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Genetic diversity for restriction fragment length polymorphisms and heterosis for two diallel sets of maize inbreds *

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Summary. Changes that may have occurred over the past 50 years of hybrid breeding in maize (*Zea maize* L.) with respect to heterosis for yield and heterozygosity at the molecular level are of interest to both maize breeders and quantitative geneticists. The objectives of this study were twofold: The first, to compare two diallels produced from six older maize inbreds released in the 1950's and earlier and six newer inbreds released during the 1970's with respect to (a) genetic variation for restriction fragment length polymorphisms (RFLPs) and (b) the size of heterosis and epistatic effects, and the second, to evaluate the usefulness of RFLP-based genetic distance measures in predicting heterosis and performance of single-cross hybrids. Five generations (parents, F_1, F_2, and backcrosses) from the 15 crosses in each diallel were evaluated for grain yield and yield components in four Iowa environments. Genetic effects were estimated from generation means by ordinary diallel analyses and by the Eberhart-Gardner model. Newer lines showed significantly greater yield for inbred generations than did older lines but smaller heterosis estimates. In most cases, estimates of additive × additive epistatic effects for yield and yield components were significantly positive for both groups of lines. RFLP analyses of inbred lines included two restriction enzymes and 82 genomic DNA clones distributed over the maize genome. Eighty-one clones revealed polymorphisms with at least one enzyme. In each set, about three different RFLP variants were typically found per RFLP locus. Genetic distances between inbred lines were estimated from RFLP data as Rogers' distance (RD), which was subdivided into general (GRD) and specific (SRD) Rogers' distances within each diallel. The mean and range of RDs were similar for the older and newer lines, suggesting that the level of heterozygosity at the molecular level had not changed. GRD explained about 50% of the variation among RD values in both sets. Cluster analyses, based on modified Rogers' distances, revealed associations among lines that were generally consistent with expectations based on known pedigree and on previous research. Correlations of RD and SRD with F_1 performance, specific combining ability, and heterosis for yield and yield components, were generally positive, but too small to be of predictive value. In agreement with previous studies, our results suggest that RFLPs can be used to investigate relationships among maize inbreds, but that they are of limited usefulness for predicting the heterotic performance of single crosses between unrelated lines.

Key words: RFLPs – Heterosis – Epistasis – Genetic distances – Prediction – *Zea mays* L.

Introduction

Heterosis is the foundation of hybrid breeding in maize, but little is known about its genetic basis. Experimental data accumulated since the early work of East (1908) and Shull (1909) suggest that heterosis for yield and other heterotic traits is a function of heterozygosity at a large number of loci. Enhancing the number of heterozygous loci by crossing less-related lines or populations generally increases the level of heterosis observed in crosses, at

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*** To whom correspondence should be addressed least over a wide range of genetic diversity (Moll et al. 1965). Conversely, reducing the initial level of heterozygosity by inbreeding usually results in decreased vigor and performance, effects known as inbreeding depression (for literature review, see Hallauer and Miranda 1988). Based on the hypothesis of a close association between heterosis and the number of heterozygous loci affecting a trait, Hallauer et al. (1988) suggested that heterosis may become predictable by differences for molecular markers.

An important question is whether the genetic improvement of maize hybrids observed over the years was a consequence of increased heterosis and/or improved inbred parents. Duvick (1984) tested five diallels, each with ten hybrids, and their parent lines, representative of the five decades from 1930 to 1980. Estimated rates of yield improvement were higher for hybrids (0.08 Mg ha^{-1} $year^{-1}$) than for inbreds (0.05 Mg ha^{-1} $year^{-1}$), indicating that heterosis and per se performance of inbreds have increased at similar rates. Meghji et al. (1984) evaluated single crosses representative of the decades 1930, 1950, and 1970, as well as their inbred parents and F_2 generations. Increases in the grain yield of inbreds were found to be smaller than those in midparent heterosis. They concluded that the increased heterosis and inbreeding depression for grain yield of the 1970's hybrids indicated that these hybrids were heterozygous at more loci affecting yield than hybrids of the previous two eras. Lamkey and Smith (1987) evaluated six populations synthesized from inbreds representative of the decades (eras) from 1930 to 1980, and their S_1 bulk populations. They reported that the increase in yield of the S_0 and S_1 populations over eras was accompanied by an increase in inbreeding depression and that the rate of inbreeding depression had doubled from era 1 to era 6. These researchers concluded that frequencies of favorable alleles were initially below 0.5 and have been increasing and/or that more recent era populations were segregating at more loci.

The advent of restriction fragment length polymorphisms (RFLPs) has made it possible to determine herozygosity of a genotype at the DNA level for a large number of molecular markers well-distributed over the maize genome. Burr et al. (1983) first suggested the use of RFLPs in estimating genetic diversity and in selecting for increased heterozygosity. Recent studies of maize have attempted to relate multilocus heterozygosity for RFLP loci to hybrid yield. In a diallel study with eight inbreds, Lee et al. (1989) reported significant correlations of grain yield and specific combining ability with modified Rogers' distance [(MRD) Goodman and Stuber 1983] determined from 33 DNA probes and 5 restriction enzymes. In contrast, Godshalk et al. (1990) found no association between MRD, based on 47 RFLP loci, and grain yield of 47 crosses between lines from different heterotic patterns. In a study involving 67 F_1 crosses and their 20 parental lines, Melchinger et al. (1990) correlated multilocus heterozygosity at 82 RFLP loci with both hybrid performance and heterosis for grain yield. They concluded that genetic distance measures based on RFLP data are not indicative of the performance of single crosses from unrelated lines.

Table 1. Lines, their parentage, and year of release included in each set

Line	Background[a]	Heterotic[b] pattern	Year of release[c]
Set 1 (older lines)			
B14A	(Cuzco × $B14^8$) rust resist. sel.	RYD	1962[c]
B37	BSSS(HT) C0	RYD	1958
L289	Lancaster Sure Crop	LSC	1936[d]
L317	Lancaster Sure Crop	LSC	1937[e]
M14	(Br10 × R8) sel.	RYD	1941[e]
Wf9	Reid Yellow Dent	RYD	1936[e]
Set 2 (newer lines)			
B73	BSSS(HT) C5	RYD	1972
B75	BSCB#3	–	1976
B76	(CI31A × B37) F_2 × B37 sel.	RYD	1974
B77	Pioneer 2-Ear Synthetic	–	1974
B79	Iowa 2-Ear Synthetic	–	1975
B84	BSSS(HT) C7	RYD	1978

[a] Henderson (1984) unless otherwise stated
[b] RYD = Reid Yellow Dent, LSC = Lancaster Sure Crop
[c] B14 was developed from BSSS(HT) C0 and released in 1953
[d] Year of release unknown, but line available prior to 1936
[e] See Stringfield (1959)

The objectives of the present study were (1) to investigate genetic variation for RFLPs in a set of older (1950's and earlier) and a set of newer (1970's) maize inbreds; (2) to compare the level of heterosis and of epistatic effects contributing to yield and yield components of single crosses produced within these two sets of inbreds; (3) to study the relation between heterosis and heterozygosity for RFLP loci; and (4) to evaluate the usefulness of RFLP-based genetic distance measures in predicting the performance of single-cross hybrids in maize.

Materials and methods

The experimental materials used in this study were produced from two sets of inbred lines of various genetic origins (Table 1). Set 1 comprised older inbreds released for commerical use before 1960. Set 2 comprised newer inbreds released from Iowa State University during the 1970's. Within each set the 6 lines were mated in a diallel series to develop the 15 possible F_1 crosses, their selfed progenies (F_2), and their first backcrosses to both parents (BC1 and BC2).

The experimental materials (parents, F_1's, F_2's, BC1's, BC2's) were grown in 1985 and 1986 at the Iowa State University Agronomy and Agricultural Engineering Research Center and at the Atomic Energy Farm, both near Ames, Iowa. An additonal location each year was planted near Ankeny, Iowa, but data from both crops were discarded because of unseasonably poor growing conditions at this location. The 132 entries were evaluated in a modified split plot, with 5 main plots arranged in a randomized complete block design with 3 replications. Main plots were the five generations (Parents, F_1's, F_2's, BC1's, and BC2's), and subplots were the genotypes of both sets within each generation. This design was employed to avoid the competitive effects of inbreeding at different levels. Two-row plots were used for all entries except for the parental lines, for which four-row plots were used.

Each experiment was machine-planted with 26 seeds per row and later thinned to 21 plants per row. The rows were 5.5 m long and spaced at 0.76 m. Thus, the final stand was approximately 50,000 plants ha^{-1}. All rows were hand-harvested by removing all ears from 15 similarly spaced plants per row. Harvested ears were dried to a uniform moisture, and data were collected for the following ear and grain traits: total ear length of primary and secondary ears, kernel-row number of primary ears, 300-kernel weight, and grain yield $plot^{-1}$ expressed as Mg ha^{-1}. Ear data were expressed on a per-plant basis.

The analysis of variance for a given environment was performed according to the analysis for a split-plot experiment, except that entries (subplot treatments) are nested within main plots (generations). Combined analyses of variance were based on least-squares estimates of entry means in individual environments. All effects in the model were considered fixed, except replications, environments, and environment × entry interactions. Within sets, the sums of squares for entries and for environments × entries were further partitioned according to the two quantitative genetic analyses described below.

First, diallel analyses were performed with the F_1 data of each set to estimate general (GCA) and specific (SCA) combining ability effects (Method 4, Model I, Griffing 1956). Parents were considered fixed, and GCA and SCA effects were interpreted accordingly (Baker 1978). Second, the model suggested by Eberhart and Gardner (1966) was used to partition the variation among all entries in each diallel set into cumulative additive (a_i^*), average heterosis ($\bar{h}$), line heterosis (h_j), specific heterosis (s_{ij}), and additive × additive (aa_{ij}) epistatic effects. Regression sums of squares were obtained by sequentially fitting more complex models. Estimates of parameters, including line mean (μ) and heterosis ($H_{ij} = \bar{h} + h_i + h_j + s_{ij} + aa_{ij}$), were obtained from the least-squares solution of the full model.

In addition to field evaluation, the 12 parental inbreds were subject to RFLP assays. Equal quantities of leaf tissue from five seedlings per line were bulked and used for DNA isolation. Procedures for the preparation of maize genomic DNA, digestion with two restriction enzymes (*Eco*RI and *Hin*dIII), electrophoresis, Southern blotting, isolation of maize clone inserts for ^{32}P-labelling by random-hexamer priming, hybridization, and autoradiography were performed as described by Lee et al. (1989). A total of 82 genomic maize clones were chosen from collections of mapped clones (Helentjaris 1987; Hoisington 1987; Burr et al. 1988) provided by T. Helentjaris (Native Plants, Salt Lake City, Utah), D. Hoisington (University of Missouri, Columbia, Mo.), and B. Burr (Brookhaven National Laboratory, Upton, N.Y.). Clones were selected on the basis of single-copy hybridization patterns (detecting differences for one band per line) and coverage of at least three clones per chromosome arm (Table 2).

RFLP patterns on autoradiographs were scored to assign RFLP variants as described by Lee et al. (1989). Each probe-enzyme combination was considered a locus and each unique RFLP pattern a distinct variant. Data analysis was confined to a data set representing only one enzyme per probe, and the restriction enzyme was selected that provided the greatest number of RFLP variants for a given probe.

Table 2. Number of clones and RFLP variants associated with each of the ten chromosomes in the maize genome for diallel sets 1 and 2

Chromosome	No. of clones	Number of RFLP variants		
		Set 1	Set 2	Set 1+2
1	11	32	32	41
2	9	24	29	35
3	7[a]	18	19	23
4	9	26	23	32
5	8	23	18	27
6	9	29	25	35
7	7	24	18	28
8	7	21	18	27
9	7	19	17	22
10	8	24	26	34
Total	82	240	225	304

[a] One of these clones was monomorphic with both restriction enzymes

For all pairs of lines in each set, multilocus Rogers' distances (RD) were calculated 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 number of loci examined. RD values pertaining to crosses within each diallel set were partitioned into general (GRD) and specific (SRD) Rogers' distances, as proposed by Melchinger et al. (1990). Simple correlations were calculated between both RD and SRD and other quantitative genetic parameters estimated from the diallel and Eberhart-Gardner (1966) analyses.

Associations among lines in each set were determined from cluster analyses based on modified Rogers' distance (MRD) estimates calculated according to the formula of Goodman and Stuber (1983). With pure-breeding lines the MRD is simply the square root of the RD, but it has the advantage of representing a Euclidian distance measure. Ward's (1963) minimum variance method was used to obtain hierarchical clustering of lines and dendrograms, i.e., clusters merged at each step were chosen so as to minimize the increase in the total within group sums of squares. Statistical Analysis System (SAS) was employed to perform cluster analyses using the PROC CLUSTER program, subroutine WARD (SAS Institute 1988).

Results

Genetic variation and diversity for RFLPs

Eighty-one of the 82 DNA clones screened revealed RFLPs with at least one of the two restriction enzymes used. The 82 DNA clones detected 304 RFLP variants, considering in all instances the enzyme that yielded the larger number of variants (Table 2). Individual DNA clones detected up to 9 variants across all 12 lines. On average, about three RFLP variants were found per clone in set 1 and slightly fewer in set 2.

Table 3. F_1 performance, estimated specific combining ability (SCA), heterosis (H_{ij}) and additive × additive (aa_{ij}) effects estimated from generation means analyses after the Eberhart-Gardner (1966) model for grain yield, and Rogers' distance (RD) between the parents of the 15 maize crosses in diallel set 1 (older lines)

Cross	F_1	Estimates of effects			Rogers' distance (RD)[a]
		SCA	Heterosis (H_{ij})	Add × add. (aa_{ij})	
	Mg ha^{-1}				
B14A × B37	8.43	−0.01	5.79	0.98**	0.60
B14A × L289	8.43	0.15	5.62	1.38**	0.67
B14A × L317	8.18	0.05	6.00	1.53**	0.60
B14A × M14	7.35	−0.11	4.71	1.64**	0.69
B14A × Wf9	8.06	0.00	4.90	0.42	0.60
B37 × L289	8.24	0.23	5.25	0.91**	0.69
B37 × L317	7.81	−0.05	5.14	1.13**	0.67
B37 × M14	7.69	0.49	4.66	0.98**	0.59
B37 × Wf9	7.21	−0.58	3.56	0.23	0.57
L289 × L317	6.68	−0.92*	3.96	1.66**	0.63
L289 × M14	7.04	0.10	4.04	1.23**	0.65
L289 × Wf9	7.96	0.43	4.41	0.32	0.68
L317 × M14	6.92	0.13	4.45	1.33**	0.62
L317 × Wf9	8.15	0.77*	4.99	−0.58	0.57
M14 × Wf9	6.10	−0.62	2.64	1.26**	0.64
Mean	7.62	–	4.68	0.96	0.63
SD[b]	0.70	0.44	0.88	0.62	0.0417
SE	0.42	0.38	0.60	0.29	–
N[c]	15	2	15	11	–

*,** Significantly different from 0.0 at the 0.05 and 0.01 probability levels, respectively

[a] Based on a total of 82 RFLP loci

[b] Standard deviation of estimates among crosses

[c] No. of crosses out of 15 showing estimates significantly different from zero at the 0.05 probability level

Table 4. F_1 performance, estimated specific combining ability (SCA), heterosis (H_{ij}) and additive × additive (aa_{ij}) effects estimated from generation means analyses after the Eberhart-Gardner (1966) model for grain yield, and Rogers' distance (RD) between the parents of the 15 maize crosses in diallel set 2 (newer lines)

Cross	F_1	Estimates of effects			Rogers' distance (RD)[a]
		SCA	Heterosis (H_{ij})	Add × add. (aa_{ij})	
	Mg ha^{-1}				
B73 × B75	9.07	0.40	5.07	1.73**	0.68
B73 × B76	8.16	0.36	4.19	1.24**	0.47
B73 × B77	8.21	0.06	4.45	2.18**	0.57
B73 × B79	7.81	0.11	3.86	1.55**	0.63
B73 × B84	6.91	−0.93*	2.47	1.62**	0.31
B75 × B76	8.17	−0.48	4.52	0.48	0.64
B75 × B77	8.66	−0.33	5.27	0.98**	0.60
B75 × B79	8.28	−0.27	4.69	0.69**	0.59
B75 × B84	9.37	0.69*	5.30	0.12	0.67
B76 × B77	8.10	−0.02	4.62	2.05**	0.63
B76 × B79	7.59	−0.09	3.93	1.69**	0.68
B76 × B84	8.05	0.23	3.85	0.35	0.44
B77 × B79	8.29	0.27	4.66	0.60*	0.67
B77 × B84	8.19	0.03	4.34	1.71**	0.59
B79 × B84	7.70	−0.02	3.51	1.50**	0.60
Mean	8.17	–	4.32	1.24	0.58
SD[b]	0.59	0.39	0.73	0.65	0.0809
SE	0.32	0.32	0.54	0.26	–
N[c]	15	2	15	12	–

*,** Significantly different from 0.0 at the 0.05 and 0.01 probability levels, respectively

[a] Based on a total of 82 RFLP loci

[b] Standard deviation of estimates among crosses

[c] No. of crosses out of 15 showing estimates significantly different from zero at the 0.05 probability level

RDs between lines in set 1 averaged 0.63, ranging from 0.57 to 0.69 (Table 3). L289 from Lancaster Sure Crop (LSC) showed consistently high RD with the lines from the Reid Yellow Dent (RYD) heterotic group. L317 was developed from the same sampling of LSC as L289, but showed below-average RD with most RYD lines, and an average RD with L289. The RD between lines in set 2 averaged 0.58 and ranged from 0.31 to 0.68 (Table 4). The lowest RDs were found among the BSSS-derived lines (B73 × B84, B73 × B76, and B76 × B84). When RD was partitioned into GRD and SRD, GRD accounted for 44.9% and 53.6% of the variation among RD in sets 1 and 2, respectively.

Associations among lines within each set, as revealed by cluster analysis of MRDs, are presented in Fig. 1. The dendrogram of set 1 revealed no close relationships between any of the lines, as indicated by the narrow range of the semi-partial R^2 values (0.18 to 0.22) associated with the various clusters. Although lines B14A and B37 (BSSS) and lines L289 and L317 (LSC) originated from the same populations, they were not clustered in the same group initially, but were aggregated with lines from other heterotic groups. Set 2 showed a much wider range (0.10 to 0.31) of semi-partial R^2 values, and the dendrogram revealed two distinct clusters: (a) the BSSS-derived lines B73, B84, and B76, and (b) the remaining three lines of diverse origins.

Analyses of generation means for yield and yield components

Generation means for yield and yield components averaged across environments, and crosses within each set are presented in Table 5. The newer lines (set 2) showed significantly ($P < 0.01$) greater generation means than did the older lines (set 1) for all traits except ear length, which was significantly longer in older lines than in newer ones. Differences in generation means between the two sets

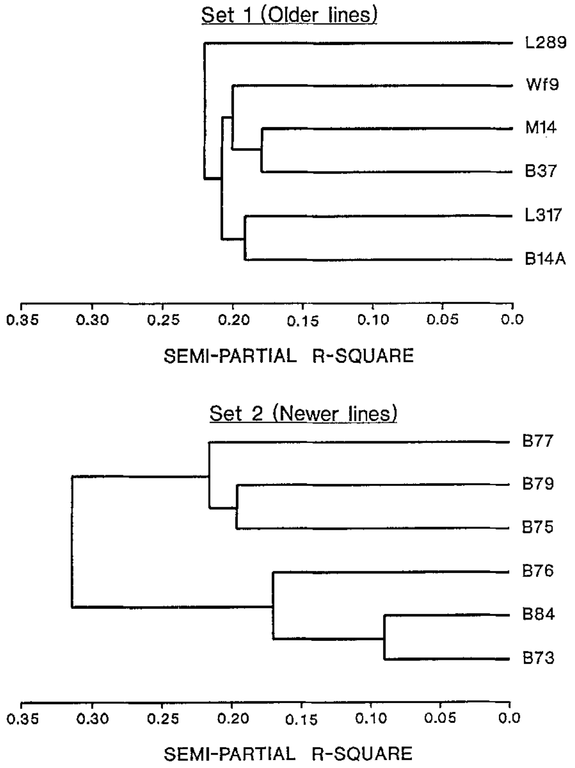

Fig. 1. Association of inbred lines in each set as revealed by cluster analysis of modified Rogers' distance (MRD) estimates calculated from RFLP data

Table 5. Generation means (±SE) averaged over the 15 crosses within each diallel set and over environments for yield and yield components

Generation	Grain yield (Mg ha^{-1})	300-Kernel weight (g)	Kernel-row number (*n*)	Ear length (cm)
Set 1 (*older lines*)				
F_1	7.62±0.11	82.3±0.9	15.47±0.07	18.02±0.12
F_1	5.67±0.08	78.0±0.5	14.74±0.06	16.00±0.12
BC^a	5.80±0.05	78.7±0.4	14.77±0.05	16.41±0.08
Parents	2.80±0.09	68.7±1.0	13.64±0.11	13.46±0.12
Midparent heterosis[b]				
Absolute	4.82±0.14	13.6±1.4	1.83±0.13	4.56±0.17
Relative (%)	172.1	19.8	13.4	33.9
Set 2 (*newer lines*)				
F_1	8.17±0.08	86.0±0.6	16.00±0.06	17.08±0.08
F_2	6.57±0.07	81.1±0.4	15.59±0.05	15.51±0.08
BC	6.51±0.05	82.3±0.3	15.66±0.05	15.20±0.05
Parents	3.76±0.11	75.9±1.2	14.64±0.10	12.62±0.09
Midparent heterosis[b]				
Absolute	4.41±0.14	10.1±1.3	1.36±0.12	4.46±0.12
Relative (%)	117.3	13.3	9.3	35.3

[a] Averaged over backcrosses to both parents (BC1 and BC2)

[b] Absolute and relative midparent heterosis were calculated as (F_1-parents) and as 100 × (F_1-parents)/parents, respectively

were consistently greater for the parental lines than for the F_1 crosses and greater for yield than for yield components. In both sets, F_2 generation means did not differ significantly ($P<0.05$) from BC means for all traits except ear length. Yields for the F_2 and BC generations were in each set significantly ($P<0.01$) greater than the mean of the F_1 and parental generations. Absolute values of midparent heterosis were smaller in set 2 than in set 1 for all traits.

The combined analysis of variance of F_1 data (data and analysis of variance not presented) indicated highly significant ($P<0.01$) GCA effects for all traits and significant ($P<0.05$) SCA effects for yield and ear length in both sets. GCA accounted for 60.4% (set 1) and 54.8% (set 2) of the variation among F_1 means for yield. Environment × GCA and environment × SCA interactions were significant for most traits in set 1 not in set 2.

The highest F_1 yields in set 1 were obtained from crosses with B14A and B37 (Table 3). Crosses with M14 were generally below average for yield. Significant estimates of positive and negative SCA were found for crosses L317 × Wf9 and L289 × L317, respectively. The highest yields in set 2 were observed for crosses with B75 (Table 4). B75 × B84 and B73 × B84 were the highest and lowest yielding crosses, respectively, and had significantly positive and negative SCA estimates, respectively.

The combined analyses of variance (not presented) after the Eberhart-Gardner (1966) model indicated that all types of genetic effects in the model, as well as deviations from the model, were significant ($P<0.01$) for most traits. Exceptions were line (a_i^*) effects for yield in both sets, specific heterosis (s_{ij}), and additive × additive (aa_{ij}) effects for 300-kernel weight in set 2, and additive × additive (aa_{ij}) effects for kernel row number in set 1. The coefficients of determination (R^2) for the nonepistatic model (μ, a_i^*, $\bar{h}$, h_i, s_{ij}) ranged between 0.88 and 0.93 (Table 6). The inclusion of aa_{ij} effects in the model increased R^2 for both yield and ear length.

Estimates of the line mean (μ) obtained from the Eberhart-Gardner (1966) analyses were greater for the newer lines (set 2) than for the older lines (set 1) for yield (29%), 300-kernel weight (9.7%), and kernel-row number (7.7%) (Table 6). Estimates of average heterosis ($\bar{h}$) were consistently greater in set 1 than in set 2. The mean ($\overline{aa}$) of the aa_{ij} effects across all crosses in each set was positive for all traits in both sets and was significant ($P<0.01$ or $P<0.05$), except for 300-kernel weight in set 2. Estimates of $\overline{aa}$ were greater in set 2 than in set 1 for all traits but 300-kernel weight.

Estimates of heterosis ($H_{ij} = \bar{h} + h_i + h_j + s_{ij} + aa_{ij}$) and aa_{ij} effects of individual crosses obtained from the Eberhart-Gardner (1966) analyses for yield are given

Table 6. Coefficients of determination (R^2) and estimates of genetic effects obtained from the combined analyses of variance of generation means in diallel sets 1 and 2 after the Eberhart-Gardner (1966) model for yield and yield components

Model/ type of effect	Grain yield	300-Kernel weight	Kernel-row number	Ear length
		Set 1 (older lines)		
$R^2(\mu, a_i^*, \bar{h}, h_i, s_{ij})$	0.93	0.89	0.90	0.88
$R^2(\mu, a_i^*, \bar{h}, h_i, s_{ij}, aa_{ij})$	0.96	0.91	0.91	0.91
		Set 2 (newer lines)		
$R^2(\mu, a_i^*, \bar{h}, h_i, s_{ij})$	0.92	0.91	0.91	0.92
$R^2(\mu, a_i^*, \bar{h}, h_i, s_{ij}, aa_{ij})$	0.96	0.92	0.93	0.96
Estimates of genetic effects[a]				
	$Mg\,ha^{-1}$	g	*n*	cm
		Set 1 (older lines)		
μ	3.03**	69.9**	13.71**	13.77**
Average heterosis ($\bar{h}$)	3.71**	9.8*	1.51**	4.26**
Epistasis ($\overline{aa}$)	0.96**	4.1**	0.32**	0.23*
		Set 2 (Newer lines)		
μ	3.92**	76.7**	14.77**	12.64**
Average heterosis ($\bar{h}$)	3.08**	9.1*	0.71*	3.03**
Epistasis ($\overline{aa}$)	1.24**	0.5	0.57**	1.37**

* $P \leq 0.05$; ** $P \leq 0.01$

μ, $\bar{h}$, and $\overline{aa}$ refer to the general mean, average heterosis, and mean of additive × additive (aa_{ij}) effects over the 15 crosses in each set, calculated by the Eberhart-Gardner (1966) model

in Tables 3 and 4. Heterosis (H_{ij}) estimates in set 1 ranged from 2.64 Mg ha^{-1} to 6.00 Mg ha^{-1} for M14 × Wf9 and B14A × L317, respectively. In set 2, H_{ij} estimates were greatest for crosses involving B75; the maximum and minimum values were 5.30 Mg ha^{-1} and 2.47 Mg ha^{-1} for B75 × B84 and B73 × B84, respectively. Estimates of aa_{ij} effects for yield were positive, with one exception (L317 × Wf9), and significant ($P<0.01$ or 0.05) in 11 and 12 of the 15 crosses in sets 1 and 2, respectively. All four nonsignificant aa_{ij} estimates in set 1 were found in crosses with Wf9.

Correlations between RFLP and yield data

Correlations of RD and SRD with F_1 performance, SCA estimates, and heterosis (H_{ij}) estimates for yield and yield components calculated across both sets of materials were generally positive and small (Table 7). Significant ($P<0.05$) correlations were found between RD and F_1 performance for ear length and H_{ij} estimates for yield and ear length, and between SRD and SCA for yield. Correlations of both RD and SRD with the various parameters were generally greater for yield than for individual yield components. SCA effects for all traits showed consistently higher correlations with SRD than with RD.

Table 7. Simple correlation of Rogers' disance (RD) and specific Rogers' distance (SRD) calculated from the RFLP data of parent lines with various parameters (Y) estimated from the analyses of generation means pooled over both diallel sets for yield and yield components

Parameter Y[a]	Grain yield	300-Kernel weight	Kernel-row number	Ear length
	r (RD, Y)			
F_1 performance	0.18	−0.05	−0.26	0.40*
SCA effects	0.26	0.09	0.08	0.22
Heterosis(H_{ij})	0.46**	0.32	0.06	0.42*
	r (SRD, Y)			
F_1 performance	0.24	0.01	0.01	0.16
SCA effects	0.40*	0.14	0.12	0.34
Heterosis (H_{ij})	0.20	0.02	0.06	0.08

* $P \leq 0.05$; ** $P \leq 0.01$

[a] Heterosis (H_{ij}) was estimated from the Eberhart-Gardner (1966) model as $H_{ij} = \bar{h} + h_i + h_j + s_{ij} + aa_{ij}$

Discussion

Almost 99% of the DNA clones employed in this study revealed RFLPs with at least one of the two restriction enzymes. The average number of RFLP variants detected per RFLP locus was 3.7 in our sample of 12 lines. This is consistent with the amount of genetic variation reported in other RFLP studies with maize (Lee et al. 1989; Lee et al. 1990; Melchinger et al. 1990).

The mean and range of RDs within the older lines (set 1) agreed with the results from four sets of Corn Belt dent inbreds reported by Melchinger et al. (1990). The smaller mean and greater range of RDs in the newer lines (set 2) were attributable to crosses B73 × B84, B73 × B76, and B76 × B84 (Table 4). The low RDs of these crosses were not unexpected because all three parental lines originated from the BSSS population. As a consequence of RFLP variants "identical by descent," related lines should have a greater proportion of RFLP variants in common than unrelated lines. Under simplified assumptions (e.g., absence of selection and unrelatedness between the progenitors of BSSS) and using formulas given by Falconer (1981), the inbreeding coefficient F (Wright 1922) of these crosses is found to be 0.265 (B73 × B84) and 0.047 (B73 × B76, B76 × B84), but F may actually be higher due to selection. When these three crosses were excluded from the analysis, there were practically no differences in genetic diversity at the molecular level between the older and newer lines.

In our materials, GRD accounted for a greater proportion of variation among RD than was previously reported by Melchinger et al. (1990). The higher coefficient of determination (R^2) of GRD in set 2 (53.6%) was surprising because relatedness among lines is expected to increase the size of SRD. However, lines B73 and B84 in set 2 had large negative GRD estimates because of low RD to all related and unrelated lines except B75.

Except for lines B14A and B37 and lines L289 and L317, the RD (Tables 3 and 4) and the cluster analyses of lines (Fig. 1) agreed with expectations based on pedigree information. In accordance with our results, cluster and principal component analyses of RFLP data in the study by Lee et al. (1990) revealed that B14A and B14, its recurrent parent, are genetically distinct from other elite lines, including B37, developed from BSSS. In addition, Lee et al. (1989) found, based on RFLP and yield data, that B77 and B79 represent germ plasm sources unrelated to BSSS lines.

Our results from molecular data support the proposal of Lee et al. (1989) that RFLP analysis provides a potential tool for the breeder to investigate relationships among maize inbreds and to assess genetic similarities among lines. Considering the deviations observed in this study and by Melchinger et al. (1990), it is questionable, however, whether inbreds of unknown origin can be unambiguously assigned to heterotic groups on the basis of RFLP data alone. To answer this question, more information is needed with respect to the genetic diversity at the molecular level within and among different heterotic groups.

Newer lines (set 2) significantly outyielded older lines (set 1) in the F_1, F_2, BC, and parental generations by 0.55 ± 0.14 Mg ha^{-1}, 0.90 ± 0.10 Mg ha^{-1}, 0.71 ± 0.07 Mg ha^{-1}, and 0.96 ± 0.14 Mg ha^{-1}, respectively (Table 5). Yield improvement was associated with increased kernel weight and more kernel rows, but was partly offset by shorter ears. If an average difference of 30 years of continued breeding efforts is assumed to exist between the development of the older and newer lines, the yearly genetic rate of gain for inbreds was 0.032 ± 0.005 Mg ha^{-1}, which is slightly smaller than the rate obtained in the study by Duvick (1984). In contrast, Meghji et al. (1984) found a considerably smaller yield increase for inbreds from the 1970's compared with those from the 1950's and 1930's. The yield increase reported for the respective F_2's, however, was similar to that reported in the current study.

The yearly genetic rate of gain for the F_1 yields was 0.019 ± 0.005 Mg ha^{-1}, which is approximately less than one-third the rates reported by previous workers using different materials (Castleberry et al. 1984; Duvick 1984; Meghji et al. 1984; Russell 1984). These authors consistently found the more recent era hybrids to show their greatest superiority under higher planting density, suggesting that the moderate plant density used in the present study was one reason for the lower rate. The F_1 yield level in set 2 was also slightly reduced by the poorer performance of the three crosses among the BSSS lines (Table 4). The results of Duvick (1984) demonstrated that evaluating genetic progress in maize over time by comparing small sets of hybrids from only two different eras can strongly deviate from the general trend, depending upon choice of parental lines. Because each set comprised six lines and set 2 included three lines from BSSS and none from the Lancaster Sure Crop heterotic pattern, our results are specific for this sample of lines and may not be representative of other materials.

The mean of heterosis (H_{ij}) effects for yield estimated from the Eberhart-Gardner (1966) analyses decreased by 0.41 ± 0.19 Mg ha^{-1} from the older to the newer lines (Tables 3 and 4). The reduction in heterosis was nonsignificant, however, when crosses among BSSS lines were excluded from the comparison. In contrast to our findings, a significant increase in midparent heterosis for yield over the past 50 years of hybrid breeding in maize was reported by Duvick (1984) and Meghji et al. (1984).

The greater yield of the inbred generations (parents, F_2, BC) in set 2 compared with that of set 1 and the reduction in heterosis indicate that the yield improvement of the newer lines was mainly attributable to the accumulation of alleles with positive additive effects for 300-kernel weight and kernel row-number. If the three crosses among the BSSS lines in set 2 are excluded, the two sets did not differ in either yield heterosis or multilocus heterozygosity for RFLPs, suggesting that single crosses of the older and newer lines had about the same number of heterozygous loci affecting yield.

In both sets, positive additive × additive (aa_{ij}) effects explained a substantial proportion of heterosis (H_{ij}) estimates for yield (Tables 3 and 4). Estimates of aa_{ij} effects for yield obtained in previous studies with maize (Eberhart and Gardner 1966; Melchinger et al. 1986, 1990) were generally smaller and predominantly negative. The negative aa_{ij} estimates in these studies were due to the higher yield of BC generations compared with the F_2's, whereas the positive aa_{ij} estimates reported here were due to a higher yield of the F_2 and BC generations compared with the mean of the F_1 and parent generations. This discrepancy is very likely the result of the good growing conditions for maize during both years of testing (1985, 1986), which favored the inbred generations more than the F_1 generation.

The low correlation between RD and F_1 yield ($r = 0.18$) and the respective plot of these variables (Fig. 2A) demonstrate that the RDs of the parent lines were of no predictive value for the yield of single crosses. The low correlation corroborates other investigations of maize that predominantly involved crosses among lines from different heterotic patterns (Godshalk et al. 1990; Melchinger et al. 1990). Lee et al. (1989) reported considereably higher correlations of MRD with F_1 yield ($r = 0.46$) and SCA effects ($r = 0.74$) for a diallel involving

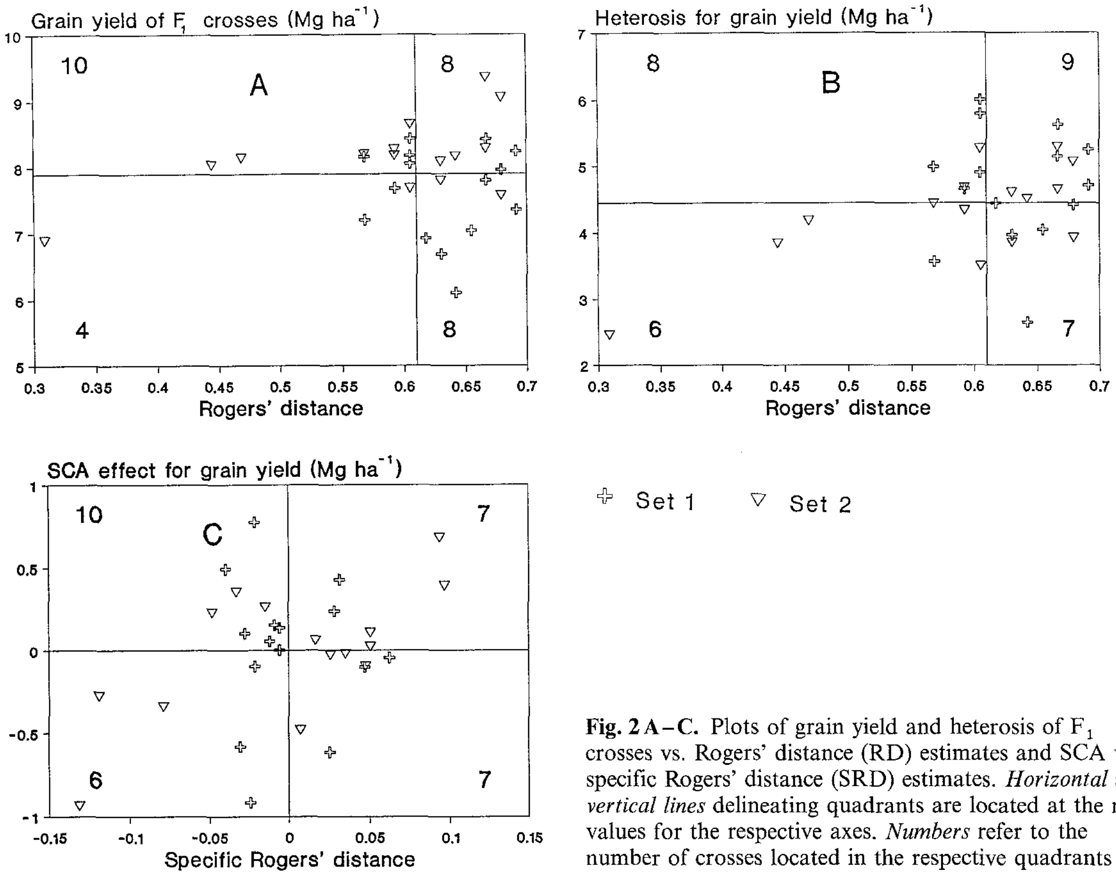

Fig. 2A–C. Plots of grain yield and heterosis of F_1 crosses vs. Rogers' distance (RD) estimates and SCA vs. specific Rogers' distance (SRD) estimates. *Horizontal* and *vertical lines* delineating quadrants are located at the mean values for the respective axes. *Numbers* refer to the number of crosses located in the respective quadrants

crosses of lines from the same and different heterotic patterns.

The correlation between RD and heterosis (H_{ij}) estimates for yield ($r=0.46^*$) was strongly influenced by the three crosses among the BSSS lines in set 2 (Fig. 2B), which combined low RD with small H_{ij} estimates (Table 4). When only crosses with a RD greater than 0.56 were considered, there was no association ($r=0.09$) between RD and H_{ij} estimates. Melchinger et al. (1990) obtained similar results and cited the following reasons for the weak association between RD and heterosis in unrelated crosses: (1) estimation of the average heterozygosity of quantitative trait loci affecting yield by means of RD determined from an arbitrarily chosen set of DNA clones may be inaccurate if (a) important quantitative trait loci (QTLs) for yield are located only in certain chromosomal regions and/or (b) no extreme gametic disequilibrium exists between QTLs and linked markers. (2) Heterosis for yield is not closely associated with heterozygosity at QTLs affecting yield when the average level of dominance differs among crosses. The first argument applies particularly to individual yield components because of the smaller number of QTLs controlling these traits as reflected in the lower correlations of RD with heterosis (H_{ij}) for yield components (Table 7). In addition, most of the correlations in Table 7 were reduced by sizeable additive × additive (aa_{ij}) effects for all traits.

In accordance with quantitative genetic expectations and experimental results presented by Melchinger et al. (1990), SCA effects for yield were more closely correlated with SRD ($r=0.46^*$) than with RD ($r=0.26$), and the same applied to all yield components (Table 7). Despite this improvement, correlations between SRD and SCA effects for all traits were too low to be useful for prediction.

In summary, our results support the conclusion of Melchinger et al. (1990) that genetic distance measures based on an arbitrarily chosen set of markers are not sufficiently associated with hybrid yield, heterosis, or SCA effects to recommend these measurements as an adjunct tool for identifying superior single-cross hybrids between unrelated lines. Instead of using a large number of RFLP markers uniformly distributed over the genome, it rather seems necessary to employ specific markers for those genomic regions actually contributing to heterosis for yield over a wide range of germ plasm. First results of dissecting the heterosis of single crosses into the contributions of individual chromosome segments were reported by Stuber et al. (1987) and Stuber (1989). Further studies are currently in progress by several research groups to investigate a greater number of crosses and to examine whether identified regions coincide in different materials.

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