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A linkage map based on information from four F_2 populations of maize (*Zea mays* L.)

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Summary. Recently, maize (*Zea mays* L.) genetic maps based primarily upon segregating restriction fragment length polymorphisms (RFLPs) have been developed by several research groups. Some of the reported maps were based upon data from a single segregating population, whereas others were based upon information from several segregating populations. Potential problems with pooling information from several segregating populations have not been reported. These include the fact that few genetic markers are polymorphic in all populations, estimates of linkage may differ among populations, and population sizes may differ. We utilize the log-likelihood statistic to genetically map partially overlapping sets of informative genetic markers, to test homogeneity of recombination among populations, and to present a composite RFLP linkage map based upon data pooled from four F_2 populations.

Key words: Multipoint linkage – Recombination – Segregation – Homogeneity test

Introduction

Several maize (*Zea mays* L.) genetic maps using RFLP and isozymic markers have been reported. Helentjaris et al. (1986) used data from four F_2 populations to construct the first reported maize RFLP map. Linkage maps from each F_2 population were combined visually to construct a composite map by inference (T. Helentjaris, personal communication). These results have been extended and refined to include the approximate locations of the centromeres and many translocation breakpoints (Weber and Helentjaris 1989). D. A. Hoisington and J. Gardiner (personal communication, Maize Genet Coop Newsl 61:49) and M. Murray et al. (personal communication) constructed similar RFLP maps, but with mostly different RFLP markers, using information from segregating populations derived from the cross TX303 × CO159 and A619Ht × Mangelsdorf-tester, respectively. Burr et al. (1988) used 45 recombinant inbred lines (RI) from the cross TX232 × CM37, and 38 RIs from TX303 × CO159 to map their RFLPs. Their map was based on pooled estimates of recombination between pairs of markers, where estimates of recombination between pairs of markers were the same for the two populations. In cases where recombination was determined to be unequal between the populations, an average of the two recombination values was reported in their composite map.

There is a need to integrate RFLP maps with maps based upon morphological markers (D. A. Hoisington, personal communication; MNL). Strategies for integrating RFLP linkage maps with morphological markers and methods for integrating cytogenetic features have been presented by Hoisington and Coe (1989a, b). There is also a need to integrate different RFLP linkage maps, but a methodology for pooling these multipoint data has not yet been suggested.

We have been constructing genetic linkage maps in several segregating F_2 populations and have encountered technical problems when attempting to pool multipoint information from more than one population. Techniques used in pooling data sampled from several populations must be capable of handling cases where not all markers are informative in all populations, and must be able to identify genomic regions with unequal recombination. Since previous reports of maize genetic linkage maps have not addressed these issues, we decided to investigate them while constructing an across-population, or composite, RFLP linkage map.

Techniques for mapping genetic markers that are not informative across populations have been developed by human geneticists. Morton (1955) recognized the problem as one of estimating missing values. Initially, the application of theory to more than a few genetic markers was computationally prohibitive (Morton et al. 1986), but an efficient EM algorithm was applied to the theory (Lander and Green 1987) and put into practice with the MAPMAKER computer program (Lander et al. 1987). We utilized MAPMAKER to develop a composite map from four F_2 populations and used its computation capabilities to extend Morton's (1956) homogeneity test to multipoint linkages among the populations. Finally, we present a composite map and individual population maps to convey the interpretation of the composite map and its limitations.

Materials and methods

Data were obtained from genetic markers that were informative in four F_2 populations (Table 1). The parents of these populations were B73, a central corn belt line derived directly from Iowa Stiff Stalk Synthetic (BSSS); V94, a central corn belt line related to BSSS; J40, a northern corn belt line related to both BSSS and Lancaster Surecrop; MO17, a central corn belt line derived from Lancaster and Krug germ plasm; G35 and W65, central corn belt lines of complex pedigree history and unrelated to BSSS; and K05, a northern corn belt line unrelated to BSSS. We refer to B73 × G35 as population 1, B73 × MO17 as population 2, K05 × W65 as population 3, and J40 × V94 as population 4.

All crosses were made by hand-pollination. Ten F_1 plants each from populations 1 and 2 were selfed in Hawaii during the winter of 1985–86. Ten F_1 plants each from populations 3 and 4 were selfed in Florida and Hawaii, respectively, during the winter of 1986–87. F_2 seed from the 10 F_1s were bulked for each population.

Data collection

Segregation data for 112 or 144 lines (Table 1) of the RFLP markers were obtained using DNA extracted from either F_2 plants or from six to ten pooled F_3 or F_4 plants. When needed, equal weights of the individual F_3 or F_4 leaf samples were pooled prior to vacuum drying and DNA extraction (Saghai-Maroof et al. 1984). The RFLP profile obtained from the pooled samples was assumed to be equivalent to that of the original F_2 individual, except in heterozygous individuals where band intensities were not always equivalent. Restriction enzyme digestions, gel electrophoresis, and transfer of the DNA to nylon membranes and DNA hybridizations were done using standard conditions (Sambrook et al. 1989). Isozyme scores were obtained as described by Stuber et al. (1988).

Most of the 209 genetic markers that we used are *Pst*I genomic DNA clones. These are named according to the conventions proposed by E. H. Coe and D. A. Hoisington (personal communication; MNL) and are preceded with a prefix that denotes the original developer: bnl – Burr et al. (1988); umc – D. A. Hoisington and J. Gardiner (personal communication; MNL); pio – this report. Where possible, we selected RFLP probes that hybridized to a single site in the genome of these inbred lines although, in several cases, we have used probes that hybridize to two sites. Probes that hybridize to more than one site in the genome are arbitrarily identified on the maps with the suffixes A and B, where A denotes the locus that was mapped first. Other genetic markers used include isozymes and DNA probes from identified genes.

Segregation and linkage analysis

Segregation of the genetic markers in each F_2 population was checked against that expected due to Mendelian inheritance with Pearson's Chi-square goodness-of-fit statistic (Snedecor and Cochran 1980). The test criterion was adjusted to an experiment-wise error rate of 0.05. Genetic markers that were informative and exhibited Mendelian inheritance were mapped in each individual population using MAPMAKER (Lander et al. 1987).

In order to create a composite map, we assumed that markers that mapped to similar chromosomal regions in different populations were identifying the same chromosomal locations. Although there are few RFLP loci that are informative in all segregating populations, it is possible to pool scores across populations because most markers were informative in more than one population (Table 2). Scores are pooled by assigning missing values to individuals in populations with monomorphic bands (Ott 1985). Thus, construction of a composite RFLP map merely entails pooling segregation scores for all RFLPs from individuals in similarly derived families, determining the likelihood equations for each of the types of families, and maximizing these for the pooled data. Because MAPMAKER (Lander et al. 1987) has likelihood equations for F_2 populations, we were able to pool data from our markers in all four populations and build a composite map with this readily available software.

For linkage groups in populations where the most likely gene order was not consistent with the composite map, the log-likelihood of the most likely gene order in each population was compared with the log-likelihood of the most likely gene order from the composite population. If the difference was not significant (difference in lod < 3.0) then the gene order of the composite map was used for the population.

Tests of homogeneity of recombination

Linkage maps for different populations consisting of a common set of genetic markers may not be equivalent, because individual markers fail to exhibit Mendelian segregation or because recombination is not homogeneous among the populations. If the genetic markers show Mendelian segregation in each population and in the pooled population, then differences in linkage maps can be attributed to differences in estimated recombination (Morton 1955).

By considering only markers that showed Mendelian segregation, we were able to investigate differences in recombination among markers that were informative in more than one population. Many studies of recombination in maize have compared estimates from different populations using the standard errors of the estimates (Stadler 1926; Rhoades, 1941; Burnham, personal communication; Phillips 1969). Homogeneity of recombination among populations can be tested formally using contingency tables (Allard 1954; Nel 1973; Robertson 1984) and Pearson's Chi-squared statistic (Snedecor and Cochran 1980), but the populations must be similarly derived, i.e., composed of the same phenotypic classes. Fisher (1949) developed a statistic that is asymptotically distributed as χ^2 with $N-1$ *df* to test homogeneity of recombination between a pair of markers:

$$\left(\sum_{i=1}^{N} \frac{L_i(r)^2}{I_i(r)} - \frac{L_p(r)^2}{I_p(r)}\right), \tag{1}$$

where L_i is the log of the likelihood equation for population i, given the maximum likelihood estimate of recombination (r) for data pooled for all N populations, I_i is the information index for r from population i and L_p and I_p are the sum of the L_i and I_i, respectively. Because the likelihood equations are determined separately for each type of population, this statistic can be used to test homogeneity of recombination among populations with different types of families. For example, Allard (1954) used this statistic to compare recombination estimates between genetic markers for growth habit and seed color in lima beans from six different types of genetic families.

Due to the ubiquitous nature of RFLP markers in the maize genome, multipoint data are used to map m (>2) linked loci. For m linked loci, it would be possible to obtain $m-1$ maximum likelihood estimates of recombination (Lander and Green 1987) and find $L_i(r)$ and $L_p(r)$. For m linked loci, I_i and I_p represent information matrices calculated from m^2 second derivatives of the likelihood for each population.

For multipoint data, Eq. 1 could be applied to all pairs of adjacent markers, but error rates for multiple two-point estimates, usually set at 0.05 for each comparison, would incorrectly identify differences among populations. An error rate associated with all markers in a linkage group would be more appropriate. However, estimates of recombination values for more than two linked markers are not independent; thus, the information matrix consists of variances of recombination estimates and non-zero covariances among recombination estimates. For multipoint data, it is not abvious how to apply Eq. 1 and control the type I error rate.

Morton (1956) proposed a homogeneity test statistic that also is asymptotically distributed as χ^2 with $N-1$ df:

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

where z_i and z_p are the respective log of the odds ratio (lod) scores, given the maximum likelihood estimates of recombination for population i and for data pooled from all N populations. This statistic is asymptotically equivalent to Eq. 1, but is more exact for small populations (Morton 1956). Morton's test statistic can be extended to multiple linked loci by replacing r with θ to denote the set of recombination parameters ($\theta_1\ \theta_2\ \theta_3 \ldots \theta_{m-1}$), where θ_j represents recombination between adjacent loci (Lander and Green 1987) in a linkage group. It is easy to show that

$$\left[\sum_{i=1}^{N} z_i(\theta) - z_p(\theta)\right] \quad (2)$$

is equivalent to

$$\left[\sum_{i=1}^{N} L_i(\theta) - L_p(\theta)\right], \quad (3)$$

where $L_i(\theta)$ and $L_p(\theta)$ are the log-likelihood values for linkage maps with the same set of m adjacent loci in population i and for data pooled from all N populations. Thus, by extending Morton's (1956) statistic to include more than two loci in a linkage group, a test for homogeneity among populations for multipoint data is possible.

It should be emphasized that in the application of this statistic, all populations have the same set of polymorphic markers. Unfortunately, there are few genetic markers that are informative across all populations. For example, among our four populations, there are 37 probes that hybridize with loci on chromosome 1 (Table 2), but only three of these are polymorphic across all four populations and only two of the three appear to be linked (Fig. 1). Thus, we could not investigate homogeneity of recombination for most of the maize genome by applying Eq. 3 to our $N=4$ populations.

In order to evaluate a larger percentage of the genome, we compared the linkage map of one population with the linkage map based on data pooled from the remaining three populations. Operationally, this was accomplished by identifying a set of linked markers, informative in one population, that have corresponding segregation data (for the same set) in any of the remaining populations. The homogeneity test statistic is then composed of $N=2$ populations, one based on data from a single population and one based on data pooled from the remaining populations. For example, of the 37 markers that were mapped to chromosome 1 in at least one of the four populations (Table 2), 23 were polymorphic and mapped in population 1 (Fig. 1). Of these, pio200855, umc83, and pio200870 are unique to population 1, and a test for linkage homogeneity between population 1 and the other populations would not include these. The log-likelihood for the linkage maps consisting of the remaining 20 markers was computed first for population 1 (Table 3), then for the same 20 markers based on data pooled from the remaining three populations. Finally, the log-likelihood of the linkage maps for these 20 markers based on data pooled for all four populations was computed so that the χ^2 statistic could be computed.

This procedure was applied to all ten linkage groups, so it is important to use a significance level for the test that is associated with the entire genome rather than for a single pair of adjacent markers. Numerical simulations are needed to determine the appropriate nominal significance levels for Eq. 3 in situations where independent linkage groups have more than two markers. However, the approximate relationship

$$1-(1-\alpha')^{M/r} \simeq \alpha \quad (4)$$

used by Lander and Botstein (1989) to describe the relationship between nominal significance levels (α') and experiment-wise error rates (α) in identification of QTL for genomes of size M with an average spacing among markers of r (cM/100) can also be applied here. For example, we tested homogeneity of recombination between population 1 and the other three populations over 18 M of the genome using 86 markers from all ten linkage groups. In order to avoid incorrect ($\alpha=0.05$) identification of unequal recombination in any genome region, we used a nominal significance level of α' of $\simeq 0.0006$.

In cases where linkage homogeneity between the population of interest and the remaining populations was rejected, we investigated recombination among adjacent markers within the linkage groups. This procedure was repeated for each of the populations.

Results and discussion

Segregation at ten RFLP loci deviated from a 1:2:1 ratio in at least one of the populations (data not shown). With the exception of bnl9.08 and bnl10.39, which showed linkage to chromosome 8 of population 1, the loci that exhibited aberrant segregation were not genetically linked. Genetic markers that did not segregate as expected in an F_2 population were not mapped in that population and data from these markers were not included in the homogeneity tests. The pooled data from all four populations showed no deviations from Mendelian segregation for any genetic markers, unless segregation of a genetic marker was non-Mendelian in an individual population. In particular, bnl6.29 and bnl9.08 did not segre-

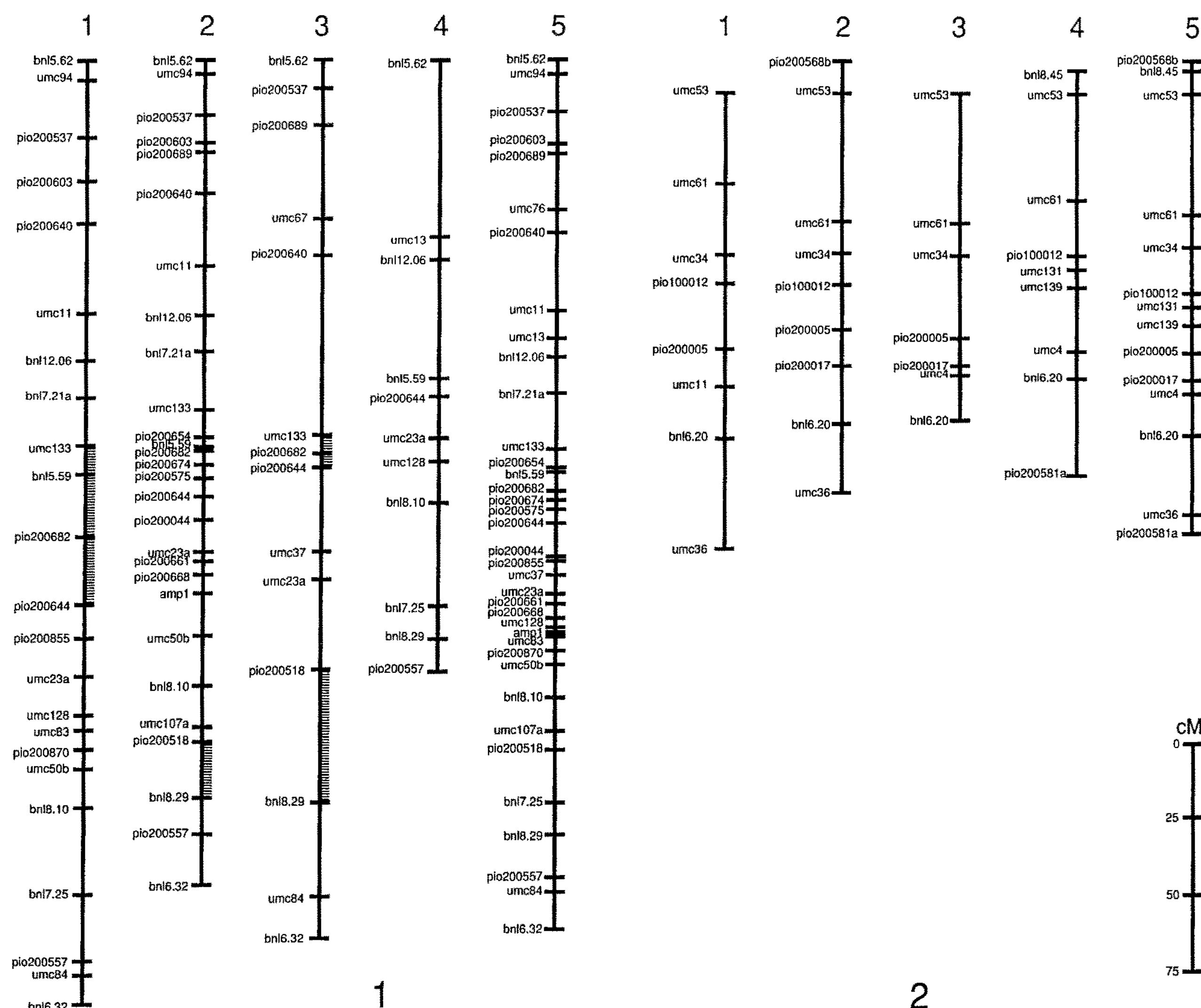

Table 1. Number of progeny, genetic markers, and estimated size of genome from four F_2 maize populations

Population	Parental cross	Number of progeny	Number of genetic markers	Estimated genome size (M)
1	B73 × G35	112	106	21
2	B73 × MO17	112	148	22
3	K05 × W65	144	78	16
4	J40 × V94	144	68	15

gate as expected in population 1 and pio200531 did segregate as expected in population 3, nor did these loci exhibit Mendelian ratios when pooled with data from other populations. Data from these populations for these three loci were treated as missing values in construction of the composite map, i.e., these loci were mapped using only data from populations exhibiting Mendelian segregation. The remaining seven genetic markers that exhibited aberrant segregation in an individual F_2 population did show Mendelian segregation when pooled with data from other populations. As with loci that exhibited normal segregation in all populations, all available data were used for these seven loci when constructing the composite map.

The estimated genetic order of RFLP loci in each of the populations was not statistically different from the composite map (Fig. 1). A consistent order is to be expected if the probes are identifying the same chromosomal locations and if there are no erroneous or missing scores. Based upon limited simulations (our unpublished data), we have found not only incorrect estimates of linkage, but also incorrect estimates of order for linkage groups with loci that are loosely linked ($r > 0.2$) to clusters of tightly linked ($r < 0.05$) loci and more than 25% of the scores missing. None of the probes in populations 1, 3, and 4 had more than a few missing scores, but seven of the loci in population 2 had more than 25% missing scores and for

3

1: bnl8.15, pio200042, pio200006, bnl13.05b, pio200802, umc26, bnl5.37, umc82, bnl6.16, pio200521, pio100080, pio200726

2: bnl8.15, e8, pio200006, bnl8.35, umc10, pio200576, pio200511, pio200558, pio200509, pio200508, umc26, bnl5.37, bnl10.24a, umc82, umc3, bnl6.16, pio150033, pio200521, umc17, umc16, umc999a, pio100080, pio200563

3: bnl8.15, bnl8.35, umc10, bnl6.16, pio150033, pio100080, pio200726

4: bnl8.15, pio1200006, bnl5.37, bnl1.24a, bnl6.16, pio150033, pio100080

5: bnl8.15, e8, pio200042, pio200006, bnl8.35, umc10, pio200576, pio200511, pio200558, pio200509, pio200802, bnl13.05b, pio200508, umc26, bnl5.37, bnl10.24a, umc82, umc3, bnl6.16, pio150033, pio200521, umc17, umc16, umc999a, pio100080, pio200726

4

1: umc87, pio200727, bnl5.46, umc999b, bnl15.45, umc66, umc19, bnl7.65, pio200071, pio200562, umc15, pio100025

2: pio200725, pio200713, umc31, bnl5.46, pio200597, bnl15.45, umc42, umc66, umc19, bnl7.65, pio200071, pio200562, umc52, pio200608, bnl8.23, pio200563

3: bnl5.46, bnl15.45, umc19, bnl7.65, pio200071, pio200562, pio100025, pio200608

4: umc31, bnl5.46, umc47, umc23b, umc66, bnl7.65, umc52, pio200608, bnl8.23

5: umc87, pio200725, pio200713, umc31, bnl5.46, umc999b, pio200597, umc47, umc23b, bnl15.45, umc42, umc66, umc19, bnl7.65, pio200071, pio200562, umc15, umc52, pio100025, pio200608, bnl8.23, pio200563

5

1: bnl8.33, bnl6.25, umc72a, umc107b, pio200898, bnl7.56, pio200872, pio060012, pio150018, pio200589, pio100014, pio200566, umc108, umc68, pio200909, pio200523, umc104

2: umc86b, bnl8.33, bnl6.25, umc107b, pgm2, bnl7.56, pio200622, umc43, pio060012, pio150024, pio150018, bnl7.71, pio200589, amp3, umc54, pio200531, pio200566, pio1200026, umc108, bnl5.24, pio200523

3: bnl8.33, bnl6.25, umc72a, bnl7.56, pio060012, pio150018, pio200715, pio200589, pio100014, pio200566, umc108, pio200523, pio100017, umc104

4: bnl8.33, umc90, bnl7.56, pio150018, pio200589, pio100014, bnl5.71, bnl5.40, umc68, pio100017, umc104

5: umc86b, bnl8.33, bnl6.25, umc90, umc72a, umc107b, pgm2, pio200898, bnl7.56, pio200872, pio200622, umc43, pio060012, pio150024, pio150018, pio200715, bnl7.71, pio200589, pio100014, amp3, bnl5.71, bnl5.40, umc54, pio200531, pio200566, pio1200026, umc108, umc68, bnl5.24, pio200909, pio200523, pio100017, umc104

6

1: pio200045, umc65, m3-4, umc46, umc138, pio200904, umc62

2: bnl6.29, pio200527, pio200528, pio200854, pio200045, umc65, umc21, umc46, pio100016, umc138, umc62, mdh2, pio200595, pio200599

3: pio200854, umc113a, umc21, umc138, umc62, pio200599

4: pio200527, umc59, pio200045, umc21, bnl15.37

5: bnl6.29, pio200527, pio200528, umc59, pio200854, pio200045, pio060007, umc65, umc113a, umc21, m3-4, umc46, pio100016, bnl15.37, umc138, pio200904, umc62, mdh2, pio200595, pio200569, pio200599

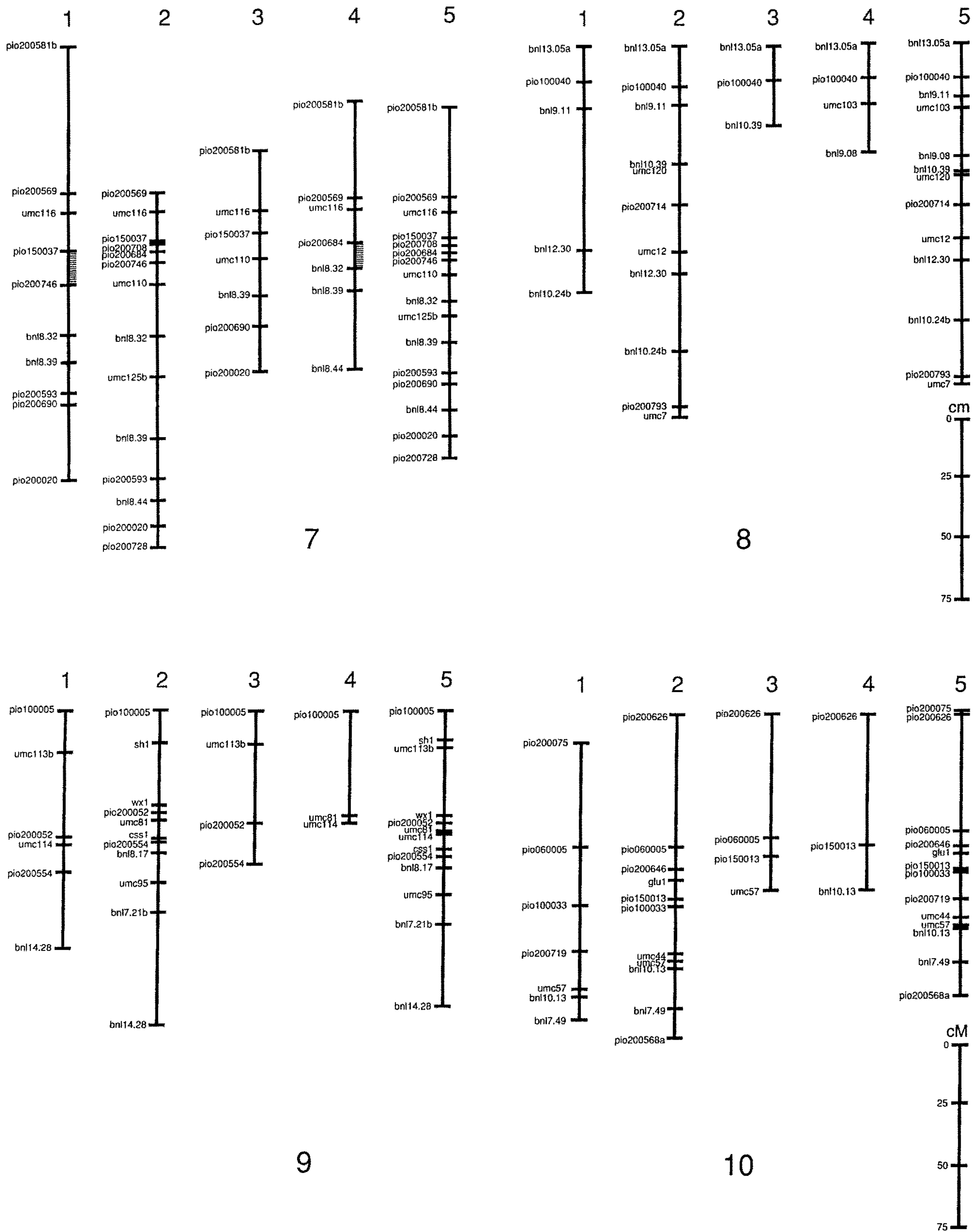

Fig. 1. Maize RFLP linkage maps listed in chromosome order for four segregating F_2 populations and the composite population. Regions of the linkage maps which exhibited recombination that was significantly different from the composite map are indicated with ≡. The F_2 populations are referenced by the parents involved in the F_1: 1 = B73 × G35, 2 = B73 × MO17, 3 = KO5 × W65, 4 = J40 × V94, 5 = the composite population

Table 2. List of genetic markers mapped to chromosome 1 in four segregating F_2 populations

Genetic marker	Populations [a]			
	1	2	3	4
bnl05.62	+	+	+	+
umc094	+	+		
pio200537	+	+	+	
pio200603	+	+		
pio200689		+	+	
umc076			+	
pio200640	+	+	+	
umc011	+	+		
umc013				+
bnl12.06	+	+		+
bnl07.21A	+	+		
umc133	+	+	+	
pio200654		+		
bnl05.59	+	+		+
pio200682	+	+	+	
pio200674		+		
pio200575		+		
pio200644	+	+	+	+
pio200044		+		
pio200855	+			
umc037			+	
umc023A	+	+	+	+
pio200661		+		
pio200668		+		
umc128	+			+
amp1		+		
umc083	+			
pio200870	+			
umc050B	+	+		
bnl08.10	+	+		+
umc107A		+		
pio200518		+	+	
bnl07.25	+			+
bnl08.29		+	+	+
pio200557	+	+		+
umc084	+		+	
bnl06.32	+	+	+	

[a] An indication (+) is given for the population(s) in which the markers were mapped

one locus, umc23, 40% of the lanes were unscorable. None of these seven probes exhibited poor segregation, and only two (pio 060005 and umc 44) were loosely linked on chromosome 10.

The composite map based upon pooled data from all genetic markers (Fig. 1) is similar to those of Burr and Hoisington reported by E. H. Coe et al. (personal communication; MNL) in that gene order is mostly consistent. There are, however, a few discrepancies. On the long arm of chromosome 1, Burr and Hoisington report the gene order: umc23, umc128, umc37. Our composite indicates the gene order: umc37, umc23, umc128 (Fig. 1). None of our populations included all three markers. In populations 1 and 4, we place umc128 distal to umc23, but our placement of umc37 proximal to umc23 is due to the map from population 3; where the most likely gene order is indicated. The reverse gene order in this population is less likely, but not by a significant amount (lod difference = 1.07). Hoisington shows umc72 as distal to umc90 on chromosome 5, and Burr shows bnl19.08 as distal to bnl10.39 on chromosome 8. None of our populations included both members of these two pairs, rather the placement of these markers was based upon proximity to flanking markers in separate populations. Resolution of these three discrepancies will have to wait until we are able to obtain two-point and three-point data for these regions from within a single segregating population. A fourth discrepancy occurs on the distal short arm of chromosome 3. Our map is based primarily upon data from population 2, where the probe pio200006 showed greater linkage to E8 than it did with bnl8.15, although in both cases linkage was weak. More probes within this linkage gap will help resolve this discrepancy.

Another observation that seems to be consistent among our maps and those of Burr and Hoisington is that on the short arm of many chromosomes (especially chromosome 10), there are large linkage gaps between terminal loci and loci that are in close proximity to the centromere. An interesting question is whether these represent recombinational "hot spots" or genomic regions, which are not sampled with probe isolation techniques.

Despite the similarities among the four Pioneer maps, they were not equivalent, primarily because different sets of informative markers were used for each map (Fig. 1). The estimated genome size (M) in each population was clearly related to the number of polymorphic markers that were mapped (Table 1). The relationship also suggests that the estimated size of the maize genome is being approached asymptotically with increasing numbers of probes. From these data it would appear that the size of the maize genome for adapted corn belt germ plasm will be estimated to be about $23M$ using these types of markers. It is still an open question as to whether or not we have adequately sampled the entire maize genome with standard probe isolation techniques.

Not only can the differences among the few maps be attributed to the use of different polymorphic markers and different numbers of markers, but there may also be differences in recombination rates. As already noted, we detected significant differences between population 1 and the other populations for 20 linked markers of chromosome one (Table 3). From a visual inspection of Fig. 1, we might infer that population 1 has greater recombination in the genomic region flanked by bnl5.62 and pio200537 and the region flanked by umc133 and pio200644. Further investigation revealed that the differences in recombination estimates for the region flanked by bnl5.62 and pio200537 were not large enough to be significant (Table 3), and that most of the detectable linkage hetero-

Table 3. Log-likelihoods and test statistics for homogeneity of recombination on chromosome 1 between population 1 and the other populations

Description of markers in the linkage group	Population(s)			
	1	2+3+4	1+2+3+4	χ^2
20 nonunique markers	−1067.0	−2256.3	−3352.5	134.6
Region flanked by bnl5.62 and pio200537	−269.12	−643.56	−914.9	10.3
bnl5.62, pio200537	−187.3	−566.7	−756.2	10.1
Region flanked by umc133 and pio200644	−390.6	−936.3	−1352.2	116.6
bnl5.57, pio200682	−185.4	−495.1	−688.9	38.6
pio200682, pio200644	−186.6	−551.9	−747.3	40.5

geneity was accounted for by differences in the region flanked by umc133 and pio200644. Indeed, much of the heterogeneity was due to differences in two-point recombination estimates, between bnl5.59 and pio200682, and between pio200682 and pio200644. The estimates of recombination between bnl5.59 and pio200682 was 0.23 in population 1, and 0.02 in data pooled from the other three populations. Note that the second estimate was based upon data from population 2, because bnl5.59 was not polymorphic in population 3 and pio200682 was not polymorphic in population 4. The estimate of recombination between pio 200682 and pio 200644 was 0.23 in population 1 and 0.05 in data pooled from populations 2 and 3. Although these differences in two-point estimates account for much of the heterogeneity between populations 1 and the other populations, some of the heterogeneity is also accounted for by differences in multipoint recombination.

We found regions of unequal recombination from all of the populations on most of the chromosomes and show these as crossed hatches of the linkage diagrams (Fig. 1). There were apparent contradictions in the analyses. For example, the region for chromosome 1 flanked by umc23 and pio 200674 was identified in population 1 and 3 (Fig. 1), but not in population 2. This can be explained by recalling that the homogeneity test is detecting differences between a single F_2 population and a "population" based upon data pooled from the remaining populations. Thus, this region for population 2 was compared with data pooled from populations 1 and 3, which had very dissimilar linkage (Fig. 1). However, when the data from populations 1 and 3 were pooled, the resulting "average" linkage was not significantly different than population 2.

We detected few differences in recombination values for linkage groups associated with chromosomes 2, 8, 9, and 10. This may be due to the fact that there were few informative markers with which to investigate homogeneity. Also, Ott (1985) has shown Eq. 3 is not a particularly powerful statistic; thus, we may not have identified all of the regions with differences in recombination.

Variability in estimated recombination values has been known in maize since the earliest genetic studies (Bregger 1918; Eyster 1921). Most studies that have reported variability in recombination estimates among maize populations were designed to detect recombination differences between male and female gametes (Bregger 1918; C. R. Burnham, personal communications MNL; Eyster 1921; Stadler 1926; Rhoades 1941, 1968; Robertson 1984). Some of the results of these studies have been contradictory for specific regions in the genome (Rhoades 1978; Robertson 1984). Several studies have shown that observed differences in estimated recombination values can be associated with heterochromatic regions (Robertson 1967; Nel 1973; Chang and Kikudome 1973; Rhoades 1978). Work is currently being done at Pioneer to determine if heterochromatic regions are associated with the observed differences in recombination values (M. Albertsen, personal communication). The differences may also be a reflection of environmental differences. Many of the early studies recognized that variable environmental conditions during gametogenesis are associated with variability of recombination estimates among plants of the same genotype. (Emerson and Hutchison 1921; Eyster 1921; Stadler 1926). Because our populations were not produced under the same environmental conditions, it is possible that differences in recombination which we detected may not be genetically based.

If genotypic sources of variability for recombination can be identified, then associations between germ plasm and recombination may be revealed. These associations would be of interest to maize geneticists and could be utilized by plant breeders. In order to estimate the repeatability of recombination estimates, it will be necessary to compare a mapping population produced in several environments. Several labs have used B73 × MO17 populations for mapping RFLPs (C. W. Stuber, personal communication; A. R. Hallauer, personal communication; W. A. Compton, personal communication; this report). If a common set of informative markers could be used in these populations, an estimate of repeatability for recombination in this population could be obtained.

Detection of unequal recombination among populations raises the question of whether or not the data should be pooled and a composite map constructed. Given a large number of populations, any given region of the genome may be associated with unequal recombination in at least one population. Thus, the actual map for a population will deviate significantly from the composite map. Despite this weakness, the composite map is useful. We have used the composite map as a reference in planning experiments that require genetic markers to be dispersed uniformly throughout the genome and to compare

quantitative trait loci identified in different genetic backgrounds. Because the information can and will be pooled, more relevant questions are how to distinguish genotypic from environmental (and $G \times E$) influences on estimates of recombination values, and how to sample populations.

When linkage information from many populations becomes available, a composite genetic map will be constructed. Before constructing a composite genetic map to represent maize, sampling strategies should be considered, because the composite map will be biased toward larger, more informative populations. For example, recall that our composite map for the region between pio200682 and pio200644 is based upon data pooled from three populations. The pooled estimate of recombination between these markers is 0.10, which is more like the value from populations 2 and 3 than it is like the value from population 1, because population 1 contributes only 30% of the information to the estimate. At this point, we do not know which estimate, if any, best represents maize germ plasm.

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