval from *Z. mays* ssp. *parviglumis* could not be amplified. A 3.9-kb sequence (GenBank accession no. AY662986) from *yz1* to the distal portion of the interloop region was assembled from the sequences of four overlapping PCR fragments of ~0.25 to 1.7 kb. Another 2.3-kb sequence (GenBank accession no. AY662987), including part of *A1-par* and its 5′ upstream region, was assembled from two overlapping PCR fragments of ~1.1 and 1.5 kb. The region between these two sequenced segments could not be PCR amplified.

Portions (part of exon 2 to part of exon 7) of the three teosinte *X1* alleles were also PCR amplified and sequenced. For each of the three *X1* alleles (GenBank accessions nos. AY656756–AY656758), sequences (3.6 kb for *X1-mex* and *X1-par* and 3.3 kb for *X1-lux*) were assembled from three overlapping PCR fragments of ~1.5 (for *X1-mex* and *X1-par*) or 1.3 (for *X1-lux*) to 1.8 kb.

**Oligonucleotides for PCR and sequencing:** Sequence comparisons between the three teosinte *A1 Sh2* haplotypes and the *a1::rdt sh2* haplotype revealed many polymorphisms, including SNPs and InDeLs, which can be used as markers to map the recombination breakpoints. Oligonucleotides were designed on the basis of sequences from the three teosinte *A1 Sh2* haplotypes as well as the maize *a1::rdt sh2* haplotype. Details regarding these primers, including their haplotype specificities, are presented in Table 1. These primers were used for PCR amplification and sequencing to map the recombination breakpoints relative to sequence polymorphisms that exist between the maize *a1::rdt sh2* haplotype and the three teosinte *A1 Sh2* haplotypes. All the sequence polymorphisms used as genetic markers cosegregate with the *a1-sh2* interval in genetic crosses, further confirming that the assemblies of the sequences of the teosinte *A1 Sh2* haplotypes are correct.

**Statistical methods:** Homogeneity $\chi^2$ tests were used to compare genetic distances/recombination rates per megabase between the *a1* and *sh2* loci among the mex, par, lux, and LC haplotypes (Table 2, Figure 1A). In these tests, the corrected numbers of recombinants and population sizes from each stock were used (Table 2). The rates of recombination per megabase in each of the subintervals defined by sequence polymorphisms (Figures 2D, 3E, and 4D) were also compared with different teosinte *A1 Sh2* haplotypes. Because not all of the recombinants between the *a1* and *sh2* loci could be mapped (*e.g.*, some were not recovered), the sizes of populations that correspond to the numbers of mapped recombinants were calculated using the following formula: actual population size × (number of mapped recombinants/number of corrected recombinants). The numbers of mapped recombinants and their corresponding population sizes were then used in the homogeneity $\chi^2$ test. These calculated population sizes were also used to obtain expected numbers of recombinants in each subinterval, assuming that the rate of recombination per megabase across the *a1-sh2* interval was equal to the genome's average (2.1 cM/Mb). Then the expected and actual numbers of recombinants mapped to a subinterval as well as the corresponding calculated population size were used in the goodness-of-fit $\chi^2$ test to compare the observed rate of recombination per megabase in a subinterval to the genome's average. Via a similar approach, the observed rate of recombination per megabase in a subinterval was compared to the average rate of recombination per megabase between the *a1* and *sh2* loci using the goodness-of-fit $\chi^2$ test. The distributions of recombination breakpoints in a given subinterval from different teosinte haplotypes were compared via the $\chi^2$ contin-

**TABLE 1**

**Oligonucleotides used as primers for PCR and sequencing**

| Primer | Sequences[a] | Haplotypes[b] | | | |
|---|---|---|---|---|---|
| | | *a1::rdt sh2* | mex | par | lux |
| rdt444 | AGCAAATAGCAATAATCAAGGCA | + | − | − | − |
| aIDPrdt4 | AATTAGTCTCTCGATCATCT | + | − | − | − |
| aIDPrdt3 | CTAAAGAAGCAAAGCAA | + | − | − | − |
| yzIDPrt5 | GCATGTTAAAAATAGAAGAAG | + | − | − | − |
| yzIDPrt4 | TTCACACAAAAAAAGGC | + | − | − | − |
| yzIDPrt3 | CTAGGAGTACATGTTTTTTC | + | − | − | − |
| IDPrdtx | TAATTCTAGTGTCCCAAC | + | − | − | − |
| QZ1001 | GATACAGAAGTATATATAAGGGCCAA | + | + | − | − |
| a1rdt2912 | AACACCCCGCTAACAC | + | + | − | − |
| a1rdt1541 | CGCTAACTATCTCGGTAACT | + | + | − | − |
| QZ1002 | TATTCGTAATGATGTTTAT | + | − | + | − |
| ajl001 | GGAGAGTCGAATAAAAAGTGT | + | + | + | − |
| a1rdt2381 | TCAACCGTGCTACCAACT | + | + | + | − |
| IrlL3 | ATCGGCAAACCCACCAA | + | + | − | + |
| ZH792 | GCGGTTGCGGCTTGT | + | + | − | + |
| IDPIRmex | GTAAGTCTCTATCCAGTC | − | + | − | − |
| YZ4725 | AAATGGTCAGGATAGCTTAGTT | − | + | − | − |
| ZH1384 | GCCATCTCTACTGTTACCTT | − | + | − | − |
| IDPyz5lr | TATCAAGCACAAGCAG | − | − | − | + |
| yzIDPmpl | AGTAGAGAGGAAATCAGAAG | − | + | + | + |
| A1.2 | GATTGTTGCTTAAGCGCCAATCGT | + | + | + | + |
| AE4EI | CGAATTCCGCCAGGGTTTTAGACA | + | + | + | + |
| XX390 | TCGGCTTGATTACCTCATTCT | + | + | + | + |
| yz3utrf | CGGGGGTTGCAGTCATTGAC | + | + | + | + |
| YZ3 | GGAAGCCTGTTTTGGTG | + | + | + | + |
| yz4127F | CATCATCTCCGTGTTCTC | + | + | + | + |
| ZH1748 | CACATCCCCGTCTCCT | + | + | + | + |
| ZH2617 | CGAACAGGGAAGAATGG | + | + | + | + |
| YZ1 | GCGGCGTTGCTGCTGTA | + | + | + | + |
| YZc85 | GGAGACGGGGATGTGG | + | + | + | + |
| XL2 | TGTTCAAAGTGGGAGG | + | + | + | + |

+, a primer that can amplify the corresponding haplotype; −, a primer that cannot amplify the corresponding haplotype.

[a] Sequences are listed 5′–3′.

[b] The mex, par, and lux haplotypes are *A1 Sh2*.

gency test. These distributions were also compared to the expected patterns obtained under the null hypothesis that recombination events resolve randomly in a given subinterval via the $\chi^2$ contingency test. The Freeman-Halton test (Freeman and Halton 1951) was used to check the reliability of the $\chi^2$ and *P*-values for subintervals that contain fewer than five recombination breakpoints. The Freeman-Halton test conducts multiple permutations of the original data to estimate the chance of obtaining a $\chi^2$ value that is equal to or greater than the value from the original $\chi^2$ contingency test. $\chi^2$ values and the resulting *P*-values obtained from the original tests were considered reliable if the chance calculated by the Freeman-Halton test (10,000 permutations) was $<0.05$. All $\chi^2$ contingency tests reported as being statistically significant had Freeman-Halton *P*-values of $<0.05$.

The level of sequence polymorphisms was calculated as the absolute number of polymorphisms (counting each SNP and InDeL one time) between a given *A1 Sh2* haplotype and the common *a1::rdt sh2* haplotype carried by all stocks per 100 bp of the *a1::rdt sh2* haplotype. The correlation coefficient of the levels of sequence polymorphisms and the rates of recombination per megabase were calculated across all three haplotypes. For these calculations, data from subintervals I-1, I-2, II, III, IV-1, IV-2, IV-3, and VI (Figure 2, D and E; Figure 3, E and F; and Figure 4, D and E) in that haplotype were pooled. The significance of the correlation coefficient was determined using Student's *t*-tests. A conservative estimate of the level of sequence polymorphisms in the partially sequenced subinterval III-par was obtained by dividing the number of sequence polymorphisms in the sequenced portion by the entire length of this subinterval in the common *a1::rdt sh2* haplotype.

## RESULTS

**Recombination rates per megabase between the *a1* and *sh2* loci differ among haplotypes:** To characterize *cis*-effects on meiotic recombination across the *a1-sh2* interval, near-isogenic mex, par, lux, and LC2 stocks that carry distinct *A1 Sh2* haplotypes (referred to as

mex, par, lux, and LC haplotypes, respectively) from three teosinte lines, *Z. mays* ssp. *mexicana* Chalco, *Z. mays* ssp. *parviglumis*, and *Z. luxurians* and from the maize inbred line C were developed (MATERIALS AND METHODS). Meiotic recombinants from each stock were isolated and confirmed (MATERIALS AND METHODS). The genetic distances between the *a1* and *sh2* loci varied approximately threefold from 0.065 ± 0.0035 cM in the lux haplotype to 0.20 ± 0.012 cM in the mex haplotype (Table 2). The resulting average rates of recombination per megabase across the *a1-sh2* intervals of these distinct haplotypes range from 0.50 to 1.5 cM/Mb (Figure 2D). On the basis of a homogeneity $\chi^2$ test, the rate of recombination per megabase in the mex haplotype is significantly different from all three others (Figure 1A). The par haplotype exhibits a recombination rate per megabase that is significantly different from that of the lux but not of the LC haplotype. The recombination rates per megabase in the lux and LC haplotypes do not differ significantly.

**Structure of the *a1-sh2* interval:** The sequences of the three teosinte haplotypes differ from each other and from the LC and *a1::rdt sh2* maize haplotypes by both large InDeLs and numerous small InDeLs and SNPs (Figure 2, A and E). The *a1-sh2* interval was divided into seven subintervals relative to the sequence polymorphisms between the maize *a1::rdt sh2* haplotype and the three teosinte *A1 Sh2* haplotypes (Figure 2A). Subinterval I consists of the 5′ two-thirds of the transcribed region of the *a1* gene. Subinterval II contains the *a1* promoter. Subinterval III consists of the intergenic region between the *a1* and *yz1* genes. Subinterval IV contains the entire transcribed region of the *yz1* gene. Subinterval V consists of the intergenic region between the *yz1* and *x1* genes. Subinterval VI contains the 3′-end of the transcribed region of the *x1* gene. Subinterval VII contains the 5′-end of the *x1* gene and the intergenic region between the *x1* and *sh2* genes. For each teosinte haplotype, the levels of sequence polymorphisms (MATERIALS AND METHODS) between the *A1 Sh2* and *a1::rdt sh2* haplotypes vary across the subintervals (Figure 2E). Within the same subinterval, such levels of sequence polymorphisms also differ among haplotypes.

**Mapping breakpoints associated with meiotic recombinants across the *a1-sh2* interval:** The recombination breakpoints associated with 99% of the confirmed recombinants (Table 2) from the mex (176/177), par (106/106), and lux (183/185) stocks were mapped to the seven subintervals relative to these sequence polymorphisms (Figure 2B). For each recombinant haplotype, only one breakpoint was detected between the *a1* and *sh2* loci, suggesting that most recombinant haplotypes resulted from simple recombination events (*i.e.*, without mosaicism).

**The distributions of recombination breakpoints across the *a1-sh2* interval differ among haplotypes:** In each of the three distinct teosinte *A1 Sh2* haplotypes,

**TABLE 2**

**Isolation of recombinants from stocks carrying distinct *A1 Sh2* haplotypes**

| | No. isolated | | | No. tested[b] | | | No. confirmed | | | No. corrected[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stocks[a] | Colored shrunken kernels | Colorless round kernels | Total | Colored shrunken kernels | Colorless round kernels | Total | Colored shrunken kernels | Colorless round kernels | Total | Colored shrunken kernels | Colorless round kernels | Total | Population size | Genetic distance (cM)[d] |
| mex | 140 | 145 | 285 | 80 | 106 | 186 | 76 | 101 | 177 | 133 | 138 | 271 | 133,040 | 0.20 ± 0.012 |
| par | 93 | 129 | 222 | 37 | 74 | 111 | 37 | 69 | 106 | 93 | 120 | 213 | 204,353 | 0.10 ± 0.0071 |
| lux | 143 | 227 | 370 | 74 | 127 | 201 | 71 | 114 | 185 | 137 | 204 | 341 | 526,806 | 0.065 ± 0.0035 |
| LC2 | 13 | 13 | 26 | 2 | 11 | 13 | 2 | 11 | 13 | 13 | 13 | 26 | 27,868 | 0.093 ± 0.018 |

[a] Recombinants associated with the mex and LC2 stocks were obtained from crosses in 1997. Recombinants associated with the par and lux stocks were obtained from crosses in 1997 and 1998.

[b] Putative recombinants were tested by genetic crosses and/or molecular analysis, *e.g.*, PCR mapping of recombination breakpoints as described by YAO *et al.* (2002).

[c] No. corrected = no. isolated × (no. confirmed/no. tested).

[d] Calculated as no. corrected/population size × 100. See MATERIALS AND METHODS.

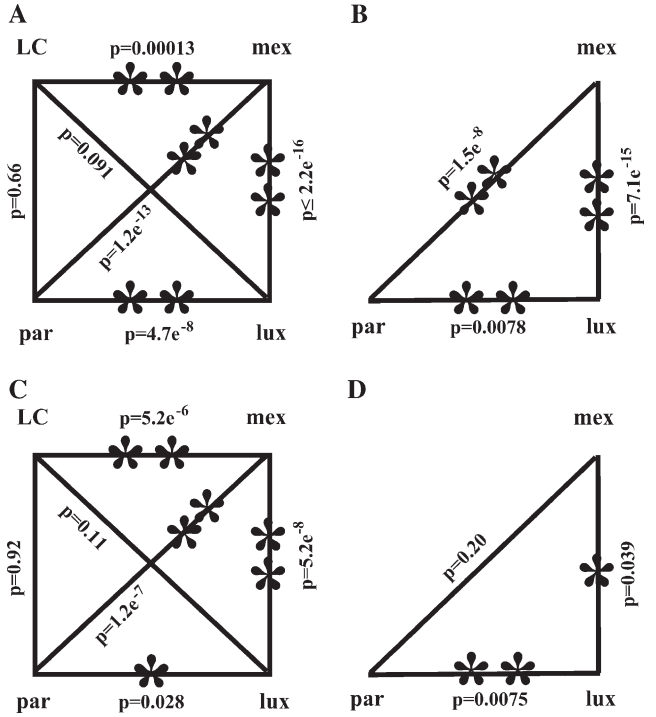



FIGURE 1.—Comparisons of recombination rates per megabase and distributions of recombination breakpoints among stocks that carry different *A1 Sh2* haplotypes. (A) Rates of recombination per megabase between the *al* and *sh2* loci. (B) Distributions of recombination breakpoints across the *al-sh2* interval. (C) Distributions of recombination breakpoints across the *a1* locus (subintervals I–II). (D) Distributions of recombination breakpoints across the *yz1* locus (subinterval IV). Rates and distributions were compared via $\chi^2$ tests and *P*-values are indicated. Statistically significant differences are indicated by asterisks. (*) Significant difference at the 0.05 level; (**) significant difference at the 0.01 level. Although the LC haplotype in A and C are identical by descent, they were analyzed in different genetic stocks (A, LC2; C, LC1; MATERIALS AND METHODS). Comparisons in D did not include recombinants that resolved in subinterval V-1 (Figure 4) because the sizes of this subinterval vary too much among haplotypes to permit fair comparisons. The distribution of breakpoints across the *yz1* gene considered only the transcribed region (subinterval IV).

the distribution of recombination breakpoints is significantly different from that expected on the basis of the null hypothesis of a random distribution across the *a1-sh2* interval (*P*-values $< 2.2e^{-16}$; Figure 2B *vs.* 2C). In each of the three haplotypes, >85% of the breakpoints mapped to the *a1-yz1* region (subintervals I–IV, Figure 2B), even though this region comprises <10% of the length of the entire *a1-sh2* interval (Figure 2A). Consistent with prior studies conducted using the maize LC and *a1::rdt sh2* haplotypes (YAO *et al.* 2002), most of the recombinants that map to the remainder of the *a1-sh2* interval (*i.e.*, subintervals V–VII) from each of the three teosinte haplotypes map to the 3′-end of the *x1* gene (*i.e.*, subinterval VI) or 5′ of the coding region of *yz1*. Even though general patterns of recombination are conserved across haplotypes, on the basis of $\chi^2$ contingency tests the distributions of recombination breakpoints across the *a1-sh2* interval differ significantly among the three teosinte haplotypes (*P*-values $< 2.2e^{-16}$). These differences exist between any two of the three haplotypes (Figure 1B); *e.g.*, within each pair of the teosinte haplotypes the distribution of hot and/or cold spots differs (Table 3, Figure 2, B and D). As shown in the analyses below, even though all seven subintervals of the three *A1 Sh2* haplotypes have divergent sequences, some subintervals are recombination hot spots in all three haplotypes; some are hot spots in only one or two haplotypes; and some are cold spots in all haplotypes. Hot spots or cold spots can be defined relative to the *a1-sh2* interval or to the entire genome. In a given stock, regions that exhibit significantly higher or lower recombination rates per megabase than the entire genome's average [2.1 cM/Mb, calculated according to the physical size of ~2500 Mb (ARUMUGANATHAN and EARLE 1991) and genetic size of 5289 cM (G. DAVIS, personal communication, cited in YAO *et al.* 2002), for the maize genome] are defined as global hot spots or cold spots; regions that exhibit recombination rates per megabase that are significantly higher or lower than that of the *a1-sh2* interval within the corresponding haplotype are defined as local hot spots or cold spots; regions that are none of the above are considered average spots (Table 3).

**Not all genes are hot spots and *cis*-modifiers can convert a genic hot spot to an average spot:** The transcribed regions of most maize genes that have been characterized are recombination hot spots (reviewed by SCHNABLE *et al* 1998). Even so, YAO *et al.* (2002) found that the transcribed region of the *x1* gene in the *a1-sh2* interval associated with the LC haplotype is not a recombination hot spot, thereby establishing that not all genic regions are hot spots in the maize genome.

To test whether *cis*-modifiers affect the recombination activity of genic regions in the *a1-sh2* interval, the rates of recombination per megabase within each genic region in each haplotype were examined. The *x1* gene is located in subintervals VI and VII (Figure 2A). Subinterval VI consists of the 3′ portion of the *x1* locus. Rates of recombination per megabase across subinterval VI were compared to the average rates across the corresponding *A1 Sh2* haplotypes and to the genome's average. These comparisons established that subintervals VI-mex and VI-lux are local recombination hot spots; subinterval VI-par is an average recombination spot (Figure 2, B–D, Table 3).

The 5′ portion of the *x1* gene is located in subinterval VII (Figure 2A). Even if all the recombination breakpoints that occurred within the ~85-kb subinterval VII (Figure 2B) map to within the transcribed region of the *x1* locus located in subinterval VII, the 5′ transcribed region of *x1* would be an average spot in the mex haplotype and global cold spots in both the par and lux haplotypes (data not shown). Correspondingly, rate of recombina-

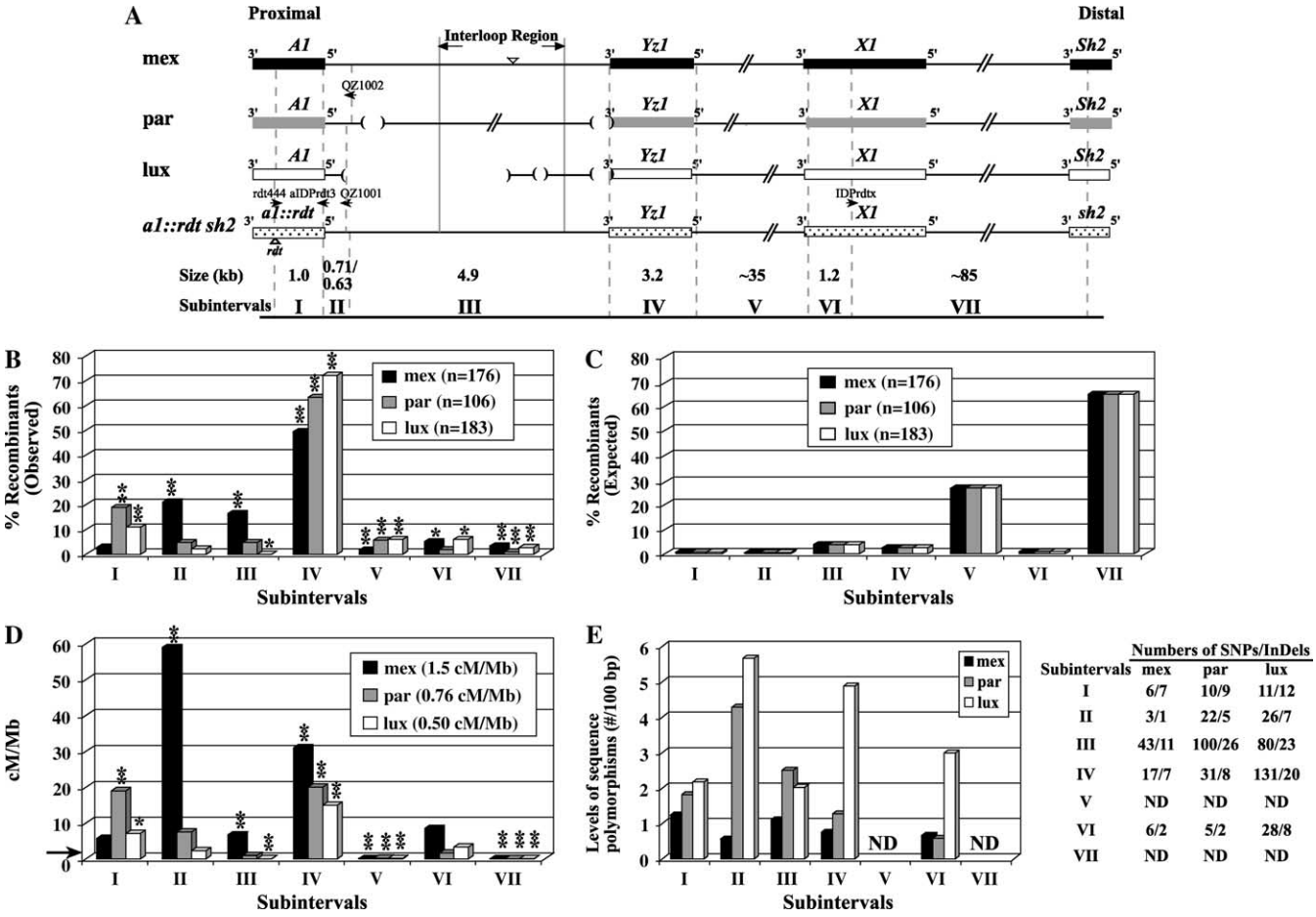



FIGURE 2.—Distributions of recombination events and sequence polymorphisms across the *a1-sh2* intervals of the mex, par, and lux haplotypes. (A) Comparisons of the structures of the three teosinte *A1 Sh2* haplotypes relative to the maize *a1::rdt sh2* haplotype. Genes are indicated as boxes. The polymorphisms shared by teosinte haplotypes relative to the *a1::rdt sh2* haplotype that were used to define the subintervals are indicated by shaded dashed lines. Subintervals I, II, IV, and VI were completely sequenced for all haplotypes. Subinterval III was completely sequenced for the mex, lux, and *a1::rdt sh2* haplotypes and partially sequenced for the par haplotype. Large InDeLs in subinterval III are indicated by triangles (insertions) and parentheses (deletions). The *rdt* transposon insertion is indicated by a triangle. Large InDeLs in other subintervals are not shown. Haplotype-specific IDP primers used to map recombination breakpoints are indicated by horizontal arrows. The sizes of each subinterval are based on those of the *a1::rdt sh2* haplotype that is common among stocks carrying the mex, par, and lux haplotypes. Because no sequence polymorphisms are shared by all three haplotypes at the distal ends of subintervals II, the size of subinterval II-mex (0.71 kb) differs slightly from the sizes of subintervals II-par and II-lux (0.63 kb). Figure not to scale. (B) Observed percentages of recombinants that resolved in each subinterval. (*) and (**) indicate significant differences between the rates of recombination per megabase based on the observed recombination breakpoints mapped to subintervals and the corresponding average rates per megabase across the *a1-sh2* interval of each haplotype at the 0.05 and 0.01 levels, respectively. (C) Percentages of recombinants expected to resolve in each subinterval based on a random distribution across the *a1-sh2* interval. (D) Recombination rates per megabase in subintervals. The indicated average rates of recombination per megabase across the *a1-sh2* interval in each of the three stocks were calculated on the basis of the physical size (~130 kb) of the common *a1::rdt sh2* haplotype carried in all stocks. The horizontal arrow indicates the average recombination rate per megabase of the maize genome (2.1 cM/Mb). (E) Levels of sequence polymorphisms (no./100 bp) between each *A1 Sh2* haplotype and the *a1::rdt sh2* haplotype. Numbers of SNPs/InDeLs in each subinterval are presented. Values for subinterval III-par were calculated using only the sequenced portions of this subinterval. ND, not determined.

tion per megabase in the entire transcribed region of the *x1* locus is only 2.9 cM/Mb in the mex haplotype, 0.47 cM/Mb in the par haplotype, and 0.96 cM/Mb in the lux haplotype. These rates are equivalent to ($P$-values = 0.52) or significantly less than ($P$-values < 0.030) the genome's average (2.1 cM/Mb) and are not significantly different from that expected if the distributions of breakpoints were random across the *a1-sh2* intervals of all three haplotypes ($P$-values > 0.16). Therefore, consistent with previous studies using the maize LC haplotype (YAO *et al.* 2002), in none of the teosinte haplotypes is the *x1* gene as a whole a recombination hot spot. Indeed, in the par and lux haplotypes the *x1* gene is a global cold spot.

The transcribed region of the *yz1* gene is a local and global hot spot in the LC haplotype (YAO *et al.* 2002).

**TABLE 3**

**Statistical analyses of recombination in the seven subintervals of the *a1-sh2* interval**

| Subintervals | Haplotypes | Comparisons to the average of *a1-sh2*[a] | Comparisons to the genome's average[a] | Comparisons among stocks[a] | Recombination activities[b] |
|---|---|---|---|---|---|
| I | mex | 0.28 | 0.40 | 0.016↓ (mex *vs.* par) | Average spot |
| | par | $6.7e^{-5}$↑ | 0.00032↑ | 0.0014↑ (par *vs.* lux) | Hot spot (local, global) |
| | lux | 0.00014↑ | 0.010↑ | 0.87 (lux *vs.* mex) | Hot spot (local, global) |
| II | mex | $1.3e^{-8}$↑ | $2.1e^{-8}$↑ | $9.9e^{-8}$↑ (mex *vs.* par) | Hot spot (local, global) |
| | par | 0.14 | 0.28 | 0.11 (par *vs.* lux) | Average spot |
| | lux | 0.34 | 0.80 | $<2.2e^{-16}$↓ (lux *vs.* mex) | Average spot |
| III | mex | 0.00033↑ | 0.0019↑ | $9.3e^{-6}$↑ (mex *vs.* par) | Hot spot (local, global) |
| | par | 0.99 | 0.25 | 0.0013 (par *vs.* lux) | Average spot |
| | lux | 0.026↓ | $1.9e^{-7}$↓ | $<2.2e^{-16}$↓ (lux *vs.* mex) | Cold spot (local, global) |
| IV | mex | $<2.2e^{-16}$↑ | $<2.2e^{-16}$↑ | 0.011↑ (mex *vs.* par) | Hot spot (local, global) |
| | par | $2.9e^{-14}$↑ | $5.5e^{-12}$↑ | 0.026↑ (par *vs.* lux) | Hot spot (local, global) |
| | lux | $<2.2e^{-16}$↑ | $<2.2e^{-16}$↑ | $1.9e^{-8}$↓ (lux *vs.* mex) | Hot spot (local, global) |
| V | mex | $1.2e^{-9}$↓ | $2.9e^{-13}$↓ | 0.67 (mex *vs.* par) | Cold spot (local, global) |
| | par | 0.00028↓ | $4.6e^{-14}$↓ | 0.58 (par *vs.* lux) | Cold spot (local, global) |
| | lux | $1.9e^{-6}$↓ | $<2.2e^{-16}$↓ | 0.89 (lux *vs.* mex) | Cold spot (local, global) |
| VI | mex | 0.049↑ | 0.083 | 0.037↑ (mex *vs.* par) | Hot spot (local) |
| | par | 0.99 | 0.85 | 0.55 (par *vs.* lux) | Average spot |
| | lux | 0.020↑ | 0.50 | 0.044↓ (lux *vs.* mex) | Hot spot (local) |
| VII | mex | $<2.2e^{-16}$↓ | $<2.2e^{-16}$↓ | 0.083 (mex *vs.* par) | Cold spot (local, global) |
| | par | $1.3e^{-15}$↓ | $<2.2e^{-16}$↓ | 0.94 (par *vs.* lux) | Cold spot (local, global) |
| | lux | $<2.2e^{-16}$↓ | $<2.2e^{-16}$↓ | 0.037↓ (lux *vs.* mex) | Cold spot (local, global) |

[a] Goodness-of-fit $\chi^2$ tests were used in the comparisons of the observed rate of recombination in a given subinterval with the average rate of recombination per megabase in each teosinte *A1 Sh2* haplotype (column three) and with the genome's average (2.1 cM/Mb) (column four), and homogeneity $\chi^2$ tests were used in the comparisons of rates of recombination per megabase in a given subinterval among the three teosinte *A1 Sh2* haplotypes (column five). Details are described in MATERIALS AND METHODS. The *P*-values obtained from these $\chi^2$ tests are listed. (↑) and (↓) indicate that an observed rate of recombination is significantly higher and lower (at the 0.05 level), respectively, than the rate of recombination per megabase to which it was compared.

[b] According to its recombination activity, a subinterval is classified as a global or local hot spot, an average spot, or a global or local cold spot. A global hot or cold spot exhibits significantly higher or lower recombination activity than the genome as a whole. A local hot or cold spot exhibits significantly higher or lower recombination activity than the *a1-sh2* interval. Recombination activity of an average spot is not significantly different from that of the *a1-sh2* interval and the genome. The cutoff level for the *P*-values is 0.05.

As discussed earlier, the majority of recombinants from the mex, par, and lux stocks (49, 63, and 72%, respectively) resolved in the transcribed region of *yz1*, subinterval IV (Figure 2B). This resulted in high rates of recombination per megabase in subinterval IV, establishing the transcribed region of the *yz1* locus as a local and global recombination hot spot in each of the distinct teosinte haplotypes (Figure 2, B–D; Table 3). Moreover, rates of recombination per megabase in this hot spot are significantly different among haplotypes.

The transcribed region of the *a1* gene is also a recombination hot spot in the LC haplotype (CIVARDI *et al.* 1994; XU *et al.* 1995; YAO *et al.* 2002). This region corresponds to subinterval I in the current study (Figure 2A). Breakpoints associated with 19 and 11% of the recombinants obtained from the par and lux stocks map to subinterval I. In contrast, only 2.8% of the recombinants from the mex stock resolved in subinterval I. Both subintervals I-par and I-lux are local and global recombination hot spots whereas subinterval I-mex is an average spot (Figure 2, B–D; Table 3).

On the basis of the existence of transcription factor binding sites between positions −130 and +1 (GROTEWOLD *et al.* 1994; TUERCK and FROMM 1994), subinterval II contains the *a1* promoter. Breakpoints associated with 21% of the recombinants from the mex stock mapped to subinterval II-mex. The corresponding rate of recombination per megabase (59 cM/Mb) in subinterval II-mex is significantly higher than the average recombination rate per megabase of the mex haplotype and the

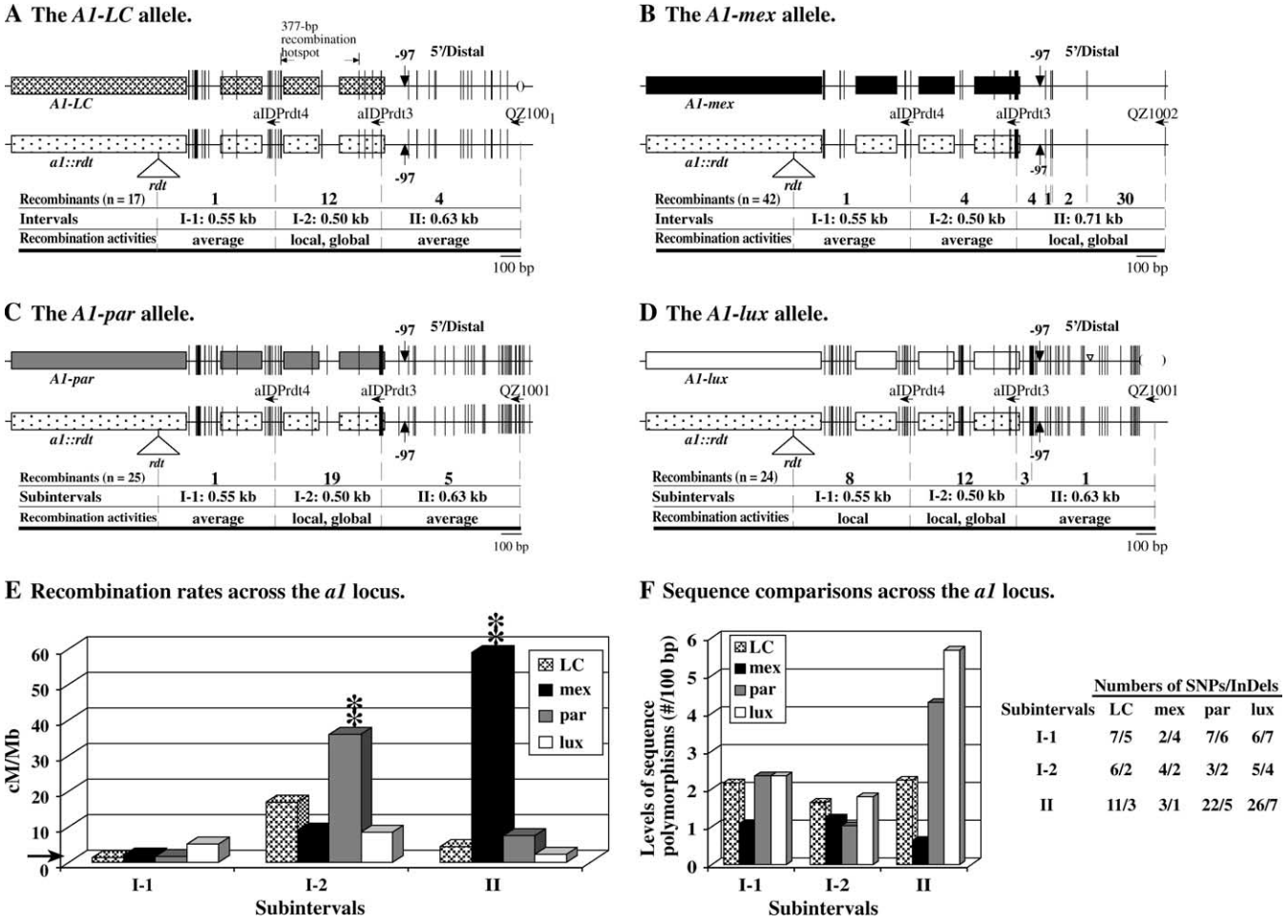



FIGURE 3.—High-resolution mapping of the recombination breakpoints that resolved in the *a1* locus of the LC, mex, par, and lux haplotypes. (A–D) Exons of the *a1* gene are shown as boxes. Short vertical lines represent sequence polymorphisms between *A1* alleles and the *a1::rdt* allele. The widths of the vertical lines are proportional to the numbers of polymorphic nucleotides. Subintervals are defined by sequence polymorphisms. Haplotype-specific primers are indicated by horizontal arrows. The numbers of recombination breakpoints that mapped to each subinterval for each haplotype are shown. Each interval is classified as being an average recombination spot (average), a local recombination hot spot (local), or a local and global recombination hot spot (local, global; see legend of Table 3 for definitions). Large InDeLs are indicated by triangles (insertions) and parentheses (deletions). (A) The positions of recombination breakpoints previously characterized by YAO *et al.* (2002), but here classified relative to subintervals I-1 and I-2. (E) Comparison of recombination rates per megabase across the *a1* locus among the LC, mex, par, and lux haplotypes. The horizontal arrow indicates the average recombination rate per megabase of the maize genome (2.1 cM/Mb). (**) indicates that the recombination rate per megabase in the labeled haplotype in the corresponding subinterval is significantly different from all others at the 0.01 level. (F) Comparison of levels of sequence polymorphisms (no./100 bp) at the *a1* locus among the LC, mex, par, and lux haplotypes. Sequence polymorphisms are between each *A1 Sh2* haplotype and the *a1::rdt sh2* haplotype. Numbers of SNPs/InDeLs in each subinterval are also listed.

genome's average (~39- and 30-fold, respectively; Figure 2, B–D; Table 3). Therefore, subinterval II-mex is both a local and global recombination hot spot. Significantly, subinterval II-mex has no overlap with the 377-bp genic *a1* hot spot identified in the maize LC haplotype (Figure 3, A and B; XU *et al.* 1995; YAO *et al.* 2002). In contrast to what is observed in subinterval II-mex, breakpoints associated with only 4.7 and 2.2% of the recombinants from the par and lux stocks, respectively, mapped to subintervals II. Both subintervals II-par and II-lux are average spots of recombination (Figure 2, B–D; Table 3).

These analyses of the *a1* gene suggest that *cis*-modifiers associated with the sequence divergence among the three *A1 Sh2* haplotypes can convert both a transcribed genic hot spot (*i.e.*, subinterval I in the par and lux haplotype) and an untranscribed genic hot spot (*e.g.*, subinterval II-mex) into average spots (*i.e.*, subinterval I-mex and subintervals II-par and II-lux).

**Not all intergenic regions are cold spots and *cis*-modifiers can convert a nongenic cold spot into a hot spot:** It has been hypothesized that almost all meiotic recombination events in eukaryotic genomes occur in genes (THURIAUX 1977). This hypothesis therefore predicts that intergenic regions are recombination cold spots.

Characterization of the maize *a1-sh2* interval did not find evidence for the presence of genes other than *a1*, *yz1*, *x1*, and *sh2* (YAO *et al.* 2002). Similar analyses of the

rice and sorghum *a1-sh2* intervals also failed to identify other genes (Chen and Bennetzen 1996; Chen *et al.* 1998). Hence, subinterval III, V, and most of subinterval VII are thought to be solely intergenic (Figure 2A). Consistent with Thuriaux's hypothesis, in all three teosinte haplotypes subintervals V and VII are local and global recombination cold spots (Figure 2, B–D; Table 3).

In contrast, subinterval III is not a uniform recombination cold spot in all three teosinte haplotypes (Figure 2, Table 3). Subinterval III contains a segment (the IR; Figure 2A) that is a recombination hot spot in the maize LC haplotype (Yao *et al.* 2002). Breakpoints associated with 16% of the recombinants isolated from the mex stock mapped to subinterval III; breakpoints associated with only 2.8% of the recombinants from the par stock mapped to subinterval III and none of the recombinants from the lux stock resolved in subinterval III (Figure 2B). Subinterval III is a local and global recombination cold spot in the lux haplotype, an average spot in the par haplotype, and a local and global hot spot in the mex haplotype (Figure 2, B–D; Table 3). Hence, *cis*-modifiers associated with sequence divergence among the *A1 Sh2* haplotypes are able to convert an intergenic cold spot to a hot spot.

**Distributions of recombination breakpoints across the *a1* and *yz1* loci differ among haplotypes:** Within maize genes, the distributions of recombination breakpoints differ. In some genes, breakpoints are randomly distributed; in others they are distributed nonrandomly (reviewed by Schnable *et al.* 1998). In the *bz1* locus, the presence of SNPs and InDeLs alters the distribution of recombination breakpoints (Dooner and Martinez-Ferez 1997). In contrast, although a large InDeL caused by a transposon insertion in the *a1* locus (position −97) decreases the rate of recombination per megabase within this gene, it does not affect the distribution of recombination breakpoints (Xu *et al.* 1995). To better understand the effects of sequence polymorphisms on patterns of intragenic recombination, the distributions of recombination breakpoints that resolved within the *a1* (subintervals I–II) and *yz1* (subintervals VI-V-1) genes from each of the three near-isogenic stocks were compared to each other and to data from the LC haplotype previously characterized by Yao *et al.* (2002) (Figures 3 and 4).

*The a1 locus:* Using the InDel polymorphism (IDP) primer, aIDPrdt4, recombinants from the mex, par, and lux stocks with breakpoints in subinterval I could be mapped to two smaller subintervals (I-1 and I-2, Figure 3). Subinterval I-2 contains the 377-bp recombination hot spot previously identified in the LC haplotype (Xu *et al.* 1995; Yao *et al.* 2002). The distribution of recombination breakpoints derived from the lux haplotype does not differ significantly from that expected if recombination occurs randomly across the *a1* locus (Table 4). In contrast, the distributions associated with the other three haplotypes do differ significantly from random. In the par and LC haplotypes, recombination breakpoints clustered in subinterval I-2; in the mex haplotype, they clustered in subinterval II (Figure 3, A–C). Significant differences were observed in the distributions of recombination breakpoints among most of the haplotypes (Figure 1C).

**TABLE 4**

**Tests for nonrandom distributions of recombination breakpoints across the *a1* and *yz1* loci**

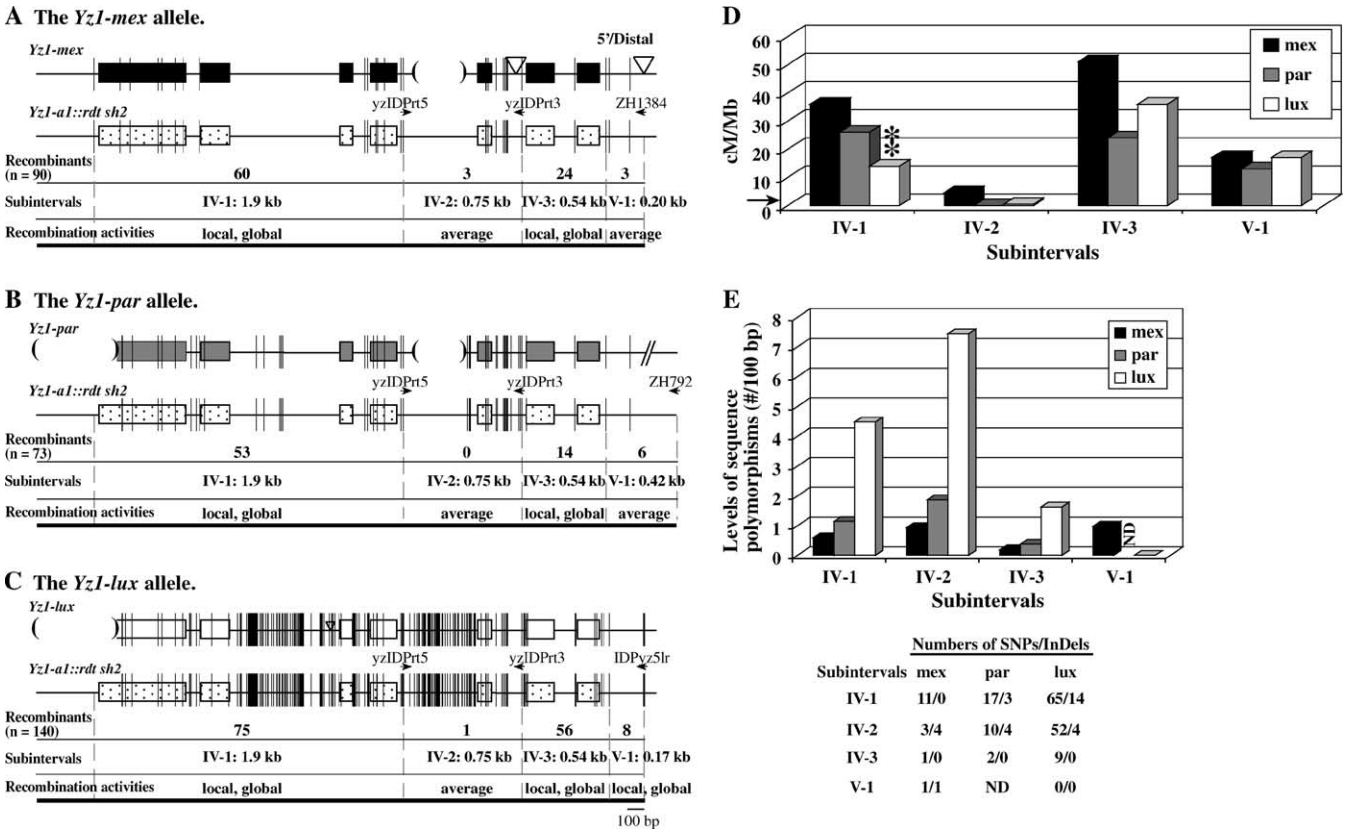
| | *P*-values[a] | |
|---|---|---|
| Haplotypes | *a1* locus | *yz1* locus |
| mex | $1.9e^{-5}$ | 0.00090 |
| par | 0.0024 | 0.00037 |
| lux | 0.20 | $4.1e^{-9}$ |
| LC | 0.032 | NA |

NA, not analyzed.

[a] Observed distributions of recombination breakpoints across the *a1* (Figure 3, subintervals I–II) and *yz1* (Figure 4, subintervals IV–V-1) loci in each *A1 Sh2* haplotype were compared to the expected distributions under the assumption of a random distribution across each locus within a haplotype using $\chi^2$ contingency tests.

*The yz1 locus:* Recombination breakpoints derived from the mex, par, and lux stocks that resolved in subintervals IV and V were mapped to higher resolution using the haplotype-specific primers indicated in Figure 4. Subintervals IV-1, IV-2, and IV-3 contain the entire coding region of the *yz1* gene and subinterval V-1 contains ~200–400 bp upstream of the beginning of the *yz1* coding region. The LC haplotype was not included in this analysis because the *yz1* markers that are polymorphic between the *a1::rdt sh2* haplotype and all of the teosinte *A1 Sh2* haplotypes are monomorphic between the *a1::rdt sh2* and the LC haplotypes. Across all of subinterval IV, no significant differences were observed in the distributions of recombination breakpoints between the par and mex haplotypes, but the distributions in both of these haplotypes differ significantly from that of the lux haplotype (Figure 1D). This is caused by the significantly lower rate of recombination per megabase in subinterval IV-1-lux as compared to the corresponding intervals of the par and mex haplotypes (Figure 4D). These high-resolution mapping experiments demonstrated that *cis*-modifiers can alter the patterns of distribution across both of the analyzed genes.

**Distributions of recombination breakpoints across an intergenic region differ among haplotypes:** Subinterval III consists of the intergenic region between the *a1* and *yz1* genes. Prior analyses of this region revealed that the LC haplotype contains two large retrotransposon insertions that are not present in the *a1::rdt sh2* haplotype (Yao *et al.* 2002). The 2.2 kb between these two insertions is termed the IR in the LC haplotype. The 800-bp proximal portion of the IR consists of repetitive




| Subintervals | Numbers of SNPs/InDels mex | par | lux |
|---|---|---|---|
| IV-1 | 11/0 | 17/3 | 65/14 |
| IV-2 | 3/4 | 10/4 | 52/4 |
| IV-3 | 1/0 | 2/0 | 9/0 |
| V-1 | 1/1 | ND | 0/0 |

FIGURE 4.—High-resolution mapping of the recombination breakpoints that resolved in the *yz1* locus in the mex, par, and lux haplotypes. (A–C) Exons of the *yz1* gene are shown as boxes. Short vertical lines represent sequence polymorphisms between each teosinte *Yz1* allele and the *Yz1* allele from the *a1::rdt sh2* stock. The widths of these short vertical lines are proportional to the numbers of polymorphic nucleotides. Subintervals are defined by sequence polymorphisms. Haplotype-specific primers are indicated by horizontal arrows. The numbers of recombination breakpoints that mapped to each subinterval are shown for each haplotype. Each interval is classified as being an average recombination spot (average), a local recombination hot spot (local), or a local and global recombination hot spot (local, global; see legend of Table 3 for definitions). Large InDeLs are indicated by triangles (insertions) and parentheses (deletions). (D) Comparison of recombination rates per megabase across the *yz1* locus among the mex, par, and lux haplotypes. The horizontal arrow indicates the average recombination rate per megabase of the maize genome (2.1 cM/Mb). (**) indicates that the recombination rate per megabase in the labeled haplotype at the corresponding subinterval is significantly different from the others at the 0.01 level. (E) Comparison of the levels of sequence polymorphisms (no./100 bp) at the *yz1* locus among the mex, par, and lux haplotypes. Numbers of sequence polymorphisms were calculated by comparing each of the teosinte *Yz1* alleles and the common *Yz1* allele from the *a1::rtdt sh2* stock. Numbers of SNPs/InDeLs in each of the subintervals are listed.

sequences. The 1.4-kb distal portion of the IR (Figure 5) is an apparently nongenic, single-copy recombination hot spot.

Subinterval III is structurally very polymorphic among haplotypes (Figure 2A). Much of the IR has been deleted from subinterval III-lux. Even though ~900 bp of the 1.4-kb single-copy distal portion of the IR has been retained, no recombinants occurred in any portion of subinterval III-lux. It was not possible to sequence all of subinterval III-par, but this haplotype retains at least 900 bp of the 1.4-kb single-copy distal portion of the IR. Even so, this region is not a recombination hot spot in the par haplotype.

In contrast, subinterval III-mex, which is structurally similar to that of the *a1::rdt sh2* haplotype, is both a local and global recombination hot spot. Recombination breakpoints from subinterval III-mex were mapped to higher resolution via PCR and sequencing (Figure 5). In contrast to what is observed in subinterval III-LC (YAO *et al.* 2002), the distribution of recombination breakpoints across subinterval III-mex is not significantly different from a random pattern (*P*-value = 0.27).

## DISCUSSION

**The highly polymorphic intergenic region between the *a1* and *yz1* loci among teosinte and maize haplotypes:** Sequence comparisons of large multigenic intervals among maize haplotypes revealed noncollinearities in both genic (FU and DOONER 2002; SONG and MESSING 2003; BRUNNER *et al.* 2005) and nongenic (FU and DOONER 2002; YAO *et al.* 2002; SONG and MESSING 2003; BRUNNER *et al.* 2005) regions. This study extends these sequence comparisons of multigenic haplotypes to teo-

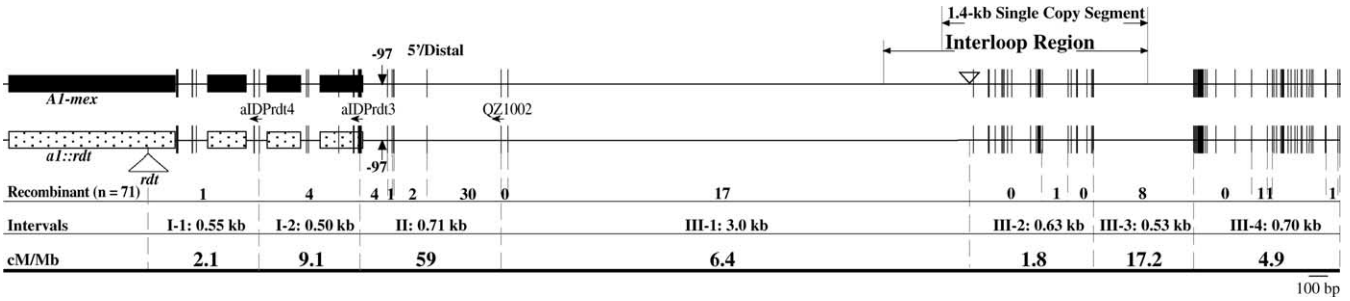



FIGURE 5.—Recombination breakpoints across the *a1*-interloop region of the mex haplotype. Exons of the *a1* gene are shown as boxes. Short vertical lines represent sequence polymorphisms between the mex *A1 Sh2* haplotype and the *a1::rdt sh2* haplotype. The widths of the vertical lines are proportional to the numbers of polymorphic nucleotides. Subintervals are defined by sequence polymorphisms. Subinterval III-1 is not drawn to scale. Haplotype-specific primers are indicated by horizontal arrows. The numbers of recombination breakpoints that mapped to each subinterval are shown. Large InDeLs are indicated by triangles.

sinte. The intergenic region (subinterval III, Figure 2A) between the *a1* and *yz1* genes is highly polymorphic among the maize and teosinte haplotypes. Subinterval III ranges in size from ~1.1 kb in the teosinte lux haplotype to ~13 kb in the maize LC haplotype (YAO *et al.* 2002). This intergenic region is ~5 kb in the maize *a1::rdt sh2* and teosinte mex haplotypes. The expansion of this region in the LC haplotype is caused by transposon and retrotransposon insertions. The reduction of this interval may be caused by deletion events. Although maize arose from *Z. mays* ssp. *parviglumis* (MATSUOKA *et al.* 2002), over the entire *a1-yz1* region the mex haplotype is more similar to the maize *a1::rdt sh2* haplotype than is the par haplotype (Figure 2E). This is consistent with the view that gene flow from ssp. *mexicana* may have contributed to the maize gene pool after domestication (MATSUOKA *et al.* 2002). Alternatively, haplotype polymorphisms present in the ancestral population of the three subspecies could still have been segregating in the ancestor of ssp. *parviglumis* and maize after the divergence of ssp. *mexicana*. If so, differential random fixation of haplotypes in the three subspecies could also explain why the mex haplotype is more similar to the *a1::rdt sh2* haplotype in the *a1-yz1* region.

**Sequence polymorphisms have *cis*-effects on meiotic recombination across the *a1-sh2* interval:** The amount, type, and distribution of sequence polymophisms between each of the four maize and teosinte *A1 Sh2* haplotypes (LC, mex, par, and lux) and the *a1::rdt sh2* haplotype differ dramatically (Figure 2, A and E). Similarly, both the rates of recombination per megabase (Figure 1A, Table 2) and the distributions of recombination breakpoints (Table 3, Figure 1B) across the *a1-sh2* interval vary significantly among these *A1 Sh2* haplotypes. Because these studies were conducted in near-isogenic stocks in which each haplotype was paired with a common *a1::rdt sh2* haplotype, it is likely that the sequence polymorphisms that exist among the *A1 Sh2* haplotypes are responsible for the observed differences in recombination rates per megabase and distribution patterns. It is also possible that inherited patterns of chromatin structure could also contribute to the differences in recombination.

The large-scale pattern of recombination across the *a1-sh2* interval is conserved among the diverse teosinte haplotypes analyzed in this study and the previously characterized LC haplotype (YAO *et al.* 2002); *i.e.*, the bulk of recombination occurs in the *a1-yz1* interval that comprises ~10% of the physical distance between *a1* and *sh2* loci. Yet, significant differences were observed in the distributions of recombination breakpoints across subintervals. It was previously established that the *a1-sh2* interval of the LC haplotype contains three recombination hot spots: the transcribed region of *a1*, the 1.4-kb single-copy proximal region of the IR, and the transcribed region of *yz1* (YAO *et al.* 2002). Although each of the three hot spots detected in the LC haplotype was also detected in at least one of the three teosinte haplotypes, two of these hot spots were not detected in at least one haplotype (Figure 2, Table 3). In addition, new hot spots were detected in some of the teosinte haplotypes.

**What causes recombination hot spots?** It has been hypothesized that the hot spots detected within maize genes are caused by the suppression of recombination in subgenic regions with higher levels of sequence polymorphisms, creating apparent hot spots in subgenic regions that have few polymorphisms (DOONER and MARTINEZ-FEREZ 1997). This hypothesis was developed on the basis of observations at the *bz1* locus, where recombination breakpoints are distributed randomly across the transcribed portion of the *bz1* locus in plants that are heterozygous for nearly identical alleles (DOONER and MARTINEZ-FEREZ 1997), but distributed in a nonrandom fashion in plants that are heterozygous for *bz1* alleles that exhibit a higher level of polymorphisms (~1/100 bp). Within many organisms, including bacteria, yeast, and mouse, recombination between polymorphic templates (*i.e.*, homeologous recombination) is suppressed, a process that involves mismatch repair proteins (reviewed by MODRICH and LAHUE 1996; BORTS *et al.* 2000; EVANS and ALANI 2000). This suppression

helps prevent deleterious ectopic recombination between repetitive sequences in a genome (reviewed by Modrich and Lahue 1996; Borts *et al.* 2000; Evans and Alani 2000). Hence, the polymorphism hypothesis is attractive because it could help to explain how a segmentally duplicated genome such as that of maize (Helentjaris *et al.* 1988; Gaut and Doebley 1997) can avoid deleterious ectopic recombination between paralogs.

Within a given haplotype, the rates per megabase and distributions of recombination events across the *a1-sh2* interval are at least partially consistent with this hypothesis. In particular, subintervals that exhibit higher recombination rates per megabase than their flanking subintervals also exhibit lower levels of sequence polymorphisms than their neighbors (Figure 2, D and E). This relationship is less clear when comparing nonadjacent subintervals. How well does the sequence polymorphism hypothesis explain the distribution of recombination breakpoints among the various *a1-sh2* haplotypes? The rate of recombination per megabase is highest in the mex haplotype and lowest in the lux haplotype (Figure 2D). Among the teosinte haplotypes, the sequenced portions of the mex and lux haplotypes are least and most, respectively, polymorphic to the *a1::rdt sh2* haplotype (Figure 2E). Considering all haplotypes together, the correlation coefficient of the level of sequence polymorphisms and rate of recombination per megabase is $-0.44$ ($P$-value $< 0.025$; materials and methods). Hence, the levels of sequence polymorphisms in subintervals of the *a1-sh2* interval do not provide a complete explanation for the nonrandom distribution of recombination breakpoints across haplotypes.

The *yz1* hot spot (subintervals IV and V-1) that was originally detected in the LC haplotype is conserved in all three teosinte haplotypes. One of the interesting features of this genic hot spot is that recombination breakpoints cluster at the 5′- and 3′-ends of the gene in all four haplotypes. Consistent with the polymorphism hypothesis, the central portion of *yz1* that experiences lower recombination rates per megabase is also the most polymorphic portion of this gene in all four haplotypes (Yao *et al.* 2002; Figure 4). In the mex and par haplotypes, the 5′- and 3′-ends of *yz1* (subintervals IV-1, IV-3, V-1) exhibit similarly low levels of sequence polymorphism (Figure 4, A, B, and E) and similar rates of recombination per megabase (Figure 4D). In contrast, in the lux haplotype, the 3′ portion of *yz1* (subinterval IV-1) is more polymorphic (Figure 4, C and E) and experiences significantly less recombination than the 5′ portion (subinterval IV-3) (Figure 4D).

The local and global *a1* hot spot (subinterval I-2) detected in the LC haplotype is conserved in the par and lux, but not mex, haplotypes (Figure 3). On the other hand, in the mex haplotype a novel local and global hot spot was detected in subinterval II, the *a1* promoter, that was not detected in the LC haplotype or in either of the other two teosinte haplotypes. In the par and lux haplotypes, the more recombinationally active subinterval I-2 is less polymorphic than the less recombinationally active subinterval II, while in the mex haplotype the less recombinationally active subinterval I-2 is more polymorphic than the more recombinationally active subinterval II (Figure 3).

Hence, analyses of the *yz1* and *a1* genes, considering only single haplotypes, are generally consistent with the polymorphism hypothesis. Even so, the rates of recombination per megabase observed within subintervals do not exhibit a linear relationship with the levels of polymorphisms within the same subintervals. This is probably because certain types of polymorphisms have greater impacts on recombination than do others, and/or interactions among different subintervals within a haplotype affect recombination rates per megabase.

The data collected on *bz1* (Dooner and Martinez-Ferez 1997) and *yz1* focused on the transcribed regions of these genes. The analysis of the *a1* hot spot in the mex haplotype extends the relationship between polymorphisms and recombination to a nontranscribed region (subinterval II).

The analysis of recombination in *a1* across haplotypes provides a less clear picture regarding the relationship between level of sequence polymorphisms and recombination (Figure 3). Subinterval I-2 from the par haplotype has fewer polymorphisms than the corresponding subinterval of the other haplotypes and also has the highest rate of recombination per megabase, which is significantly higher (four times) than that experienced by subinterval I-2-mex. This occurs even though subinterval I-2-par has only one fewer SNP than subinterval I-2-mex. Similarly, although I-2-mex has fewer polymorphisms than I-2-lux, I-2-lux and I-2-mex have similar rates of recombination per megabase.

Likewise, a correlation between levels of sequence polymorphisms and recombination rates per megabase across haplotypes is not observed in the *x1* gene. Although *x1* is not a recombination hot spot in the LC haplotype, and the 5′-end of *x1* is not a recombination hot spot in any of the haplotypes, the 3′-end of *x1* (subinterval VI) is a local hot spot in the mex and lux haplotype (Figure 2, Table 3). The polymorphism hypothesis would predict that the 3′-ends of the *x1*-mex and *x1*-lux alleles should exhibit a lower level of sequence polymorphisms to the *x1* allele from the *a1::rdt sh2* haplotype than the 5′-ends of these two alleles and the 3′-end of the *x1*-par allele do. Exactly the opposite is observed. The 5′-ends of *x1*-mex and *x1*-lux are more similar to the *x1* allele derived from the *a1::rdt sh2* haplotype (with 0.2 and 1.2 sequence polymorphisms/100 bp, respectively) than are the 3′-ends (with 0.7 and 3 sequence polymorphisms/100 bp, respectively). In addition, the 3′-ends of the *x1*-mex and *x1*-par alleles are less similar to the *a1::rdt sh2* haplotype than is the corresponding region of the *x1*-par allele (with 0.6 sequence polymorphisms/100 bp). These results demonstrate that the

polymorphism hypothesis cannot by itself explain the distribution of all genic recombination hot spots.

This hypothesis is further weakened by our analysis of an apparently nongenic region. The apparently nongenic subinterval III can be subdivided into four subintervals (Figure 5). In the mex haplotype, but not in the other haplotypes, subinterval III is both a local and a global recombination hot spot (Figure 2, Table 3). Even though levels of polymorphisms vary dramatically among these four subintervals, there is no statistical evidence for a nonrandom distribution of recombination events in this haplotype. For example, although subintervals III-1 and III-4 exhibit similar rates of recombination per megabase (6.4 and 4.9 cM/Mb), they have quite different levels of sequence polymorphisms; the 3-kb subinterval III-1 has only a single SNP, while the 0.7 kb subinterval III-4 contains multiple SNPs and InDeLs relative to the *a1::rdt sh2* haplotype.

Comparisons between the nongenic hot spot in subinterval III-1-mex and the adjacent genic hot spot in *a1* strengthen the argument against the polymorphism hypothesis. Although the 0.7-kb genic subinterval II-mex (three SNPs and one small InDeL) has a higher level of polymorphisms than the adjacent 3-kb nongenic subinterval III-1-mex (one SNP), the former has a ninefold higher rate of recombination per megabase (59 *vs.* 6.4 cM/Mb; Figure 5).

Because the levels of sequence polymorphisms within the *a1-sh2* interval are not by themselves sufficient to explain the observed patterns of recombination and, by virtue of the experimental design, *trans*-acting factors are unlikely to have contributed to these differences, we conclude that other types of *cis*-factors, *e.g.*, region-specific chromatin structure (see below) and/or interactions among subintervals, may affect the rates and distribution of recombination across the *a1-sh2* interval. Moreover, regions surrounding the *a1-sh2* interval could vary among the *A1 Sh2* haplotypes due to linkage drag. Therefore, we cannot rule out the possibility that *cis*-factors outside of the *a1-sh2* interval may also contribute to the patterns of recombination in this interval. Even so, within genes there often is a relationship between the level of polymorphism and the rate of recombination per megabase.

**Domestication and recombination:** Domestication bottlenecks reduce genetic diversity. Consequently, all other factors being equal, genome-wide rates of recombination per megabase would be expected to increase following domestication because in general the level of sequence polymorphisms is negatively correlated with the recombination rate per megabase. Such an increase in recombination rate per megabase could impact various evolutionary processes, *e.g.*, faster fixation of agronomically important alleles during domestication (Kimura and Ohta 1969; Wang *et al.* 1999).

**How do polymorphisms suppress recombination?** Any model to explain the mechanism by which polymorphisms suppress recombination needs to take into account the finding that small changes in the level of polymorphisms may dramatically alter recombination rates per megabase (*e.g.*, subintervals I-2-par *vs.* I-2-mex) and that the relationship between levels of polymorphisms and recombination does not apply in all regions (*e.g.*, *x1* and subinterval III).

It has been proposed that polymorphism-mediated suppression occurs at the level of DSB initiation (Dooner and Martinez-Ferez 1997). The absence of data regarding the distribution of DSB in plants makes it very difficult to test this hypothesis. Even so, the finding that mutations in yeast genes that encode mismatch repair enzymes inhibit the suppression of homeologous recombination (reviewed by Modrich and Lahue 1996; Borts *et al.* 2000; Evans and Alani 2000) provides a significant clue. If the suppression of homologous recombination in polymorphic regions of plant genomes is also dependent upon mismatch repair enzymes, then, because the substrates for mismatch repair are produced after DSB initiation, it is unlikely that polymorphism-mediated suppression of recombination occurs at the level of DSB initiation, but instead occurs by altering the relative outcomes of DSB repair.

**Why do recombination events cluster in genes?** On the basis of the observation that among eukaryotes the physical sizes of genomes vary more than the sizes of genetic maps and that the numbers of genes are fairly constant, Thuriaux (1977) hypothesized that recombination events occur primarily within genes. Consistent with this hypothesis, maize genes are usually recombination hot spots (reviewed by Puchta and Hohn 1996; Schnable *et al.* 1998) and many of the hot spots in the *a1-sh2* interval are associated with genes.

As discussed above, recombination hot spots often exhibit high levels of sequence similarity. Hence, the high level of sequence conservation in genes probably favors the occurrence of recombination in genes. But it has also been observed that repetitive retrotransposon sequences in intergenic regions exhibit low rates of recombination per megabase (Yao *et al.* 2002) even when these sequences are homozygous (Fu *et al.* 2002). Hence, a low level of sequence polymorphisms cannot by itself explain the existence of genic hot spots.

**Do region-specific chromatin structures affect meiotic recombination?** Even though polymorphisms can suppress recombination in some, but not all, intervals, our results also establish that a high degree of sequence similarity is not sufficient to create a recombination hot spot (*e.g.*, *x1* and subinterval I-mex). We hypothesize that the failure of the *x1* gene to act as a recombination hot spot in most haplotypes, even though it exhibits low levels of polymorphism, could be explained by the presence of local chromatin structure that does not support high rates of DSB initiation. If this is true, then some features of the mex and lux haplotypes must alter chromatin structure in the vicinity of the *x1* gene to

allow the 3′-end of this gene to function as a local hot spot. Differences in chromatin structure could also explain the ninefold lower rate of recombination per megabase within the 3-kb nongenic subinterval III-1-mex and the adjacent subinterval II-mex (Figure 5). For example, even though the more recombinationally active subinterval II exhibits a higher level of polymorphisms than does subinterval III-1, it contains the *a1* promoter, perhaps making it more accessible to recombination machinery than subinterval III-1, which contains mostly repetitive sequences. Therefore subinterval II-mex may be similar to α-hot spots of yeast (reviewed by Petes 2001). If this is true, it is clear that region-specific chromatin structure is not sufficient to stimulate recombination because subinterval II is not a hot spot in the more polymorphic lux and par haplotypes. Alternatively, the differences in the rates of recombination per megabase in subintervals I and II in the mex haplotype could be the consequence of competition between these two regions for DSB initiation or resolution sites.

***Cis*-modifiers of recombination can affect linkage disequilibrium (LD):** Whole-genome association mapping based on LD is an efficient tool to identify variant alleles of quantitative trait loci. Recombination shapes the genomic pattern of LD (reviewed by Gaut and Long 2003). In humans, the pattern of LD is correlated with rates of recombination per megabase; high LD blocks with low rates of recombination per megabase are interspersed with recombination hot spots that exhibit rapid decay of LD (reviewed by Goldstein 2001; Rafalski and Morgante 2004). The genome-wide pattern of LD in maize may have a similar structure (reviewed by Rafalski and Morgante 2004). Our data suggest that *cis*-genetic modifiers of recombination may affect the genome-wide pattern of LD in maize. For example, maize or teosinte populations that contain high frequencies of haplotypes in which the region between the *a1* and *yz1* loci is a recombination cold spot would be expected to exhibit a high degree of LD, whereas in other populations LD would be expected to decay rapidly in this interval due to the high frequencies of haplotypes in which this interval is a recombination hot spot.

High levels of LDs across maize genes are often thought to be associated with strong selection (reviewed by Gaut and Long 2003; Rafalski and Morgante 2004). This relationship, however, is complicated by the fact that alleles of a given gene can exhibit different rates of recombination per megabase. Hence, it is not possible to conclude that just because a gene exhibits a high degree of LD that it has been under selection. Consequently, additional characterization of genetic modifiers of meiotic recombination is likely to enhance our understanding of the genomic patterns of LD and thus help us to better interpret LD data.

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