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Genetic diversity of maize inbred lines within and among heterotic groups revealed by RFLPs

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Summary. The objectives of this study were (1) to investigate genetic diversity for RFLPs in a set of important maize inbreds commonly used in Italian breeding programs, (2) to compare genetic similarities between unrelated lines from the same and different heterotic groups, and (3) to examine the potential of RFLPs for assigning maize inbreds to heterotic groups. Forty inbreds were analyzed for RFLPs with two restriction enzymes (*Eco*RI and *Hin*dIII) and 82 DNA clones uniformly distributed over the maize genome. Seventy clone-enzyme combinations gave single-banded RFLP patterns, and 79 gave multiple-banded RFLP patterns. The average number of RFLP patterns detected per clone-enzyme combination across all inbreds was 5.8. RFLP data revealed a wide range of genetic diversity within the two heterotic groups assayed, Iowa Stiff Stalk Synthetic (BSSS) and Lancaster Sure Crop (LSC). Genetic similarity (GS) between lines was estimated from binary RFLP data according to the method of Nei and Li (1979). The mean GS for line combinations of type BSSS × LSC (0.498) was substantially smaller than for unrelated line combinations or type BSSS × BSSS (0.584) but almost as great as for unrelated line combinations of type LSC × LSC (0.506). Principal coordinate and cluster analyses based on GS values resulted in the separate groupings of lines, which is consistent with known pedigree information. A comparison between both methods for multivariate analyses of RFLP data is presented.

Key words: Maize – RFLPs – Genetic diversity – Heterotic groups

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Introduction

Accurate descriptions of the relationships among currently and historically important maize (*Zea mays* L.) inbred lines and cultivated varieties are important for their identification and for the recognition and exploitation of heterotic patterns among germ-plasm pools. To this end, several criteria, or sets of characters, have extensively been used, including morphological traits, the electrophoretic separation of isozymes (Goodman and Stuber 1983; Smith et al. 1985a, b), and storage proteins such as zeins (Nucca et al. 1979; Smith and Smith 1986) and globulins (Cross and Adams 1984).

An increasing amount of evidence suggests that restriction fragment length polymorphisms (RFLPs) reveal more polymorphisms between genotypes than protein markers (Helentjaris et al. 1986; Helentjaris 1987; Burr et al. 1983). The RFLP markers are practically unlimited in number, developmentally stable, and are mostly inherited as co-dominant Mendelian markers free of pleiotropic effects (Evola et al. 1986; Helentjaris et al. 1985). The large number of polymorphic RFLP loci in the maize genome (Burr et al. 1983; Helentjaris et al. 1985; Evola et al. 1986) has permitted rapid construction of genetic linkage maps (Helentjaris et al., 1986; Coe et al. 1988; Burr et al. 1988). Practical applications of these maps include the tagging or tracking of major genes of agricultural importance, such as those for disease resistance (Murray et al. 1988), as well as the genetic dissection of complex traits with a quantitative mode of inheritance (Stuber 1990).

RFLP analyses have considerable potential for exploring the evolutionary relationships among populations and for studying the genetic similarity of inbred lines (Burr et al. 1983). They permit, in fact, a precise estimation of genetic distances between genotypes due to

the feasibility of a complete and uniform sampling of the genome (Walton and Helentjaris, 1987). Recent studies in maize (Lee et al. 1989; Smith et al. 1990; Melchinger et al. 1991) indicate that RFLPs can be used to investigate pedigree relationships among inbreds and to assign them to heterotic groups. Moreover, Lee et al. (1989) and Smith et al. (1990) reported a close association of hybrid performance of maize single crosses to RFLP-based genetic distances between their parents. In contrast, Godshalk et al. (1990) and Melchinger et al. (1990a, b) concluded that RFLP-based genetic distances are of limited value in predicting heterotic performance between lines of different heterotic groups.

In the study presented here we (1) investigated the genetic diversity for RFLPs in a set of important maize inbreds, (2) compared genetic similarities between lines from the same and different heterotic groups, (3) considered the potential of RFLPs for assigning maize inbreds to heterotic groups, and (4) compared the grouping of lines obtained from multivariate analyses of RFLP data with expectations based on their breeding history.

Materials and methods

Plant materials

Forty inbred lines were chosen to represent diverse maize germ plasms. All these inbreds have been used extensively in the production of hybrid seed and in maize breeding programs, particularly in Italy. The inbreds and their pedigrees are given in Table 1. Pedigree information was primarily obtained from Henderson (1984), Bertolini et al. (1991) and from maize breeders working with these materials. Based on pedigree information and on heterotic behavior in crosses, 16 inbreds are included into the Iowa Stiff Stalk Synthetic (BSSS) heterotic group, 15 into the Lancaster Sure Crop (LSC), 4 (Lo932, Lo944, A12, A13) are related to line W153, 3 (A11, H55, H96) are related to line Hy (1 of the 16 progenitors of BSSS), and 2 (A9, Pa91) are related to line Wf9.

DNA preparation, restriction and genomic blot analysis

Genomic DNA was isolated from a bulk of 20–30 shoots of 7- to 9-day-old dark-germinated seedlings. DNA was purified by phenol extractions followed by equilibrium centrifugation in cesium chloride and afterwards separately restricted with fourfold excess units of restriction enzymes *Eco*RI and *Hin*dIII (Bethesda Research Laboratory) for 4 h or overnight according to the manufacturer's instructions.

Digested DNA samples (8.5 µg) were loaded onto 0.7% agarose gels, electrophoresed, and transferred to nylon membranes (Amersham, Hybond N) according to the Southern blot procedure as described by Sambrook et al. (1989). DNA was covalently bound to the membranes by UV irradiation at 302 nm in a transilluminator for 3–5 min. Molecular weight markers consisted of lambda fragments of 2.0, 2.3, 3.7, 4.4, 4.7, 6.6, 9.4, and 21.3 kb.

Eighty-two DNA clones, providing a fairly uniform coverage of the maize genome with several markers per chromosome arm (Table 2), were selected from collections of mapped maize clones (Burr et al. 1988; Coe et al. 1988) kindly provided by Dave Hoisington (University of Missouri, Columbia, M.). Recombinant plasmids were prepared according to Birnboim and Doly (1979). The inserts of genomic clones were isolated by electroelution from agarose gels and labelled to a high specific activity with the random-primer method of Feinberg ad Vogelstein (1983). Filters were prehybridized, hybridized, and washed as described by Motto et al (1988). Autoradiographs were prepared by exposing Kodak X-OMAT AR5 films with intensifying screens at −70 °C for appropriate time periods. After film development, the filters were stripped for reprobing according to manufacturer's instructions. DNA clones were hybridized with both restriction enzyme digests apart from few exceptions (Table 2). Altogether we analyzed RFLP data from 149 clone-enzyme combinations.

RFLP profiles for inbreds in autoradiographs were scored by assigning a number to each band according to its positions. Bands were considered to be different when their borders did not overlap on a gel. Data were binary coded, i.e., the presence of absence of a band in a line was coded by 1 or 0, respectively.

Statistical analyses

Genetic similarities were calculated among all possible pairs of inbreds by the measure devised by Dice (1945) and first suggested for RFLP data by Nei and Li (1979):

$$GS\ (i,j) = 2N(i,j)/[N(i) + N(j)],$$

where $GS(i,j)$ is the measure of genetic similarity between lines i and j, $N(i,j)$ is the total number of bands common to i and j, and $N(i)$ and $N(j)$ is the number of bands for lines i and j, respectively. The GS value reflects the proportion of RFLP bands that cannot be distinguished between two inbreds. A GS value of 1 indicates that two lines have identical RFLP patterns, whereas a GS value of 0 indicates that two lines have no RFLP bands in common for all clone-enzyme combinations considered.

Co-ancestry coefficients, (Malecot 1948), between lines related by pedigree were calculated according to the rules described in Falconer (1981) using the assumptions listed in detail by Melchinger et al. (1991).

Associations among inbreds were determined from principal coordinate analysis (PCOA) (Gower 1966) and from cluster analysis based on GS values. The UPGMA clustering algorithm (or „group average" or „average linkage" cluster analysis) was used for hierarchical clustering, and the necessary computations for both types of multivariate analyses were performed with appropriate subroutines of program NTSYS-pc (Rohlf 1989).

Results

RFLP profiles

Four out of the 82 DNA clones (UMC115, UMC52, UMC111, UMC117) assayed yielded monomorphic RFLP patterns across all of the inbreds for both restriction enzyme digests; 2 DNA clones (UMC26, UMC89) detected polymorphism only for one of the two restriction enzymes. Seventy out of the 149 clone-enzyme combinations gave RFLP patterns with a single band per line, whereas 79 gave multiple-banded RFLP patterns, suggesting the presence of sequence repetition in the genome (Table 2).

The average number of RFLP patterns per clone-enzyme combination was 5.8. Clone-enzyme combinations

Table 1. Inbreds considered in the RFLP analysis

Line[a]	Background[c]	Line	Background
BSSS[b] related lines		LSC[e] related lines	
B14A	(Cuzco × B14[8]) rust. res. selection[d]	C103	Lancaster Sure Crop
B37	BSSS(HT)C0	C123	(C102 × C103) selection
B68	(41.2504B × B14[3]) selection	H99	Illinois Syn. 60C
B73	BSSS(HT)C5	Lo881	Syn. C103
B84	BSSS(HT)C7	Lo924	H99[2] × Mo17
CM109	V3 × B14[2]	Lo976	Mo17[2] × LA16215
Lo950	Pioneer 3183	Mo17	CI.187-2 × C103
Lo951	Pioneer 3183	Va22	Va17 × C103[2]
Lo999	(B37 × Teosinte) × B73	Va26	Oh43 × K155
N28	Nebraska Stiff Stalk Synthetic	Va35	C103 × T8[2]
A1	50% B14	Va59	(C103 × T8[2]) × (K4 × C103[2])
A2	50% A1	Va85	Va. Long Ear Synthetic
A3	Commercial hybrid	A6	75% Oh43
A4	Commercial hybrid	A7	75% Oh43
A5	B3 recovered selection	A10	75% Oh43
A8	Commercial hybrid		
Miscellaneous origin		*Miscellaneous origin*	
H55	Hy[2] × Mo21A	A9	Wf9[2] × B14
H96	H55 × H56	A11	75% Hy
Lo932	Syn. BS5	A12	75% A385, 25% flint
Lo944	Syn. BS5	A13	75% W153, 25% flint
Pa91	[(Wf9 × Oh40B) S_4 × (Ind38-11[2] × L317) S_4]		

[a] Lines designated A1 to A13 are private property
[b] BSSS = Iowa Stiff Stalk Synthetic heterotic group
[c] Anonymous (1989), Hallauer et al. (1983), Henderson (1984), Bertolini et al. (1991)
[d] Power refers to the number of backcross generations
[e] LSC = Lancaster Sure Crop heterotic group

yielding single-banded RFLP patterns were, as expected, less powerful in distinguishing inbreds than those clones yielding multiple-banded RFLP patterns. The average number of patterns per clone-enzyme combination for the former and the latter set was 3.8 and 7.7, respectively, and the maximum number of different patterns for a given clone-enzyme combination was 7 and 25, respectively.

Genetic similarities among unrelated lines

Table 3 shows summary statistics of GS values for various groups of unrelated inbreds. Two lines were considered unrelated by pedigree when their co-ancestry coefficient was lower than 0.10. GS values across all 718 pairs of unrelated lines averaged 0.508 and ranged from 0.385 to 0.773. GS estimates for line combinations of type BSSS × BSSS and LSC × LSC ranged from 0.468 to 0.773 and from 0.426 to 0.694, respectively, and averaged 0.584 and 0.506, respectively. Line combinations of type BSSS × LSC had a similar range (0.424–0.622) and mean (0.498) of GS estimates as combinations of type LSC × LSC. Furthermore, GS values among inbreds of miscellaneous origins were of similar size, suggesting that these lines originated from unrelated germ plasm sources.

GS estimates for individual combinations of public lines of type BSSS × LSC are given in Table 4. Lines from BSSS differed considerably in their mean GS from LSC lines and vice-versa. Lo881 and Va26 were the LSC lines with the greatest (0.512) and smallest (0.467) mean GS to the BSSS lines, respectively. The large mean GS value of Lo881 was attributable to its increased GSs with two BSSS-related lines (Lo950, Lo951) developed, as Lo881, at the breeding station of Bergamo, Italy. Among BSSS lines, Lo950 and B68 had the greatest (0.515 and 0.510, respectively) and B73 had the smallest (0.460) mean GS to the LSC lines.

The mean and range of GSs for combinations of lines of miscellaneous origins with 10 public BSSS and 12 public LSC lines are shown in Table 5. H55 and H96 had considerably greater GSs to BSSS than to LSC lines; the reverse was true for Pa91. Lo932 had small mean GSs to both heterotic groups, whereas Lo944 had increased GS to the BSSS lines even thought both inbreds originated from the same population (BS5).

Multivariate analyses of RFLP data

Associations among lines revealed by PCOA based on GSs are presented in Fig. 1. Principal coordinates 1 and

Table 2. Chromosomal location of DNA clones assayed and number of clone-enzyme combinations yielding single-banded or multiple-banded RFLP patterns for each chromosome in the 40 inbreds

Chromosome	Clone designation[a]	No. of clone-enzyme combinations	
		Single banded	Multiple banded
1	BNL5.62[b], UMC115, UMC76, UMC11, UMC167, UMC119[E], UMC58, UMC23, UMC83, UMC140[H], UMC106[E], UMC84	13	8
2	UMC53, UMC5, UMC61, UMC34, UMC131, UMC55, UMC6, UMC4[E], UMC36	5	12
3	UMC32, UMC10, UMC50, UMC26, UMC60, UMC3, UMC16, UMC63, UMC111	10	8
4	UMC87, UMC31, UMC42, UMC66, UMC19, UMC15, UMC52, UMC111	6	10
5	BNL6.25, UMC90, UMC27, UMC1[H], BNL5.71, UMC54, UMC68, UMC35	5	10
6	UMC85, UMC59, UMC65[E], UMC21, UMC46, UMC38, UMC132, UMC62, UMC134	8	9
7	BNL15.40, UMC136, UMC116[E], UMC110,BNL14.07, UMC168[H], BNL8.44[E]	6	5
8	BNL13.05[E], BNL9.11, UMC103, BNL9.44, UMC89[E], UMC117, UMC30, UMC48, UMC7[H]	8	7
9	UMC109[E], UMC113, UMC81, UMC20, UMC114, BNL5.09	7	4
10	BNL3.04, UMC130[H], UMC64[E], UMC146, UMC44	2	6
Total	82	70	79

[E] Clone only used in combination with *Eco*RI; [H] clone only used in combination with *Hin*dIII
[a] Clone designations according to the maize RFLP linkage maps of Coe et al. (1988)
[b] Clones were used in combination with *Eco*RI and *Hin*dIII unless stated otherwise

Table 3. Mean, minimum, maximum, and standard deviation (SD) of Dice genetic similarity coefficient (GS × 100) calculated from RFLP data of 149 clone-enzyme combinations for various groups of unrelated[a] maize inbreds

Group	N	GS × 100			
		Mean	Minimum	Maximum	SD
All unrelated lines	718	50.8	38.5	77.3	5.31
Unrelated lines from BSSS	103	58.4	46.8	77.3	6.06
Unrelated lines from LSC	68	50.6	42.6	69.4	4.43
Unrelated lines of miscellaneous origin	32	50.5	40.5	73.8	5.95
BSSS × LSC line combinations	24.0	49.8	42.4	62.2	3.58

[a] Lines were considered unrelated if their co-ancestry coefficient f was less than 0.10

2 encompassed only 10.7% and 8.1% of the total variation, respectively. Despite this, the first two principal coordinates grouped the lines in accordance with their genetic background. Lines from BSSS and LSC formed two clear clusters, particularly with respect to principal coordinate 1. Each clusters was however, spread in the dimension of the principal coordinate 2. Within the BSSS lines, inbreds related to B14 (B14A, B68, A1, CM109) and B73 and its relatives (A3, A4, A8, Lo950, Lo951) formed two distinct subgroups. B37, its derivate Lo999, and B84 occupied a position midway between subgroups B14 andB73. Within LSC lines, loose subgroupings were apparent for lines related to Mo17 and its parent C103 (Lo976, Lo881, C123), Oh43-related lines (A6, A7, A10, Va26, H99), and C103-related lines (Va22, Va35, Va59, Va85) developed at the Virginia Polytechnic Institute (VPI). Lo924 mapped adjacent to Mo17 but rather away from its recurrent backcross parent H99. Inbreds of miscellaneous origin fell within or adjacent to the subgroup of Oh43-related lines and close to the B14-related lines.

The dendogram obtained from average linkage cluster analysis (UPGMA) of RFLP-based GSs among the 40 inbreds is presented in Fig. 2. With few exceptions this analysis revealed similar associations among lines as

Table 4. Dice genetic similarity coefficient (GS × 100) calculated from RFLP data of 149 clone-enzyme combinations for line combinations between public maize inbreds from the Iowa Stiff Stalk Synthetic (BSSS) and the Lancaster Sure Crop (LSC) heterotic groups

Inbred	B14A	B37	B68	B73	B84	CM109	Lo950	Lo951	Lo999	N28	Mean
C103	45.9[a]	48.1	50.4	42.4	47.7	50.7	52.1	51.8	47.6	45.9	48.3
C123	47.3	50.1	50.3	47.8	51.1	49.2	55.2	56.6	49.8	45.6	50.3
H99	48.9	44.8	49.7	49.6	47.7	48.6	50.6	50.5	51.2	45.2	48.7
Lo881	50.5	50.1	54.1	47.5	51.7	50.1	54.7	55.9	48.9	48.8	51.2
Lo924	45.7	46.7	50.1	46.5	48.3	43.1	53.1	53.6	47.2	50.4	48.5
Lo976	50.0	50.7	53.0	43.7	49.0	48.9	52.8	50.1	48.5	49.6	49.6
Mo17	49.3	49.5	50.9	50.0	47.6	44.0	54.8	51.3	49.6	45.5	49.3
Va22	45.0	51.2	50.5	44.4	44.4	51.4	44.9	47.3	48.8	49.5	47.7
Va26	49.5	50.1	51.9	44.0	43.7	47.9	48.6	42.9	43.1	45.2	46.7
Va35	47.2	48.9	47.5	42.7	46.9	45.7	49.9	49.5	47.3	54.2	48.0
Va59	49.9	45.3	49.6	46.3	44.8	48.8	52.4	49.0	48.1	55.8	49.0
Va85	53.1	48.7	53.9	47.3	48.8	55.1	48.9	50.1	46.7	46.3	49.9
Mean	48.5	48.7	51.0	46.0	47.6	48.6	51.5	50.7	48.1	48.5	48.9

[a] Standard errors for individual GS estimates calculated by the jackknife method (Miller 1974) ranged between 3.9 and 4.1

Table 5. Mean minimum, and maximum of Dice genetic similarity coefficient (GS × 100) calculated from RFLP data of 149 clone-enzyme combinations for inbreds of miscellaneous origins in combination with 10 public inbreds from the Iowa Stiff Stalk Synthetic (BSSS) and 12 public inbreds from the Lancaster Sure Crop (LSC) heterotic groups

Inbred	Dice genetic similarity coefficient (GS × 100) to					
	10 BSSS inbreds			12 LSC inbreds		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
H55	51.2[a]	47.5	54.4	46.7[a]	38.6	50.7
H96	52.9	49.6	57.2	48.2	40.0	51.3
Lo932	48.1	44.8	50.4	46.5	43.1	49.6
Lo944	52.1	49.1	55.4	47.0	42.7	50.0
Pa91	48.8	41.5	54.2	53.0	50.5	60.3

[a] Standard errors for means of GS estimates calculated by the jackknife method (Miller 1974) were 1.3

PCOA. One main cluster comprised exclusively lines derived from or related to BSSS and included to subclusters of B14-related and B73-related lines. Although this is inconsistent with its pedigree records, Lo999 was joined with B37 before being merged into the B73 subcluster. N28 resulted in being the least related to the other BSSS-related lines. In contrast to PCOA, all C103-related lines were classified into a single cluster with three sub-clusters composed of lines from crosses or synthetics with C103, lines from crosses with C103 and T8 selected at VPI, and Mo17 and selections from backcrosses with Mo17, including Lo924. The Oh43-related lines formed a loose cluster that also included Pa91. H99, often classified as being related to OH43, was joined independently to this cluster and its backcross derivative Lo924.

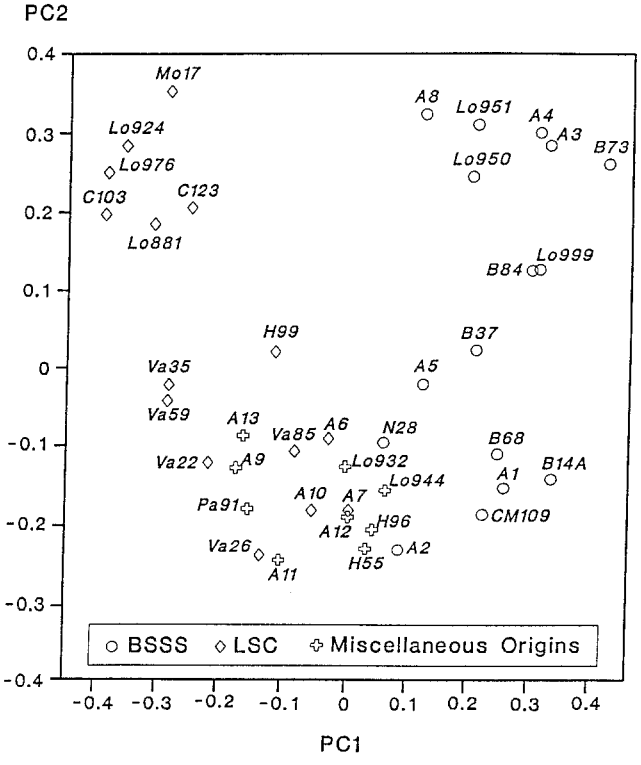

Fig. 1. Associations between lines on the basis of the first two principal coordinates (PC1, PC2) from principal coordinate analysis of Dice genetic similarity (GS) coefficients calculated from RFLP data of 149 clone-enzyme combinations for 40 maize inbreds

Discussion

One of the practical uses of RFLP technology is genetic fingerprinting. DNA fingerprints may soon play an important role in establishing line identity, plant variety

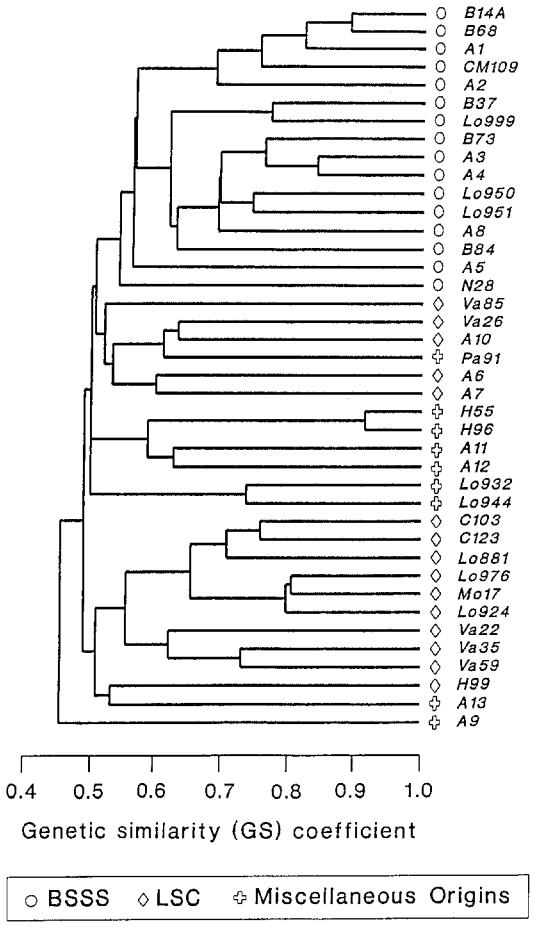

Fig. 2. Associations among lines revealed by UPGMA cluster analysis of Dice genetic similarity (GS) coefficients calculated from RFLP data of 149 clone-enzyme combinations for 40 maize inbreds

protection, and the patent protection of genes, (Smith and Smith 1989). Additional and promising applications of RFLP are marker-assisted selection for qualitative and quantitative traits (Stuber 1989), assessment of the genetic similarity of related and unrelated lines, and grouping of inbreds according to their genetic background and origin (Melchinger et al. 1991). In maize breeding, this information should be useful for sharply defining maize heterotic pools, for assigning inbreds developed from inter-pool crosses, and for choosing tester(s) when evaluating the combining ability of lines (Beckmann and Soller 1986).

Genetic variation and diversity for RFLPs

In agreement with earlier reports (Helentjaris et al. 1985; Evola et al. 1986), a high degree of polymorphism for RFLPs was present in the 40 inbreds assayed in this study. More than 95% of the DNA clones revealed polymorphic RFLP patterns with at least one of the two restriction enzymes employed. Most of the 149 clone-enzyme combinations yielded more than 4 different RFLP patterns across the 40 inbreds. Each inbred had a unique RFLP profile, and even highly related lines (such as C103 and Mo17 with a co-ancestry coefficient of $f = 0.5$) differed in their RFLP pattern in 52 clone-enzyme combinations. This confirms the high discriminatory power of RFLPs in establishing line identity for plant variety protection (Soller and Beckmann 1983).

In the present study the average number of RFLP patterns per clone-enzyme combination was greater than that found in comparable investigations with U.S. Cornbelt materials (Godshalk et al. 1990; Melchinger et al. 1991). The main reason for this discrepancy is that the latter studies employed DNA clones with single-banded RFLP patterns, whereas about half of our DNA clones yielded multiple-banded RFLP patterns. These clones are superior for line identification because they enable inbreds to be distinguished with fewer clones and hybridizations.

Molecular diversity of inbreds within and between heterotic groups

Most of the maize hybrids grown in the temperate areas are crosses between inbred lines of the BSSS and LSC heterotic groups (Hallauer et al. 1988). However, future breeding progress and reduction in the genetic vulnerability of the maize crop require that a sufficient genetic variation among elite lines within each heterotic pool is conserved. According to the average GS values presented in Table 3, unrelated lines from LSC were more diverse than those from BSSS. In particular, Oh43-related lines were relatively loosely related with C103-related lines, including Mo17, as evidenced by PCOA analysis. Together, the low means and wide ranges of GS values for combinations of the type BSSS × BSSS and LSC × LSC suggested that both groups encompass a fairly wide range of genetic diversity, a conclusion supported by previous studies from isozyme (Smith et al. 1985a, b) and RFLP analyses (Melchinger et al. 1991; Messmer et al. 1991). Additionally, it was interesting to note that, although widely spread, BSSS and LSC germ-plasms formed two clearly separated groups.

The mean GS value for combinations BSSS × LSC (0.498) was considerably smaller than for unrelated ($f < 0.10$) line combinations of type BSSS × BSSS (0.584). This is consistent with recently published data (Melchinger et al. 1991) and with the expectation that lines originating from different heterotic groups are, on average, more divergent than those from the same heterotic group. In contrast to this expectation, unrelated line combinations of type LSC × LSC had an almost identical mean GS (0.506) as those of type BSSS × LSC, mainly because of the low GS values among Oh43- and C103-related lines. Thus, the latter result might be atypical for a genetically broader sample of lines from LSC.

Our RFLP analyses identified Lo932 and Lo944 as being diverse from the BSSS and LSC lines. This is consistent with the high general combining ability of both lines toward BSSS materials (Bertolini et al. 1991) and with known pedigree information about their parent population BS5. This population was synthesized from 23 early flint and dent inbreds (Eberhart et al. 1972) and is a germ plasm source clearly divergent from BSSS and LSC.

Assignment of maize inbreds to heterotic groups

According to Hallauer et al. (1988), the currently dominating heterotic groups are neither the result of systematic breeding efforts nor are they clearly defined. LSC may serve as a paradigm. Because a single closed reference breeding population does not exist, the LSC heterotic pool consists of an unsettled collection of prominent inbreds, such as Oh43, C103, and Mo17, developed from different LSC strains. Mo17, for example, was developed from the cross CI.187-2 × C103, the former line originating from Krug and the latter from a strain of LSC (Stringfield 1959). For this reason, Smith et al. (1985a) hesitate to assign Mo17 to the LSC heterotic group, although in crosses with lines from BSSS or Reid Yellow Dent (RYD) it behaves like a "typical" LSC line. Our RFLP assays revealed that there is a high similarity of Mo17 with its parent C103 (GS = 0.653) at the molecular level, as also shown by the adjacent placement of both lines in PCOA. This supports the breeders' experience of classifying Mo17 as a LSC line. In contrast to LSC, the BSSS heterotic pool can be sharply defined because of the well-documented synthesis and selection procedures of the BSSS population (Hallauer et al. 1983).

In practical breeding programs new lines are often developed from commercial hybrids, i.e., from crosses between heterotic pools. Under such circumstances two questions arise. Which established heterotic groups should these lines be assigned to? Which unrelated tester(s) should be used in testing the combining ability of these lines? In the present study, Lo950 and Lo951 represent two examples. On the basis of available information about their breeding behaviour, they were assigned to the BSSS heterotic group. Both lines have relatively large GS with B73 (0.694 and 0.711, respectively), but only moderate GS with all LSC lines. Thus, the RFLP data support the inclusion of Lo950 and Lo951 into the BSSS heterotic pool and suggest the use of LSC lines for identifying high-yielding crosses.

The similarity of H55 and H96 to the BSSS lines, particularly the B14 – related lines, is consistent with known pedigree information. Both inbreds have Hy, 1 of the 16 progenitors of the BSSS population (Hallauer et al. 1983) as the predominant ancestor. Moreover, recent RFLP assays revealed a high molecular similarity between B14 and Hy (Melchinger et al. 1991).

Pa91 was developed from a cross between RYD and LSC germ plasm but showed high GS values with Oh43-related lines, as supported by the results of principal coordinate and cluster analyses. This suggests that Pa91 inherited a larger proportion of its genome from Oh40B, one of the parents of Oh43, than expected on the basis of its pedigree.

Comparison of methods for multivariate analyses of RFLP data

PCOA and cluster analyses each resulted in a grouping of lines largely consistent with previous classifications of lines into heterotic groups based on their breeding history and/or breeding behavior. Both methods start out from GS values among lines but use different procedures for the graphical representation of relationships among taxonomic units. Cluster analysis is known for its faithful representation of GS among similar taxonomic units (Sneath and Sokal 1973). In fact, we found lines closely related by pedigree to be tightly clustered together in most cases (B14A–B68; B73–A3–A4; H55–H96; Lo976–Mo17). An exception was Lo924, in which case the pedigree ($H99^2 \times Mo17$) suggests a closer relationship with H99 than with Mo17. However, selection during line development of Lo924 stressed the recovery of agronomic traits typical for Mo17, suggesting that a greater proportion of the Mo17 genome was retained than expected from the pedigree.

PCOA provides a two-or three-dimensional graph with the objective of giving a presentation of the pairwise similarity among all lines. Thus, the distance among subgroups of lines from different main clusters can be estimated, and this often reveals unexpected relationships among breeding materials such as the positioning of B37 and B84 midway between the B14- and B73-related lines. In some instances, however, results from PCOA-RFLP data can be misleading because only a small proportion of the total variation is generally explained by the first two or three principal coordinates. Examples in the present study are Lo932 and Lo944, which were positioned adjacent to N28, A7, and A6, although their GS with these lines is fairly small. When the third and fourth principal coordinates are considered, this problem is reduced, but not solved. Based on our data experience, we recommend using cluster and principal coordinate analyses as complementary rather than competitive tools for extracting a maximum of information from RFLP data.

In conclusion, the results presented here indicate that RFLPs are useful in assigning maize inbreds to heterotic groups and in assessing pedigree relationships among inbred lines. The assignment of maize inbreds to heterot-

ic groups before field testing may allow the breeder to curtail costs by avoiding crosses within groups. Moreover, it should be possible to select divergent parents for establishing new source populations for line development that have good chances of yielding transgressive segregants.

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