Published May, 1991

Diversity and Relationships among U.S. Maize Inbreds Revealed by Restriction Fragment Length Polymorphisms

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ABSTRACT

Restriction fragment length polymorphisms (RFLPs) have been proposed as molecular markers for characterizing the genetic diversity in maize (*Zea mays* L.). The objectives of this study were to evaluate the usefulness of RFLP data for (i) elucidating heterotic patterns among maize inbreds and (ii) assessing genetic similarity among related and unrelated lines. Thirty-two maize inbreds from the U.S. Corn Belt were analyzed for RFLPs with two restriction enzymes and 83 DNA probes distributed over the maize genome. Eighty-two probes detected polymorphisms with at least one enzyme. On average, 4.3 variants were found per probe-enzyme combination across all 32 inbreds. Genetic distances among lines, estimated from RFLP data as Rogers' distance (RD), revealed considerable diversity among lines from Iowa Stiff Stalk Synthetic (BSSS), Reid Yellow Dent (RYD), and Lancaster Sure Crop (LSC). Lines from different heterotic groups had a slightly greater RD mean than unrelated lines from the same heterotic group, yet differences were small when compared with the wide range of RDs for individual lines combinations within each group. RDs between related lines agreed well with expectations based on coancestry coefficients determined from pedigree data with few exceptions. Principal component analyses of RFLP data resulted in a separate grouping of lines from BSSS/RYD and LSC. Dispersion of lines of miscellaneous origins was generally consistent with expectations based on known breeding behavior and pedigrees. Results from this study suggest that RFLP data can be used for assigning inbreds into heterotic groups and quantifying genetic similarity between related lines, but it seems that a large number of probe-enzyme combinations are required to obtain reliable estimates of genetic distance.

INFORMATION about germplasm diversity and relationships among elite breeding materials is fundamental in the improvement of agricultural plants (Hallauer and Miranda, 1988). In hybrid breeding of maize, knowledge of genetic relationships among inbreds is useful in planning crosses for hybrid and line development, assigning lines to heterotic groups, and identification of inbreds for plant variety protection. Assessment of genetic similarity (or distance) between lines, populations, or races may be based on analysis of pedigree and heterosis data, morphological traits, or molecular markers such as isozymes, and more recently, RFLPs.

The coancestry coefficient (Malecot, 1948) between genotypes has been widely used to estimate levels of genetic diversity as well as genetic relationships between cultivars in autogamous crops such as oat (*Avena sativa* L.: Rodgers et al., 1983; Souza and Sorrells, 1989), wheat (*Triticum aestivum* L.: Cox et al., 1985b; Murphy et al., 1986), and soybean [*Glycine max* (L.) Merr.: Cox et al., 1985a]. For allogamous crops, including maize, coancestry coefficients have not been as easily determined because pedigree data of lines are often unobtainable or unreliable, especially when selections were made from broad-based populations. Moreover, estimates of relationship based on the coancestry coefficient might be inaccurate because of inadequate simplifications in the underlying model that assumes equal parental contributions and no selection.

Morphological data traditionally have been used in plant variety protection and registration for description of identity and distinctness of cultivars and inbreds under the guidelines of the Union de Protection Obtention Végétale (UPOV, 1980); however, morphological characters often do not reliably portray genetic relationships because of environmental interactions and the largely unknown genetic control of these traits (Smith and Smith, 1989a). In addition, classification of maize breeding materials into heterotic groups based on endosperm types (e.g., flint vs. dent) has been recognized as inadequate, because some endosperm types differ only by one gene (Coe et al., 1988).

Biochemical data obtained by separating proteins by using electrophoresis or reversed-phase high-performance liquid chromatography provide superior descriptors of the genotype because they are not significantly affected by environment and their genetic bases are generally well understood (see Stuber et al., 1988; Smith and Smith, 1987). Allozymes have been used extensively in maize to characterize genetic variation among elite inbred lines (Stuber and Goodman, 1983; Smith et al., 1985a,b), commercial hybrids (Smith, 1984, 1988), open-pollinated and exotic populations (Kahler et al., 1986; Smith, 1986), and germplasm collections (Goodman and Stuber, 1983; Doebley et al., 1983). Isozyme data for 21 loci from 72 historically important U.S. maize inbreds revealed considerable genetic diversity, especially among lines from Reid Yellow Dent and Iowa Stiff Stalk Synthetic (Smith et al., 1985a). However, associations among lines obtained from principal component analysis of isozyme data generally were incongruent with their heterotic groups. Combined use of isozymic and chromatographic data allowed unique characterization of 95% of 62 widely used U.S. maize inbreds, and only lines closely related by pedigree through backcrossing had indistinguishable profiles (Smith et al., 1987). Multivariate and cluster analysis of isozymic and chromatographic data were able to reveal subgroups

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Published in Crop Sci. 31:669–678 (1991).

Abbreviations: BSSS, Iowa Stiff Stalk Synthetic; cM, centimorgan; kb, kilobase (1000 base pairs); LSC, Lancaster Sure Crop; RD, Rogers' distance, and GRD, SRD, general and specific Rogers' distance, and MRD, modified Rogers' distance; RFLP, restriction fragment length polymorphism; RYD, Reid Yellow Dent.

of lines related by pedigree (Smith and Smith 1987, 1988), yet biochemical data provided poorer estimates of genetic relatedness among elite lines when compared with distance measures based on pedigree and heterosis data (Smith and Smith, 1989b). Although biochemical genetic markers provided new insights into genetic diversity and associations among elite maize germplasm, their usefulness for certain breeding problems is limited by the insufficient sampling of the genome due to (i) the small number of marker loci available and (ii) the reduced number of polymorphic loci generally found in elite breeding materials with a narrow genetic base.

Restriction fragment length polymorphisms have the potential to overcome limitations associated with morphological traits and biochemical markers (Tanksley, 1983). The major advantage provided by RFLPs in maize is the relatively large number of polymorphic loci found within breeding materials, which has made it possible to develop well-populated genetic linkage maps (Helentjaris, 1987; Burr et al., 1988; Coe et al., 1988). Use of RFLPs for measuring genetic diversity and for strain identification was first proposed by Burr et al. (1983). Results from a study by Lee et al. (1989) suggested that RFLP analysis may provide an alternative to field testing when attempting to assign maize inbreds lines to heterotic groups. Although the abundance of RFLPs in maize is well established, no reports are hitherto available concerning the genetic diversity for RFLPs in breeding materials from different heterotic groups. Herein, we (i) investigate the genetic variation for RFLPs in a sample of maize inbred lines adapted to the U.S. Corn Belt, (ii) determine the level of genetic diversity for RFLPs found within and between different heterotic groups, (iii) compare estimates of genetic similarities among related lines based on RFLP and pedigree data, and (iv) examine the usefulness of RFLPs for assigning maize inbred lines to heterotic groups.

MATERIALS AND METHODS

Maize Inbred Lines Examined

Thirty-two public maize lines of current or historic importance in the U.S. Corn Belt were analyzed (Table 1). Nine of these lines have been either selected directly or derived from lines or populations selected directly from Iowa Stiff Stalk Synthetic. Eight lines have been selected directly from or following hybridization to lines from the open-pollinated variety Lancaster Sure Crop. Four lines belonged to the Reid Yellow Dent heterotic group, and 11 lines had miscellaneous origins. The genetic background and year of release of the lines are listed in Table 1. The lines were all highly inbred and maintained by self-pollination from seed of individual ears and rogueing for off-type plants for >10 generations; phenotypic appearance in field observation plots and RFLP patterns of the inbreds (obtained with single-copy clones yielding only one or two bands per inbred) showed no evidence of remnant heterozygosity or seed-stock contamination.

RFLP Analyses

The 32 inbreds were analyzed for their respective RFLP patterns. Equal quantities of leaf tissue harvested from 10 seedlings per line were lyophilized and bulked. The techniques for plant genomic DNA isolation, separate digests with restriction enzymes *Eco*RI and *Hin*dIII, gel electrophoresis, Southern transfer, filter hybridization, and probe labeling by random-primer synthesis were as described previously (Lee et al., 1989). At every eighth lane in the gel, a set of molecular-weight markers was loaded, which was composed of lambda fragments of 2.3, 4.3, 6.7, 9.4, 13.3, and 23.1 kb obtained from single digests of lambda with restriction enzymes *Hin*dIII and *Bgl*II. The 32 lines were loaded on two different gels; lambda markers and two common inbreds (B73 and Mo17) were used for comparing banding patterns of lines on autoradiographs tracing back to different gels. A total of 83 genomic DNA clones (Table 2) were used as DNA probes from collections of mapped maize clones provided by B. Burr (Brookhaven National Laboratory, Upton, NY), T. Helentjaris (Native Plants, Inc., Salt Lake City, UT), D. Hoisington (University of Missouri, Columbia, MO), and D. Grant (Pioneer Hi-Bred International, Inc., Johnston, IA).

The DNA probes were chosen on the basis of their single-copy hybridization patterns and to provide a fairly uniform coverage of the genome with at least six probes per chromosome (Table 2). The average map distance between adjacent markers was ≈30 cM. The RFLP profiles for inbreds were recorded for each probe-enzyme combination by assigning a number to each unique band on the two autoradiographs. Each probe-enzyme combination was considered as an RFLP locus and each unique RFLP banding pattern as a distinct variant. Data were binary coded; i.e., presence or absence of a band in a line was coded by 1 and 0, respectively.

Table 1. Inbreds and their parentage and year of release†.

Line	Source/Pedigree	Year of release
Iowa Stiff Stalk Synthetic (BSSS)		
B14	BSSS C0	1953
B37	BSSS C0	1958
B38	(HO × B10) × B10	1958
B39	BSSS C0	1959
B44	BSSS C0	not released
B46	(W22 × B10) selected	1960
B73	BSSS C5	1972
B76	(CI.31A × B37)F_2 × B37 sel.	1973
B84	BSSS C7	1978
Reid Yellow Dent (RYD)		
I159	Iodent	not released
I205	Iodent	1937‡
Hy	Illinois High Yield	1937‡
Wf9	Reid Yellow Dent	1936‡
Lancaster Sure Crop (LSC)		
Oh43	Oh40B × W8	1949
A619	(A171 × Oh43) × Oh43	1961
B55	(Oh45 × W92) sel.	1963
B86	(Oh43 × B52) sel.	1979
L289	LSC	—§
L317	LSC	1937‡
Mo17	CI.187-2 × C103	1964
H99	Illinois Syn. 60C	1974
Miscellaneous		
38-11	Funk 176A	1936§
M14	(Br10 × R8) sel.	1941§
B50	(M14 × A206) × Oh4c	not released
B52	MR164	1959
B54	BSCB#1	1963
B57	Midland-125-2-1	1963
B75	BSCB#3	1975
B77	Pioneer 2-Ear Synthetic (BS11)	1974
B79	Iowa 2-Ear Synthetic (BS10)	1975
R177	Snelling Corn Borer Syn.	1960
De811	approx. 23% B14 and other sources	1984

† Henderson (1976, 1984).
‡ See Stringfield.
§ Year of release unknown, but line available before 1936.

Statistical Analyses

Statistical analyses of RFLP data were performed on a data set that represented only one enzyme per probe by selecting the restriction enzyme that yielded the greater number of variants for a given probe. Multilocus Rogers' distances were calculated for all pairs of lines according to the formula given by Rogers (1972). Because pure-breeding lines were used in this study, the RD is equal to the ratio of the number of loci for which two lines differ to the total number (83) of loci examined. Hence, a RD value of 0.0 indicates no diversity between a pair of inbreds, whereas a value of 1.0 represents maximum diversity for the RFLP loci considered. Estimates of the variance of RD estimators were obtained by the jackknife procedure (Miller, 1974), making use of the variation among RFLP loci.

Principal component analysis was performed with the binary data matrix of RFLP variant frequencies for reduction of dimensions for graphical analysis of relationships among inbreds. Calculations of principal components were based on the correlation matrix of RFLP variant frequencies, which led to a substantially better discrimination among lines than using the covariance matrix. Thus, the degree to which each allele contributes to the discrimination among lines depends inversely on the variance of its frequency in the entire set of lines. By this method, differences for variants with intermediate frequencies contribute relatively little to the discrimination among lines, whereas differences for variants with a high or low frequency receive a greater weight. All computations were performed by using appropriate procedures of SAS (SAS Institute, 1988).

Table 2. Number of DNA clones and average number of restriction fragment length polymorphism (RFLP) variants associated with each chromosome.

Chromosome	Clone designation†	Clones	Average variants per clone
		no.	
1	BNL5.62, UMC76, UMC11, UMC13, BNL12.06, BNL5.59, NPI429, UMC23, UMC107, UMC86, BNL6.32	11	4.6
2	BNL8.45, UMC53, UMC5, UMC34, UMC55, UMC98, UMC4, UMC22, UMC36	9	5.4
3	UMC32, NPI249, UMC10, UMC26, BNL5.37, UMC60, UMC2	7	2.7
4	UMC31, UMC47, UMC19, BNL7.65, UMC15, UMC52, PIO1025, UMC111, BNL8.23, BNL15.07	10	4.5
5	UMC72A, UMC27, BNL6.10, BNL4.36, BNL5.71, UMC54, UMC104, UMC35	8	3.5
6	NPI235, UMC85, BNL6.29, UMC65, UMC21, UMC46, NPI280, UMC134	8	3.9
7	BNL15.40, UMC116, UMC56, BNL14.07, UMC168, UMC80	6	4.5
8	NPI220, BNL9.11, UMC103, BNL9.08, UMC120, UMC89, UMC30, NPI268, UMC7	9	4.7
9	UMC109, PIO105, UMC81, BNL5.04, UMC114, NPI209, BNL5.09	7	4.0
10	BNL3.04, NPI105, UMC64, PIO1033, NPI232, BNL10.13, UMC57, BNL7.49	8	4.6
Total		83	4.2

† Clone designations beginning with letters BNL, NPI, UMC, and PIO refer to restriction fragment length polymorphism linkage maps of Burr et al. (1988), Helentjaris (1987), Coe et al. (1988), and Pioneer Hi-Bred International, Inc. (D. Grant, 1986, personal communication), respectively.

Genealogical Relationships

Pedigrees of lines are given in Table 1. The coancestry coefficient, f (Malecot, 1948), was used to quantify the degree of relatedness of inbred lines used in this study. The coancestry coefficient (= coefficient of kinship or parentage) is the probability that two homologous genes drawn at random, one from each of two individuals, will be identical by descent. Calculations of f between related lines were performed according to the rules described in Falconer (1981), by using the following assumptions: (i) All lines, including parental and ancestral lines, are homozygous and homogeneous; (ii) lines without known common parentage are unrelated to each other ($f = 0$); and (iii) a line derived from a cross obtained half of its genes from each parent.

The coancestry coefficient f of two inbreds derived from Cycle 0 of BSSS was $f = 1/16$, because the original BSSS population had been synthesized by crossing 16 unrelated homozygous lines (Sprague, 1946). The f values for B73 and B84, which originated from the fifth and seventh cycle, respectively, of a recurrent selection program with BSSS, were derived by using equations [3.11] and [3.12] in Falconer (1981) and by assuming an effective population size (N_e) of 10.5, which corresponds to the recombination of 10 S_1 lines in each cycle, with a mating scheme avoiding selfing (Eberhart et al., 1973).

Relationships between RFLP-Based and Genealogical Distance Measures

To evaluate the usefulness of RFLP data for investigating relationships among inbreds, the relationship between genetic distance, measured by the RD calculated from RFLP data and the traditional measure of relatedness, obtained by the coancestry coefficient, was derived. By applying Malecot's (1948) concept of alleles *alike in state* (ais) and *identical by descent* (ibd) (for definitions, see Falconer, 1981) to RFLP variants, it can be shown that an estimate of the Rogers' distance between two related homozygous inbreds i and j can be obtained as

$$\hat{RD}_{ij} = (1 - f_{ij})\,\overline{RD} \qquad [1]$$

where f_{ij} is the coancestry coefficient of i and j, and $\overline{RD}$ is the average Rogers' distance between unrelated homozygous lines from the same heterotic group(s) as i and j. Equation [1] allows adjustment for possible differences in the average heterozygosity level of RFLP variants within different heterotic groups and can be applied without knowledge of the RFLP genotype of any direct progenitors of i and j.

A better estimate can be obtained if the RFLP genotype of a complete set of progenitor lines is known. For example, if the RFLP genotype of Line j as well as that of parental Inbreds k and l of i are known, a more accurate estimate of RD_{ij} is given by

$$\hat{RD}_{ij} = (RD_{kj} + RD_{lj}) / 2 \qquad [2]$$

Applying this equation to the case that Inbred i was derived from the hybridization of Inbreds j and k with known RFLP genotypes, we obtain

$$\hat{RD}_{ij} = \hat{RD}_{ik} = RD_{jk} / 2 \qquad [3]$$

Equations [1] to [3] were derived under the following assumptions: (i) each parent contributes equally to the genetic composition of a line, and (ii) selection is absent through all generations of inbreeding.

RESULTS

Genetic Variation for RFLPs

All but one of the 83 DNA probes used in this study revealed polymorphisms among the 32 inbreds with at least one of the two restriction enzymes assayed. Altogether, 357 RFLP variants were observed among the 83 DNA probes employed, considering in all instances only the enzyme yielding the greater number of variants. Most DNA probes detected between two and five RFLP variants across all 32 lines (Fig. 1). The maximum number of RFLP variants detected by a single probe was 10. There was also considerable variation in the degree of polymorphism among the 10 chromosomes (Table 2); Chromosome 2 was about twice as polymorphic as Chromosome 3.

Fifty-five (15%) of the 357 variants occurred only in one of the 32 inbreds. More than 60% of all variants had a frequency below 0.2, and <14% of all variants had a frequency >0.5. The eight LSC lines were slightly more polymorphic (249 variants) than the nine BSSS lines (237 variants); both groups of lines had 170 variants in common.

Genetic Distances among Unrelated Inbreds

Figure 2 shows histograms for RDs between unrelated lines from BSSS, RYD, and LSC. Two lines were considered unrelated by pedigree, unless their coancestry coefficient f was >0.0625 (related pairs of lines are listed in Table 3). The RD values reflect the proportion of the 83 RFLP loci for which different variants are found between two inbreds. RDs for line combinations of type BSSS × BSSS and LSC × LSC ranged from 0.40 to 0.67 and 0.43 to 0.67, respectively (averaging 0.54 and 0.57, respectively). The RDs for BSSS × LSC type line combinations had a similar range (0.48–0.74) and a slightly higher mean (0.60). The RDs for line combinations of the type RYD × BSSS and RYD × LSC both had a narrower range (0.51–0.66 and 0.52–0.70, respectively) and averaged 0.58 and 0.60, respectively.

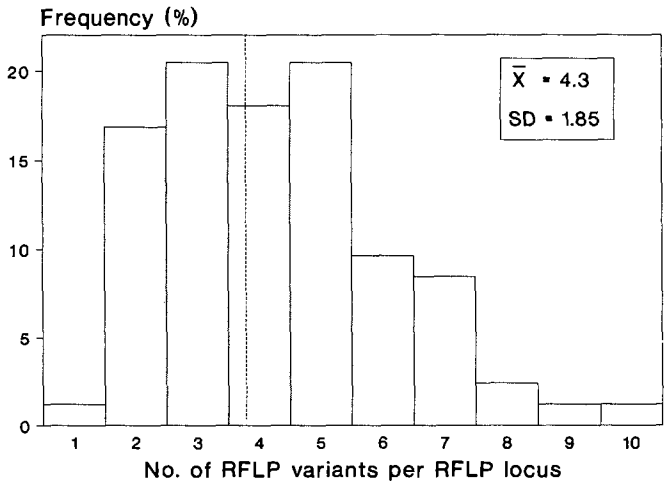

Fig. 1. Histogram of number of restriction fragment length polymorphism (RFLP) variants detected per RFLP locus with 83 DNA probes in 32 maize inbreds from the U.S. Corn Belt. For all DNA probes, only the maximum number of variants detected with restriction enzyme *Eco*RI or *Hin*dIII was considered.

Rogers' distance for individual line combinations of the type BSSS × LSC and RYD × LSC are presented in Table 4. Lines from BSSS and LSC differed substantially in their mean RD to lines from the other heterotic group. Mo17 and B86 were the LSC lines with greatest (0.65) and smallest (0.56) mean RD to the BSSS lines, respectively. The high mean RD of Mo17 to the BSSS lines was unexpected, because one of its parents, CI.187-2, is also a progenitor of BSSS

Table 3. Coancestry coefficient (f) for pairs of related inbreds, their estimated Rogers' distance (RD) calculated from Eq. [1] and [3], and their Rogers' distance (RD) determined from restriction fragment length polymorphism data of 83 DNA probes.

Inbred i	Inbred j	f_{ij}	$\hat{RD}_{ij}$†	RD_{ij}	SE(RD)‡
BSSS × BSSS					
B38	B46	0.375	0.34	0.44	0.05
B37	B76	0.750	0.14	0.14	0.04
B73	B84	0.265	0.40	0.28	0.05
LSC × LSC					
Oh43	A619	0.750	0.14	0.22	0.04
Oh43	B55	0.250	0.43	0.41	0.05
Oh43	B86	0.500	0.26§	0.24	0.05
A619	B55	0.375	0.36	0.41	0.05
A619	B86	0.375	0.36	0.37	0.05
B55	B86	0.125	0.50	0.45	0.05
Others					
M14	B50	0.250	0.45	0.40	0.05
B52	B86	0.500	0.26§	0.29	0.05
De811	B14	0.230	0.46	0.41	0.05

† In calculating $\hat{RD}$ according to Eq. [1], it was assumed that $\overline{RD}$ is 0.54, 0.57, and 0.60 for the BSSS × BSSS, LSC × LSC, and other line combinations, respectively.
‡ Standard error of RD estimates, calculated by the jackknife method (Miller, 1974).
§ Based on Eq. [3] and a Rogers' distance of 0.52 between Oh43 and B52, the parents of B86.

Table 4. Rogers' distance (RD) calculated from RFLP data of 83 DNA probes for lines derived from the Iowa Stiff Stalk Synthetic (BSSS) population and the Reid Yellow Dent (RYD) heterotic group in combination with lines from the Lancaster Sure Crop (LSC) heterotic group.

Inbred	Oh43	A619	B55	B86	H99	L289	L317	Mo17	Mean†
				BSSS × LSC					
B14	0.60	0.63	0.54	0.49	0.63	0.64	0.53	0.61	0.58
B37	0.67	0.67	0.59	0.62	0.63	0.64	0.63	0.71	0.65
B38	0.56	0.60	0.52	0.59	0.57	0.62	0.53	0.61	0.57
B39	0.48	0.55	0.57	0.49	0.61	0.63	0.55	0.66	0.57
B44	0.48	0.56	0.61	0.51	0.62	0.62	0.56	0.66	0.58
B46	0.62	0.67	0.60	0.56	0.74	0.69	0.61	0.68	0.65
B73	0.64	0.67	0.63	0.56	0.56	0.61	0.59	0.60	0.61
B76	0.66	0.63	0.59	0.62	0.64	0.68	0.63	0.69	0.64
B84	0.63	0.66	0.60	0.55	0.54	0.59	0.55	0.61	0.59
Mean†	0.59	0.63	0.58	0.56	0.62	0.64	0.58	0.65	0.60
				RYD × LSC					
I159	0.67	0.64	0.62	0.55	0.61	0.62	0.61	0.62	0.62
I205	0.67	0.70	0.59	0.59	0.55	0.55	0.69	0.60	0.62
Hy	0.56	0.56	0.63	0.59	0.63	0.67	0.64	0.68	0.62
Wf9	0.55	0.60	0.55	0.54	0.53	0.61	0.57	0.52	0.56
Mean†	0.61	0.63	0.60	0.57	0.58	0.61	0.63	0.60	0.60
SE(RD)‡ = 0.05									

† Mean Rogers' distance of the respective inbred in combination with all inbreds from the other heterotic group.
‡ Standard error of RD estimates, calculated by the jackknife method (Miller, 1974).

(Sprague, 1946). Among the BSSS lines, B37 and B46 had the greatest (0.65) and B38 and B39 had the smallest (0.57) mean RD to the LSC lines. The RYD lines did not differ in their mean RD to LSC lines, except for Wf9, which had a low RD to most LSC lines, especially Mo17 and H99. Standard errors of the RD estimates in Table 4, calculated by the jackknife method (Miller, 1974), were 0.05 in all instances.

When RD values were partitioned into general (GRD) and specific (SRD) Rogers' distances according to the factorial model proposed by Melchinger et al. (1990), GRD accounted for 62 and 45% of the variation among RDs in the BSSS × LSC and RYD × LSC line combinations, respectively. This indicates that the RD of a specific combination of lines differed in many instances considerably from the value expected on the basis of the mean RD of the respective lines in combination with all lines from the other heterotic group. A noteworthy example is B73 × Mo17, which had the smallest RD of all BSSS lines with Mo17, although this cross is known for its outstanding hybrid performance and heterotic response (Lee et al., 1989; W.A. Russell, 1989, personal communication).

The mean and range for RDs of lines of miscella-

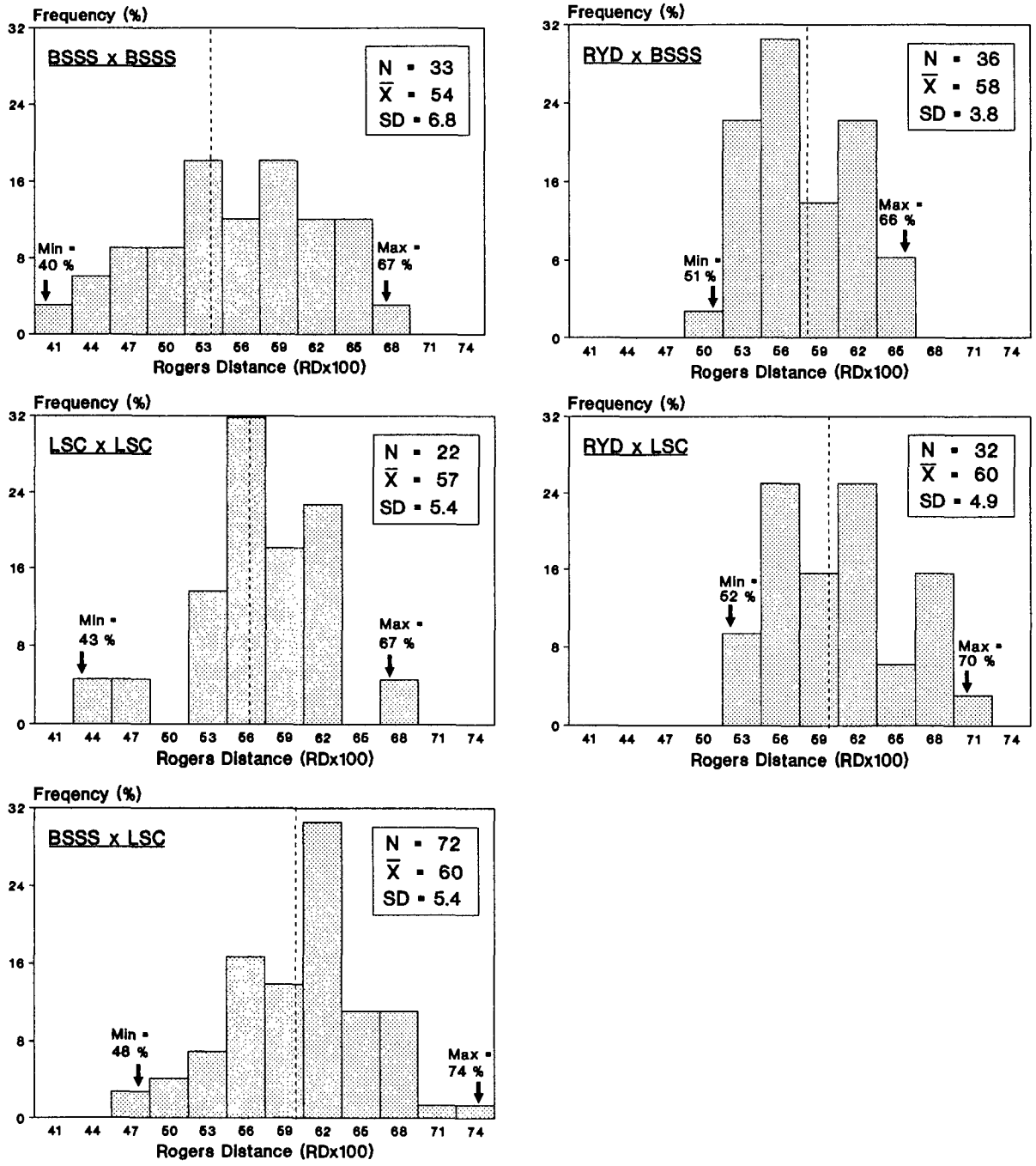

Fig. 2. Histograms of Rogers' distances (RD × 100) calculated from restriction fragment length polymorphism data of 83 DNA probes between unrelated ($f \leq 0.0625$) inbreds derived from the Iowa Stiff Stalk Synthetic (BSSS) population and the Lancaster Sure Crop (LSC) and Reid Yellow Dent (RYD) heterotic groups. *N* refers to the number of line combinations considered in each category.

Table 5. Mean, minimum, and maximum Rogers' distances (RD) calculated from restriction fragment length polymorphism data of 83 DNA probes for inbreds of miscellaneous origins in combination with nine inbreds derived from the Iowa Stiff Stalk Synthetic (BSSS) population and eight inbreds from the Lancaster Sure Crop (LSC) heterotic group.

	Rogers' distance to					
	BSSS inbreds			LSC inbreds		
Inbred	Mean	Min.	Max.	Mean	Min.	Max.
M14	0.57	0.53	0.62	0.59	0.52	0.67
B50	0.57	0.51	0.61	0.57	0.51	0.66
B52	0.59	0.53	0.64	0.55†	0.51†	0.60
B54	0.62	0.59	0.68	0.58	0.54	0.62
B57	0.63	0.54	0.70	0.65	0.60	0.70
B75	0.59	0.49	0.64	0.50	0.40	0.57
B77	0.60	0.54	0.64	0.58	0.52	0.62
B79	0.58	0.48	0.66	0.55	0.43	0.69
R177	0.65	0.59	0.70	0.62	0.55	0.68
De811	0.62‡	0.53‡	0.74	0.63	0.55	0.69

† Disregarding line B86, which was derived from B52 (Table 1).
‡ Disregarding line B14, the predominant parent of De811 (Table 1).

neous origins to the nine lines from BSSS and eight lines from LSC are given in Table 5. B57, R177, and De811 had a high mean RD to both heterotic groups, indicating that these lines and their respective parental populations represent germplasm sources unrelated to the BSSS and LSC lines. B52, B54, and B75 seemed to be more closely related to LSC germplasm than to BSSS germplasm. With the exception of B75, however, differences in the mean RD to each heterotic group were small compared with the wide range of RDs within each group, which overlapped in all instances. All other lines, including B77 and B79, showed similar mean RDs of medium size to both heterotic groups in harmony with the results reported by Lee et al. (1989).

Genetic Distances among Related Inbreds

Among the 32 maize inbreds analyzed in this study, 12 pairs of lines were related by pedigree with an estimated coancestry coefficient f ranging from 0.125 to 0.75 (Table 3). In most instances, RDs determined from the RFLP genotype of the lines were in close agreement with RD estimates calculated from Eq. [1] or [3]. Significant deviations between RD and $\hat{RD}$ (greater than twice the estimated standard error of the RD estimator obtained by the jackknife method) were found for line combinations B73 × B84, B38 × B46, and Oh43 × A619. For B73 × B84, the discrepancy was very likely attributable to an underestimation of f. Both lines originated from advanced cycles of a recurrent selection program, but selection, which is known to cause a further reduction in the actual effective population size (Harris et al., 1984), was ignored in calculating f for lack of suitable treatment. Rank correlations of RD with $\hat{RD}$ and $1 - f$ were almost identical (r_s=0.71 and 0.74, respectively) and significantly ($P < 0.01$) greater than zero.

Principal Component Analyses of RFLP Data

Figure 3 presents the results from principal component analysis of RFLP data for all 32 inbreds. The

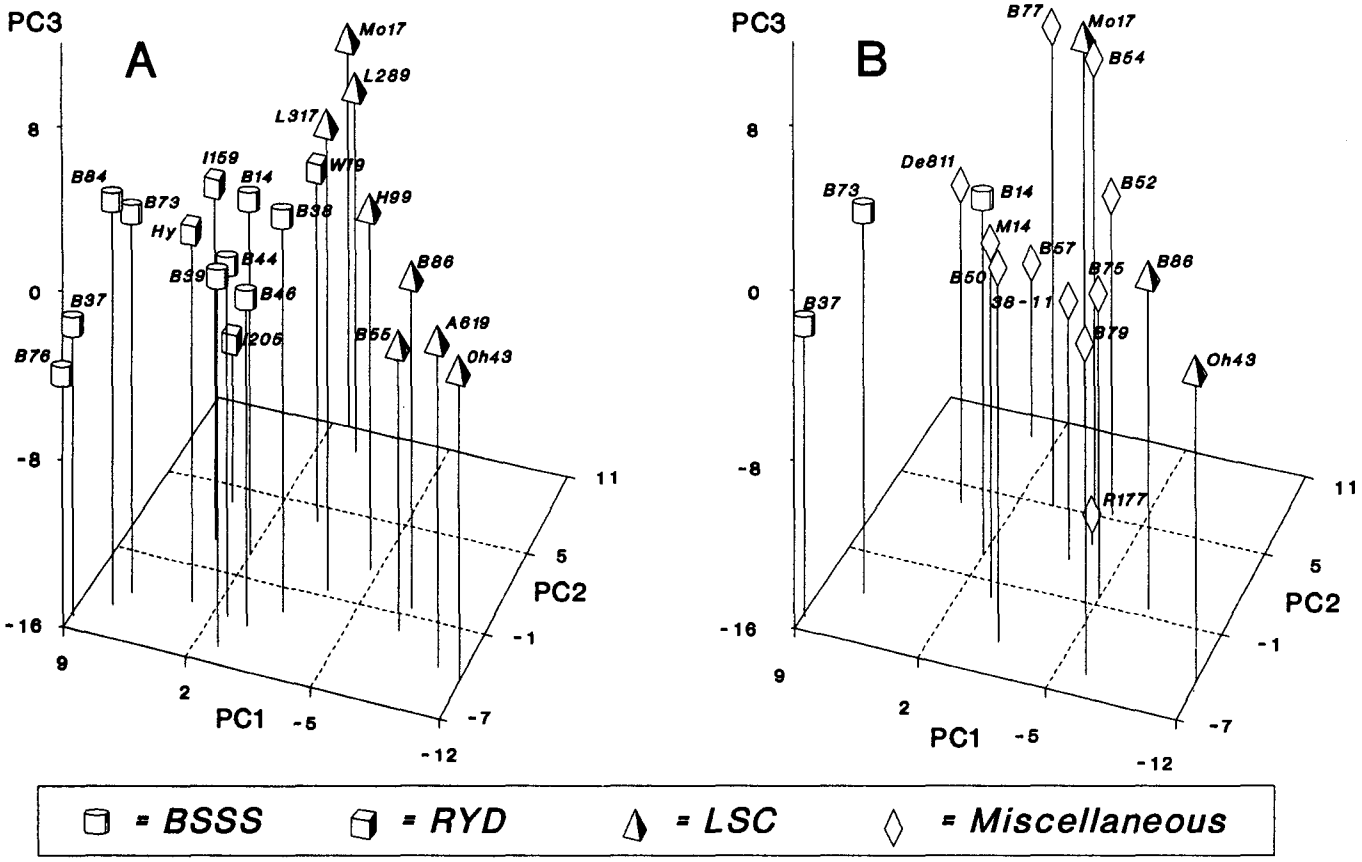

Fig. 3. Associations between lines on the basis of the first three principal coordinates (PC1, PC2, PC3) from multivariate analysis of restriction fragment length polymorphism data: (A) Inbreds from BSSS, RYD, and LSC; (B) Inbreds of miscellaneous origins together with some reference lines included in (A).

first, second, and third principal component accounted for 7.1%, 5.9%, and 5.5% of the total variation, respectively. As illustrated by the different symbols (Fig. 3A), grouping of inbreds from BSSS, RYD, and LSC was largely consistent with their known phylogenetic relationships. Lines from BSSS and LSC formed two clearly separated groups. BSSS-derived lines were more tightly grouped together, whereas LSC-derived lines were more widely spread, with Oh43 and Mo17 representing the most distant lines. This may reflect a greater degree of genetic homogeneity among the BSSS lines, possibly because most of them were directly derived from the BSSS population. In contrast, five of the eight LSC lines were recoveries from the crosses of elite lines, some of which originated from germplasm sources other than LSC. Three of the four RYD lines were positioned within or adjacent to the distribution of the BSSS lines: Hy and I159, two of the progenitor lines of BSSS, close to the center of the distribution, and I205 somewhat more remote. The remaining RYD line, Wf9, was grouped apart from the BSSS lines and grouped more closely to LSC lines L317, L289, and Mo17.

Lines of miscellaneous origins positioned outside the spread of the BSSS and LSC lines were B57, B77, B79, and R177 (Fig. 3B). B77 and B79 were widely spaced from each other and occupied positions approximately intermediate to the BSSS and LSC lines: B77 was closer to Mo17, and B79 closer to the Oh43 related lines. B52 and B75 fell within the spread of the LSC lines, the latter being adjacent to B55, one of the 16 progenitor lines of its parent population BSCB#3. B50 and its grandparental line M14 were positioned among the BSSS lines, and De811 was placed adjacent to B14, its predominant parent.

Within the group of LSC lines, Oh43 and its relatives A619, B55, and B86 formed a distinct subgroup (Fig. 3A). Also, most other pairs of related lines listed in Table 3 were tightly grouped together. An exception was B86, which occupied a position almost exactly midway between its two widely separated parental lines, B52 and Oh43 (Fig. 3B).

DISCUSSION AND CONCLUSIONS

In the research reported here, a set of 32 inbreds was used as a paradigm for examining potential applications of RFLP analyses in hybrid maize breeding for (i) assessment of genetic similarity of related and unrelated lines and (ii) assignment of inbreds of unknown genetic background to established heterotic groups. In addition, our results provided information about the genetic diversity for RFLPs in a larger set of breeding materials than hitherto reported.

Genetic Diversity among Maize Inbreds for RFLPs

Almost 99% of the DNA clones used in this study revealed RFLPs with at least one of the two restriction enzymes. The average number of RFLP variants detected per probe–enzyme combination was 4.3 in our sample of 32 lines (Fig. 1). This level of polymorphism is consistent with the amount of genetic variation reported in other RFLP studies with maize (Godshalk et al., 1990; Lee et al., 1990) and is approximately twice the amount observed in comparable isozyme studies (Stuber and Goodman, 1983; Smith et al., 1985a, 1985b).

A large proportion of RFLP variants occurred in only one or a few lines. While this could be indicative either of abundant molecular genetic diversity in the maize germplasm or instability of RFLPs, the latter can be ruled out, because RFLPs in maize have been demonstrated to be stably inherited over several generations (Evola et al., 1986).

The high mean and wide range of RDs observed for line combinations of type BSSS × BSSS and LSC × LSC (Fig. 2) indicate that unrelated lines from BSSS and LSC are diverse and vary considerably in their genetic similarity at the molecular level. Regarding the BSSS lines, this result was not unexpected, because BSSS is known to represent a genetically broad population, on the basis of its breeding history (Sprague, 1946), estimates of genetic variation for agronomic traits (Hallauer et al., 1983), and estimates of genetic diversity based on isozyme and RFLP data (Smith et al., 1985a; Lee et al., 1991). With respect to LSC, the result might be specific to the lines studied: H99 was found to be similar to Oh43 and its relatives (A619, B55, and B86), whereas Mo17 and L289 were distinct from these lines, as reflected in Fig. 3A.

The lower mean RD of the BSSS × BSSS (0.54) compared with the RYD × BSSS (0.58) line combinations could be attributable to the minor inbreeding present in BSSS. The BSSS population generally is regarded as subgroup of RYD because it was synthesized by intermating 16 unrelated (by pedigree) lines, 10 of which had RYD background (Hallauer et al., 1983). Two randomly selected inbreds derived from the original BSSS population (BSSS C0) are, therefore, expected to have RFLP variants identical by descent in one out of 16 RFLP loci, resulting in a reduced RD estimate. Actually, inserting $f = 1/16$ into Eq. [1] accounts exactly for the observed difference in the mean RD between the two groups.

The slightly greater mean RD for the BSSS × LSC and RYD × LSC line combinations in comparison with the other three types of line combinations presented in Fig. 2 is in harmony with the expectation that lines from different heterotic groups are, on average, more divergent than those originating from the same heterotic group. However, the relative increase in heterozygosity for RFLPs observed in line combinations between heterotic groups over those within heterotic groups was moderate (<8%), especially when compared with the 20 to 30% increase in heterosis for grain yield generally observed in single crosses between as opposed to within the RYD and LSC heterotic groups (A.R. Hallauer, 1989, personal communication). This corroborates recent results reported by Melchinger et al. (1990), indicating that the degree of heterozygosity at RFLP loci is not associated with heterosis for yield for crosses among unrelated lines.

A direct measure for the genetic diversity of different groups of lines is provided by the modified Rogers' distance, calculated after the formula of Goodman and Stuber (1983), which is a function of the differences in variant (or allele) frequencies. The MRD between the nine BSSS and eight LSC lines calculated

from the RFLP data was 0.38. By comparison, the MRD between the RYD and LSC open-pollinated varieties calculated from isozyme data of 13 and 18 loci published by Kahler et al. (1986) and Smith (1986) was 0.17 and 0.20, respectively. The MRD is expected to decrease with an increasing number of variants (alleles) per locus and consequently should be smaller for RFLP than for isozyme studies. Comparison of these MRD values, therefore, suggests that the BSSS and LSC lines analyzed in the present study were more genetically divergent from each other than the original RYD and LSC populations, possibly as a result of selection for testcross performance in combination with lines from the other heterotic group.

Among the lines of miscellaneous origin, RFLP analyses identified only B57, R177, and De811 as representing divergent germplasm sources from the RYD and LSC heterotic groups (Table 3). Midland, the parent population of B57, has previously been described as a distinct heterotic group, based on its heterosis in crosses with other populations including RYD and LSC (Kauffmann et al., 1982). De811 has predominantly non-RYD parents in its pedigree. All other lines of miscellaneous origins were either closely related to the BSSS or LSC heterotic pattern. For lines B75 and B79, this was not unexpected, because several progenitors used in the synthesis of their parent populations BSCB#3 and BS10, respectively, belonged to the LSC and BSSS/RYD heterotic pattern, respectively.

Assessment of Genetic Similarity among Related Inbreds

Although f and RD (or 1 − RD = similarity index) have both been used to estimate the degree of similarity between related lines, the two statistics do not measure exactly the same biological relationship and are also subject to different sources of error. The coancestry coefficient f is an indirect measure based on the pedigree of the lines and represents the expected proportion of loci with alleles identical by descent. For a given pair of lines, the actual proportion, f^*, of loci carrying alleles identical by descent may differ from f due to random drift (Cockerham and Weir, 1983), and selection during inbreeding in line development. Also, f underestimates f^* if supposedly unrelated ancestors are, in fact, related. Last, but not least, f ignores possible differences in genetic similarity between lines, which are attributable to different proportions of loci, that carry alleles alike in stale but not identical by descent. Variation in the latter proportion is likely to occur if unrelated ancestors differ greatly in their degree of similarity, as suggested by the wide range of RDs even within the same heterotic group (Fig. 2). This source of bias is expected to be of greater importance for less related lines than for closely related lines (i.e., it increases with decreasing values of f).

By comparison, the RD statistic calculated from a large number of molecular markers involves sampling of the genomes to be compared and, hence, provides a direct measure of the genetic similarity of two lines. However, RFLP-based RDs are subject to sampling effects in that they depend on the specific set of probe–enzyme combinations employed and possible errors in scoring of autoradiograms. Therefore, RDs for different line combinations are directly comparable only if they were determined with the same probe–enzyme combinations and with identical laboratory procedures.

Two advantages of RD over f are that it presupposes neither complete pedigree data nor any simplifying assumptions. Therefore, comparison of RDs determined from RFLP data with RD estimates obtained from Eq. [1] to [3] could provide information as to what extent selection, random drift, and other sources of error may invalidate similarity estimates based on f. The results presented in Table 3, especially the comparisons involving B86 and its two parents B52 and Oh43, suggest that f generally provides fairly accurate estimates of genetic similarity but that, for some line combinations, greater discrepancies do occur. Investigation of the associations between f, RD, $\hat{\text{RD}}$, and heterosis with a larger data set is recommended to examine whether RFLP-based RDs frequently provide substantially better estimates of genetic similarity so as to justify the expenditures for RFLP assays even, when f can be reliably determined from pedigree data.

In agreement with our results, Atchley et al. (1988) also found a close association between the degree of genetic similarity determined by 95 molecular-marker loci and genealogical distances determined for 10 inbred strains of mice. Cox et al. (1985a,b) described similar analyses for 43 hard red winter wheat cultivars and 115 soybean cultivars, respectively, yet with a considerably smaller number of marker loci. Both the soybean and wheat analyses had smaller levels of associations between molecular and genealogical data than those reported here and by Atchley et al. (1988). Cox et al. argued that a combination of selection and random drift was responsible for reducing the correlation between the two measures of similarity. In addition, our results concerning the precision of RD estimates suggest that the number of marker loci employed in these two studies was insufficient for obtaining reliable estimates of similarity.

Assignment of Inbred Lines to Heterotic Groups

Although heterotic groups are of great concern to maize breeders, the currently dominating heterotic groups have not been established systematically, nor are they clearly defined (Hallauer et al., 1988). Because the RYD (including BSSS) vs. LSC heterotic pattern has received greatest use in the U.S. Corn Belt, lines of miscellaneous genetic backgrounds are often classified to these heterotic groups based on pedigree information and breeders' experience. A more objective criterion for defining heterotic groups, such as genetic similarity at the molecular level, could enhance the efficiency of hybrid breeding programs.

From a theoretical point of view, the problem of assigning inbreds to heterotic groups is closely related to the problem of separation and classification addressed in discriminance analysis. As outlined in texts on this subject matter (e.g., Johnson and Wichern, 1988), the discriminatory power of a classification criterion depends on (i) the difference in the population means and (ii) the dispersion of the observations within each group. Regarding RFLP-based genetic distance measures, the first property could be improved by restricting the analysis only to probe–enzyme combinations that actually do exhibit significant differences in variant frequencies between the two heterotic

groups. The second property is a function of the genetic variation within each group and of the sampling error associated with individual RD estimates, which depends on the number of probe–enzyme combinations analyzed.

A major result of the present study with respect to these properties was that RDs for line combinations from different heterotic groups (BSSS × LSC, RYD × LSC) had only slightly higher means than those from the same heterotic groups (BSSS × BSSS, LSC × LSC), especially when compared with the wide range of RDs within each group (Fig. 2). From this, two conclusions can be drawn: (i) reliable heterotic grouping of a line of unknown heterotic pattern requires determination of its mean RD to a large number of representative lines from each heterotic group; (ii) the number of probe–enzyme combinations employed in this study seems to represent a lower limit. Considering the low level of genetic differences between the BSSS/RYD and LSC lines and also the sizeable standard error of RD ($\approx$0.05 in most cases), one probably has to employ a larger number of markers to reduce the changes of misclassification.

Despite these limitations and the small proportion (18.4%) of the total variation explained by the first three principal components, principal component analysis resulted in a clear separation of the BSSS (and most RYD) lines from the LSC lines (Fig. 3A). Moreover, lines of miscellaneous origins were mostly grouped in agreement with known breeding behavior or pedigree information (Fig. 3B): Mo17 and Oh43 were found to represent distinct germplasm within the LSC heterotic group, as described by Dudley (1984); B52, B54, B75, 38-11, and Wf9 from RYD were positioned within or adjacent to the spread of the LSC lines, which is in harmony with the assignment of these lines to the LSC heterotic groups based on breeders' experience [A.R. Hallauer, Report of the North Central Corn Breeding Res. Conf. (NCR-2 Meeting, Rosemount, IL; 24–25 Feb. 1987]. Deviating from this source of information, however, our results suggested that B57 and R177 represent germplasm sources diverse from LSC.

In conclusion, our results support the proposal of Lee et al. (1989) that RFLP-based genetic distance estimates are useful for assigning maize inbreds to established heterotic groups and investigating relationships among inbred lines. Principal component analysis of RFLP data provided a fairly accurate portrayal of associations among lines according to their origin from different heterotic groups and pedigree relationships. However, our data suggest that a large number of probe–enzyme combinations is needed to measure the genetic distance between maize inbreds with sufficient precision. Once an RFLP data base for a large number of lines has been established, it could assist the breeder in (i) partitioning breeding materials into well-defined or new heterotic groups, (ii) rapid and systematic integration of new lines of unknown genetic background into these heterotic groups, (iii) quantifying the genetic similarity between related and unrelated inbreds, and (iv) choice of divergent parents for creating new source populations in line development that have good chances of yielding transgressive segregates (Melchinger et al., 1988).

ACKNOWLEDGMENTS

We thank Iris E. Melchinger for typing the manuscript. This work was supported by grants from the Iowa Biotechnology Council and Pioneer Hi-Bred International.

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