Development of a Core RFLP Map in Maize Using an Immortalized F_2 Population

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ABSTRACT

A map derived from restriction fragment length polymorphisms (RFLPs) in maize (*Zea mays* L.) is presented. The map was constructed in an immortalized Tx303 × CO159 F_2 mapping population that allowed for an unlimited number of markers to be mapped and pooled F_3 seed to be distributed to other laboratories. A total of 215 markers consisting of 159 genomic clones, 16 isozymes and 35 cloned genes of defined function have been placed on 10 chromosomes. An examination of segregation data has revealed several genomic regions with aberrant segregation ratios favoring either parent or the heterozygote. Mapping of cloned genes and isozymes that have been previously mapped by functional criteria has provided 29 points of alignment with the classical maize genetic map. Screening of all mapped RFLP probes against a collection of U.S. Corn Belt germplasm using *Eco*RI, *Hin*dIII and *Eco*RV has resulted in a set of 97 core markers being defined. The designation of a set of core markers allows the maize genome to be subdivided into a series of bins which serve as the backbone for maize genetic information and database boundaries. The merits and applications of core markers and bins are discussed.

MAIZE (*Zea mays* L.) is a plant of agronomic importance that embodies many of the features of a model genetic system. It has a well developed genetic map that encompasses over 600 genetic loci, many of which are expressed as seedling or kernel factors that are easily diagnosed and manipulated (COE, NEUFFER and HOISINGTON 1988). The cytogenetics of maize is also well developed and has played a pivotal role in defining many key aspects of chromosome behavior in addition to equipping the maize geneticist with a rich resource of tools for addressing genetic problems (CARLSON 1988). Recessive genes can be mapped to 19 of the 20 chromosome arms using B-A translocations (BECKETT 1978) and dominant genes can be localized using waxy-marked translocation stocks (ANDERSON 1956; BURNHAM 1966). Compound translocations have been constructed that allow gene dosage experiments to be conducted (BIRCHLER 1982). Last, several well characterized transposon systems are available for tagging and cloning genes of interest (WALBOT and MESSING 1988).

The application of restriction fragment length polymorphisms (RFLPs) to genetic mapping in maize has helped to further characterize the maize genome. This is due, at least in part, to the fact that maize is highly polymorphic (EVOLA, BURR and BURR 1986). Using pooled data from several F_2 populations, HELENTJARIS *et al.* (1986) constructed the first published RFLP map for maize. WEBER and HELENTJARIS (1989) refined this RFLP map using B-A translocations to define regions of the centromeres. In the process of developing the linkage map, HELENTJARIS, WEBER and WRIGHT (1988) observed that 29% of their cloned maize sequences hybridized to mappable duplicate nucleotide sequences. Comparison of these duplicate loci residing on pairs of chromosomes indicated that they were generated by a nonrandom process that preserved their linear order. BURR *et al.* (1988) developed an RFLP map based on segregation data in two recombinant inbred populations (Tx303 × CO159 and T232 × CM37) deriving map distances as the average of the recombination values in the two populations. Another RFLP map has been developed by the Agrigenetics Company using two F_2 populations and a recombinant inbred population (MURRAY *et al.* 1988; SHOEMAKER *et al.* 1992). Further, BEAVIS and GRANT (1991) applied a statistical treatment designed to test for homogeneity of recombination among populations, and have presented comparative maps and a composite RFLP map based on pooled data sets and common markers from four F_2 populations.

Previously, an RFLP map was constructed in a Tx303 × CO159 F_2 population that had not been immortalized and was not scored for isozyme segregation (HOISINGTON and COE 1990). This paper presents an RFLP map constructed in an immortalized

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Tx303 × CO159 F_2 (IF_2) population. Immortalized F_2 (IF_2) signifies a set of mapping strains derived by a procedure that maintains (*i.e.*, "immortalizes") the heterozygous alleles in each individual F_2 plant by pooled random mating in the F_3 and subsequent generations. Because the genotype of the F_2 is maintained in each pooled IF_2 family and allows the distribution of RFLP-typed IF_2 seed, one of the main disadvantages to RFLP mapping in a transient F_2 population is overcome. Additionally, all molecular markers mapped in this study have been characterized for allelic variation in nine inbred maize lines representing a range of U.S. Corn Belt germplasm. This has allowed for the designation of a subset of RFLP probes as core markers that are relatively polymorphic and evenly spaced throughout the genome yet yield an easily interpretable hybridization pattern. This is crucial because this core marker set allows the minimal genome coverage necessary to map any quantitative or qualitative trait. The designation of a set of core markers has facilitated the dissection of the maize genome into a series of computer-sortable and subdividable bins that serve as collection points for mapped genetic loci. It is anticipated that these core markers and subsequently defined bins will form the genomic backbone necessary for the integrated mapping project ongoing in this laboratory.

MATERIALS AND METHODS

Materials: All chemicals were reagent grade or better and purchased from Sigma Chemical Company, St. Louis, Missouri, or Fisher Scientific, St. Louis, Missouri. Restriction enzymes and agaroses were from Bethesda Research Laboratories, Gaithersburg, Maryland. Radioisotope ($dCT^{32}P$, 3000 mCi/mol) was from New England Nuclear, Boston, Massachusetts.

Plant materials: ATx303 × CO159 F_2 population was produced using C0159 as the male parent. Leaf material was sampled from each of 56 F_2 plants, quick frozen in liquid nitrogen, and freeze dried for 3–5 days. Care was taken in sampling to leave enough leaf area to allow an ear to be produced. When F_3 leaf material was used, equal amounts from 12 to 20 F_3 plants from a single F_2 ear were sampled and bulked as one sample just prior to freeze drying. After freeze drying, the samples were ground to a fine powder in a Tecator Cyclotec sample mill (Fisher Scientific cat. no. TC1093-002) and stored at −20° until used for DNA extraction. Alternatively, after freezing in liquid nitrogen, leaves were placed in a −80° freezer until space was available on the freeze dryer. This did not affect the quality or yield of DNA obtained. The heterozygosity of the F_2 population was "immortalized" as follows. Forty F_3 seeds from each F_2 plant were planted in two rows of 20. On successive pollinating days, pollen from five plants in one row was bulked and used to pollinate five plants in the corresponding row of plants from that family. Both rows were used as pollen parents to the corresponding row. A minimum of 20 ears were bulked to immortalize the F_2.

DNA isolations: Total maize genomic DNA was prepared using the method of SAGHAI-MAROOF *et al.* (1984) with the following modifications. DNA was treated with RNAase A (0.5 mg/500 mg dried tissue) just prior to isopropanol precipitation. After isopropanol precipitation the DNA was spooled out using a glass hook and resuspended in TE (10 mM Tris pH 8.0, 1 mM EDTA) and extracted with phenol followed by a chloroform extraction. The DNA was brought to 0.25 M NaCl and precipitated with 2.5 volumes of cold ethanol. Spooled DNA was washed in 76% ethanol, 0.2 M sodium acetate, followed by 76% ethanol, 10 mM ammonium acetate and resuspended in TE at a final concentration of 0.6 mg/ml. DNA was stored at 4° for short times and at −20° for longer periods.

Plasmids were isolated from 10-ml cultures using a scaled down version of the method of BIRNBOIM (1983). Inserts were obtained by digesting 20 μg of each plasmid with the appropriate enzyme and electrophoresing in TAE (40 mM Tris, 5 mM sodium acetate, 0.5 mM EDTA, pH 8.0) gels prepared using low melting point (LMP) agarose. Agarose plugs containing the excised insert in TE were diluted to a final concentration of 10 ng/μl.

Sources of RFLP probes: Probe sources are listed in Table 1. University of Missouri, Columbia (UMC) probes were developed from a *Pst*I genomic library kindly provided by TIM HELENTJARIS. Native Plants Incorporated (NPI) probes were supplied by SCOTT WRIGHT, Salt Lake City, Utah. Pioneer (PIO) probes were provided by DAVID GRANT at Pioneer Hi-Bred International, Johnston, Iowa. Brookhaven National Laboratory (BNL) probes were kindly provided by BENJAMIN BURR at Brookhaven National Laboratory, Upton, New York. Sources of other RFLP probes representing DNA sequences with known functionality are described in Table 2.

Restriction digestion, agarose gel electrophoresis and Southern transfer: Maize genomic DNA (40–80 μg) was digested in a total volume of 300 μl with 2.5 units of restriction enzyme/μg DNA for 4 hr at 37° to ensure complete digestion. DNA was precipitated and resuspended in a smaller volume, which allowed DNA to be loaded at a concentration of 10 μg/lane. Agarose TAE gels (0.7%) were run overnight until the bromphenol blue tracking dye had migrated 5.5 cm. Gel dimensions were 20 cm × 25 cm, which allowed four sets of combs with 25 or 30 wells to be used on a single gel. After electrophoresis, gels were stained in ethidium bromide (1 μg/ml), briefly destained in distilled H_2O and photographed. Gels were then denatured in 3 volumes of 0.4 N NaOH, 0.6 M NaCl for 40 min, followed by neutralization in 3 volumes of 0.5 M Tris, pH 7.5, 1.5 M NaCl for 40 min. DNA was then blotted onto nylon membranes (MSI Magnagraph, Fisher Scientific) with 25 mM $NaPO_4$, pH 6.5, using a method modified after SOUTHERN (1983) which utilized cellulose sponges for wicking. After blotting overnight with one change of paper towels, membranes were briefly washed in 2 × SSC (1 × SSC; 150 mM NaCl, 15 mM sodium citrate), dried, and UV-cross-linked with a Stratalinker according to manufacturer's recommendations (Stratagene, San Diego, California). Membranes were then baked at 92° for 2–3 hr.

Oligolabeling, hybridizations and autoradiography: Oligolabelings were done using 50 ng of probe insert DNA and 5.0 μl of $dCT^{32}P$ for a 250-cm^2 membrane (FEINBERG and VOGELSTEIN 1984). Incorporation levels were high enough so that no additional purification of the radiolabeled insert was necessary. Filters were prehybridized at 65° in a buffer which consisted of 5 × SSC, 50 mM Tris, pH 8.0, 0.2% sodium dodecyl sulfate (SDS), 10 mM EDTA, pH 8.0, 0.1 mg/ml denatured sonicated salmon sperm DNA and 1 × Denhardt's solution (0.02 g Ficoll 400, 0.2 g polyvinylpyrollidone 40000, 0.02 g bovine serum albumin, fraction V). After 4 hr, the prehybridization solution was removed

TABLE 1

Sources of RFLP probes and markers used in map construction

Source	Laboratory designator	No. of loci mapped	No. of loci in core map	Reference
Brookhaven National Laboratories[a]	BNL	18	12	BURR *et al.* (1988)
Native Plants Incorporated[b]	NPI	20	12	HELENTJARIS *et al.* (1986)
Pioneer Hi-Bred International[b]	PIO	15	5	BEAVIS and GRANT (1991)
University of Missouri-Columbia[a]	UMC	99	68	This paper
Rhone-Poulenc Agrochimie[c]	RPA	7	0	G. FREYSSINET (personal communication)
Functional Probes	UMC	40	0	Table 2
Isozymes	NA[d]	16	0	STUBER and GOODMAN (1983)
Total		215	97	

[a] BNL and UMC probes are available from this laboratory.
[b] PIO and NPI probes are available from DAVID GRANT at Pioneer Hi-Bred International, Des Moines, Iowa.
[c] RPA probes are available from G. FREYSSINET, Rhone Poulenc Agrochimie, Lyon, France.
[d] Isozyme loci are defined and designated by their gene product.

and replaced with hybridization buffer (3.0 ml/250 cm^2) which contained 10% dextran sulfate (Pharmacia, Piscataway, New Jersey) and denatured radiolabeled probe. Membranes were hybridized overnight at 65°. Membranes were washed briefly in 2 × SSC, 0–5% SDS to remove the bulk of the radioactivity, followed by three 1-hr washes in 0.1 × SSC, 0–1% SDS at 65°. After washing membranes were exposed to x-ray film (Kodak X-Omat brand) with the aid of an intensifying screen. Exposure times were generally overnight to several days.

Isozyme analysis: Isozyme analysis was conducted by Biogenetic Services, Brookings, South Dakota, using the methods of STUBER *et al.* (1988). Ten individual etiolated F_3 seedlings were typed to reconstitute the genotypes of each of the 56 F_2 individuals.

Linkage analysis: Multipoint maximum likelihood linkage analysis was performed at a Sun 4.0 workstation (Sun Microsystems, Palo Alto, California) using MAPMAKER (LANDER *et al.* 1987). Initially, unordered linkage groups were generated using a minimum log likelihood difference (LOD) score of 3.0 and a recombination fraction of 0.4. A three-point map was constructed using the "three point and orders" command. Markers that could not be fitted to the three-point framework were mapped to an interval using the multipoint "try" and "compare" commands. Once a consistent order was derived for a particular chromosome, the "ripple" command was used to determine which final order was the most likely for adjacent pairs of loci.

RESULTS

Choice of probes and enzymes: A *Pst*I genomic library was used as a source of probes (1–2 kb) because we and others (HELENTJARIS *et al.* 1986; BEAVIS and GRANT 1991; BURR *et al.* 1988) have found it to be relatively rich in low copy sequences. Tx303 and CO159 parents whose genomic DNA had been cut with *Eco*RI, *Hin*dIII, *Eco*RV and *Bam*HI restriction endonucleases displayed polymorphisms with 60% of random *Pst*I inserts used as probes with one or more enzymes. Those probes that did not detect RFLPs with the first four enzymes were further screened with *Xba*I, *Dra*I, *Xho*I and *Bgl*II, among which 50% of the probes hybridized to polymorphic bands. In general, only the first four restriction enzymes were employed to screen the *Pst*I library, with the second four enzymes being used to detect polymorphisms for random genomic *Pst*I or functional clones that were regarded as critical for alignment to other RFLP maps. Using this approach, 199 RFLP loci were mapped (Table 1, Figure 1). Additionally, 16 isozyme loci were mapped.

RFLP map generation and evaluation: A 215-locus data set was loaded into MAPMAKER, and evaluated, which generated 11 unordered linkage groups using a minimum LOD of 3.0 and a recombination fraction of 0.4. Eleven linkage groups were obtained due to the failure to establish linkage between the three most distal markers on chromosome arm *2S* (*NPI239*, *BNL8.45*, *UMC53*) and *UMC6*. This is most likely due to missing data in key individuals and the large distance involved. The three most distal markers on *2S* were assigned on the basis of a previous map (COE, NEUFFER and HOISINGTON 1988) and comparison to other published RFLP maps (BURR *et al.* 1988; BURR and BURR 1991; HELENTJARIS *et al.* 1986).

There were several chromosomal regions that exhibited distorted segregation ratios as diagnosed by a chi square goodness of fit test for the expected 1:2:1 segregation ratio for codominant loci (Table 3). Two genomic regions were skewed in favor of the CO159 parent. One region on chromosome *2* centered around *UMC4* and was significant at the 0.01 probability level. This region was bounded proximally by *PIO200005* and *UMC125A* and distally by *UMC122* and *UMC137*, each of which were significantly skewed at the 0.05 probability level. The second region was on chromosome *5*, bounded by the proximal and distal markers *UMC126* and *UMC108*, respectively. *UMC126*, *UMC54* and *UMC141* were significantly skewed at the 0.01 probability level. *UMC51* in this region was difficult to evaluate because it was scored as presence *vs.* absence and critical genotypes (homo-

TABLE 2

Mapped loci of functional significance

Locus	Clone (name/size)	Chromosome	Apparent function	Reference
UMC194(GPR)	pZmGprotein/1.2 kb	*1*	G protein subunit	C. HAN (personal communication)
UMC195(HSP26)[a]	pHSP26/1.0 kb	*1*	26-kD heat shock protein	NIETO-SOTELO, VIERLING and HO (1990)
UMC185(P)	p-VB.4/2.8 kb	*1*	Pericarp color (*P1* gene)	LECHELT *et al.* (1989)
UMC196(UNT)	pZm7gg/0.9 kb	*1*	Unidentified transcript	W. CAMPBELL (personal communication)
UMC181(Bz2)	pbz2pP300/0.3 kb	*1*	*Bz2* gene	MCLAUGHLIN and WALBOT (1987)
UMC197(b32)	pb32/1.0 kb	*1*	Endosperm albumin (*Alb1* gene)	DIFONZO *et al.* (1988)
UMC184A/B(Glb)	pGlb1S/2.0 kb	*1/2*	Embryo globulin (*Glb1* gene)	BELANGER and KRIZ (1989)
UMC198(Whp)	pC2c46/1.5 kb[b]	*2*	Chalcone synthase (*Whp1* gene)	WIENAND *et al.* (1986)
UMC210A/B(SSU)	pZmSSU/1.0 kb	*2/4*	RuBP carboxylase small subunit	BROGLIE *et al.* (1984)
UMC208(cpPGK)	pPGK/1.0 kb	*3*	Chloroplast phosphoglycerate kinase	LONGSTAFF *et al.* (1989)
UMC199(A1)	pA1-upstream/1.0 kb	*3*	Anthocyaninless (*A1* gene)	O'REILLY *et al.* (1985)
UMC24(CAB)	pZLH5/1.2 kb	*3*	Chlorophyll *a/b* binding protein	MAYFIELD and TAYLOR (1984)
UMC200(Adh2)	pZmL841/1.0 kb	*4*	Alcohol dehydrogenase (*Adh2* gene)	SACHS *et al.* (1985)
UMC191(GPC)	pGAPC1S, pZm9/0.3 kb[c]	*4*	Glyceraldehyde-3-phosphate dehydrogenase (*Gpc1* gene)	BRINKMANN *et al.* (1987), RUSSELL and SACHS (1989)
UMC201(NR)	pZmnr1/2.1 kb	*4*	Nitrate reductase	GOWRI and CAMPBELL (1989)
UMC202(PRH)	pZmPP1/0.2 kb[d]	*4*	Type I protein phosphatase	SMITH and WALKER (1991)
UMC193A/B/C(Orp)	pTrpB/1.5 kb	*4/10/7*	Tryptophan synthase B subunit	K. CONE (PERSONAL COMMUNICATION)
UMC209(PRK)	pPRK/1.0 kb	*5*	Phosphoribulokinase	RAINES *et al.* (1989)
UMC203(GPC)	pGAPC2S/0.3 kb	*6*	Glyceraldehyde-3-phosphate dehydrogenase	RUSSELL and SACHS (1989)
UMC172(OEC33)	p12-6/0.6 kb	*6*	33-kD oxygen evolving polypeptide	SHEEN, SAYRE and BOGORAD (1987)
UMC204(Bz1)	pBz2.1/2.1 kb[e]	*6*	*Bz1* homolog	K. CONE (personal communication)
UMC205(Pl)	p*Sal*I/*Pst*I 1.0 kb[f]	*6*	Purple plant (*Pl1* gene)	CONE, BURR and BURR (1986)
UMC180(PEP1)	pPEPC1/2.8 kb	*6*	Phosphoenolpyruvate carboxylase	HUDSPETH *et al.* (1986)
UMC170(RAB17)[g]	pM3-4/0.9 kb	*6*	Responsive to abscisic acid	CLOSE, KORTT and CHANDLER (1989)
UMC173(PDK)	pPPDK4/0.6 kb	*6*	Pyruvate orthophosphate dikinase	SHEEN and BOGORAD (1987)
UMC189(A1)	pA1-downstream/0.9 kb[h]	*9*	*A1* homolog	N. FEDOROFF, personal communication
UMC206(HSP70)	pHSP70/4.0 kb	*8*	70-kD heat shock protein	ROCHESTER, WINTER and SHAH (1986)
UMC186A/(Bs1)	pDW20/0.6 kb	*8/5*	Barley stripe retrotransposon	JOHNS *et al.* (1989)
UMC207(Sh1)	p246A/2.5 kb	*9*	Shrunken kernel (*Sh1* gene)	BURR and BURR (1982)
UMC192(Bz1)	pBz1cDNA/1.8 kb	*9*	Bronze kernel (*Bz1* gene)	FEDOROFF, FURTEK and NELSON (1984)
UMC025(Wx)	pBF225, pWx5/3.4 kb[i]	*9*	Waxy kernel (*Wx1* gene)	SHURE, WESSLER AND FEDOROFF (1983) and EVOLA, BURR and BURR (1986)
UMC190(CSS)	p21.2/6.0 kb	*9*	Sucrose synthase (*Css1* gene)	MCCARTY, SHAW and HANNAH (1987) and GUPTA *et al.* (1987)
UMC188(GPA)	pGAPA1S,pZm57/0.4 kb[j]	*10*	Glyceraldehyde-3-phosphate dehydrogenase (*Gpa*1 gene)	BRINKMANN *et al.* (1987) and RUSSELL and SACHS (1989)
UMC182(R)	pR-nj:1/0.6 kb	*10*	Seed color (*R1* gene)	DELLAPORTA *et al.* (1988)

[a] Previously designated *UMC195(HSP33)*.

[b] A 1.5-kb probe for the *C2* locus on chromosome *4* revealed an RFLP that maps to the *Whp1* locus on chromosome arm *2L*, a region of known DNA sequence homology to *C2* (WIENAND *et al.* 1989).

[c] pGAPC1S is a 260-bp 3′UTR subclone of pZm9, obtained from M. SACHS.

[d] *UMC202(PRP)* locus was detected using a 150-bp 5′UTR subclone of pZmPP1.

[e] pBz2.1 is a 2.1-kb *Pst*I fragment from *Bz1* that gives a complicated hybridization pattern with a single RFLP that maps to chromosome *6*.

[f] p*Sal*I/*Pst*I is a 1.0-kb subclone of the *C1* probe. It detects an RFLP that maps to *Pl1* on chromosome *6*, a region of known DNA homology to *C1* (CONE and BURR 1988).

[g] Previously designated *UMC170(DHN)*.

[h] pA1-downstream contains most of the *A1* coding region and hybridizes with two loci, one of which detects an RFLP on chromosome arm *8L*.

[i] pBF225 is a 3.4-kb *Pvu*I genomic subclone of pWx5 which contains an 11-kb *Eco*RI insert that spans the transcribed region.

[j] pGPA1S is a 400-bp *Xho*I/*Eco*RI subclone derived from the 3′UTR of pZm57, obtained from M. SACHS.

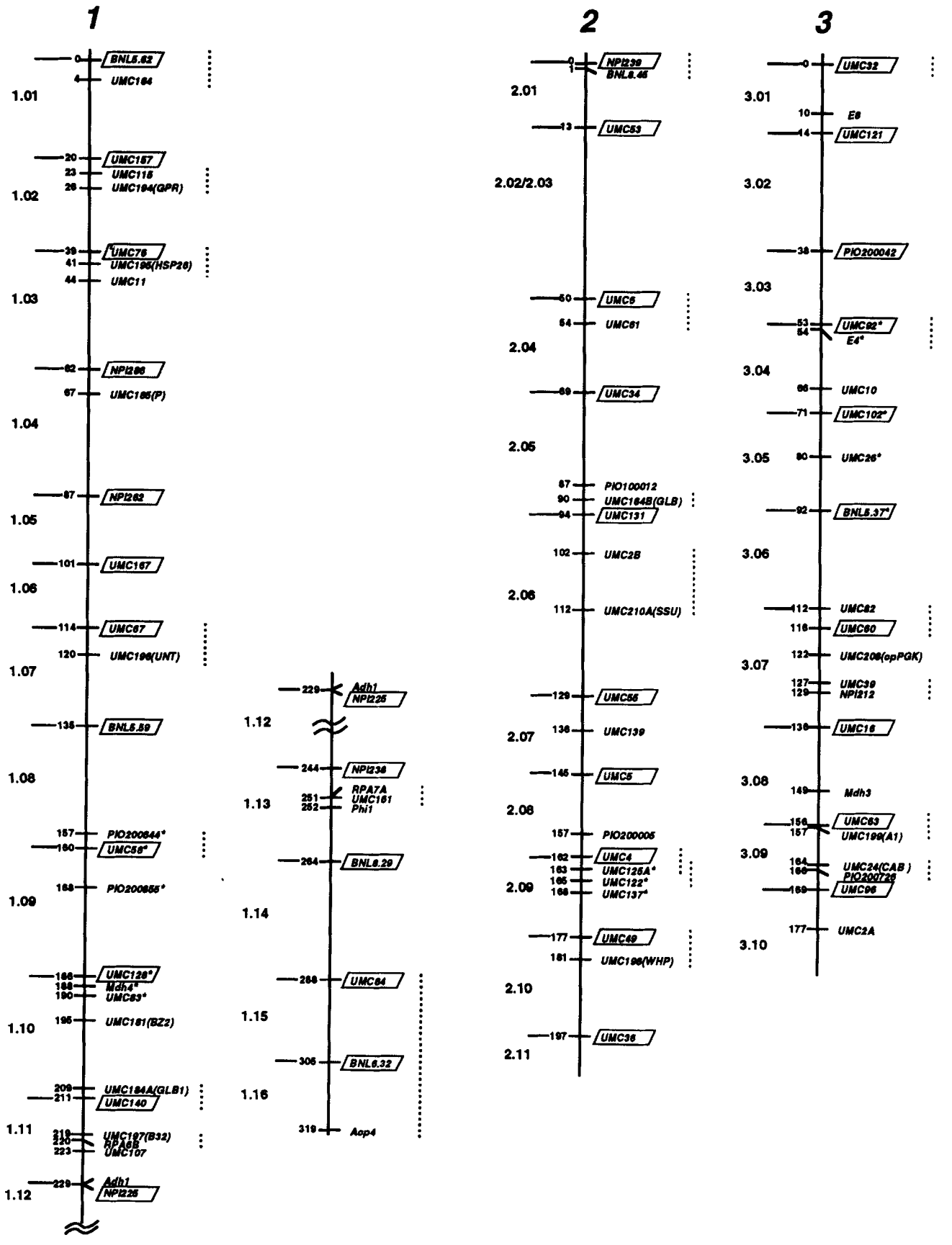
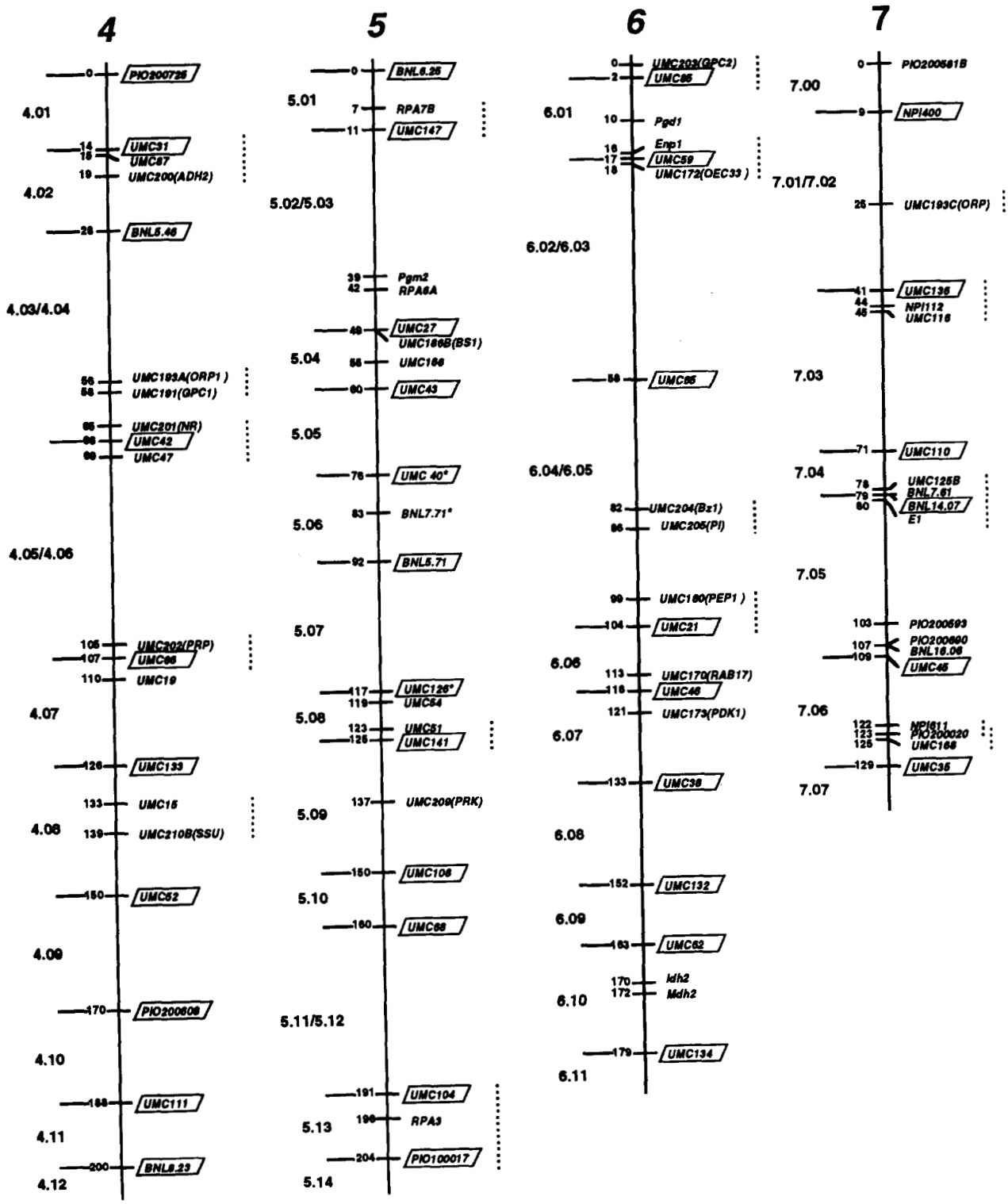


FIGURE 1

(continued on page 922)


4
0 PIO200726
4.01
14 UMC31
15 UMC87
19 UMC200(ADH2)
4.02
26 BNL5.46
4.03/4.04
56 UMC193A(ORP1)
58 UMC191(GPC1)
65 UMC201(NR)
66 UMC42
69 UMC47
4.05/4.06
105 UMC202(PRP)
107 UMC66
110 UMC19
4.07
126 UMC133
133 UMC15
4.08
139 UMC210B(SSU)
150 UMC62
4.09
170 PIO200608
4.10
188 UMC111
4.11
200 BNL8.23
4.12
5
0 BNL6.25
5.01
7 RPA7B
11 UMC147
5.02/5.03
39 Pgm2
42 RPA6A
48 UMC27
UMC186B(BS1)
5.04
55 UMC166
60 UMC43
5.05
76 UMC 40*
83 BNL7.71*
5.06
92 BNL5.71
5.07
117 UMC126*
119 UMC84
5.08
123 UMC61
126 UMC141
5.09
137 UMC209(PRK)
150 UMC106
5.10
160 UMC68
5.11/5.12
191 UMC104
5.13
196 RPA3
204 PIO100017
5.14
6
0 UMC203(GPC2)
2 UMC85
6.01
10 Pgd1
16 Enp1
17 UMC59
18 UMC172(OEC33)
6.02/6.03
58 UMC65
6.04/6.05
82 UMC204(Bz1)
86 UMC205(PI)
99 UMC160(PEP1)
104 UMC21
6.06
113 UMC170(RAB17)
116 UMC46
121 UMC173(PDK1)
6.07
133 UMC36
6.08
152 UMC132
6.09
163 UMC62
170 Idh2
172 Mdh2
6.10
179 UMC134
6.11
7
0 PIO200681B
7.00
9 NPI400
7.01/7.02
26 UMC193C(ORP)
41 UMC136
44 NPI112
45 UMC116
7.03
71 UMC110
7.04
78 UMC125B
79 BNL7.61
80 BNL14.07
E1
7.05
103 PIO200593
107 PIO200600
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UMC45
7.06
122 NPI611
123 PIO200020
125 UMC168
129 UMC35
7.07

FIGURE 1

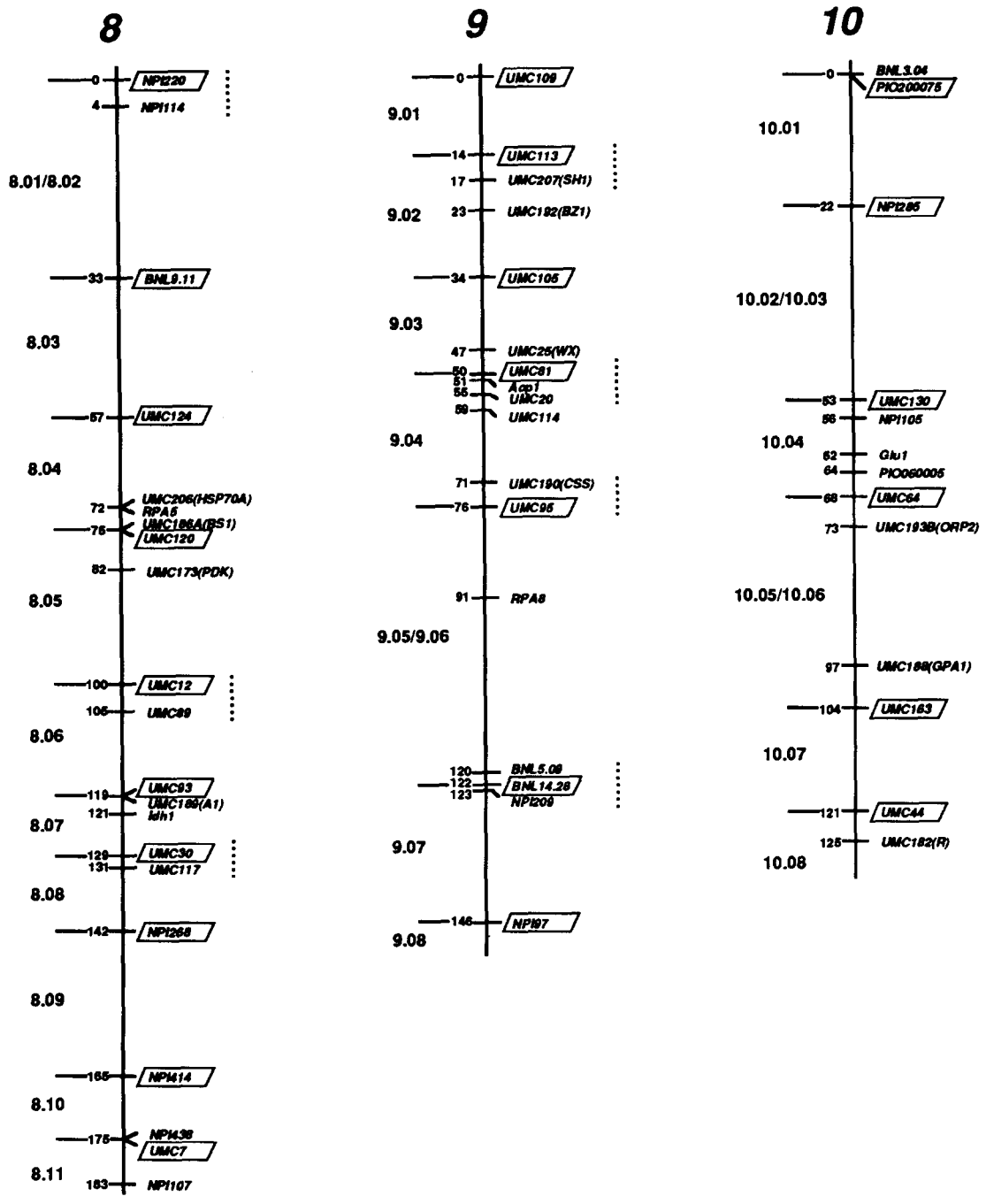

FIGURE 1.—Maize core RFLP map. Maize chromosomes *1* through *10* are shown with the short arm toward the top of the page. Chromosome *1* has been split in order for it to be fit into space limitations. Symbols for loci are shown on the right with their cumulative distance from the most distal marker on the short arm shown on the left. Markers that could not be placed with a LOD of 3.0 or greater are denoted by a dotted line on the right. Discrepancies relative to RFLP maps of other laboratories are discussed in the text. Loci with distorted segregation ratios are denoted with an asterisk, see Table 3 for exact details. Core markers are boxed, generally not greater than 30 cM apart. Core bins are shown on the left, spaced between core markers that delineate the boundaries for that bin. Bins are labeled from the end of the short arm to the tip of the long arm such that its chromosome location is denoted and followed by a decimal pointing system that allows for further subdivisions and computer sorting. Distances greater than 30 cM are double-binned and await an appropriate marker to divide them into two separate bins.

zygous Tx303) were indistinguishable from heterozygotes in the pooled class. *UMC209(PRK)* and *UMC108* were significantly skewed at the 0.05 probability level. A region on chromosome *1* bounded proximally by *PIO200644* and distally by *UMC83* was skewed toward the Tx303 parent. All markers in this region were significantly deviant at the 0.01 probability level with the exception of the proximal and distal markers, which were significantly deviant at the 0.05 probability level. Additionally, two regions were skewed toward the heterozygote at the 0.01 probability level. The first region, on chromosome *3*, was

TABLE 3

Loci showing distorted segregation

Locus	Chromosome	Genotypes[a] CO	Tx/CO	Tx	χ^{2b}
PIO200644	*1*	7	29	20	6.1*
UMC58	*1*	6	28	22	9.2**
PIO200855	*1*	7	24	25	12.1**
UMC128	*1*	6	28	22	9.2**
Mdh4	*1*	6	28	22	9.2**
UMC83	*1*	6	30	20	6.2*
UMC125A	*2*	15	34	5	7.3*
PIO200005	*2*	15	34	5	7.3*
UMC4	*2*	15	37	4	9.3**
UMC122	*2*	16	35	5	6.8*
UMC137	*2*	16	34	6	6.2*
UMC92	*3*	6	41	9	12.4**
E4	*3*	7	40	9	10.4**
UMC102	*3*	10	40	6	9.7**
UMC26	*3*	9	41	6	12.4**
BNL5.37	*3*	4	41	3	24.0***
UMC40	*5*	10	40	6	10.8**
BNL7.71	*5*	6	32	6	9.1*
UMC126	*5*	19	32	3	11.3**
UMC54	*5*	22	31	3	13.2**
UMC141	*5*	20	24	2	12.9**
UMC209(PRK)	*5*	18	33	5	7.7*
UMC108	*5*	21	29	6	8.1*

[a] The number of individuals for each of the three genotypic classes. CO = CO159 homozygote, Tx = Tx303 homozygote, Tx/CO = Tx303/CO159 heterozygote.

[b] Chi square values, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

defined by *UMC92* and *BNL5.37*, but was bisected into two smaller regions by *UMC10* whose codominant segregation ratio did not deviate from the expected. A second region of preferential heterozygosity on chromosome *5* was defined by *UMC40* and *BNL7.71*.

In general, the map generated (Figure 1) compared well with the other published RFLP maps (BEAVIS and GRANT 1991; BURR *et al.* 1988; HELENTJARIS *et al.* 1986). There are, however, a few discrepancies worth noting. The terminal pairs of markers, *UMC111* and *BNL8.23*, *UMC104* and PIO100017, and *NPI220* and *NPI114*, for chromosome arms *4L*, *5L* and *8S* respectively, are inverted on the present map as compared to the most recent version of the BNL map (B. BURR, personal communication). The *8S* markers *NPI220* and *NPI114* and *5L* markers *UMC104* and *PIO100017* are also inverted on the present map relative to the NPI (HELENTJARIS, WEBER and WRIGHT 1988) and Pioneer (BEAVIS and GRANT 1991) maps. There are several regions where the order of a cluster of tightly linked markers on the present map does not reconcile with that given in the most recent version of the BNL map (B. BURR, personal communication). This was not surprising, since these cases generally deal with a cluster of markers spaced only a few centimorgans apart unflanked by any proximal or distal markers. An example of this type of situation is seen in the centromeric region on *10S* (Figure 1). A general order of *UMC130*, *NPI105*, *Glu1* and *UMC64* is consistent in both maps. Adding *PIO06005* and *UMC193B(ORP2)* to these markers results in different orders for the two maps. The Pioneer map (BEAVIS and GRANT 1991) supports the BNL map in that *PIO060005* is more distal on the short arm than *Glu1*. There were two possible inversions on chromosome *4* that are also worth noting. The *UMC42* and *UMC47* loci, while consistent on both the BNL and UMC maps, have a more distal location with respect to the short arm than *UMC193A(Orp)* on the BNL map. The Pioneer map indicated a reverse order for *UMC42* and *UMC47*, but provided no information on the *orp1* locus (BEAVIS and GRANT 1991). Another possible inversion on *4L* involved *UMC66*, *UMC19* and *UMC210B(SSU)*, which were in reverse order on the BNL map. The Pioneer and NPI maps indicate an order consistent with the UMC map as does a teosinte-maize (*Z. mays* ssp. *mexicana* × *Z. mays* ssp. *mays*) map generated by DOEBLEY *et al.* (1990) and DOEBLEY and STEC (1991).

Mapping DNA sequences of functional significance: Particularly important to the construction of this RFLP map was the chromosomal localization of 35 probes (40 loci) that represent DNA sequences defined by either their gene product or molecular function (Table 2). These loci were of crucial importance because they not only allowed alignment with other RFLP maps, but alignment with the maize genetic map as well (COE, NEUFFER and HOISINGTON 1988). In nearly all cases, mapped locations of probes with known functional identities corresponded with locations that had previously been genetically defined. In several cases, additional loci were revealed in addition to those that had been genetically defined. One example of this was the orange pericarp (tryptophan synthase B) loci, which are duplicate factors genetically mapped by NEUFFER and CHANG (1986) to chromosomes *4S* and *10L* on the basis of an orange pericarp phenotype in *orp1*/*orp2* kernels. Loci *UMC193A*, *B* and *C* detected by the cloned probe revealed, in addition to the two genetically defined loci, a third locus on *7S*, which may or may not produce a functional product. A similar situation was seen for *A1*, which has been mapped genetically to *3L* by aleurone color expression (EMERSON 1918). Molecular mapping with a probe for the coding sequence of the *A1* gene product indicated a second locus on 8L in addition to the one on *3L*. A comparable but more extended result for *A1* was seen by WRIGHT and HELENTJARIS (1988), who mapped RFLP loci on *1L*, *2L*, *5L* and *7S* in addition to the *3L* locus. A third point of interest was possible evidence for a duplication on *6L* of the *C1 Sh1 Bz1* region on *9S*. This was indicated by tight linkage (4.0 cM) between *UMC204(BZ1)* and *UMC205(P1)* on *6L*. Probes for these loci arose from

cDNA clones for *Bz1* and *C1* genes. While *C1* and *Pl* are known to be duplicate loci (CONE and BURR 1988), *Bz1* has not been previously linked to *Pl* on *6L*. This suggested the presence of a small duplicated region common to *6L* and *9S*. These findings are consistent with those found by other researchers (C. J. DOWTY, personal communication).

Selection of core RFLP markers: A set of 97 RFLP markers (Table 1, Figure 1) was selected, which should in principle be distributed relatively evenly throughout the maize genome and allow any quantitative or qualitative trait to be mapped. In addition to the initial prerequisite of detecting an RFLP between the CO159 and Tx303 mapping parents, the selection criteria for core markers were as follows. First, a core marker should be a low copy probe that gives an easily interpretable pattern and is polymorphic across a variety of inbred lines. The vast majority of probes included in this study were screened with *Eco*RI, *Hin*dIII and *Eco*RV (S. JOHNSON, manuscript in preparation) against nine inbred lines (Oh40B, Oh43, W8A, W153R, C103, Mo17, CI187, B73 and B37) that represent a distribution of U.S. Corn Belt germplasm. For the most part, core markers are low copy probes that detect three to four alleles utilizing one of the three enzymes. A few represent the only probe available in a sparsely marked region of the genome. This may mean that the genomic organization of these sparsely marked regions consists of islands of low-copy DNA flanked by regions of highly recombinogenic or repetitive DNA. Secondly, core markers were chosen so as not to have a spacing greater than 30 cM (22% recombination) in most cases, with an optimal spacing of 15–20 cM. In 12 cases, lack of a suitable core marker necessitated regions greater than 30 cM to be defined between a pair of core markers. It is anticipated that these regions will be subdivided by additional core markers that are in the process of being determined. Last, a strong preference has been given to those probes (BNL, UMC) that are available to researchers in both the public and private sectors. Probes for loci defined by NPI or PIO are available from Pioneer Hi-Bred International by arrangement.

DISCUSSION

Choice of probes and enzymes: The results using size-selected *Pst*I genomic clones as a source of RFLP probes were similar to those obtained by other laboratories. Over 90% of the *Pst*I clones hybridized to one or two bands in maize, which was similar to results obtained for tomato (*Lycopersicum esculentum* L.) (TANKSLEY *et al.* 1987) but differs significantly from rice (*Oryza sativa* L.) where only 58% of the *Pst*I clones hybridized to low copy sequences. Using the present F_2 mapping parents, approximately 60% of the *Pst*I inserts detected an RFLP with at least one of the four enzymes tested, comparable to results obtained by BURR *et al.* (1988). In rice, 78% of the *Pst*I clones detected an RFLP, but a direct comparison to maize is not possible since 11 restriction enzymes were surveyed. Using tomato genomic clones that consisted of both *Eco*RI and *Pst*I clones and eleven different enzymes, BONIERBALE *et al.* (1988) found at least one RFLP between parents of an interspecific cross in potato (*Solanum tuberosum* L.) for 60% of the probes tested. In barley (*Hordeum vulgare* L.), the majority of *Pst*I clones were polymorphic with at least one of five enzymes in an interspecific cross between barley and *H. vulgare* ssp. *spontaneum* C. Koch emend Bacht. (GRANER *et al.* 1991). Using 85 prescreened *Pst*I clones from wheat (*Triticum aestivum* L.), HEUN *et. al* (1991) determined that approximately 50% revealed polymorphisms in each of two doubled-haploid populations of barley. It therefore seems reasonable to assume, from the higher plants surveyed to date, that *Pst*I libraries are a good source of low copy RFLP probes.

RFLP mapping in an immortalized F_2 population: An RFLP linkage map constructed in an IF_2 mapping population encompasses many of the advantages of both recombinant inbred and F_2 mapping populations. RFLP linkage maps have been constructed from pooled F_3 individuals in *Arabidopsis thaliana* (CHANG *et al.* 1988; NAM *et al.* 1989), barley (GRANER *et al.* 1991; SHIN *et al.* 1990), wheat (M. GALE, personal communication) and maize (BEAVIS and GRANT 1991). One advantage of working with bulked F_3 plants is that large amounts of tissue can be collected for preparing DNA. This is particularly important for *A. thaliana*, which contains a relatively small amount of vegetative material. Even the leaf tissue from an F_2 maize plant is eventually exhausted, which means that ultimately a new, untyped F_2 mapping population will be needed. Pooling of leaf material from F_3 individuals while at the same time systematically crossing F_3 individuals within each F_3 pool has allowed the production of representative seed for distribution to other laboratories. All a laboratory needs to do is plant 10–20 kernels and pool to reconstitute with reasonable assurance the genotype of an F_2 individual. By probing DNA from each of the 56 F_3 pools and comparing the resulting RFLP pattern to our IF_2 database using MAPMAKER (LANDER *et al.* 1987), any molecular marker can easily be mapped. The immortalization of individual F_2 plants using staggered planting dates and pollen bulking to produce pooled seed has allowed maintenance of individual F_2 genotypes with a minimum of genetic drift. Using this approach the main disadvantages of mapping in an F_2 population have been overcome. Additionally, mapping in IF_2 populations using MAPMAKER allows a multipoint (as opposed to a two-point) approach to be

taken that makes the most efficient use of codominant data. In this study, full resolution of 112 meiotic products is approached by the application of the multipoint treatment. For infinitely small intervals, each F_2 individual approximates two meiotic strand products, whereas each recombinant inbred approximates 1.67 strand products (HALDANE and WADDINGTON 1931). For a population size of 56 individuals, standard errors of 3.0 and 4.3 can be calculated for 10 and 20 percent recombination, respectively (ALLARD 1954). Doubling this population size to 112 individuals would decrease the standard errors to 2.1 and 3.1, respectively. The present study does not have the benefits of two distinct, combined mapping populations (BURR *et al.* 1988). In the uncommon case of a presence-absence (*i.e.*, not codominant) polymorphism at the end of a linkage group, the resolution of order is poorer in an F_2 as compared to a recombinant inbred population.

Distorted segregation ratios: A number of genomic segments with segregation ratios that are distorted in favor of the Tx303 parent (chromosome *1*), the CO159 parent (chromosomes *2* and *5*) and the heterozygote (chromosomes *3* and *5*) were observed (Table 3). Distorted segregation ratios are not unexpected in any genetic cross where large numbers of markers are mapped, but are of particular interest when sizeable genomic regions encompassing several genetic markers are distorted. Utilizing an interspecific backcross in tomato, PATERSON *et al.* (1988) reported distorted ratios for 48 of 70 markers (68%) that fell into 21 distinct regions. In rice, MCCOUCH *et al.* (1988) observed two large regions skewed toward one parent. Using an interspecific cross in potato monitored by genomic and cDNA tomato probes, BONIERBALE, PLAISTED and TANKSLEY (1988) detected large genomic regions on several chromosomes that displayed aberrant segregation ratios. In barley, using doubled-haploid mapping populations (GRANER *et al.* 1991; HEUN *et al.* 1991) distorted ratios have been reported, presumably at least in part due to the fact that the parent exhibiting the favored alleles responds better to *in vitro* culture (GRANER *et al.* 1991). It is noteworthy that Beavis and GRANT (1991) did not observe aberrant segregation ratios of linked markers in any of four maize F_2 populations studied. This may be attributable to the closely related pedigrees of the central and northern U.S. Corn Belt lines used in their F_2 mapping populations. The parental inbred lines used in the present study were ones that had previously been chosen on the basis of maximal isozyme differences (STUBER and GOODMAN 1983) and represent an early Canadian line derived from Pioneer Brand hybrid 6124 and a Texas line derived from 'Yellow Surecropper.' Given the diversity of these two groups, despite full fertility of the hybrid and progeny, it does not seem unreasonable that gametic and/or pairing factors may have played a role in the distortion of genetic ratios in particular regions. In a similar situation, WENDEL, EDWARDS and STUBER (1987) observed segregation distortion on 7 of 10 chromosomes for 11 of 17 isozyme loci in an F_2 cross involving two maize inbreds with divergent pedigrees. The region of chromosome *1* (*PIO200644* to *UMC83*) that had an aberrant ratio favored the Tx303 parent. This genomic region also displayed aberrant segregation in the previous UMC mapping population with the same parents (HOISINGTON and COE 1990). It is interesting to note, however, that this genomic segment on chromosome *1* displayed normal segregation in recombinant inbreds of the same genetic cross (B. BURR, personal communication). A region on chromosome *5* flanked proximally by *UMC126* and distally by *UMC108* was distorted in favor of the CO159 parent. This region was also shown by DOEBLEY *et al.* (1990) and DOEBLEY and STEC (1991) to be distorted toward the teosinte (*Z. mays* L. ssp. *mexicana*) parent in a maize-teosinte F_2 population. In this genomic region of the integrated genetic map (COE 1992) is the widely distributed gametophytic factor, *ga2* (BURNHAM 1936), for which *Ga2* pollen grains are competitively superior to *ga2*. A disturbed genetic ratio favoring CO159 homozygotes could occur if Tx303 carried the *ga2* allele and CO159 carried the *Ga2* allele. It is unclear what genetic mechanism could account for a significant biasing toward heterozygosity on chromosomes *3* and *5*, unless the present study unknowingly applied selection toward heterozygotes within this genomic segment. Previous mapping data from an identical population (HOISINGTON and COE 1990) did not indicate a bias towards heterozygotes in these regions though it is worth noting that the genomic segment flanked by *UMC26* and *UMC102* on chromosome *3* was distorted towards the Tx303 homozygote.

Differences in gene order(s): Not surprisingly, the map contained some genomic segments that differed in gene order compared to other RFLP maps constructed in maize (BEAVIS and GRANT 1991; BURR *et al.* 1988; HELENTJARIS *et al.* 1986). That the present map differed in assessment of the most probable gene order for the terminal markers of chromosome arms *4L*, *5L* and *8S* is most likely due to the unflankable nature of terminal markers. This phenomenon is further compounded for chromosome arms *5L* and *8S* by the fact that the two terminal markers were tightly linked but the next proximal marker was 30 cM away. The uncertainty of order of terminal markers has also been noted in *A. thaliana* (CHANG *et al.* 1988; NAM *et al.* 1989) and rice (MCCOUCH *et al.* 1988). Development of telomeric RFLP probes that are simultaneously defined by functional, recombinational and physical criteria would aid in the resolution of termi-

nal marker orders (BURR *et al.* 1992). The inversion of a region on chromosome arm *4L* involving *UMC66*, *UMC19* and *UMC210B(SSU)* (equivalent to *BNL17.05*) relative to the most recent version of the BNL map (B. BURR, personal communication) was of concern since it involved several RFLP probes that had been designated as core markers. In an attempt to resolve the inconsistency between the UMC and BNL maps, two approaches were taken using the multipoint mapping capabilities of MAPMAKER (LANDER *et al.* 1987). First, the flanking proximal markers *UMC42* and *UMC47* and distal marker *UMC111* common to both maps were fixed and all possible orders (6) of *UMC66*, *UMC19*, and *UMC210B(SSU)* (=*BNL17.05*) placed between these flanking markers were tested. The log likelihood score for the UMC order was −126.7 with a total map distance of 121 cM as compared to a log likelihood score of −131.2 and total map distance of 240 cM for the order specified by the BNL map. Based on these data, the UMC map order is $10^{(131.2 - 126.7 = 4.5)}$ times more likely. The second multipoint approach taken was to again fix a set of proximal (*UMC42* and *UMC47*) and distal (*UMC52* and *PIO200608*) markers while comparing all orders of *UMC66*, *UMC19*, *UMC133*, *UMC15* and *UMC210B(SSU)* between the flanking sets of markers. The UMC order was preferred with a log likelihood score of −186.1, while a log likelihood score of −195.4 was required to bring *UMC210B(SSU)* into a proximal position relative to *UMC19* and *UMC66*, an order that reflects the BNL map. The map order shown for this region is based on this approach to the analysis of the data. Mapping of additional RFLP markers should help to resolve this descrepancy. Any changes in map order for this or any other region will be reflected yearly in the *Maize Genetics Cooperation Newsletter*. As with all recombinational maps, our map is subject to the same "pending" status.

Alignment to the genetic map: Using a combination of isozymes and cloned genes whose location on the classical genetic map had been previously defined by functional criteria, it was possible to establish 29 direct contact points between the RFLP map and the classical genetic map (COE, NEUFFER and HOISINGTON 1988; COE 1992). These contact points are critical not only for correlating linkage groups with particular chromosomes, but also as landmarks for choosing RFLP probes to flank any genetic trait of interest. In maize it is fortunate that there are several other RFLP maps (BURR *et al.* 1988; HELENTJARIS *et al.* 1986; BEAVIS and GRANT 1991; SHOEMAKER *et al.* 1992) that have incorporated identical isozymes and cloned genes and allow for the assignment of linkage groups to chromosomes and alignment of the RFLP maps. Additionally, B-A translocations (BURR *et al.* 1988; WEBER and HELENTJARIS 1989) and monosomics (HELENTJARIS, WEBER and WRIGHT 1986) have been used to assign linkage groups to chromosomes when cloned genes or isozymes have not been available. In *A. thaliana*, alignment to the genetic map was achieved by using multiple-marked stocks to construct the F_2 mapping population in addition to using cloned genes (CHANG *et al.* 1988; NAM *et al.* 1989). In tomato (TANKSLEY *et al.* 1987) and rice (MCCOUCH *et al.* 1988) primary trisomics played an important role in assigning linkage groups to chromosomes while isozyme markers provided direct contact points for the RFLP and genetic maps in tomato (BERNATZKY and TANKSLEY 1986). In barley, multiple-marker stocks were used to construct F_2 mapping populations (SHIN *et al.* 1990) that allow contact points with the barley genetic map. Linkage groups have been assigned to specific barley chromosomes (GRANER *et al.* 1991; HEUN *et al.* 1991) using wheat-barley addition lines or chromosome arms using wheat nullisomic-tetrasomic and ditelosomic aneuploid stocks (HEUN *et al.* 1991). Establishing contact points between the genetic and RFLP maps allows access to a wealth of cytological and cytogenetic information.

Development of core markers and bins: The several RFLP linkage maps in maize jointly encompass over 1200 molecular markers. Many of these maps have numerous markers in common, which allows for an approximate correlation of the various public and private sector maps. Still, there is not a complete correlation between maps and choosing the proper RFLP probes to flank a trait of interest is often a trial and error process dependent upon finding a suitable probe-enzyme combination. Clearly, a standardized set of chosen RFLP markers would be a useful starting point for any maize researcher wanting to map a particular trait. The designation of 97 core markers that have been selected on the basis of their usability, spacing and availability has allowed the maize genome to be divided into a series of "bins" that define a core map. Each bin is defined by a pair of RFLP markers. For example, bin 1.01 is defined by *BNL5.62* and *UMC157*. Bins are numbered according to the chromosome on which they reside, with further subdivisions following the decimal point being created by numbering sequentially from the most distal short arm core marker to the most distal long arm core marker. This bin system has the advantage that any genetic trait can be localized to a small region of the genome by using a limited number of agreed-upon markers. At the present time, we are engaged in an integrated mapping project that is directed toward the genomic localization of large numbers of previously unmapped morphological markers. One potential aid to achieving this goal is a mapping strategy that encompasses the speed of random amplified polymorphic DNA (RAPD) marker systems (WILLIAMS *et*

al. 1990) and the resolving power of previously mapped core markers with a bulking strategy that allows monitoring of genomic segments using DNA pools (MICHELMORE, PARAN and KESSELI 1991; GIOVANNONI *et al.* 1991). Core markers are critical in assigning RAPD linkage groups to chromosomes in addition to defining a series of bins into which morphological markers are placed after mapping. Once localized to a particular bin, the bin number and its left and right markers serve as a computer-sortable tag which will allow all markers in the same bin to be identified, should more precise map order within a bin be desired. Another advantage of a bin system for collection, organization and retrieval of genetic information is that it is expandable and allows bins to be infinitely subdivided should mapping technologies become available that allow an even finer dissection of the genome than is presently possible. We are hopeful that this approach, utilizing core markers and bins, will allow the maize researcher to contribute to, as well as benefit from, the wealth of maize genetic information available.

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