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Comparative Recombination Distances Among *Zea mays* L. Inbreds, Wide Crosses and Interspecific Hybrids

Claire G. Williams,* Major M. Goodman,† and Charles W. Stuber‡

**Department of Forest Science, Texas A&M University, College Station, Texas 77843, †Department of Crop Science, North Carolina State University, Raleigh, North Carolina 27695 and ‡USDA-ARS, North Carolina State University, Raleigh, North Carolina 27695-7614*

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ABSTRACT

Recombination distances and linkage heterogeneity were compared among a wide range of maize inbreds, wide crosses and maize × teosinte hybrids. Twelve maize and four teosinte races were backcrossed to stocks fixed for rare marker alleles on chromosome arm *1L*. Recombination fraction estimates were higher for exotic germplasm than for either U.S. maize or maize × teosinte crosses. Serrano, Tuxpeño and a US-adapted inbred line of tropical origin, NC300, exhibited enhanced recombination. Three of the four maize × teosinte hybrids had little or no recombination between two loci. The observed recombination "shrinkage" resulted from an apparent inversion in the vicinity of the *Ampl* locus. Average recombination distances among common marker loci for composite maps were highly variable, even when map construction was restricted to maize germplasm of similar origins.

THE advent of syntenic molecular markers has kindled new approaches to the study of meiotic recombination, a process that generates new variability in recurrent plant breeding and provides the foundation for genetic map construction. Recombination occurs during meiosis I and crossovers are inferred from recombinants among marker loci. Recombination or map distances are then inferred using a map function that embodies assumptions about the phenomenon of recombination. Using markers, substantial genetic variability in recombination distances has been detected for many eucaryotic organisms (KOROL *et al.* 1994).

Genetic variability in recombination distance was first reported for maize more than 75 years ago (BREGGER 1918; STADLER 1926). Since then, chromosomal and genic mechanisms affecting recombination distances have been identified (NEL 1973; ROBERTSON 1984; CARLSON 1988; BROWN and SUNDERSAN 1991; DAS *et al.* 1994). Another phenomenon, cryptic structural differentiation (CSD), deters efficient introgression and compounds linkage drag (STEPHENS 1950; LONNQUIST 1974). CSD limits recombination between genetically distant parents, leading to reduced chiasma frequency or production of inviable or inferior recombinant products. Reduced chiasma frequency leads to "recombination shrinkage" in progeny from wide or interspecific crosses. Recombination shrinkage tends to decrease in later generations of backcrossing (RICK 1969).

Heterogeneity in recombination distances for maize may be common enough to preclude certain uses of a composite genetic map. Heterogeneity among maps has been reported for Corn Belt inbreds (BEAVIS and GRANT 1991; FATMI *et al.* 1993), which represent <2% of all maize germplasm (KIDD 1993), and for a composite population of adapted × exotic germplasm sampled five generations after the initial wide cross (TULSIERAM *et al.* 1992). Previous studies have been stymied by imbalance across linkage maps occurring as a result of monomorphic loci; this problem can be circumvented through backcrossing to a common parent fixed for rare marker alleles.

This report describes results of comparative mapping of recombination distances for 16 widely diverse populations representing maize and its wild relative, teosinte. It also addresses (1) some of the causes of variability in recombination distances and (2) the hypothesized relationship among distorted segregation ratios, recombination shrinkage and map distances in wide crosses and interspecific hybrids.

MATERIALS AND METHODS

New World maize germplasm was represented by 16 maize and maize × teosinte hybrid parents (Table 1). The entries were grouped into three categories based on genetic similarity: (1) U.S. populations (the public BSSS-derived inbred line B73 and its ultimate progenitors, Gourdseed and Longfellow), (2) exotic maize races and an adapted exotic inbred line and (3) several wild relatives of maize, the teosintes (see review by WILKES 1977). In all backcrosses except NC300, the chromosomal stock was the maternal parent to the F_1 as well as the recurrent maternal parent for BC1. For NC300, the recurrent maternal parent for BC1 was NC300 due to timing differences in flowering. The reciprocal cross of B73 used chromosome *1L* stock as paternal parent and then as the maternal parent for the backcross.

Corresponding author: Claire G. Williams, Department of Forest Science, Texas A&M University, College Station, TX 77843-2135.
E-mail: claire_williams@tamu.edu

TABLE 1

Description of parents of tropical maize, adapted maize and maize × teosinte backcrosses surveyed for recombination distances on Chromosome arm *1*L

Sample	Species	Origin	Race	Pedigree	Description
200	*Zea mays* L.	U.S. (A)	B73	Public inbred	Midwestern
25	*Zea mays* L.	U.S. (A)	B73 reciprocal	Public inbred	Midwestern
199	*Zea mays* L.	U.S. (A)	NC300	Public inbred	All-tropical origin
200	*Zea mays* L.	U.S. (A)	Longfellow	Founder OP race	Northeastern flint; Corn Belt founder
200	*Zea mays* L.	U.S. (A)	Gourdseed	Founder OP race	Southern dent; Corn Belt founder
200	*Zea mays* L.	Mexico (T)	Tuxpeño	OP race	Lowland dent
200	*Zea mays* L.	Guatemala (T)	Serrano	OP race	Highlands
200	*Zea mays* L.	Venezuela-Colombia (T)	Costeño	OP race	Caribbean flint
172	*Zea mays* L.	Mexico (T)	Cónico	OP race	Highlands
199	*Zea mays* L.	Cuba, West Indies (T)	Cuban Flint	OP race	Caribbean flint
200	*Zea mays* L.	Bolivia (T)	Coroico	Relic, indigenous population	Interior tropical lowlands
200	*Zea mays* L.	Bolivia (T)	Confite Puneño	OP race	Very high-elevation Andean highlands
200	*Zea mays* L.	Guatemala (T)	Tepecintle	OP race	Low elevation
196	*Zea luxurians*	Guatemala (H)	Guatemala	Wild maize relative	Rarely introgresses with maize
172	*Z. mays* ssp. *mexicana*	Mexico (H)	Central Plateau	Wild maize relative, $2N = 20$	Frequently crosses with maize
199	*Z. mays* ssp. *mexicana*	Mexico (H)	Balsas	Wild maize relative, $2N = 20$	Occasionally introgresses with maize
198	*Z. diploperennis*	Mexico (H)	*Zea diploperennis*	Wild maize relative, $2N = 20$	Poor crossability with maize compared with other teosintes

T, tropical maize; A, adapted maize; H, maize × teosinte backcross.

Chromosome arm *1*L was chosen for this study for several reasons. It is the longest chromosome arm in the maize genome and has a long interstitial region outside the immediate influence of the chiasmata-suppressing centromeric region. Variability in recombination distances was expected because a number of "hot spots" for enhanced recombination had been previously reported on chromosome arm *1*L (BEAVIS and GRANT 1991; TULSIERAM *et al.* 1992). Of the available rare-allele stocks, the chromosome *1*L stock offered a greater range of recombination distances, which allowed sample sizes (and standard errors) to be kept to a minimum.

The chromosome arm *1*L stock was developed over many generations from tropical and U.S. germplasm carrying rare alleles. All F_1 crosses were made in southern Florida; backcrosses were all made in Clayton, NC. Parent 6039-18b was used for all backcrosses except Longfellow, Coroico and Guatemala teosinte because the latter flowered when 5761-12# was not available. Stocks 5761-12 and 6039-18 were developed through recurrent sibbing; they share a common F_1 parent and have a coancestry of the same rare alleles.

Each of 16 backcrosses was based on 172 to 200 BC1 offspring. The *a priori* sample size was chosen based on the approximate variance of recombination fractions, estimated as $V_r = r(r-1)/n$ (BAILEY 1961; LORIEUX *et al.* 1995); this represents approximate standard errors of 1.1 to 2.1 cM for intervals 15 to 30 cM apart. The actual estimate of standard errors was used in cases of segregation distortion (BAILEY 1949; LORIEUX *et al.* 1995):

$$V_r = r(1-r)[(uv+1)(1-r)+(u+v)r] \times [(uv+1)r+(u+v)(1-r)]/4nuv \quad (1)$$

Using BAILEY's (1949) notation, u is the viability of the phenotypes carrying the A allele relative to the a allele and v is the viability of the B allele relative to the b allele. The recombination estimate is shown as r and the population size is n. If $u = v = 1$, then there is no segregation distortion and the standard error simplifies to $V_r = r(r-1)/n$.

We chose markers that were expressed early, exhibited codominant inheritance, showed conserved locus order among maps and represented transcribed gene products. The latter is important, given that recombination appears to occur near or around actively transcribed regions in maize (CIVARDI *et al.* 1994). Eight isozyme loci, *Amp1, Mdh4, mmm, Pgm1, Phi1, Adh1, Dia2* and *Acp4* spanned roughly 86 cM along the length of chromosome arm *1*L (Figure 1). Etiolated coleoptile tissue from 5-day-old seedlings was sampled and used for starch gel electrophoresis as described by STUBER *et al.* (1988). All gels were scored by two independent observers.

Segregation ratios: Segregation of the markers in each backcross was checked against the expected Mendelian 1:1 ratio using Pearson's chi-square goodness-of-fit statistic (SNEDECOR and COCHRAN 1980). The individual test criterion was adjusted upward using an experimentwise error rate of 0.05 (WEIR 1990, p. 109) to preclude false acceptance of the alternative hypothesis and to parallel data analysis for previous composite map construction (BEAVIS and GRANT 1991). For all 16 entries, segregation distortion was tested for all loci to detect an excess or deficiency of heterozygotes and for individual loci. For teosinte × maize hybrids, the number of recombinants were tested using a Poisson distribution with Yates' correction for continuity (SNEDECOR and COCHRAN 1980). As used here, this is effective as a test for inversions,

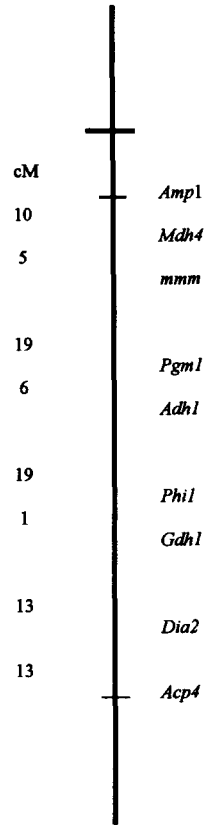

FIGURE 1.—Gene order for eight linked isozyme loci on chromosome arm *1*L for *Zea mays* L. based on WENDEL (1989).

where loci were classed into three separate regions and each region tested separately.

Recombination frequency and crossover distribution: A binary score was used for a relative comparison of crossover frequency among loci because the number of syntenic polymorphisms is balanced across nearly all pedigrees for a single linkage group. If a crossover was present in an interval between any pair of adjacent markers, it was assigned a score of 1. Each backcross received a total crossover score that was subsequently standardized so that scores had a total mean of 0 and a variance of 1.

Linkage map construction and test for linkage heterogeneity: To construct the actual linkages maps, we used MAPMAKER 3.0's F_2 backcross option (LANDER *et al.* 1987; LINCOLN *et al.* 1992) and verified map distance output using classical methods. Individual and pooled linkage maps were reported using the Kosambi map function to allow comparison with a previous composite map (WENDEL 1989). Maximum likelihood values for the test of linkage hetereogeneity in recombination distances were obtained from MAPMAKER.

The linkage heterogeneity was based on the maximum likelihood approach for two loci as shown by MORTON (1956). The M-test statistic is asymptotically distributed as χ^2 with $N - 1$ degrees of freedom (MORTON 1956; RISCH 1988; OTT 1991, p. 200):

$$\chi^2 = (2 \ln 10)\left[\left(\sum_{i=1}^{N} z_i(r)\right) - z_p(r)\right] \qquad (2A)$$

where z_i and z_p are the respective log 10 of the odds ratio (LOD) scores based on the maximum likelihood estimates of recombination for population i and for pooled (p) data from all N populations. The chromosome *1*L interval *Amp1-Acp4*'s recombination fraction is defined as θ where θ represents recombination among adjacent loci.

This M-test has been extended to multipoint linkage maps (OTT 1991, pp. 200–202; BEAVIS and GRANT 1991) where $L_i(\theta)$ and $L_p(\theta)$ are the log-likelihood values for linkage groups with the same set of adjacent loci in backcross i and for data pooled from all N backcrosses:

$$\chi^2 = 4.605\left[\left(\sum_{i=1}^{N} L_i(\theta)\right) - L_p(\theta)\right] \qquad (2B)$$

A significance level of 1% was appropriate for our study of a single linkage group. The chromosome arm *1*L linkage group had all markers in common so the direct M-test for linkage heterogeneity for each backcross was appropriate.

Composite maps were first constructed for all backcrosses, then separately for all backcrosses of U.S. origin, all maize × teosinte crosses, and all backcrosses of exotic origin. In addition to these latter, three backcrosses with increased recombination (Serrano, Tuxpeño and NC300) were used to create a separate composite map denoted as a "high recombination" map.

RESULTS

Variability in recombination distances: Recombination distances were clustered according to origin (Figure 2; Table 2). The exotic races, particularly Serrano, exhibited high crossover frequencies. NC300, a U.S.-adapted inbred line of tropical origin, showed recombination distances similar to those of its main progenitor race, Tuxpeño. B73 and its ancestral progenitors Longfellow and Gourdseed had low crossover frequencies relative to other maize × maize backcrosses. Balsas teosinte was similar to the maize × maize backcrosses although the three remaining maize × teosinte backcrosses showed extreme recombination shrinkage (Figure 2). In all regions except *Amp1-Mdh4* there was continuous variation for recombination frequency. The *Amp1-Mdh4* region exhibited a bimodal distribution for recombinants; one group had from 0 to 6.3 cM, and the remainder ranged from 8.8 to 16.9 cM (Table 2). The clustering of recombination distances within geographic origins for the maize × maize crosses is unlikely to be due to cryptic structural differentiation (CSD). The chromosomal *1*L stock had mixed temperate and tropical origins and thus is not distantly related to the maize parents. Only the interspecific maize × teosinte crosses showed substantial recombination shrinkage (Table 2).

Construction of a composite genetic map: None of the composite maps had statistically homogeneous recombination distances (Table 2). Even composite maps for smaller subsets of maps such as U.S.-origin germplasm and the "high recombination" exotic races had large chi-square values. These composite maps for maize had conserved locus order, but composite recombination distances were inaccurate estimators of target gene proximity. Any of these composite maps would probably be highly inaccurate for the purpose of using a genetic map to begin positional cloning along the physical map.

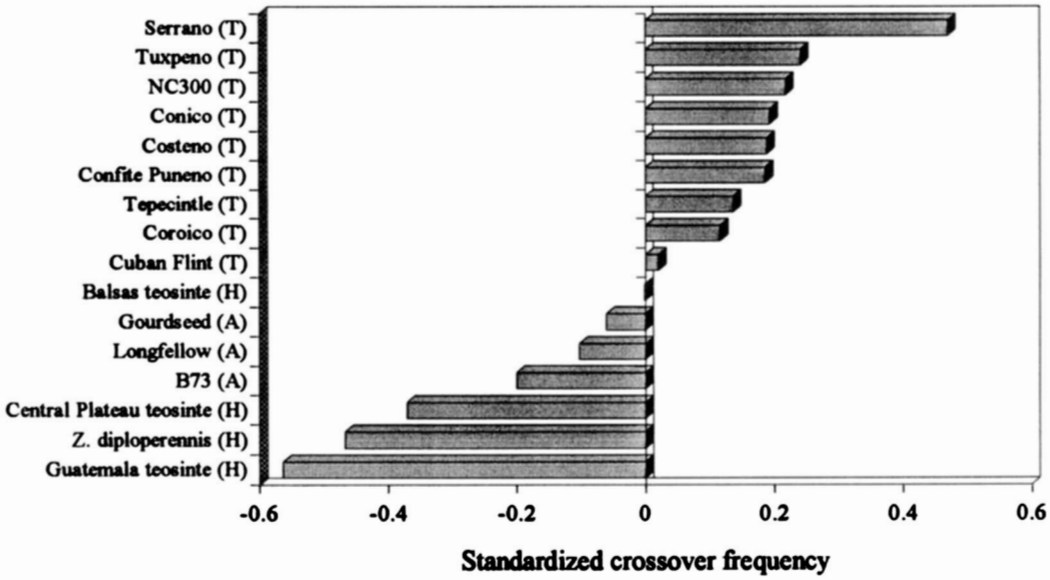

FIGURE 2.—Crossover frequencies in the *Amp1-Acp4* interval on chromosome arm *1*L for tropical (T) maize, adapted (A) maize and maize × teosinte hybrids (H) were transformed using the standardized normal distribution for relative comparison. The nontransformed overall mean and variance for total crossovers are 0.694 and 0.480 respectively.

There was a 43% spread in total map distance for chromosome arm *1*L among maize backcrosses. For single intervals, map distances varied two- to threefold (Table 2). For the *Amp1-Mdh4* interval, Cónico and Confite Puneño BC1 had recombination fractions of 6 and 17%. On the average, a 1% recombination fraction corresponds to an average of 1460 kb in maize (CIVARDI *et al.* 1994). If one assumed that this average ratio of cM to kb is indeed accurate for all pedigrees in the chromsome *1*L interval, then the difference in these estimates was threefold (8760 *vs.* 24,820 kb). Similarly, the composite map interval for exotic maize backcrosses alone was nearly 12%, suggesting an average physical distance of 17,520 kb as the appropriate spacing for

TABLE 2

Composite map of all backcrosses except Longfellow and Guatemala teosinte which were imbalanced with respect to polymorphic markers

MAP	*amp1-mdh4*	*mdh4-pgm1*	*pgm1-phi1*	*phi1-dia2*	*dia2-acp4*	Total cM (K)	*L* (θ)	χ^2 value (d.f.) Pr > 1%
Wendel (1989)	**15.0**	**19.0**	**25.0**	**14.0**	**13.0**	**86.0**	**NA**	**NA**
Composite	**9.7**	**15.9**	**17.4**	**14.4**	**12.7**	**70.2**	**−3240.34**	**282.93 (13)**
Composite- U.S.	**12.4**	**19.3**	**15.0**	**9.3**	**10.5**	**66.5**	**−460.14**	**68.06 (1)**
B73	8.5	16.2	11.7	11.6	9.4	57.4	−215.91	
Gourdseed	6.9	17.5	19.2	11.1	12.3	66.9	−227.52	
Composite-Exotic	**11.8**	**19.1**	**17.6**	**15.7**	**12.9**	**77.2**	**−2179.79**	**121.23 (8)**
NC300	12.1	17.4	18.9	14.7	15.0	78.1	−247.16	
Serrano	13.0	25.1	20.6	22.8	13.0	94.5	−265.58	
Tepecintle	11.0	17.9	12.6	20.9	12.3	74.7	−238.69	
Tuxpeño	12.5	21.2	21.6	8.0	15.1	78.3	−244.38	
Confite Puneño	16.7	23.0	18.7	7.1	11.5	76.9	−241.41	
Cónico	6.3	17.7	11.1	19.1	20.4	74.6	−202.02	
Coroico	15.4	13.2	15.6	19.9	10.8	74.9	−242.67	
Costeño	10.4	24.0	22.2	15.0	9.0	80.6	−244.41	
Cuban Flint	8.8	13.9	17.0	17.8	12.3	69.6	−236.01	
Composite "High"*	**12.5**	**21.2**	**20.3**	**14.9**	**14.3**	**83.2**	**−760.80**	**25.02 (2)**
Composite Maize × teosinte	**4.8**	**6.3**	**18.6**	**12.7**	**13.5**	**56.0**	**−597.14**	**89.24 (2)**
Balsas teosinte	14.0	9.3	24.5	12.2	9.8	69.8	−230.25	
Z. diploperennis	0	3.9	11.8	15.2	18.1	49.0	−184.58	
Central Plateau teosinte	0	5.6	20.7	10.6	12.7	49.7	−165.24	

* Composite "High" refers to a composite map of Serrano, Tuxpeño and NC300. Markers *Gdh1* and *mmm* were omitted here because they were not polymorphic for all races. Boldface refers to composite maps. NA, not available.

TABLE 3

Segregation distortion patterns for maize and tesosinte testcrosses to chromosome *1*L tester

Parent	*Amp1*	*Mdh4*	*mmm*	*Pgm1*	*Phi1*	*Gdh1*	*Dia2*	*Acp4*	Total %
B73 (A)	0.43	0.43	0.42	0.44	0.45	0.44	0.43	0.44	***38.0***
B73 reciprocal (A)	0.58	0.63	—	0.50	0.46	0.42	—	0.63	57.5
NC300 (A)	0.45	0.47	—	0.47	0.51	0.50	0.54	0.53	49.0
Longfellow (A)	0.51	0.47	—	0.49	0.52	0.51	0.54	—	50.0
Gourdseed (A)	0.56	0.55	0.56	***0.60***	0.56	0.56	0.57	0.54	***64.5***
Tuxpeño (T)	0.54	0.52	0.51	0.49	0.46	0.48	0.51	0.51	49.0
Serrano (T)	0.47	0.48	0.47	***0.38***	0.45	—	0.55	0.59	44.5
Costeño (T)	***0.60***	***0.59***	***0.59***	0.55	0.54	0.55	0.54	0.55	***63.0***
Cónico (T)	0.42	0.43	0.46	0.45	0.48	0.47	0.46	0.47	***43.0***
Cuban flint (T)	***0.59***	0.56	0.56	0.56	0.51	0.50	0.53	0.51	***58.0***
Coroico (T)	0.45	0.47	0.54	0.50	0.48	0.47	0.44	0.43	***43.0***
Confite Puneño (T)	0.51	0.49	0.48	0.44	***0.40***	***0.40***	***0.38***	***0.38***	***38.0***
Tepecintle (T)	0.49	0.49	0.47	0.50	0.50	0.51	0.50	0.45	48.5
Balsas teosinte (H)	0.46	0.50	0.52	0.52	0.47	0.44	0.46	0.44	46.0
Guatemala teosinte (H)	***0.40***	***0.41***	***0.41***	***0.42***	***0.44***	***0.41***	—	***0.36***	***35.0***
Central Plateau teosinte (H)	***0.39***	***0.40***	***0.40***	***0.40***	***0.40***	***0.40***	***0.40***	***0.46***	***34.0***
Zea diploperennis (H)	***0.26***	***0.25***	***0.26***	***0.26***	***0.30***	***0.31***	***0.36***	0.47	***22.0***

Chi square tests are based on monogenic and total segregation ratios for isozyme loci on the long arm of chromosome *1*. The values shown are the proportions of heterozygotes at individual loci followed by the total proportion (%) of heterozygotes summed across all loci in the *Amp1-Acp4* interval. ***Italicized bold*** proportions indicate values deviating from expected 0.50 with a probability of greater $\chi^2 > 5\%$. T, tropical; A, adapted; H, maize × teosinte backcross.

the *Amp1-Mdh4* interval. Inaccuracy increases with the inclusion of maize × teosinte hybrids. These interspecific crosses increased the range in total map distance along chromosome arm *1*L from 49 to 95.9 cM (Table 2). Such differences for an adjacent pair of loci were greatest near the inversion at *Amp1-Mdh4* ranging from 0 for the *Zea diploperennis* backcross to 16.9 cM for Confite Puneño (Table 2).

Distorted segregation ratios and recombination shrinkage: There was no support for a causal relationship between increased recombination distance and segregation distortion (Figure 3). There was a slight tendency for the converse, *i.e.,* an excess of homozygosity which coincided with severe recombination shrinkage for two of the maize × teosinte backcrosses, *Z. diploperennis* (78%) and Central Plateau teosinte (66%) (Table 3; Figure 3). Inversion heterozygotes produce recombinant gametes with unbalanced chromosomes, fertilization results in inviable zygotes and few, if any recombinants, are recovered in seedlings (MORGAN 1950; RHOADES and DEMPSEY 1953). Reduced map distances for three of the four teosinte parents suggested that the F_1 parents were inversion heterozygotes (Table 4).

The importance of inversions, rather than distorted ratios, as a cause of recombination shrinkage is also supported by a previous study (DOEBLEY and STEC 1991). They report a map distance of 6.6 between markers *umc107-umc83* for maize × teosinte using Chalco teosinte (a maize mimetic) crossed to a Mexican maize race. This is fourfold less than reported for the U.S. domestic maize composite map (27.5 cM). Despite the extreme recombination shrinkage, they report no segregation distortion at an adjacent locus, *bnl 5.59* in the maize × teosinte cross (DOEBLEY and STEC 1991).

Maize backcrosses exhibited distorted segregation ratios that were random with respect to origin. Deviations from the expected 1:1 segregation ratios varied from a deficiency of homozygotes for Gourdseed (35.5%) and Cuban Flint (42%) to a consistent excess of homozygotes for three of the teosintes (as low as 25% at the *Mdh4* locus for *Z. diploperennis*) (Table 3). The types of segregation distortion appeared to differ among backcrosses. For example, B73 has a excess of homozygotes but its reciprocal F_1 cross had a deficiency of homozygotes (Table 3). In this case, the reciprocal referred to the parental cross rather than the backcross so it was evidence for sex-specific gametic selection. In most of the teosintes, especially in *Z. diploperennis,* there was also evidence for selective elimination of the teosinte parent. In the region from *Amp1-Dia2* the frequency of teosinte-derived alleles varied from 25 to 36%. This was substantially less than the expected gametic contribution of 50%. The pedigrees in this study were not sufficient to discriminate among types of segregation distortion models.

DISCUSSION

This is the first comparative survey of recombination distances in New World maize. Linkage heterogeneity among pedigrees implies that composite maize maps are mostly useful for ordering loci. This comparative mapping effort suggests some taxon- and pedigree-specific mechanisms profoundly alter recombination distances and distort segregation ratios.

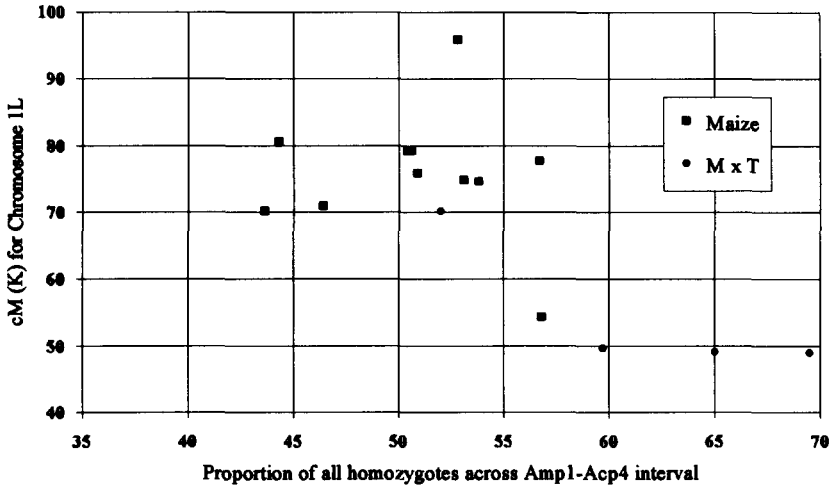

FIGURE 3.—Relationship between distorted segregation ratios and map distance for chromosome arm *1*L. Distorted ratios are expressed as proportion of total homozygotes across eight loci. Data are based on 15 maize backcrosses with open-pollinated races, inbred lines and maize × teosinte hybrids.

Implications for map-based cloning and composite map construction: Map-based cloning has been successful when a locus can be mapped to a chromosomal position adjacent to segments of DNA that have already been cloned using RFLP or microsatellite markers (TANKSLEY *et al.* 1995). In theory, a chromosome walk is initiated from the closest marker, then a series of overlapping clones are isolated along the physical length of the chromosome. The greatest deterrent to chromosome walking in maize is the large amount of interspersed repetitive DNA. The cM to kb ratio is often used as a likely measure of success for map-based cloning but the genome-wide average is unreliable because there is a nonlinear relationship between genetic map distance and physical lengths. Another approach is to estimate the cM to kb ratio within a restricted length of chromosome (CIVARDI *et al.* 1994). The comparative mapping results presented here suggest that the cM to kb ratio is not restricted to a chromosome length but may also be specific to a pedigree because cM intervals vary widely. Our data support the need for alternatives to map-based cloning for large, complex genomes such as maize (*e.g.*, FRANCES *et al.* 1995; TANKSLEY *et al.* 1995).

There is a tendency to merge genetic maps to simplify available information. Algorithms designed for merging maps with imbalance in polymorphic loci explicitly assume that recombination distances are constant (STAM 1993). This assumption is violated for maize; the variability in recombination fractions was often twofold, even among closely related pedigrees. Recombination fractions are altered by nonrandom crossover distribution, a number of chromosomal factors and a few genic mechanisms (*e.g.*, NEL 1973; ROBERTSON 1984; LALOUEL *et al.* 1986; BROWN and SUNDERSAN 1991; DAS *et al.* 1994).

No one pair of composite maps had homogeneous estimates of recombination distance. The inaccuracy of recombination distances in composite mapping was further magnified by the existence of hot spots in distal and interstitital chromosomal regions. The existence of hot spots compounds the inaccuracy of average recombination distance of composite maps in relation to the physical map distance. The correlation between genetic and physical distances even among related pedigrees is poor; making composite maps among divergent pedigrees compounds the inaccurate relationship. Thus the value of composite maps based on wide crosses or interspecific maize hybrids is largely synteny, *i.e.*, gene order.

Selecting on recombination distances: The variability in recombination distances raises the question of how to select for genetic backgrounds with enhanced or restricted recombination rates. Selection for genes that

TABLE 4

Deficiency of recombinants for maize × teosinte backcrosses

			Amp1-Mdh4-mmm observed			
Genotype	Class	Expected	Dip	Gua	CP	Bal
ABC/abc	NCO1	84	50	77	74	82
abc/abc	NCO2	84	149	115	114	87
Abc/abc	SCO1	10	0	1	0	9
aBC/abc	SCO2	10	0	3	1	18
Abc/abc	SCO3	5	0	0	0	0
abC/abc	SCO4	5	0	0	0	3
AbC/abc	DCO1	0	0	0	0	0
aBc/abc	DCO2	0	0	0	1	0

modify crossover frequencies has been proposed for decades (*e.g.*, DETLEFSEN and ROBERTS 1921). Many studies show a pattern of continuous variation in recombination distances between any two marker loci, and this has been construed as evidence for polygenic inheritance for genes controlling recombination rates. If the genes controlling recombination distances are truly polygenic with additive effects, then bidirectional selection should yield a linear response to selection on observed recombination distances across generations. For maize, alternatives to classical bidirectional selection on observed recombination distances are needed.

First, selection on observed recombination distances is unlikely to yield a linear selection response across generations. Observed recombination distance is a complex product of chromosomal and genic mechanisms, most of which do not display classic Mendelian inheritance. Supernumerary chromosomes encourage preferential segregation (*e.g.*, NEL 1973), chromosomal rearrangements exert arbitrary effects on recombination (*e.g.*, ROBERTSON 1984) and movement of transposable elements increases intragenic recombination throughout the genome (*e.g.*, BROWN and SUNDERSAN 1991; CIVARDI *et al.* 1994). These types of mechanisms serve as a genetic background that can obscure or bias selection for modifier genes. Developing a reliable genic model and selecting on tightly linked markers in a standardized genetic background may prove to be a better approach for modifying recombination rates in maize.

Second, there is likely to be a lower bound on restricted recombination that will result in a plateau in selection response in the early generations. This is hypothesized because there appears to be a minimum requirement of at least one chiasma per chromosome arm and this requirement is genetically conserved. One chiasma per arm ensures orderly disjunction of chromosomes during anaphase; without it gametes can receive unequal, and perhaps inviable, chromosomal complements. The requirement for one chiasma per arm may place a lower limit on selection for restricted recombination.

There is wide variability in the recombination rates studied here, especially among the tropical maize open-pollinated races. The maize × maize backcrosses show continuous variation for recombination distances, but this does not constitute an argument for polygenic inheritance. Continuous variation is not proof of genic inheritance; here it represents an aggregate value for combined chromosomal and genic mechanisms. Modifier genes with major effects on recombination have been reported, but the evidence is definitive only if based on patterns of variation across generations. For example, DAS *et al.* (1994) report segregation at a locus showing a twofold increase in recombination. The locus exhibits dominant-recessive inheritance and has a demonstrated effect across more than one generation.

A genic model for modifying recombination would be useful for testing for this lower limit to selection and for testing whether enhanced recombination rates improve efficacy of recurrent plant breeding. However, selecting directly on observed recombination rates does not appear to be realistic for maize.

Recombination shrinkage and segregation distortion: These data did not support a causal relationship between increased recombination distances and segregation distortion. This was first proposed as an explanation for recombination shrinkage in Lycopersicon (PATERSON *et al.* 1990), and it was based on the assumption that base-sequence homology determines where crossing-over will occur (BORTS and HABER 1987). According to this assumption, base sequence-identical homologues initiate reciprocal genetic exchange during meiosis I. Hence reciprocal genetic exchanges are more likely to occur between sequence-identical homologues in homozygous regions than between sequence-divergent strands in heterozygous regions. If so, more crossovers should be recovered along a chromosome arm if there is an excess of homozygotes. Segregation distortion mechanisms that produce an excess of homozygotes are predicted to have more crossovers, thus recombination fractions (and distances) will be higher than expected. Conversely, an excess of heterozygotes would be expected to exhibit recombination shrinkage.

These results do not support the explanation hypothesized by PATERSON *et al.* (1990). This can be attributed to the fact that the base-sequence homology model (BORTS and HABER 1987) is poorly supported by more recent experiments. Later studies show no effect of heterozygosity on crossover or gene conversion frequencies (MALONE *et al.* 1994). Also, there is growing evidence supporting the importance of chromatin structure and epistatic control rather than base sequence homology as the prompt for initiation of genetic exchange events (WU and LICHTEN 1994, 1995).

It is not necessary to have an exact knowledge of distortion factors to estimate recombination distances although segregation distortion can mildly bias estimates of recombination distance (BAILEY 1961, pp. 49–52). The bias depends on whether zygotic or gametic selection is distorting the ratios, whether markers are codominant, dominant or a mixture, and what type of pedigree is used (BAILEY 1961, pp. 49–52; HEUN and GREGORIUS 1987; WAGNER *et al.* 1992).

In our case of codominant markers and backcross pedigrees, there was a slight bias in recombination fractions proportional to distortions in gene frequencies. For example, the maize race Serrano had distorted ratios for the *Pgm1* and *Phi1* loci (Table 3) for which there are four gametic classes: two parental (*A1/B1*, *A2/B2*) and two recombinant (*A2/B1*, *A1/B2*). In the absence of distortion, the combined gene frequencies for recombinant types are expected to be 0.50 (0.25 for *A2/B1* and 0.25 for *A1/B2*). Distortion altered the combined gene frequencies of recombinant gametic

types to 0.49 (0.28 for *A2/B1* and 0.21 *A1/B2*). This reduced the probability of recovering recombinant gametes, thus very slightly biasing the true recombination distance downward.

Normal segregation in a diploid plant depends on euploidy, on regular chromosome disjunction at anaphase I, and on equal viability at both gametic and zygotic stages (GRANT 1975, pp. 228–250). These pedigrees were not sufficient to discriminate among the types of segregation distortion models, but there are several testable hypotheses. For example, there was some evidence for selective elimination of the teosinte parent (Table 3); Guatemala, Central Plateau teosinte and *Z. diploperennis* had a deficiency of heterozygotes at all observed loci, a phenomenon associated with chromosomal elimination in interspecific crosses (KASHA and KAO 1970). Loss of the teosinte chromosomal complement in the F_1 parent can be tested with pachytene squashes and with marker analysis of F_2 offspring where both teosinte homozygote and heterozygote classes would be under-represented for some linkage groups.

A second factor that can cause segregation distortion is gametic selection associated with a zygotic lethal transmitted by the exotic parent. If the lethal was partially dominant, this would cause a slight deficiency of heterozygotes, as in the case of Coroico, Confite Puneño and Cónico (Table 3). This hypothesis could be tested with marker analysis of F_2 offspring (*e.g.*, FU and RITLAND 1994). In the case of partial dominance ($0 < h < 0.5$), the heterozygote class would be under-represented, and the exotic maize homozygote would be nearly absent if tightly linked to the lethal allele. Many zygotic lethals have been reported for exotic maize; EMERSON (1939) found a zygotic lethal allele on chromosome arm *1S* in Bolivian maize of unspecified origin.

In the present study, completely recessive lethal alleles from exotic maize parents can be ruled out. The chromosome arm *1L* stock were used as the common recurrent parents for all backcrosses except NC300, and the homozygote class is composed of alleles from the chromosome arm *1L* inbred parent. If lethal alleles from the chromosome arm *1L* parent were distorting segregation ratios, then all backcrosses would show a similar excess of heterozygotes.

In summary, recombination distances among pedigrees were highly variable and clustered according to geographic origins; only the interspecific maize × teosinte crosses exhibited recombination shrinkage. Linkage heterogeneity precluded map merging but gene order was conserved in all pedigrees. Distorted segregation ratios did not cluster according to geographic origin and appeared to arise from diverse causes.

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