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Molecular Genetic Diversity among Progenitors and Derived Elite Lines of BSSS and BSCB1 Maize Populations

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Abstract

The maize (*Zea mays* L.) populations Iowa Stiff Stalk Synthetic (BSSS) and Iowa Corn Borer Synthetic No. 1 (BSCB1) have undergone reciprocal recurrent selection (RRS) since their establishment in 1949. This study focused on molecular genetic variation of the progenitor inbred lines used to synthesize BSSS and BSCB1 as well as elite inbred lines derived from different cycles of selection. Our objectives were to investigate changes in allele frequencies and genetic diversity from progenitors to derived lines and evaluate trends in genetic diversity among elite lines derived from early and advanced selection cycles. Genotypic data for 105 restriction fragment length polymorphism (RFLP) loci were collected from four groups: 16 progenitors and 18 elite lines derived from BSSS, 12 progenitors and 7 elite lines derived from BSCB1. Each progenitor group had a broad genetic base but both were genetically similar. The groups of derived lines diverged substantially from each other. A larger Roger's distance was found between the groups of lines derived from advanced cycles than between the groups of lines derived from Cycle 0. Allelic variation within each group of lines, however, decreased just slightly with the elite lines capturing almost 75 and 67% of the allelic variation present in the progenitor lines of BSSS and BSCB1, respectively. The results of this study confirm the long-term potential of this RRS program and the importance of the choice of broadly based progenitor materials.

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Molecular Genetic Diversity among Progenitors and Derived Elite Lines of BSSS and BSCB1 Maize Populations

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ABSTRACT

The maize (*Zea mays* L.) populations Iowa Stiff Stalk Synthetic (BSSS) and Iowa Corn Borer Synthetic No. 1 (BSCB1) have undergone reciprocal recurrent selection (RRS) since their establishment in 1949. This study focused on molecular genetic variation of the progenitor inbred lines used to synthesize BSSS and BSCB1 as well as elite inbred lines derived from different cycles of selection. Our objectives were to investigate changes in allele frequencies and genetic diversity from progenitors to derived lines and evaluate trends in genetic diversity among elite lines derived from early and advanced selection cycles. Genotypic data for 105 restriction fragment length polymorphism (RFLP) loci were collected from four groups: 16 progenitors and 18 elite lines derived from BSSS, 12 progenitors and 7 elite lines derived from BSCB1. Each progenitor group had a broad genetic base but both were genetically similar. The groups of derived lines diverged substantially from each other. A larger Roger's distance was found between the groups of lines derived from advanced cycles than between the groups of lines derived from Cycle 0. Allelic variation within each group of lines, however, decreased just slightly with the elite lines capturing almost 75 and 67% of the allelic variation present in the progenitor lines of BSSS and BSCB1, respectively. The results of this study confirm the long-term potential of this RRS program and the importance of the choice of broadly based progenitor materials.

Reciprocal recurrent selection was proposed by Comstock et al. (1949) as a cyclical method for simultaneous improvement of two parent populations used for line development in a hybrid breeding program. The primary goal of RRS is to improve the cross performance between two populations while maintaining the genetic variation for further progress in subsequent selection cycles. Following this proposal, G.F. Sprague initiated a RRS program in 1949 with two synthetic maize populations, 'Iowa Stiff Stalk Synthetic' (BSSS) and 'Iowa Corn Borer Synthetic No. 1' (BSCB1). Fifteen cycles of RRS in BSSS and BSCB1 have been completed by Iowa's Cooperative Federal–State maize breeding program. Selection was for increased grain yield as first priority and reduced grain moisture at harvest as well as increased resistance to root and stalk lodging as second priority.

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Selection progress for RRS with BSSS and BSCB1 was evaluated in several studies (Penny and Eberhart, 1971; Martin and Hallauer, 1980; Smith, 1983; Keeratinijakal and Lamkey, 1993a; Schnicker and Lamkey, 1993). After 11 selection cycles, grain yield of the interpopulation cross showed an increase of 77% compared with Cycle 0 (C0), with concurrent favorable responses in the other traits (Keeratinijakal and Lamkey, 1993a). Moreover, the program has been the source of numerous elite inbred lines, which have been widely used as parents in commercial hybrids (Darrah and Zuber, 1986).

On the basis of theory, RRS is expected to accumulate the most favorable allele in each population at quantitative trait loci (QTL) displaying partial to complete dominance. In contrast, RRS should in the long run fix different alleles in the two populations at loci displaying overdominance (Comstock et al., 1949). Consequently, the genetic distance between the two populations is expected to decrease in the former case but increase in the latter case and this should be reflected at both the population level and inbred lines derived from advanced selection cycles. However, it is also possible and likely that populations diverge with partial to complete dominance, depending on the gene frequencies in the opposite population and the amount of random genetic drift (Keeratinijakal and Lamkey, 1993b; Hanson and Moll, 1986).

Until a decade ago, trends in the genetic diversity within and between populations were mostly evaluated with morphological markers or biometric analyses of quantitative variation (Melchinger, 1999). With the advent of molecular markers such as RFLPs, it is possible to examine genetic diversity directly at the level of DNA and test the above hypotheses concerning dominance or overdominance as reason for the heterotic increase. Using RFLPs, Labate et al. (1997) evaluated the genetic diversity within and between C0 and C12 of the RRS program with BSSS and BSCB1 as well as their progenitor inbred lines. While the initial populations were genetically fairly similar, they substantially diverged in C12 accompanied by a loss of genetic variation within the populations. Messmer et al. (1991) examined genetic diversity among progenitors and five elite lines derived from BSSS, focusing on a comparison of allozyme and RFLP data. Inbreds used for the synthesis of BSSS were found to be genetically diverse and genetic variation had been lost in the elite lines as a result of selection and drift during recurrent selection and line development. However, a comparison between the genetic diversity of all progenitors and a representative set of derived elite lines of both populations is lacking hitherto.

Abbreviations: BSSS, Iowa Stiff Stalk Synthetic; BSCB1, Iowa Corn Borer Synthetic No. 1; PMPH, panmictic midparent heterosis; RFLP, restriction fragment length polymorphism; RRS, reciprocal recurrent selection.

The objectives of our study were to (i) investigate changes in allele frequencies from progenitors of BSSS and BSCB1 to derived elite lines and examine especially the contribution of individual progenitors; (ii) compare the genetic diversity within and between the groups of progenitors and derived lines; and (iii) evaluate trends in genetic diversity among elite lines derived from early and advanced selection cycles.

MATERIALS AND METHODS

Maize Inbred Lines Examined

We analyzed a total of 53 maize inbred lines from the U.S. Cornbelt, consisting of four groups. Group 1, subsequently denoted as pBSSS, comprised 14 out of the 16 progenitor lines of BSSS plus both parents (Fe and IndB2) of one missing progenitor (F1B1). Regrettably, seed of F1B1 and CI617, another progenitor of BSSS, is no longer available. Group 2, denoted as pBSCB1, included all 12 progenitor lines of BSCB1. Group 3 comprised 18 elite lines derived from BSSS and they are denoted as dBSSS, but a few inbred lines (B73, B78 and B84) were derived from the half-sib recurrent selection program of BSSS (for further details see Eberhart et al., 1973). They were assumed to fit well in the group of dBSSS. Group 4 included seven elite lines derived from BSCB1; they are denoted as dBSCB1. The origin of progenitor lines and the selection history of the derived lines are described in Table 1. Seed of all inbreds was obtained from Cooperative Federal-State Breeding Program at Ames, IA. One plant from each inbred line was sampled for DNA extraction.

RFLP Analyses

RFLP analyses were performed by Biogenetic Services, Inc., (Brookings, SD, USA), following the protocols described by Ausubel (1994) with minor modifications. DNA was isolated by a CTAB (hexadecyltrimethylammonium bromide) extraction method. Gel electrophoresis was conducted with a TAE buffer. Southern blots were made with nylon membranes and the resulting complex of fragments was subject to hybridization techniques (Budowle and Baechtel, 1990). After washing, the membranes were placed on Kodak AR X-ray films and exposed for up to 14 d depending on the optimal exposure time. Data from individual probes were scored by first determining the total number of variants at a locus and then assigning each band a molecular weight on the basis of internal lane standards (λ 2.0- and 24-kb fragments). Data from 99 probes were scored. All probes were previously characterized as single copy and used in other assays. Chromosomal locations are based on published maps (Davis et al., 1996; Matz et al., 1994) and information obtained from the Maize Genome Database (http://www.agron.missouri.edu; verified 14 Oct. 2002). Presence or absence of a band was recorded for each individual. Two probes were eliminated because their banding pattern could not be interpreted unambiguously. Each retained RFLP probe was assumed to be a single locus and variants at each locus were assumed to be allelic. Eight probes were found to carry two loci and both loci were scored. Hence, altogether 105 loci were scored. When individual bands could not be scored unambiguously in a line, they were treated as missing data in the subsequent numerical analyses, but this occurred infrequently. Probes, chromosomal locations, and numbers of alleles at each locus identified in this study are shown in Table 2.

Table 1. Origin of the maize inbred lines analyzed in this study.

Line	Source/Pedigree
Progenitor lines of Iowa Stiff Stalk Synthetic (pBSSS)	
A3G-3-1-3	[BL345B × IAI129] (both strains of RYD)§
CI.187-2	Krug-Nebraska Reid strain × Iowa Gold Mine‡
CI.540	Illinois Two Ear Variety‡
I159	Iodent (a strain of RYD)§
I224	Iodent (a strain of RYD)§
Ill.12E	Source of line unknown§
Ill.Hy	Illinois High Yield§
Ind.461-3	Reid Medium (Duddelston No. 461)§
Ind.AH83	Funks 176A (a strain of RYD)§
Ind.B2	Troyer Reid (Late Butler)††
Ind.Fe2 1073	Troyer Reid (Early)††
Ind.Tr 9-1-1-6	Reid Early Dent (Troyer Strain)§
LE23	Illinois Low Ear§
Oh3167B	Echelberger Clarage†
Os420	Osterland Yellow Dent (a strain of RYD)§
WD456	Walden Dent (strain of RYD)§
Progenitor lines of Iowa Corn Borer Synthetic No. 1 (pBSCB1)	
A340	4-29 × 64
CC5	Golden Glow (renamed W23)§
Ill.Hy	Illinois High Yield
I205	Iodent
K230	From 1947, no origin listed but probably from Midland Yellow Dent§
L317	LSC
Oh07	C.I.540 × III.L
Oh33	Clarage
Oh40B	Eight line composite of LSC lines
Oh51A	[(Oh56 × Wf9) Oh56]
P8	Palin Reid
R4	Funk Yellow Dent
Elite lines derived from Iowa Stiff Stalk Synthetic (dBSSS)	
B10	BSSSC0
B11	BSSSC0
B14	BSSSC0
B14A	Cuzco × B14
B17	BSSSC0
B37	BSSSC0
B39	BSSSC0
B40	BSSSC0
B43	BSSSC0
B44	BSSSC0
B67	BSSSC0
B69	BSSSC0
B72	BSSS(HT)C3
B73	BSSS(HT)C5
B78	BSSS(HT)C8
B84	BS13(S)C0
B89	BSSS(R)C7
B94	BSSS(R)C8
Elite lines derived from Iowa Corn Borer Synthetic No. 1 (dBSCB1)	
B42	BSCB1C0
B54	BSCB1C0
B90	BSCB1(R)C7
B91	BSCB1(R)C8
B95	BSCB1(R)C7
B97	BSCB1(R)C9
B99	BSCB1(R)C10

† Baker, 1984.
‡ Henderson, 1984.
§ Hallauer, 1984.
†† Parental lines of F1B1-7-1 (progenitor line of BSSS, not available).

Genetic Data Analysis

Gene diversity, also referred to as expected heterozygosity, is the probability that two randomly chosen alleles at a locus within a population will be different (Nei, 1987, p. 177). It was estimated for locus l and sample size n as:

$$D_l = 2n(1 - \Sigma x_i^2)/(2n - 1), \quad [1]$$

where x_i is an estimate of the observed frequency of the ith allele:

$$x_i = x_{ii} + \Sigma_{i \neq j} x_{ij}/2, \quad [2]$$

Table 2. RFLP probes, their chromosomal location, and average number of alleles at each locus identified in progenitors and derived elite lines of BSSS and BSCB1.

Chromosome	RFLP probe	Number of loci	Group			
			pBSSS	dBSSS	pBSCB1	dBSCB1
			average no. of alleles			
1	asg045†, asg62†, bnl5.62†, bnl6.25, bnl6.32, csu164†, npi97, umc76†, umc84, umc107, umc128, umc140†, umc157	13	4.38	3.31	3.62	2.46
2	bnl12.09, umc4, umc5†, umc34, umc55†, umc61, umc131, umc137	9	3.88	3.75	3.63	2.00
3	asg24, bnl5.14, bnl6.06, bnl7.26, bnl8.35, umc26, umc32, umc60†, umc97, umc121†	11	3.73	2.82	4.45	2.64
4	bnl5.46, bnl5.67, bnl15.07, phi20725†, umc15, umc19, umc31, umc42, umc52, umc156, umc158	13	3.38	2.85	3.23	2.23
5	bnl5.24, bnl5.71, bnl7.71, bnl8.33, phi10017, umc27, umc43, umc54, umc90, umc108, umc147	11	4.18	3.63	4.09	2.18
6	bnl3.03, bnl5.47, phi10016, phi20854, umc21†, umc38, umc46, umc59, umc62, umc65, umc85, umc134, umc152	14	3.86	3.14	3.64	2.71
7	asg8, bnl8.32, bnl15.40, umc7, umc35, umc80, umc110	7	5.14	4.14	4.86	3.29
8	bnl9.11, bnl9.44, bnl12.30†, bnl13.05, npi414, umc30, umc89, umc120	10	3.00	2.30	3.40	1.60
9	bnl3.06, bnl5.09, bnl5.10, csu61†, csu147, phi10005†, umc113, umc81, umc95, umc153	10	4.60	3.10	4.00	2.80
10	bnl3.04, bnl7.49, ksu5†, phi6005, umc57, umc159	7	3.71	2.71	3.57	2.14
Total		105	3.95	3.13	3.77	2.40

†Restriction enzyme *Eco*RI was used for DNA digestions; for all other probes *Hin*dIII was used for DNA digestion.

and x_{ii} is the frequency of genotype A_iA_i, x_{ij} is the frequency of genotype A_iA_j in the observed sample. Total gene diversity, sometimes referred to as average gene diversity (Nei, 1987, p. 179) was calculated as:

$$D = \Sigma_{l=1}^{m} D_l/m, \qquad [3]$$

for m loci. Gene diversity is a more appropriate measure of variability than observed heterozygosity for inbred populations (Weir, 1996).

The average number of alleles per locus was determined from single-locus values. For percent polymorphic loci, a locus was regarded as polymorphic when the frequency of the most common allele was smaller than 0.99. Fisher's Exact Test (Fisher, 1934) was used to investigate the significance of allele frequency changes between groups of progenitors and derived lines. Left-tailed P-values were calculated at a significance level of $\alpha = 0.05$.

For all pairs of lines or groups of lines, Rogers' (1972) distance (RD) was calculated according to

$$\mathrm{RD} = \frac{1}{m}\sum_{i=1}^{m}\sqrt{\frac{1}{2}\sum_{j=1}^{a_i}(p_{ij} - q_{ij})^2}, \qquad [4]$$

where a_i is the number of alleles at the ith locus, and p_{ij} and q_{ij} are the allele frequencies of the allele j of this locus in the respective pair or group of lines. For homozygous lines, RD corresponds to the proportion of marker loci for which two lines differ and is equal to the Nei-Li distance (Nei and Li, 1979). The standard deviation of RD was obtained as the square root of a jackknife estimator (Shao, 1999, p. 330) of its variance determined by resampling over marker loci:

$$\hat{V}ar(RD) = \frac{m-1}{m}\sum_{j=1}^{m}(RD_{-j} - RD_m)^2 \qquad [5]$$

where RD_{-j} is the estimator obtained by omitting the jth marker locus and RD_m is the mean of the m estimates RD_{-j}.

Associations among the lines were determined from principal component analysis (PCA) based on the covariances between allele frequencies.

The genetic data analysis program GDA (Lewis and Zaykin, 2001) was used for calculating diversity statistics. Fisher's Exact Test was performed by means of the FREQ procedure of SAS (SAS Institute, 1990). Distance measures and PCA were calculated with the statistical software R (Ihaka and Gentleman, 1996).

RESULTS

Progenitor and derived lines were homozygous at most loci with the exception of line K230, which was found to be heterozygous at 47 out of the 105 marker loci (data not shown).

A decreasing trend in the number of alleles per locus was observed from progenitors to derived lines (Fig. 1). On average, we found a decrease of almost one allele per locus from progenitors to derived lines in BSSS and more than one allele in BSCB1 (Table 3). Accordingly,

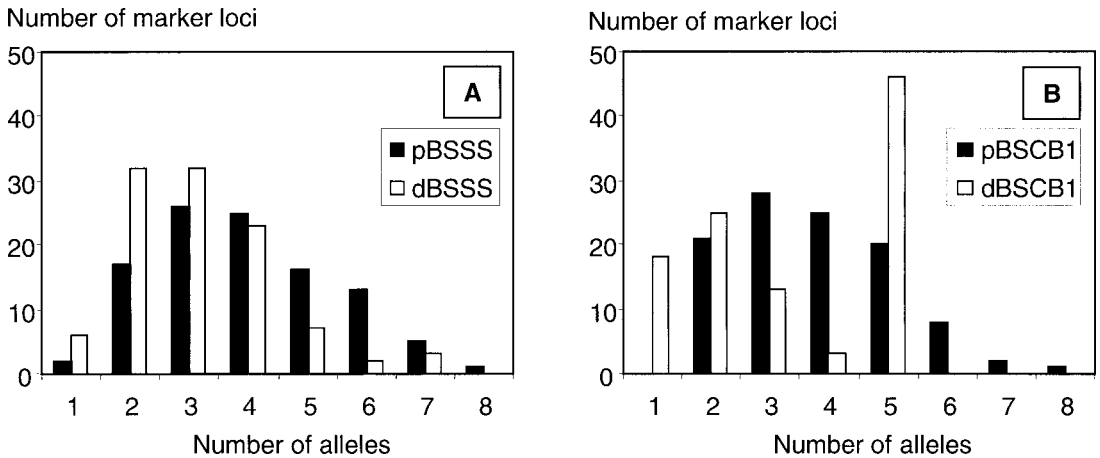

Fig. 1. Number of alleles detected per RFLP locus at 105 marker loci in progenitors (pBSSS, pBSCB1) and derived elite lines (dBSSS, dBSCB1).

Table 3. Diversity statistics for 105 RFLP loci in progenitors and derived elite lines from BSSS and BSCB1.

Group	N†	Percentage of polymorphic loci	Average number of alleles per polymorphic locus	Average gene diversity D_l
pBSSS	16	0.97	4.03	0.57
pBSCB1	12	0.99	3.79	0.60
dBSSS	18	0.94	3.26	0.50
dBSCB1	7	0.83	2.69	0.42

† No. of lines in respective group.

frequency distributions of gene diversity D_l across all RFLP loci shifted toward lower values between progenitors and derived lines in both BSSS and BSCB1 (Fig. 2). Average gene diversity D decreased by 0.07 from progenitors to derived lines in BSSS and by 0.18 in BSCB1 (Table 3).

More than 80% of the alleles had frequencies lower than 0.5 in both progenitor and derived lines of each population (Fig. 3). In dBSSS lines, extreme allele frequencies ($P < 0.2$ or $P > 0.7$) occurred more often than in pBSSS lines. We found the same trend in BSCB1 but the allele frequency distribution of dBSCB1 lines was even more extreme than the allele frequency distribution of dBSSS lines.

In pBSSS and pBSCB1, 27.0% of the alleles were unique, i.e., they occurred in exactly one of the progenitors of the respective group. In dBSSS lines, 5.8% of the alleles were novel, i.e., they were not detected in any of the pBSSS lines; their frequency ranged between 0.03 and 0.28. By comparison, 3.5% novel alleles were found in dBSCB1 lines with frequencies ranging from 0.07 to 0.71. Six alleles were consistently found in all dBSSS lines, three of them being monomorphic in pBSSS lines. A total of 17 alleles was found in all dBSCB1 lines with one of them being monomorphic in the pBSCB1 lines.

Fisher's Exact Test showed a significant ($P < 0.05$) increase in the frequency of 30 alleles from progenitors to derived elite lines in BSSS and 25 alleles in BSCB1 (Table 4). Allele frequency changes ranged from 0.28 to 0.83 in BSSS and from 0.41 to 0.77 in BSCB1 (data not shown). In BSSS, the corresponding marker loci showing increased allele frequencies were distributed over all chromosomes, whereas in BSCB1, six of these loci were located on Chromosome 5 but none on Chromosomes 2 and 10. In both BSSS and BSCB1, each progenitor line contributed at least two alleles with a significant ($P < 0.05$) increase in frequency. In BSSS, most alleles with a significant ($P < 0.05$) increase in frequency were contributed by progenitor lines CI.540 and I159. In BSCB1, progenitor lines K230 and L317 contributed most alleles with a significant ($P < 0.05$) frequency increase. Frequencies of 19 alleles decreased significantly ($P < 0.05$) from pBSSS to dBSSS; at 12 of these alleles, the initial allele frequencies ranged between 0.38 and 0.65 and dropped by 0.32 to 0.54. Seven alleles with a frequency of 0.20 to 0.62 in the pBSSS lines were not recovered in any of the dBSSS lines (data not shown). Eleven alleles showed a significant ($P < 0.05$) decrease in frequency from pBSCB1 to dBSCB1; four of them had initial frequencies between 0.63 and 0.74 and decreased in a range between 0.48 and 0.58. Seven alleles with initial frequencies between 0.44 and 0.67 were not recovered in any of the dBSCB1 lines (data not shown).

The ratio of the number of alleles with a significant increase and decrease in allele frequencies was taken as an indication of the contribution of individual progenitors to the derived elite lines (Table 4). Accordingly, line CI.540 made by far the largest contribution to the dBSSS lines, carrying 10 alleles with a significant increase but only four alleles with a significant decrease. In contrast, Ill.12E and Ind.461-3 had the lowest ratio and, thus, contributed least to the dBSSS lines. Altogether, there was a trend that progenitors from Reid Yellow Dent (RYD) contributed more alleles to dBSSS lines than those from other sources. Oh07 and P8 made the largest contributions to the dBSCB1 lines, whereas Oh40B, Oh33, and Oh51A contributed little. Ill.Hy, the progenitor line in common between both populations, carried a large number of alleles with a significant frequency increase in each group. Nevertheless, its contribution to dBSSS and dBSCB1 lines was only moderate.

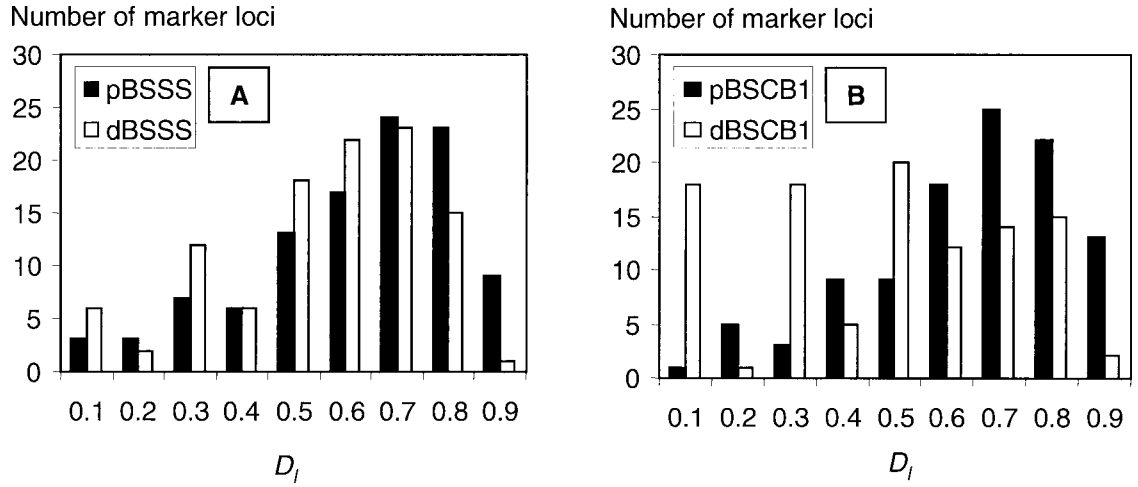

Fig. 2. Histogram of gene diversity (D_l) across all RFLP loci in progenitor (pBSSS, pBSCB1) and derived (dBSSS, dBSCB1) elite lines. Each value along the *x* axis refers to the upper boundary of the corresponding class interval.

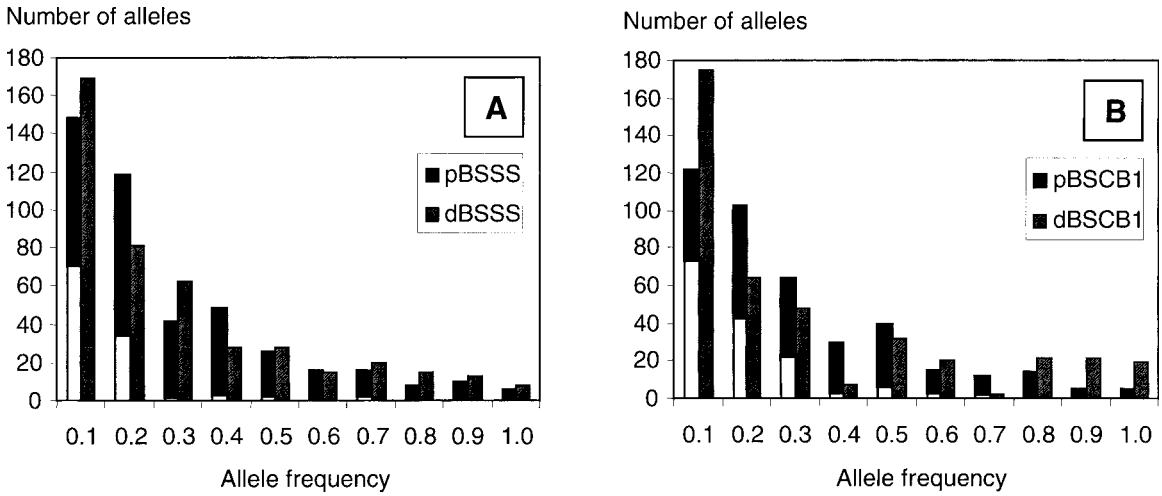

Fig. 3. Allele frequencies versus number of alleles in progenitors and derived elite lines of (A) BSSS and (B) BSCB1. The white columns indicate the number of alleles present in the progenitor lines but not recovered in the derived elite lines. Each value along the *x* axis refers to the upper boundary of the corresponding class interval.

Mean pairwise RD within the progenitors was similar in pBSSS (0.59) and pBSCB1 (0.61), but the range was much larger in the former (0.12 to 0.74) than in the latter group (0.50 to 0.70) (data not shown). By comparison, mean pairwise RD within dBSSS (0.51) and dBSCB1 (0.45) were substantially smaller. Rogers' distance for groups of lines was 0.23 between pBSSS and dBSSS lines derived from C0 and 0.31 between pBSSS and dBSSS lines derived from C3 to C8 (Table 5). There was no significant difference in RD between pBSCB1 and the early and advanced cycles of dBSCB1 lines. Rogers' distance between BSSS and BSCB1 increased from 0.19 for progenitors to 0.41 for lines derived from C0 to 0.50 for lines derived from advanced cycles.

Principal component analysis revealed a relatively strong genetic similarity of pBSSS and pBSCB1 lines in that the two groups largely overlapped (Fig. 4). Progenitors of BSSS located outside the main cluster of pBSSS lines, e.g., Ill.12E, CI.540, Ill.Hy, and A3G were all Non-RYD lines. The groups of elite lines derived from BSSS and BSCB1 diverged in opposite directions and formed separate clusters. dBSSS lines derived from C0 were rather widely spread, most of them being adjacent to the progenitors but a few (B14, B14A, B17) being located fairly remote. The elite inbred lines derived from the BSSS(HT) and BS13(S) populations fit well in the grouping of all dBSSS lines. By comparison, dBSSS lines derived from C3 to C8 were more tightly grouped together. The two dBSCB1 lines derived from C0 were positioned in the cluster of the pBSCB1 lines, whereas all lines derived from C7 to C10 formed a widely remote but tight cluster.

DISCUSSION

BSSS and BSCB1 were originally developed through a series of single, double, or three-way and double-double crosses (Lamkey et al., 1991) tracing back to the inbred progenitor lines examined in this study. The initial populations of the RRS program were formed by random-

Table 4. Number of alleles in progenitor lines with significant ($P < 0.05$) increase and decrease in allele frequency from pBSSS to dBSSS and from pBSCB1 to dBSCB1 as revealed by Fisher's Exact Test. The ranking is based on the ratio between increased and decreased alleles.

BSSS			BSCB1		
	Number of alleles			Number of alleles	
Progenitor lines	Increased	Decreased	Progenitor lines	Increased	Decreased
CI.540 (Non-RYD†)	10	4	Oh07	7	4
I159 (RYD‡)	8	7	P8	7	6
Ind.Tr9-1-1-6 (RYD)	7	8	L317	8	7
CI187-2 (RYD)	6	8	K230	8	7
I224 (RYD)	6	9	CC5	6	7
Ill.Hy (Non-RYD)	7	11	Ill.Hy	6	8
Os420 (RYD)	5	8	I205	5	7
WD546 (RYD)	5	8	A340	5	7
LE23 (Non-RYD)	4	7	R4	3	6
A3G-3-1-3 (Non-RYD)	4	8	Oh40B	3	7
Oh3167B (Non-RYD)	6	12	Oh33	3	7
Ind.AH83 (RYD)	6	13	Oh51A	2	6
Ind.Fe2 + Ind.B2 (RYD)	7	17			
Ind.461-3 (RYD)	4	12			
Ill.12E (Non-RYD)	2	13			

† Non-Reid Yellow Dent.
‡ Reid Yellow Dent.
§ Contributions of the parental lines of the BSSS progenitor F1B1-7-1 (Ind.Fe2 and Ind.B2) are summed up.

Table 5. Roger's genetic distance (RD) between groups of progenitors of BSSS and BSCB1 and their derived elite lines. The elite lines are divided in groups derived from Cycle 0 and from advanced cycles.

	BSSS			BSCB1		
Group	**pBSSS**		**dBSSS C0**	**dBSSS C3-C8**	**pBSCB1**	**dBSCB1 C0**
dBSSS C0	**0.23ab†**	**0.22‡**				
dBSSS C3-C8	**0.31cd**		**0.26abc**			
pBSCB1	**0.19a**		**0.26bc**	**0.33cde**		
dBSCB1 C0	**0.39defg**		**0.41defg**	**0.47fg**	**0.37def**	**0.31**
dBSCB1 C7-C10	**0.26defg**		**0.45fg**	**0.50g**	**0.39defg**	**0.45efg**

† Means with different superscript letters are not significantly ($P < 0.10$) different.
‡ Roger's genetic distance between progenitors and all derived lines.

mating bulked seed from the double-double crosses for five to six generations. Cycle 1 to C8 were generated by recombining 10 selected S_1 lines in each population, whereas starting with C9, 20 selected S_1 lines were employed for recombination (Keeratinijakal and Lamkey, 1993a).

Our estimates for the average number of alleles and gene diversity (D) for both group of progenitors were similar to those obtained with other RFLP studies in maize (Dubreuil et al., 1996; Livini et al., 1992). Since the number of alleles is expected to increase as more individuals are sampled, this provides a simple explanation for the higher average number of alleles per locus detected in pBSSS relative to pBSCB1. In contrast, gene

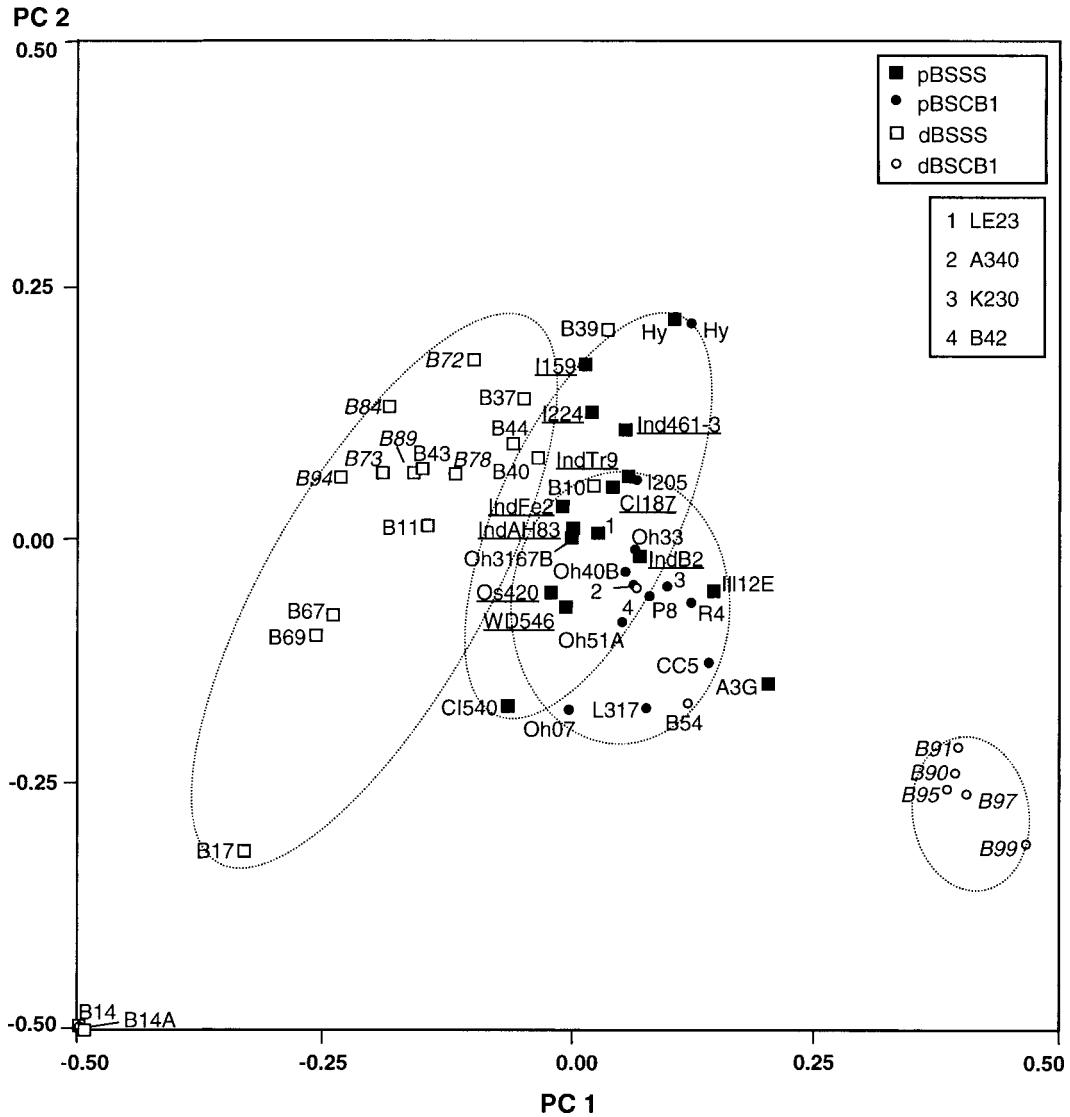

Fig. 4. Associations among the inbred lines revealed by principal component analysis performed on covariances between allele frequencies calculated from RFLP data of 105 loci. pBSSS and dBSSS lines are designated by solid and open squares, respectively; pBSSS lines originating from Reid Yellow Dent are underlined. pBSCB1 and dBSCB1 lines are indicated by solid and open circles, respectively. Lines derived from advanced cycles are given in italics. PC1 and PC2 are the first and second principal components. The dashed ellipses describe the clustering of the groups.

diversity *D* was higher in pBSCB1 than in pBSSS, reflecting a smaller variance in the gene frequencies of different alleles at a given locus. The large estimates of *D* in the progenitor groups relative to the derived groups were attributable to the large number of alleles that occurred only in one or a few progenitor lines. While in general this could reflect either large genetic diversity for RFLPs in the progenitor lines or unstable inheritance of these markers, the latter can be ruled out in our study because RFLPs in maize have been demonstrated to be stably inherited over several generations (Evola et al., 1986). Nevertheless, precaution in the interpretation is necessary, because we are studying variation at marker loci but not allelic variants of functional genes. Under this restriction, our results suggest that the materials chosen for the foundation of the BSSS and BSCB1 populations were broadly based. This is in agreement with the results of Messmer et al. (1991) on the group of pBSSS lines. While BSSS was mostly composed of lines originating from various strains of RYD, the results from PCA illustrate that the large genetic variance within pBSSS was strongly influenced by several lines (Ill.12E, CI.540, Ill.Hy, LE23) that did not originate from RYD.

Rogers' distance at population level between the groups of pBSSS and pBSCB1 lines was 0.19. In combination with the results from PCA, we conclude that the two groups of progenitor lines were not highly divergent. Several members of one progenitor group were relatively similar to members of the other group and Ill.Hy was even identical. The genetic distance between the groups of progenitors calculated by Labate et al. (1997) supports our findings. Almost 70% of the alleles occurring in any progenitor line of BSSS or BSCB1 was also detected in at least one progenitor line of the other group. If these findings also apply to QTL for yield, this could explain the low panmictic midparent heterosis (PMPH) found in the interpopulation crosses of the C0 populations (Eberhart et al., 1973; Hallauer et al., 1983).

The smaller average number of alleles per locus within dBSCB1 in comparison with dBSSS results from the larger set of lines sampled in BSSS. The comparatively high *D* values are attributable to a large number of alleles occurring with low frequencies. In both groups of derived elite lines, novel alleles contributed little to the genetic variation. In BSSS, some of the novel alleles may have originated from the missing progenitor CI617. While migration due to contamination with foreign pollen and errors in the lab assay cannot be ruled out entirely, common mutation rates estimated for eukaryotes (10^{-8} to 10^{-4} per generation) are insufficient to generate this amount of new genetic variation with the small effective population size employed in this RRS program (Lacy, 1987).

Each progenitor appears to have made some contribution to the derived elite lines. We found that each progenitor had a few alleles with a significant increase in frequency in the derived elite lines. pBSSS lines carried different numbers of alleles with a significant frequency increase or decrease (Table 4). CI.540 in comparison to Ill.12E and Ind.461-3 seemed to make strong contributions to the dBSSS lines. Nevertheless, because each allele is not unique to a progenitor we cannot determine from which progenitor an allele descended in the derived elite lines. In BSCB1, there were no substantial differences in the number of alleles with a frequency increase or decrease between the progenitor and derived elite lines. For BSCB1, our results support the findings of Labate et al. (1997) that no single progenitor made excessive contributions to the derived lines. Single nucleotide polymorphisms (SNPs) seem to be a promising tool to investigate in detail the descent of each chromosome segment from individual progenitors, because they are highly polymorphic. For example, Tenaillon et al. (2001) observed in maize a polymorphism every 28 base pairs.

Rogers' distance between BSSS and BSCB1 increased significantly ($P < 0.10$) from the progenitors to the elite lines derived from C0 and the difference was even greater for lines extracted from advanced selection cycles. PCA graphically revealed the divergence of the dBSSS and dBSCB1 groups. In agreement with our results, Labate et al. (1997) showed substantial divergence of the BSSS and BSCB1 populations after 12 cycles of selection. The divergence of the populations can be attributable to selection, genetic drift, or new variation through mutation or migration from outside sources. As stated above, mutation and migration were likely only minor causes for divergence of BSSS and BSCB1.

Recurrent selection may lead to divergence of two populations, depending on the mode of gene action at the loci affecting the trait under selection. In the biallelic case, the accumulation of two different alleles in the two populations is expected through selection at overdominant loci, where as accumulation of the same allele in both populations is expected under dominance (Comstock et al., 1949). We observed a frequency increase of different alleles in the two groups of derived lines. Additionally, many alleles with fairly similar frequencies in the progenitor lines increased in frequency in one group and decreased in the other group (Fig. 5). This observed divergence may be caused by overdominance or by linked genes mimicking pseudo-overdominance. However, Moll et al. (1978) showed that the allele frequency change at a locus in one population is related to the allele frequencies at the locus in the other population and, consequently, for multiple alleles the accumulation of different alleles can also occur in the absence of overdominance. Keeratinijakal and Lamkey (1993a,b) and Hanson (1987) concluded that (i) selection for complementary alleles at loci with partial to complete dominance is the reason for the increasing performance of the interpopulation crosses and (ii) there is no evidence for overdominance for grain yield in BSSS and BSCB1. The results from our study neither support nor exclude dominance, overdominance, or pseudo-overdominance due to linked loci as major reasons for the performance increase of the interpopulation crosses.

Loss of genetic diversity because of random genetic drift is caused by sampling. The early cycles of selection in BSSS and BSCB1 led to inbreeding due to genetic

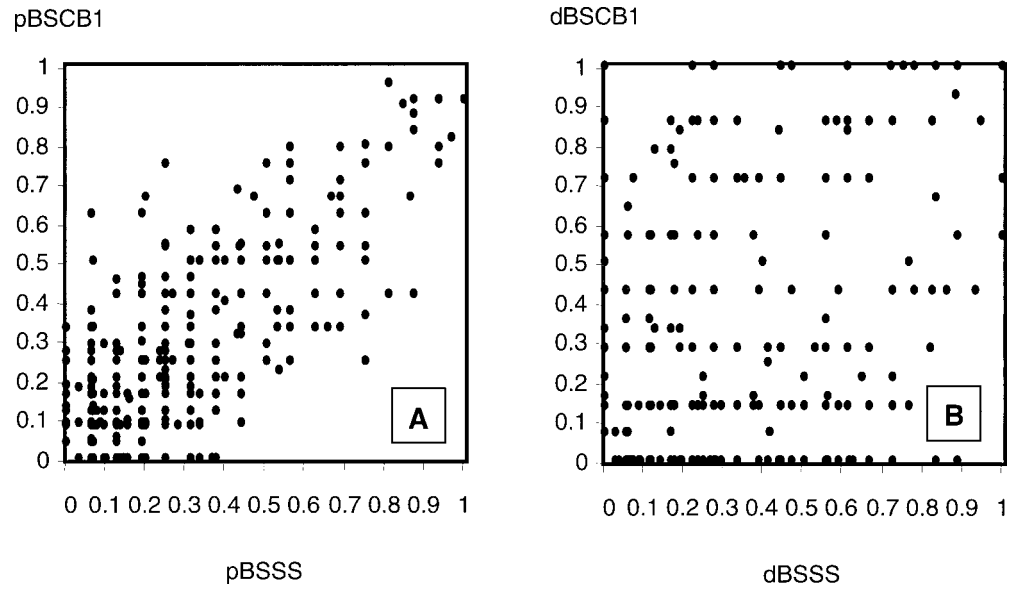

Fig. 5. Allele frequencies in (A) pBSSS versus pBSCB1 and (B) dBSSS versus dBSCB1.

drift because only 10 parents were used in recombination (Keeratinijakal and Lamkey, 1993a). Smith (1983) and Helms et al. (1989) reported significant inbreeding depression due to random genetic drift in BSSS and BSCB1. Keeratinijakal and Lamkey (1993a) estimated an inbreeding level of at least 37% in both C11 populations. On the molecular genetic level, Labate et al. (1997) reported substantial drift during the maintenance of BSSS until C0 due to a small effective population size. In agreement with these results, genetic diversity measured by *D* and RD within groups of lines decreased from progenitors to derived elite lines in both BSSS and BSCB1. Regarding allele frequencies, we observed more alleles either near extinction or near fixation within the groups of derived lines than within the groups of progenitor lines. Nevertheless, we found relatively few alleles with a significant frequency increase or decrease from progenitors to derived elite lines as well as alleles not recovered in the derived lines. In total, about 75% of all alleles occurring in the pBSSS lines were recovered in the dBSSS lines and almost 67% of all alleles in the pBSCB1 lines were recovered in the dBSCB1 lines. This showed that the derived lines are fairly diverse within each group and captured a large amount of the genetic variation present in the progenitors.

The average RD between pairs of lines from the two populations of an RRS program is expected to remain constant over selection cycles if only genetic drift but no selection occurs (A.E. Melchinger, 2001, personal communication). We observed an increase of the average pairwise RD, however, between lines derived from advanced cycles of selection (0.50) compared with lines derived from C0 (0.41). Under the assumption that the allele frequencies in the lines represent those in the parental populations, this indicates that not only random genetic drift but also selection was responsible for the divergence of the two populations.

Quantitative genetic theory predicts that PMPH will increase with increasing divergence of the parental populations. In interpopulation crosses of BSSS and BSCB1, grain yield increased 6.46% per cycle after 11 cycles of selection, grain moisture increased 0.85 g/kg per cycle, stalk lodging decreased 1.64% per cycle (Schnicker and Lamkey, 1993). These results indicated that RRS has been effective in increasing the mean performance of the population cross while maintaining genetic variance in the synthetic populations. Examining field data to investigate hybrid improvement after RRS in BSSS and BSCB1, Betrán and Hallauer (1996) reported that the hybrid population progress by RRS was directly reflected in superior single crosses. Accordingly crosses between lines randomly derived from improved populations of BSSS and BSCB1 provided hybrids with greater grain yield and improved agronomic traits than crosses between lines from C0. Our results support the conclusions of these authors demonstrating the divergence of the elite lines derived from BSSS and BSCB1 on the molecular genetic level. It might be rewarding to investigate whether the genomic regions showing the largest divergence also account for a large proportion of the heterosis observed in the cross between the two populations, as hypothesized by Smith et al. (2000).

Reciprocal recurrent selection is an effective selection method for improving population crosses and inbred lines derived from single crosses; its efficiency increases with the number of cycles, once favorable complementary allele combinations are created in both parental populations during the initial cycles (Betrán and Hallauer, 1996). Our study showed at the molecular genetic level that the requirements for a successful RRS breeding program were fulfilled in BSSS and BSCB1. First, the progenitors used to form the synthetic populations were broadly based and had a large variation. Second, we found changes in allele frequencies mostly in opposite directions in dBSSS and dBSCB1 from advanced cycles. Furthermore, BSSS and BSCB1 diverged substantially from each other from progenitors to derived lines. Despite permanent selection and genetic drift

since the establishment of the original synthetics, we observed slow overall changes in allele frequencies from progenitors to derived lines. We found the derived lines to represent a large proportion of the genetic variation occurring in the progenitors. Since the derived lines captured a large amount of the original variation, the future prospects seem to be promising for further success in finding superior elite lines through this RRS program. The elite lines proved to be genetically diverse, especially those resulting from different cycles of selection. Therefore, it seems promising to use different elite lines derived from each population as parents for recycling breeding programs.

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The authors would like to dedicate this paper to Dr. A.R. Hallauer on the occasion of his 70th birthday. Dr. Hallauer has dedicated his life to breeding maize and developing germplasm that makes studies like ours possible. For these contributions and being a friend we are much indebted.

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