Comparative Genome Mapping of Sorghum and Maize

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ABSTRACT

Linkage relationships were determined among 85 maize low copy number nuclear DNA probes and seven isozyme loci in an F_2 population derived from a cross of *Sorghum bicolor* ssp. *bicolor* × *S. bicolor* ssp. *arundinaceum*. Thirteen linkage groups were defined, three more than the 10 chromosomes of sorghum. Use of maize DNA probes to produce the sorghum linkage map allowed us to make several inferences concerning processes involved in the evolutionary divergence of the maize and sorghum genomes. The results show that many linkage groups are conserved between these two genomes and that the amount of recombination in these conserved linkage groups is roughly equivalent in maize and sorghum. Estimates of the proportions of duplicated loci suggest that a larger proportion of the loci are duplicated in the maize genome than in the sorghum genome. This result concurs with a prior estimate that the nuclear DNA content of maize is three to four times greater than that of sorghum. The pattern of conserved linkages between maize and sorghum is such that most sorghum linkage groups are composed of loci that map to two maize chromosomes. This pattern is consistent with the hypothesized ancient polyploid origin of maize and sorghum. There are nine cases in which locus order within shared linkage groups is inverted in sorghum relative to maize. These may have arisen from either inversions or intrachromosomal translocations. We found no evidence for large interchromosomal translocations. Overall, the data suggest that the primary processes involved in divergence of the maize and sorghum genomes were duplications (either by polyploidy or segmental duplication) and inversions or intrachromosomal translocations.

GENETICISTS and evolutionary biologists have a long-held interest in the mechanisms involved in chromosomal evolution. Until recently, the primary means of addressing questions surrounding this issue has been via cytological analysis of interspecific hybrids and surveys of naturally occurring chromosomal diversity within populations (STEBBINS 1971; JACKSON 1984; GRANT 1987). Comparative genome mapping adds a powerful new technique for investigating the mode and tempo of chromosomal evolution. This approach involves the use of molecular markers such as restriction fragment length polymorphisms (RFLPs) to map the genomes of two species for a common set of markers (loci). Although a labor intensive and expensive method, comparative genome mapping allows one to determine the extent and nature of chromosomal rearrangements between cross-incompatible species. This method thus opens up comparisons among distantly related genomes which are not amenable to analysis by traditional cytogenetic techniques. This approach was pioneered by TANKSLEY and co-workers using tomato RFLP probes to map the tomato, chili pepper and potato genomes (BONIERBALE, PLAISTED and TANKSLEY 1988; TANKSLEY *et al.* 1988). These studies demonstrated that more distantly related species accrue a greater number of rearrangements between their genomes than do closely related species. In the case of tomato and potato, BONIERBALE, PLAISTED and TANKSLEY (1988) were able to determine the location of three paracentric inversions differentiating potato from tomato.

This paper presents a comparative genome mapping analysis of sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays* L.) using RFLP probes of maize and isozymes. Both species are members of the grass tribe Andropogoneae but are distantly related within the tribe, which has a base chromosome number of $x = 5$ (CELARIER 1956). Because sorghum and maize have a haploid chromosome number of ten, they are thought to represent ancient polyploids derived from an $n = 5$ ancestor (ANDERSON 1945; GARBER 1950; RHOADES 1951; CELARIER 1956). Duplicated isozyme loci (GOODMAN and STUBER 1983; WENDEL *et al.* 1986, 1989; MORDEN, DOEBLEY and SCHERTZ 1989), and RFLP loci (HELENTJARIS, WEBER and WRIGHT 1988; HULBERT *et al.* 1990) have been demonstrated in both species and would appear to support the hypothesis of ancient polyploidy. It is not known whether these putative ancient polyploids resulted from a single round of chromosome doubling prior to the divergence of sorghum and maize or independent events after their divergence.

The present study was conducted to construct a linkage map for sorghum and to compare this map to

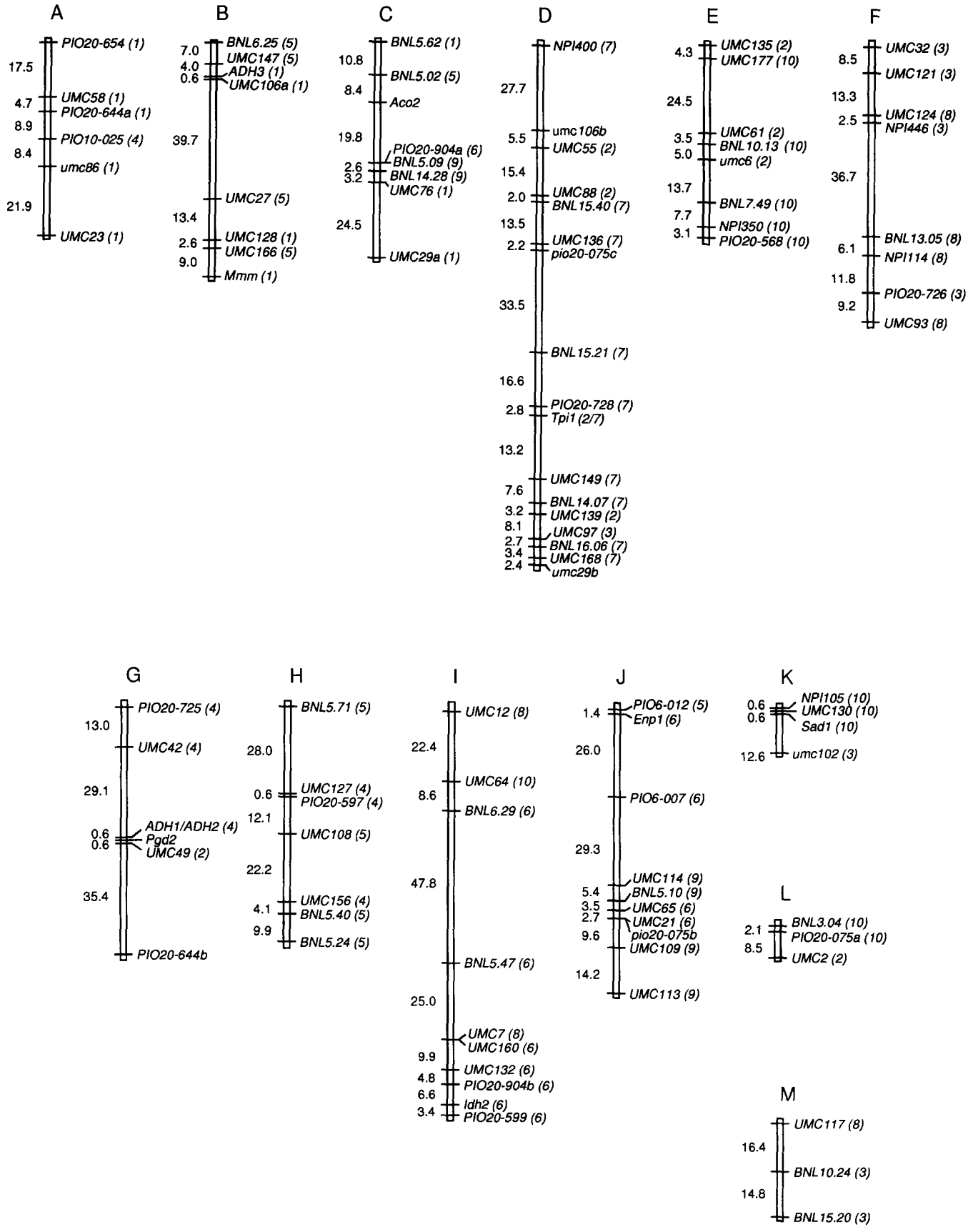

FIGURE 1.—Sorghum linkage map produced from maize RFLP genomic probes. Numbers on left of bar are map distances in centiMorgans (Haldane estimates). Locus names appear at right of bar followed by chromosomal location of the corresponding locus in maize in parentheses.

the published map of maize. Two major questions addressed are: (1) Have the maize and sorghum genomes maintained colinearity with respect to RFLP and isozyme loci? If not, can we tell the nature and number of rearrangements? (2) Do the two genomes share duplicated loci and the same constellation of duplicated linkage groups, or has the independent loss of duplicated sequences (random diploidization) eliminated previously shared duplicated genomic regions?

MATERIALS AND METHODS

Sorghum cross: The F_2 mapping population was derived by self-pollinating a single F_1 hybrid plant from a cross between *S. bicolor* ssp. *bicolor* race bicolor (IS2482C) and *S. bicolor* ssp. *arundinaceum* race virgatum (IS18809). Caryopses were planted in 9.5-cm square pots at a density of four per pot in standard potting soil and germinated under ambient light and temperature in glasshouses at the University of Minnesota. After 8 days individual seedlings were transplanted to 14.7-cm diameter pots in standard potting soil.

Isozymes: Crude protein extracts were made from young leaf tissue of the individual F_2 plants. Methods for extraction, starch gel electrophoresis and isozyme staining follow MORDEN, DOEBLEY and SCHERTZ (1987). The following enzyme systems were analyzed for loci known to segregate in this cross: aconitase (*Aco2*), endopeptidase (*Enp1*), isocitrate dehydrogenase (*Idh2*), 6-phosphogluconate dehydrogenase (*Pgd2*), shikimate dehydrogenase (*Sad1*) and triosephosphate isomerase (*Tpi1*). The enzyme modifying locus *modifier of malate dehydrogenase* (*Mmm*) was also assayed.

Genomic DNA isolation: Total genomic DNA was extracted using a modification of the procedure of SAGHAI-MAROOF *et al.* (1984). Between 2 and 4 g of leaves or young inflorescences were ground to a fine powder in liquid nitrogen using a mortar and pestle. For each gram of fresh weight material, 2.5 ml of extraction buffer [100 mM Tris-HCl (pH 8), 2% w:v mixed alkyltrimethyl-ammonium bromide (Sigma), 700 mM NaCl, 20 mM EDTA (pH 8), 1% v:v β-mercaptoethanol, 1% w:v sodium bisulfite] was added and the tissue was ground for approximately 1 min to form a thin slurry. The slurry was incubated at 60° for 30 min with one mixing, followed by cooling to room temperature and extracting twice with chloroform:octanol (24:1). Genomic DNA was precipitated by adding an equal volume of ice-cold isopropanol. The DNA was hooked on a glass pasture pipette and transferred to 2 ml of 76% ethanol/0.2 M sodium acetate for 20 min. A final wash of 76% ethanol/10 mM ammonium acetate for about 2 min preceded air drying to remove traces of alcohol and resuspension in 200–400 μl of 10 mM Tris-HCl (pH 8), 1 mM EDTA (pH 8). The final volume was adjusted to an apparent concentration of 2 μg/μl as determined by UV spectrophotometry (260 nm absorbance).

Filter preparation: Genomic DNA was digested in a 20-μl volume containing 10 μg of genomic DNA, 1 μg RNAase A (Sigma), 1 × buffer (BRL) and 16–20 units of restriction endonuclease [*Eco*RI, *Eco*RV, *Hin*dIII or *Sst*I (BRL)], and incubated at 37° for 3 hr. Digested DNAs were loaded in 0.8% agarose gels and run at 1.76 volts/cm for 9.5 hr. Gel and tank buffer was 1 × TAE [0.1 M Tris, 1 mM EDTA (pH 8), acetic acid to pH 8.1]. Gels were stained with 0.5 mg/ml of ethidium bromide and photographed. The DNA was not depurinated nor UV-nicked prior to Southern transfers. Southern transfers were carried out by washing each gel for 30 min in 0.4 N NaOH/0.6 M NaCl, rinsing in distilled water, washing for 30 min in 0.5 M Tris-HCl (pH 8)/1.5 M NaCl and transferring with 20 × SSC (0.3 M sodium citrate, 3 M NaCl) overnight onto nylon filters (MSI Magna). Filters were washed for 20 min in 2 × SSC/0.2 M Tris-HCl (pH 8), UV cross-linked (0.12 joule), air dried and vacuum baked at 80° for 2 hr.

Clones: Plasmid clones of low copy number maize nuclear sequences were obtained from Brookhaven National Laboratory (BNL), University of Missouri, Columbia (UMC), Pioneer Hi-Bred International, Inc. (PIO), and Native Plants, Inc. (NPI). Clones were digested in a restriction reaction using the appropriate enzyme and enough of the clone to yield approximately 1 μg of insert. The restriction digest was loaded into a 0.8% TAE low melting point agarose gel (BRL) and run at 1.76 volts/cm for 8 hr. The insert band was identified by staining with ethidium bromide and viewing under UV light. The isolated fragment was cut from the gel, added to 3 volumes of distilled water, boiled for 10 min, mixed and stored as a stock in a microcentrifuge tube at −20°.

Hybridizations: Filters were prehybridized overnight in excess of 60 μl/cm^2 of prehybridization buffer [5 × SSC, 50 mM Tris-HCl (pH 8), 1% sodium dodecyl sulfate (SDS), 5 × Denhardt's solution, 10 mM EDTA (pH 8) and 100 μg/ml of single-stranded, fragmented salmon sperm DNA (ssDNA)] in heat-seal pouches at 65°.

A 50–100-ng aliquot of clone insert DNA was prepared by heating the stock for 2 min at 60° and withdrawing the appropriate volume. Radioactive labeling of the insert followed the primer extension procedure of FEINBERG and VOGELSTEIN (1983), modified by allowing the labeling reaction to proceed for 5 hr at 37°, followed by spin columns to separate unincorporated nucleotides and agarose (SAMBROOK, FRITSCH and MANIATIS 1989). Labeled probes were boiled for 10 min prior to adding to hybridization buffer.

Prehybridization buffer was removed from the heat seal pouch, hybridization buffer containing the labeled probe was added, and the pouch was resealed and returned to 65° as quickly as possible. Hybridization buffer was either fresh prehybridization buffer (for new filters or probes prone to give high background) or 10% dextran sulfate, 5 × SSC, 50 mM Tris-HCl (pH 8), 10 mM EDTA (pH 8), 1 × Denhardt's solution, 0.2% SDS, and 0.1 mg/ml of ssDNA. Hybridizations were carried out overnight. Filters were washed three times for 30–45 min at 65° in 0.5 × SSC/0.2% SDS, wrapped in plastic, and read with a GM survey meter to determine time of exposure for autoradiography.

Data gathering and analysis: Survey for polymorphic enzyme/probe combinations were carried out with filters containing DNA from both parents and seven F_2 individuals restricted with *Eco*RI, *Eco*RV, *Hin*dIII and *Sst*I. Once a probe proved polymorphic, it was mapped using 81 F_2 individuals. Individuals were genotyped by comparing their banding pattern to the pattern in the parents. Linkage among pairs of loci and tests for expected segregation frequencies were carried out using the LINKAGE-1 program (SUITER, WENDEL and CASE 1983). Maximum likelihood estimates of linkage groups and Haldane map distances were determined using the MAPMAKER program (LANDER *et al.* 1987). Linkages were determined with a recombination value of 0.3 and a LOD score of 3.0.

Information on duplicate loci was gathered by preparing filters with seven inbred lines of sorghum digested for the

same four enzymes used in the polymorphism survey. These filters were probed at the same time as polymorphism filters. When a probe produced a single band across all four enzymes, it was interpreted to result from a single locus. If two bands or more were observed across all four enzymes a minimum of two loci were counted. Information on which RFLP loci are duplicated in maize was taken from several sources (HELENTJARIS, WEBER and WRIGHT 1988; COE, HOISINGTON and NEUFFER 1990; HULBERT *et al.* 1990; DOEBLEY and STEC 1991; BEAVIS and GRANT 1991; BURR and BURR 1991; J. DOEBLEY, unpublished).

Locus designations: Many RFLP probes hybridized to more than a single locus in sorghum (see below). When a single locus was mapped for a probe, we followed the convention of designating the locus with uppercase letters if it represented a "strong" locus (dark band on the autoradiograph) and lowercase letters if it was a "weak" locus (weaker hybridization signal). In cases where a probe hybridized to two or more segregating loci in our population, a suffix (a, b, or c) was used to distinguish among them.

RESULTS

Sorghum map: A total of 146 maize RFLP probes were analyzed for polymorphism in the cross bicolor × virgatum. Eighty-five RFLP probes and seven sorghum isozyme loci (*Aco2, Enp1, Idh2, Mmm, Pgd2, Sad1, Tpi1,*) revealed polymorphisms and the resulting loci were mapped. Our sorghum linkage map (Figure 1) consists of 98 loci in 13 linkage groups that range in size from 10.6 cM (Figure 1L) to 159.8 cM (Figure 1D) with a total map distance of 949 cM. The average distance between loci is 11.2 cM, determined by dividing the total map distance by the number of loci minus the number of linkage groups.

The sorghum map is based on a set of loci which cover nearly the entire maize RFLP linkage map (Figure 2). Several regions from the maize genome are not as well represented in the sorghum map as others. These regions are the short arm of maize chromosomes *1* and *2* and the long arm of maize chromosomes *3* and *4* (Figure 2). More complete coverage in these regions may lead to both a greater density of loci in the sorghum map as well as an increase in the total map distance. The total length of the sorghum map is 53% of the approximately 1800 cM of the maize linkage map (COE, HOISINGTON and NEUFFER 1990).

Significant segregation distortion ($P < 0.05$) was only found in sorghum linkage group B with a greater than expected recovery of alleles from the bicolor parent. For marker loci *UMC147, ADH3* and *UMC106a,* the probability of observing the data under the null hypothesis of normal Mendelian segregation was less than 0.001. For *BNL6.25,* this probability was between 0.01 and 0.001, and, for *Mmm,* it was between 0.05 and 0.01.

Conservation of linkage groups: An examination of Figure 1 reveals that no sorghum linkage group consists entirely of loci from a single maize chromosome, although two linkage groups nearly meet this criterion. Group A is composed of loci from maize chromosome *1* with a single locus (*PIO10–025*) from maize chromosome *4*. Group G is composed of loci from maize chromosome *4* with a single locus (*UMC49*) from maize chromosome *2*. The sorghum enzyme locus *Pgd2* in this linkage group encodes plastid 6-phosphogluconate dehydrogenase. The gene encoding this enzyme in maize has not been mapped.

Figure 1 also demonstrates that the sorghum linkage groups are not composed of random assemblages of loci from several maize chromosomes. Rather, the clear pattern is that most sorghum linkage groups contain loci from two maize chromosomes. For example, sorghum linkage group B is composed solely of loci from maize chromosomes *1* and *5*, group E from maize chromosomes *2* and *10*, group F from maize chromosomes *3* and *8*, and group H from maize chromosomes *4* and *5*. Similarly, the majority of the loci in linkage group D map to maize chromosomes *2* and *7*, the majority in group I map to maize chromosomes *6* and *8*, and the majority in group J map to maize chromosomes *6* and *9*. Only group C presents an apparently random mixture of loci from several maize chromosomes.

Conservation of gene order: Figure 3 compares the maize and sorghum genomes with regard to the linear order of the loci within the conserved linkage groups. As discussed above, most sorghum linkage groups possess loci from two separate maize chromosomes, and thus, both of these chromosomes (or segments thereof) are aligned with their sorghum counterpart. Since we studied loci that have not all been placed on a single maize linkage map, it was necessary to synthesize the maize chromosome maps shown in Figure 3 from several maize linkage maps (COE, HOISINGTON and NEUFFER 1990; BEAVIS and GRANT 1991; BURR and BURR 1991; DOEBLEY unpublished). For this reason, map distances between loci on the maize chromosomes depicted in Figure 3 are in some cases approximate. Because the marker loci we have analyzed are generally only loosely linked (>10 cM) in maize and because many of the loci are located on at least two of the three maize maps, it was possible to combine the maize maps with very little ambiguity as to gene order.

Examination of Figure 3 reveals that many linkage groups have been substantially conserved between maize and sorghum, although there is also clear evidence for rearrangements. Gene order in sorghum group C is identical to the corresponding portions of maize chromosome *1*. Gene order in sorghum group E is identical to a corresponding portion of maize chromosome *2*, but appears to exhibit a small rearrangement relative to maize chromosome *10*. Gene

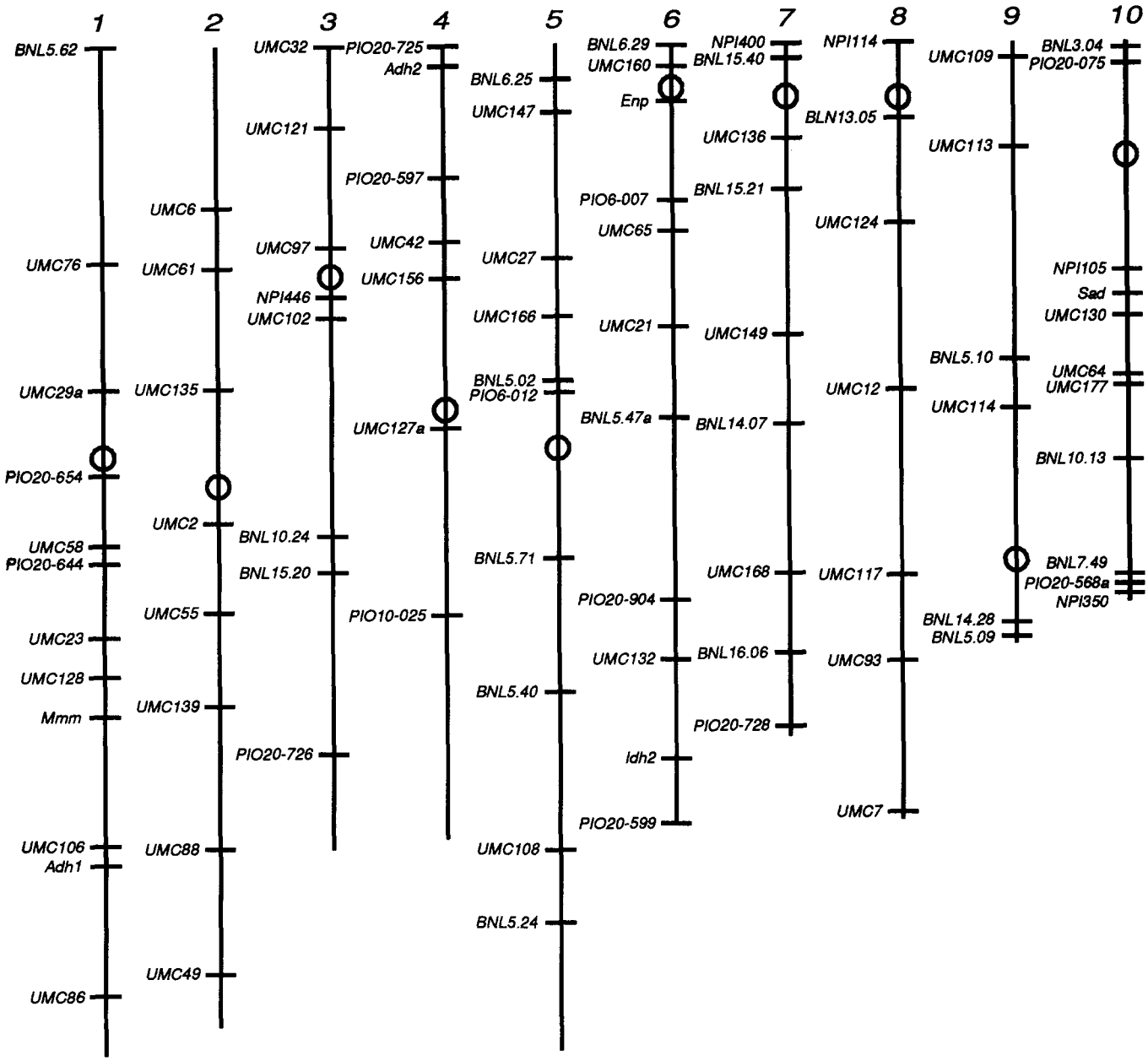

FIGURE 2.—Approximate map location in maize of RFLP loci used to map the sorghum genome.

order in sorghum group B is identical to a portion of maize chromosome *5*, but shows one rearrangement relative to maize chromosome *1*. Gene order in sorghum group A and a segment of maize chromosome *1* is the same except that the order of *UMC23a* and *UMC86* is inverted. Gene order in sorghum group J is identical to the corresponding segment of maize chromosome *6*, but shows one rearrangement relative to maize chromosome *9*. Similarly, gene order in sorghum group F is identical to the corresponding segment of maize chromosome *3*, but shows one rearrangement relative to maize chromosome *8*. Other rearrangements are seen between sorghum D and maize *2* and *7*, sorghum G and maize *4*, sorghum H and maize *4* and *5*, and sorghum I and maize *6*. Altogether, there are 14 cases in which the order of linked loci in sorghum is inverted or otherwise rearranged relative to the corresponding segment in maize. These may represent either intrachromosomal translocations or inversions.

Conservation of map distances: In addition to examining the extent of conservation of gene order, one may assess the degree to which map distances between linked loci are conserved. Table 1 lists linkage groups conserved between the maize and sorghum genomes. In assembling Table 1, we excluded conserved linkage groups which include apparent inversions between maize and sorghum because for these linkage groups the map distances would clearly not be calculated over the same physical distance. There are a total of 16

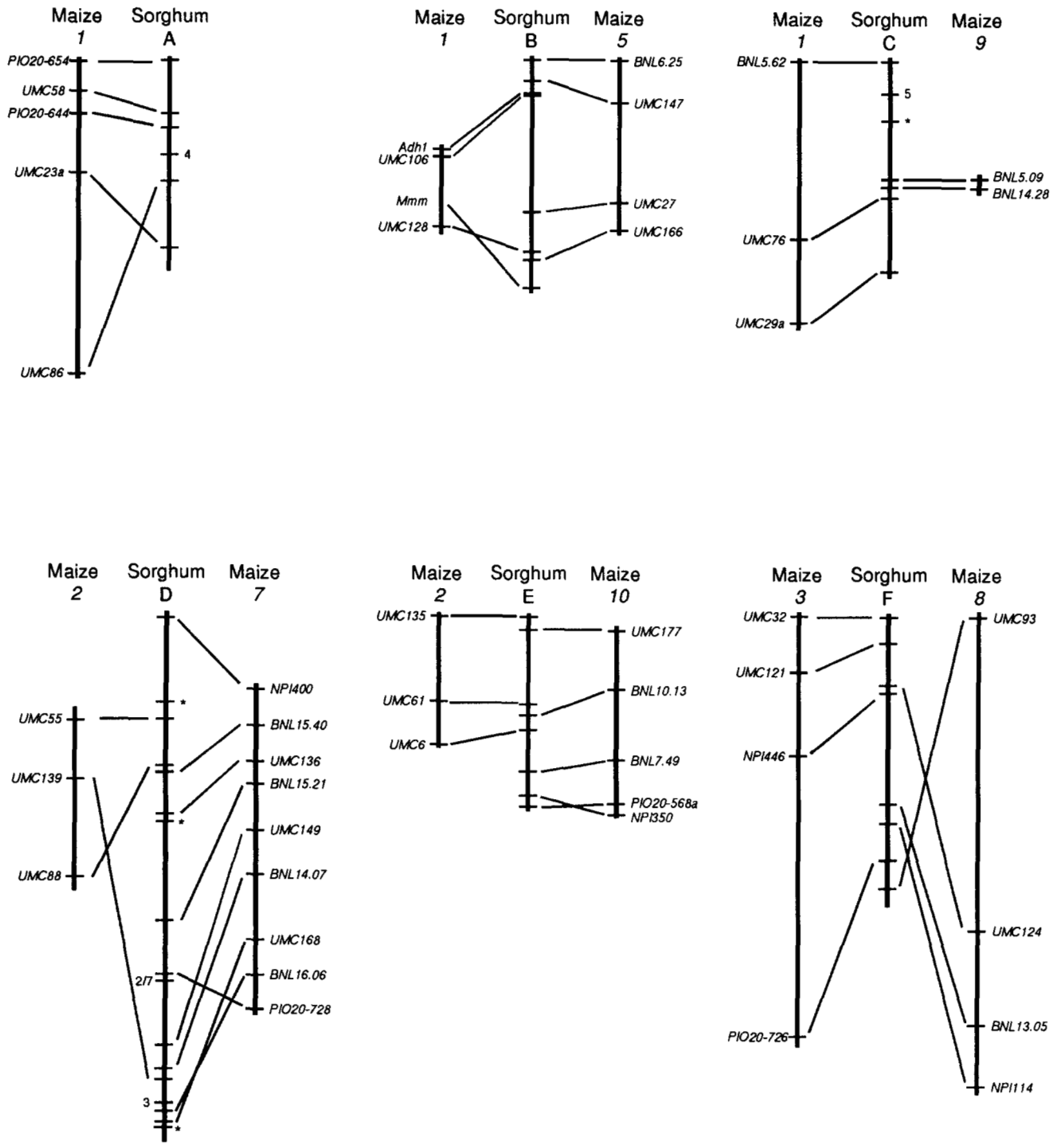

FIGURE 3

groups that collectively represent 835 cM in the sorghum map. Maize linkage groups corresponding to these 16 cover 862 cM. In general, map distances are roughly equivalent in maize and sorghum. The average size of these conserved linkage groups is 52.2 cM in sorghum versus 53.9 cM in maize.

Duplicated loci: The cloned RFLP probes can hybridize to two or more loci in maize (HELENTJARIS, WEBER and WRIGHT 1988) or sorghum (HULBERT *et al.* 1990). We also observed this phenomenon in our study. Fifty-five of the 146 probes (38%) hybridized to two or more loci in sorghum. These duplicated loci include (1) those hybridizing to two loci with equal hybridization intensity (8.2%), (2) those hybridizing to one locus strongly and a second locus weakly (22%), and those hybridizing to three or more loci (7.5%). We had information from both maize and sorghum regarding the number of loci to which a common set

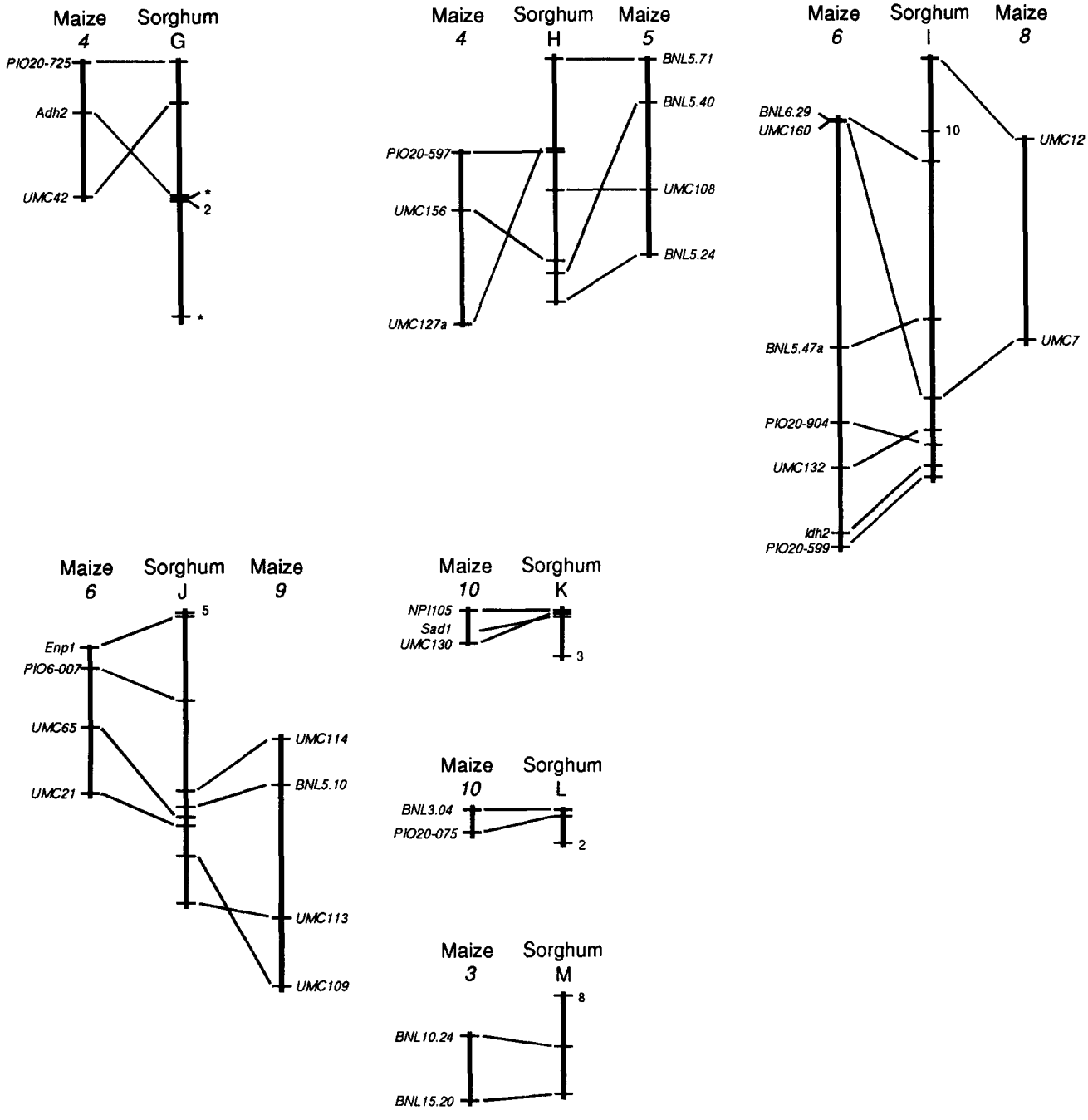

FIGURE 3.—Alignment of sorghum linkage groups with corresponding chromosome segments from maize. Maize locus names are shown along the maize chromosomal segments. The diagonal/horizontal lines between the maize and sorghum chromosomal segments indicate the positions of corresponding (either orthologous or paralogous) loci. An asterisk (*) on a sorghum linkage group indicates a locus not mapped in maize. A number on the sorghum linkage group indicates the maize chromosomal location corresponding to that sorghum locus. The location of the isozyme loci, *Mmm* and *Sad1*, are approximate in maize chromosomes *1* and *10*, respectively.

of 89 probes hybridize (Table 2). This information comes from both published sources (HELENTJARIS, WEBER and WRIGHT 1988; COE, HOISINGTON and NEUFFER 1990; HULBERT *et al.* 1990; BEAVIS and GRANT 1991; BURR and BURR 1991) and our own analyses. Of these 89 loci, 38% were duplicated in the sorghum genome versus 72% in the maize genome. Thirty-one percent showed evidence of duplication in both sorghum and maize. Forty percent show duplication in maize but not in sorghum, and 6.7% are duplicated in sorghum but not in maize. Twenty-one percent were specific for a single locus in both sorghum and maize.

DISCUSSION

Sorghum map: Our sorghum map contains thirteen linkage groups, although sorghum has only ten chromosomes. The smallest linkage groups, K, L and M,

TABLE 1

Conserved linkages between sorghum and maize

Sorghum segment	Sorghum linkage group	Map distance[a]	
		Sorghum	Maize[b]
PIO20-654–PIO20-644a	A	22.2	19.0
BNL6.25–UMC166	B	67.3	56.2
ADH3–UMC106a	B	0.6	2.0
BNL5.09–BNL14.28	C	2.6	2.0
BNL5.62–UMC29a	C	69.3	75.1
NPI400–BNL14.07	D	140.0	83.2
UMC135–umc6	E	37.3	41.7
UMC177–BNL7.49	E	51.0	41.7
UMC32–PIO20-726	F	78.9	142.4
UMC124–NPI114	F	45.3	52.2
UMC12–UMC7	I	103.8	63.3
BNL6.29–PIO20-599	I	97.5	134.2
Enp1–UMC21	J	66.9	46.6
UMC114–UMC113	J	35.4	76.0
BNL3.04–PIO20-075a	L	2.1	7.1
BNL10.24–BNL15.20	M	14.8	19.6

[a] MAP distances in centiMorgans (Haldane estimates).
[b] Maize map distances derived from smallest reported distances in COE, HOISINGTON and NEUFFER (1990), BEAVIS and GRANT (1991) and BURR and BURR (1991).

may represent unlinked portions of the other groups. For example, groups K and L may be parts of group E, based on the shared loci from maize chromosomes *2* and *10*. Linkage group M may be a portion of group F, based on the shared loci from maize chromosomes *3* and *8*. Additional markers selected from maize chromosome arms *2S* and *8L* should allow these segments to linked together if these inferences are correct.

Genome length: The total length of our sorghum map is 949 cM. We estimate that it would require minimally an additional 150 cM to join sorghum linkage groups K, L and M to other sorghum linkage groups and thus reduce the number of linkage groups to 10. Thus, a minimal length for the sorghum genome is 1099 cM or 61% of the length of the maize genome. At least two factors may contribute to the smaller estimate for the size of the sorghum genome relative to the maize genome. First, our markers clearly do not cover the entire sorghum genome since we did not find 10 linkage groups corresponding to the 10 sorghum chromosomes. Complete coverage of the sorghum genome by molecular markers may bring the genome size of sorghum closer to that of maize. Second, the choice of a relatively wide cross to produce the sorghum map may have led to a reduction of recombination and thus smaller map distances. Evidence from conserved linkage blocks (see below) however, argues against this idea.

The relative lengths of the maize and sorghum genomes can also be assessed by comparing map lengths in conserved (homoeologous) linkage blocks. Comparison of the genetic lengths for the 16 conserved linkage blocks shared by the maize and sorghum genomes (Table 1) demonstrates that these blocks represent 835 cM in the sorghum genome and 862 cM in the maize genome. These two values are remarkably similar, differing by only 3.1%. If one examines the lengths of the 16 conserved segments individually (Table 1), the similarity of the values is equally as striking, especially considering the small populations sizes employed in making both the maize and sorghum maps.

Linkage group conservation: The most striking feature to emerge from the comparative mapping of the maize and sorghum genomes is that most sorghum linkage groups possess loci that map predominately to two maize chromosomes. The pattern of similarity between sorghum and maize linkage groups follows very closely the pattern of duplicated isozyme and RFLP loci (GOODMAN *et al.* 1980; WENDEL *et al.* 1986, 1989; HELENTJARIS, WEBER and WRIGHT 1988). These authors documented numerous duplicated loci on the maize chromosome pairs *1L* and *5S*, *2L* and *7*, *3S* and *8*, and *6* and *8L*. Loci from each of these pairs largely map to single sorghum linkage groups, B, D, F and I, respectively. HELENTJARIS, WEBER and WRIGHT (1988) also showed some duplicated loci to be shared between maize chromosome pairs *1S* and *9L*, *2S* and *10L*, and *4L* and *5*. RFLP loci from each of these pairs map to single sorghum linkage groups, C, E, and H, respectively (Figure 3), strengthening the argument that they represent or contain duplicated segments in the maize genome.

The fact that most sorghum linkage groups are composed of RFLP loci predominately from two maize chromosomes may reflect either the putative polyploid ancestry of these species from a common ancestor with $n = 5$ chromosomes or chromosomal segment duplication without polyploidy in the lineage leading to maize (RHOADES 1951; WENDEL *et al.* 1986; HELENTJARIS, WEBER and WRIGHT 1988). If segmental duplication in the maize lineage were the cause, then one anticipates that duplicated linkage groups should be found only in maize (model A, Figure 4). If polyploidy in the ancestry of both maize and sorghum were the cause, then one anticipates that duplicated linkage groups should be found in both species (model B, Figure 4). Under both models, loci on duplicated segments in maize can map to a single segment in sorghum such as we have observed; however, if both species are ancient polyploids as their chromosome numbers ($n = 10$) seem to suggest, then it should be possible to detect a series of duplicated segments in sorghum as well. We have not detected these, however we have mapped too few duplicated loci in sorghum to enable us to do so. Thus, the issue remains unresolved. An argument against the segmental duplica-

TABLE 2

Minimum locus number for RFLP probes in sorghum and maize

Probe	Locus no. in sorghum	Locus no. in maize	Loci mapped in sorghum	Probe	Locus no. in sorghum	Locus no. in maize	Loci mapped in sorghum
ADH (aerobic)	2	1	2	UMC31	1	2	0
ADH (anaerobic)	1	1	1	UMC32	1	2	1
rDNA	1	1	0	UMC35	1	2	0
Sh1	2	2	0	UMC36	1	2	0
BNL3.04	2	1	1	UMC38	2	2	0
BNL5.09	1	2	1	UMC39	1	2	0
BNL5.24	1	2	1	UMC42	2	2	1
BNL5.37	2	2	0	UMC50	2	2	0
BNL5.47	1	2	1	UMC53	1	2	0
BNL5.59	1	1	0	UMC54	1	1	0
BNL6.29	2	1	1	UMC55	1	2	1
BNL7.21	2	2	0	UMC61	1	1	1
BNL7.49	2	2	1	UMC65	1	1	1
BNL7.71	2	2	0	UMC72	1	2	0
BNL8.45	1	2	0	UMC76	1	2	1
BNL10.13	2	1	1	UMC83	1	2	0
BNL10.17	1	2	0	UMC85	1	1	0
BNL10.24	1	2	1	UMC93	1	2	1
BNL13.05	2	2	1	UMC94	1	2	0
BNL14.07	1	1	1	UMC95	1	1	0
BNL14.28	1	2	1	UMC97	1	2	1
BNL15.20	2	2	1	UMC102	1	2	1
BNL15.21	1	1	1	UMC106	2	2	2
NPI114	1	2	1	UMC107	1	2	0
NPI253	2	2	0	UMC108	2	2	1
NPI259	2	1	0	UMC109	1	2	1
NPI289	2	2	0	UMC110	1	2	0
NPI350	1	2	1	UMC111	1	1	0
NPI400	2	2	1	UMC113	2	2	1
NPI412	2	2	0	UMC115	1	2	0
NPI425	1	2	0	UMC117	1	1	1
NPI426	1	2	0	UMC121	1	2	1
PIO20-075	2	2	3	UMC122	1	1	0
PIO20-568	2	2	1	UMC127	2	2	1
PIO20-581	1	2	0	UMC129	1	2	0
PIO20-597	2	1	1	UMC130	1	1	1
UMC2	2	2	1	UMC136	1	2	1
UMC5	2	2	0	UMC139	1	1	1
UMC6	1	1	1	UMC141	2	2	0
UMC7	2	2	1	UMC152	1	1	0
UMC8	2	2	0	UMC154	2	2	0
UMC12	1	2	1	UMC156	2	2	1
UMC18	1	2	0	UMC168	1	1	1
UMC19	1	1	0	UMC177	1	2	1
UMC29	2	2	2				

tion hypothesis (model A) is that the extensive nature of the duplication in the maize genome would require numerous independent segmental duplications if the polyploidy hypothesis is rejected. The polyploidy hypothesis seems more parsimonious, especially when coupled with the cytotaxonomic evidence for the ancient polyploid origin of maize and sorghum (ANDERSON 1945; GARBER 1950; RHOADES 1951; CELARIER 1956).

Comparative locus order: There are 17 linkage groups of three or more loci that are shared between maize and sorghum (Figure 3). For five of these, locus order is the same in maize and sorghum. Of the remaining 12, 10 show a single case of inverted locus order, and two (sorghum D *vs.* maize 7, and sorghum 1 *vs.* maize 6) show two cases of inverted locus order within a single aligned segment. This gives a total of 14 apparent cases of inverted locus order between the sorghum and maize genomes. Five of these cases involve closely linked loci: *UMC128* and *Mmm* on sorghum group B, *UMC168* and *BNL16.06* on sorghum D, *PIO20–568* and *NPI350* on sorghum E,

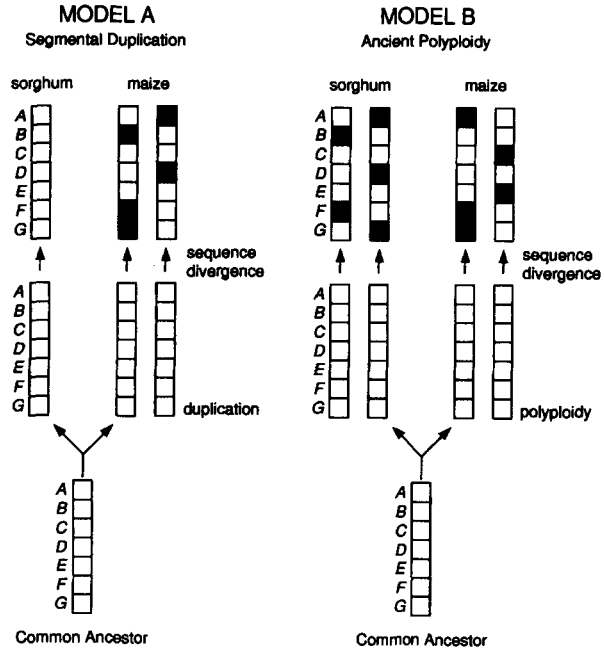

FIGURE 4.—Two possible models for the origin of duplicated linkage groups in the maize and sorghum genomes. Model A involves segmental duplication in the lineage leading to maize. Under this model, there would be duplicated linkage groups in maize but not in sorghum, and loci on the duplicated maize linkage groups would map to a single sorghum linkage group. Model B involves polyploidy in the ancestry of both maize and sorghum. Under this model, duplicated linkage groups would occur in both species, and loci from a single linkage group in one species could map to separate linkage groups in the other species. Each bar represents a chromosomal segment or linkage group. *A–G* represent RFLP loci in each of the linkage groups. Loci that have been deleted or substantially altered by the accumulation of base substitutions over time are darkened.

PIO20–904b and *UMC132* on sorghum I, and *Sad1* and *UMC130* on sorghum K. Because locus order for these loci may be inaccurate in either the sorghum or maize map, we cannot be certain that the order is truly inverted. Thus, a more conservative estimate of the number of cases of inverted locus order between the sorghum and maize maps is nine. These nine differences in locus order could result from intrachromosomal translocations, duplications or inversions. Inversions are known to occur with reasonable frequency in teosinte, a wild relatives of maize, supporting the view that they may have been important in the differentiation of the maize and sorghum genomes (TING 1964; KATO 1976). There are also several known cases of intrachromosomal gene duplications in maize (zein genes on chromosomes *4*, *Tpi3* and *Tpi5* on chromosome *8*, and *Sh1* and *Css* on chromosome *9*). These duplications could appear as inversions of locus order in a comparative mapping analyses if the locus mapped in maize was paralogous to that mapped in sorghum. Thus, none of the possible mechanisms can be excluded at this time. However, placement of a larger number of marker loci in these regions of the sorghum and maize maps could resolve this question.

Evidence for large interchromosomal translocations during the evolution of maize and sorghum would appear as a sorghum linkage group composed of a contiguous block of loci from one maize chromosome linked to a contiguous block of loci from another maize chromosome. We observed no such case, although it should have been detected if large translocations were an important component in the differentiation of these genomes. In all cases where loci from two maize chromosomes map to a single sorghum linkage group, the loci are interspersed rather than in two contiguous blocks. Although this pattern could be explained by a large interchromosomal translocation followed by extensive intrachromosomal rearrangements, our data are easier to reconcile with segmental duplication or polyploidy as discussed above. From this evidence, we infer that large interchromosomal translocations were not a prominent mechanism in the evolution of the maize and sorghum genomes. This contrasts with the evolution of the pepper and tomato genomes during which large interchromosomal translocations were common (TANKSLEY *et al.* 1988). Comparative mapping of a larger number of loci would be necessary to test this inference more thoroughly. The lack of evidence for large interchromosomal translocations is consistent with prior research which failed to find any cytological evidence for translocations in surveys of 155 maize landraces from Latin America (COOPER and BRINK 1937; RHOADES and DEMPSEY 1953).

There are a few cases in which a single locus from one maize chromosome is embedded within a sorghum linkage group composed largely of loci from other maize chromosomes. For example, *PIO10–025*, which maps to maize chromosome *4*, maps to sorghum group (A) that is otherwise entirely composed of loci from maize chromosome *1*. Similarly, *UMC64*, which maps to maize chromosome *10*, maps to sorghum group I which is otherwise composed of loci corresponding to maize chromosomes *6* and *8*. These occurrences can be explained by interchromosomal transfer of small chromosome segments, perhaps even single genes. If such events occurred in the evolution of the maize and sorghum genomes, they must be relatively uncommon as they have not obscured the clear pattern that sorghum linkage groups are predominately composed of loci homologous to two and only two maize chromosomes.

Duplicated loci: Several authors have previously reported extensive duplication of RFLP loci in maize or sorghum (HELENTJARIS, WEBER and WRIGHT 1988; HULBERT *et al.* 1990). While duplications are readily detected by Southern hybridization, there are several factors that influence the ability to estimate accurately the proportion of duplicated loci. First, it is well known that stringency of the hybridization and post-hybridization washes critically affect the ability to

detect homologous sequences (ZAMIR and TANKSLEY 1988). Second, the condition of the Southern filter has a significant effect. We observed that many probes hybridize to one locus strongly and a second locus weakly. On the first use of a filter, a probe may easily detect both the strong and the weak locus; however, after multiple filter reprobings, the same probe may only detect the stronger locus. Third, under moderate stringency, probes that hybridize to a single locus intensely can hybridize to other loci with a continuum of intensities. In some cases, this requires that a judgment be made as to whether the weaker bands are intense enough relative to the stronger bands to be scored as duplicated loci.

Comparison of a common set of 89 RFLP probes for maize and sorghum indicated that only 38% were duplicated in sorghum as compared to 72% in maize. This suggests that there are nearly twice as many duplicated loci in maize. This observation agrees with isozymes analyses which also show fewer duplicated isozyme loci in sorghum than in maize (MORDEN, DOEBLEY and SCHERTZ 1989; J. DOEBLEY, K. F. SCHERTZ, P. ALDRICH and C. W. MORDEN, unpublished). For example, there are two loci of phosphoglucomutase in maize and one in sorghum. For mitochondrial malate dehydrogenase, there are three loci in maize and two in sorghum. For triosephosphate isomerase, there are five loci in maize and three in sorghum. For aconitase, there are at least four loci in maize and two in sorghum. For cytosolic 6-phosphogluconate dehydrogenase, there are two loci in maize and one in sorghum. For eight other enzymes, the isozymes of maize and sorghum are encoded by the same number of loci. Only for alcohol dehydrogenase does maize possess fewer loci than sorghum (two *vs.* three). Thus, although duplicated loci are found in both maize and sorghum, a greater degree of duplication appears to characterize the maize genome.

The greater proportion of duplicated loci in maize relative to sorghum agrees with estimates that the DNA content of the maize genome is three to four times larger than that of the sorghum genome (LAURIE and BENNETT 1985). This difference in DNA content does not specify whether sorghum possesses less high or middle repeated DNA, or less low copy number DNA. Our evidence that the low copy number RFLP loci are more frequently duplicated in maize than sorghum suggests that at least some of the differences in genome size occurs in the latter portion of the genome.

CONCLUSION

Previous studies involving comparative genome mapping in plants have demonstrated two patterns of chromosomal evolution. Comparative mapping of the tomato and potato genomes revealed essentially complete conservation of linkage groups with only a small number of inversions differentiating these species (BONIERBALE, PLAISTED and TANKSLEY 1988). In contrast, comparative mapping of the tomato and pepper genomes revealed extensive rearrangements including many interchromosomal translocations. Our results from comparative mapping of the maize and sorghum genomes in conjunction with previously published analyses revealed yet another pattern of chromosomal evolution. Among these grasses, duplication of chromosomal segments followed by inversions or intrachromosomal translocations appears to have been the principal mechanisms of chromosomal evolution. We found no evidence for larger interchromosomal translocations such as differentiate the tomato and pepper genomes. We also found that the proportion of duplicated loci is greater in maize than in sorghum. If maize and sorghum are both ancient polyploids, then the larger proportion of duplicated loci in maize may indicate that the loss of duplicated segments has occurred more rapidly in the lineage leading to sorghum. Alternatively, if the occurrence of duplicated loci is the result of segmental duplication without polyploidy, then one may infer that segmental duplication has proceeded more rapidly in the lineage leading to maize.

Finally, comparative mapping studies can have utility outside of evolutionary biology. A practical aspect of this study is evident for maize molecular genetics. Some chromosome walking strategies in maize may be more efficiently executed in sorghum, given the conservation of gene order, fewer duplicated loci and smaller size of the sorghum genome.

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