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Pedigree analysis and haplotype sharing within diverse groups of *Zea mays* L. inbreds

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Abstract The objectives of this study were the examination of genetic similarities in a diverse group of maize inbreds and an investigation of the incidence of shared haplotypes within closely related groups. Size polymorphisms from 218 mapped simple-sequence repeats (SSR) for 57 entries were detected with the ABI377 Prism system and scored with Genotyper software. The standard error for the estimated size of identical PCR products was 0.13 base pairs. Size estimates were used to examine genetic relationships among the Iodent, flint, corn belt dent, sweet corn and popcorn groups in *Zea mays* L. inbreds. Cluster analysis of SSR distance data from 57 entries showed similarity between the European flints (F2, F7 and EP1), CO109 and the *su1* sweet corns developed in the United States. The inbred F64 from Argentina was distinct from all other entries. Close examination of two sources of B37 revealed that the Purdue University version of B37 contains a set of alleles characteristic of B73. Five groups (Iodents, European flints, the B73 group of corn belt dents, *su1* sweet corn, popcorn) show persistent within-group haplotypes.

Keywords Maize pedigree SSR

Introduction

Estimation of genetic relationship using pedigree information depends on the assumption that parental contributions to progeny are equal to their expected values (Bernardo 1996). In the simplest case, inbred parents *a* and *b* contribute equally to an F_2-derived individual. This expectation is actually the mean of a distribution of parental contributions in an F_2 population in which both chromosome assortment and recombination occur at random in the absence of selection. In plant-breeding programs, small populations and unpredictable growing conditions can result in deviation from expected values due to genetic drift and natural selection alone, even if no deliberate selection is applied. Strategies involving multiple parents, multiple selection schemes and multiple plant breeders blur lines of descent.

Molecular markers provide a means to estimate actual parental contribution without knowledge of the intervening process. Simple-sequence repeats (SSR) have demonstrated utility for the estimation of genetic relationships between maize (*Zea mays* L.) inbreds (Smith et al. 1997). Both restriction fragment length polymorphism (RFLP) and SSR markers are sufficiently polymorphic, and densely distributed enough in maize to distinguish different inbreds extracted from selfing the F_2 progeny of a single hybrid parent (Bernardo et al. 2000).

DNA markers can also provide an accurate and objective determination of the identity of a given inbred or hybrid if the marker system used is reproducible under standard conditions and the markers can be scored independently of the context in which they were produced. Examples of context independence are automated band-size estimates or actual DNA sequence. Marker alleles that are scored manually, or given arbitrary names without reference to reproducible standards (alleles A, B and C, for example), are context-dependent and, as such, are problematic in situations in which the identity of given variety, hybrid or inbred is at issue. In a study of 13 maize inbreds in which three were the progenitors of the other ten inbreds, RFLP and SSR markers gave similar estimates of parental contributions, but RFLP markers showed a higher incidence of apparent nonparental bands (Bernardo et al. 2000). The SSR data were scored automatically, while the RFLP data were scored manual-

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ly. Internal quality control studies have shown that the largest difference between the RFLP datasets produced by different people scoring the same set of autoradiograms was not the estimated size of the band but whether or not the band was scored at all (J. Romero-Severson, unpublished results).

Linkage disequilibrium results in shared haplotypes among close relatives. Recombination in successive generations results in the decay of haplotype sharing (DHS). McPeek and Strahs (1999) have shown that the lengths of an inherited ancestral segment in the descendants are distributed as exponential random variables with rate τ, where τ is the number of generations from the ancestor, assuming random mating, no selection and no interference. However, recombination does not disrupt shared haplotypes once the ancestral segment becomes fixed in the population. In maize breeding, elite inbred lines arise from intense selection among inbred lines derived by selfing out of segregating populations. Under these regimes, one might expect a considerable degree of haplotype sharing among inbreds that arose from the same founder population. Shared haplotypes permit detection of essential derivation, a circumstance in which inbred lines are extracted directly from the population produced by selfing a single hybrid.

Our objective in this study were (1) to examine the genetic relationships among maize inbreds with a large set of densely distributed and well-characterized SSR markers and (2) to detect the incidence of shared haplotypes within closely related groups.

Material and methods

Maize inbreds

Fifty seven maize inbreds were chosen to represent the major sources of elite maize inbreds in North America and Europe: the Iodent, Iowa Stiff Stalk Synthetic (BSSS), and Lancaster heterotic groups, flints, popcorns and sweet corns. Three separate sources of B73 and two sources of H84 and B37 were included to provide quality control data and to examine the effect of source on genetic divergence within a given inbred line. Most of the BSSS and all of the Lancaster inbreds were obtained in 1997 from the U.S. Department of Agriculture North Central Regional Plant Introduction Station (Ames, Iowa). Purdue University provided the second source of B73, H84 and B37 (P_B73, P_H84 and P_B37) from stocks maintained in long-term cold storage. Linkage Genetics (Salt Lake City, Utah) provided a source of B73 from seed stored on site since 1992. Inbred entries from Pioneer Hi-bred International included representative lines from the Iodent, BSSS and Lancaster heterotic groups and are labeled P-1 through P-10. The Institut National de la Recherche Agronomique, the University of Wisconsin-Madison, and the Orville Redenbacher Popcorn Company provided the European flint, sweet corn and popcorn inbreds, respectively.

SSR production and detection

Leaf tips were harvested from 30–50 seedlings of each of the 57 inbreds. The DNA was extracted from the bulked sample of leaf tips using a CTAB procedure (Saghai-Maroof et al. 1984). Each inbred was screened with 218 SSR primer pairs, 176 of which were developed jointly by Brookhaven National Laboratory (New York, USA), Celera AgGen, and a consortium of 15 private companies. The remaining 41 primers were developed by Brookhaven National Laboratory (8), Pioneer Hi-bred International (28), DuPont (2) and North Carolina State University (3) (Chin et al. 1996; Taramino and Tingey 1996; Senior et al. 1998). The Brookhaven-Maize Consortium SSRs have the prefix ‘bnlg’, the Pioneer SSRs have the prefix ‘phi’, the DuPont SSRs have the prefix ‘dup’ and the North Carolina State SSRs have the prefix ‘nc’. In this set of SSRs, 180 were AG repeats and four were CT repeats. The rest were simple and complex longer repeats. Repeat classes and all of the primer sequences are available from the Maize Genome Database website at ftp://ftp.agron.missouri.edu/pub/Probe/SSR.txt.

For SSR detection, the primer pairs were labeled with one of three phosphoramidite fluorescent dyes (6-FAM, VIC, NED) and amplification products produced as described by Bernardo et al. (2000). The amplified DNA samples were electrophoresed at 200 W for 2.5 h on an ABI Prism 377 DNA Sequencer equipped

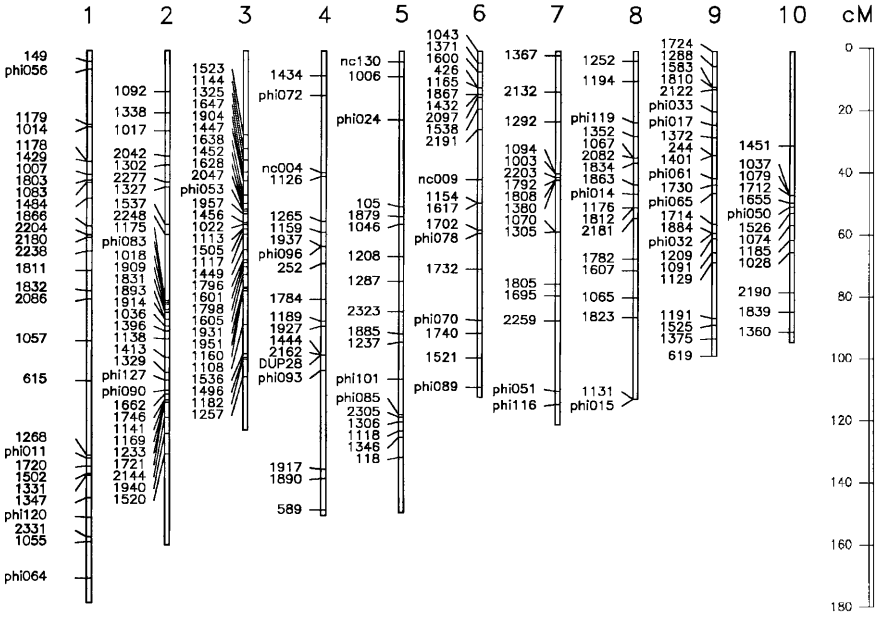

Fig. 1 SSR markers and map locations. All SSR markers lacking a letter prefix are bnlg markers

with ABI Prism 377-96 Collection software (v 2.5 ABI Prism 377-96 Collection). The DNA fragments were sized automatically and assigned to specific alleles based on binning a range of sizes (±0.5 base pair), as determined by Genotyper software using the local Southern algorithm (Elder and Southern 1987). All of SSRs included in this study showed the expected patterns under the three-point test in which two inbred parents show different DNA fragment sizes ("bands") and the F_1 hybrid shows both bands.

Data quality control

We examined the effect of time and DNA extraction by comparing bands sizes for B73, P_B73, H84, P_H84, B37 and P_B37 in analyses done 6 months apart. The B73, H84 and B37 data sets for this comparison included 37, 49 and 28 SSR respectively. For these data, analyses done at one time mean that DNA samples were subjected to PCR-amplification in a common plate and gel-electrophoresis in the same gel. We compared the estimated band sizes as reported by Genotyper, not the binned allele sizes. SSR band sizes were highly correlated between one inbred source and another, and one run time with another for the same inbred source (r>0.99 for all possible comparisons except P_B37 with B37). We also examined the precision of the estimated band sizes by comparing the size estimates of 30 SSR alleles for one sample of B73 and one sample of P_B73 on each of 2 different days. The average standard deviation for the estimated size of the B73 alleles was 0.14 base pairs (bp) with a range from 0.71 to 0.01 bp. The average standard deviation for the estimated size of the P_B73 alleles was 0.12 bp with a range from 0.71 to 0.01 base pairs. Thus the Genotyper software assignment to specific alleles based on binning a range of sizes (±0.5 base pair) appears to be conservative.

Data analysis

Polymorphic index content (PIC) values were calculated using the formula:

$$PIC = 1 - \sum_{i=1}^{n} f_i^2$$

where f_i is the frequency of the ith allele. For a given number of alleles, PIC gives the highest value when allele frequencies are equal. Genetic similarities were calculated from comparisons of band size estimates using a modified Nei's distance (Nei and Li 1979). Cluster analysis was performed on the similarity matrix using the connected or single-linkage method (Nei et al. 1983). Chromosomal locations of SSR markers (Fig. 1) were determined by consensus mapping in five populations as described previously (Bernardo et al. 2000 and the Maize Genome Database at http://www.agron.missouri.edu:80/cgi-bin/sybgw_mdb/mdb3/Panel+of+Stocks/145836).

Shared haplotypes were detected by direct examination of all the the SSR data for all of the entries within each of the following clusters: Iodent, Lancaster Surecrop/Oh43, B73, B37, European flints, sweet corn and popcorns. The clusters were chosen on the basis of distinctness from other clusters, known pedigrees and historical interest. The binned band sizes were used for a count of allele frequencies. The map positions of all of the SSRs showing only one band size for all of the entries within a cluster were examined. A shared haplotype within the cluster was assumed if these SSRs were adjacent to one another on the linkage map.

Results and discussion

SSR distance data

The genetic relationships revealed by cluster analysis of SSR similarity data were similar to results obtained in previous studies for the same entries (Smith et al. 1985a, b; Messmer et al. 1992a, b; Gerdes et al. 1993; Gerdes and Tracy 1994; Kantety et al. 1995; Smith et al. 1997; Dubreuil and Charcosset 1999). The major heterotic groups included in the analysis, the Iodents, BSSS and Lancasters, occurred in distinct clusters consistent with known pedigrees (Fig. 2). SSR similarity data also correctly distinguished the three main groups within the stiff stalks: B14, B73 and B37. The placement of P_B37 was not consistent with the pedigree records. B76 arose from the cross CI31A×B37, with backcrossing to B37. H84 was a selection out of the cross B37×GE440. P_B37, a different source of B37, should cluster closest to B37 itself.

The relationship between three European flints (F2, F7 and EP1), CO109 and sweet corn inbreds is consistent with the isozyme evidence and historical records that indicate a common derivation from the Northern

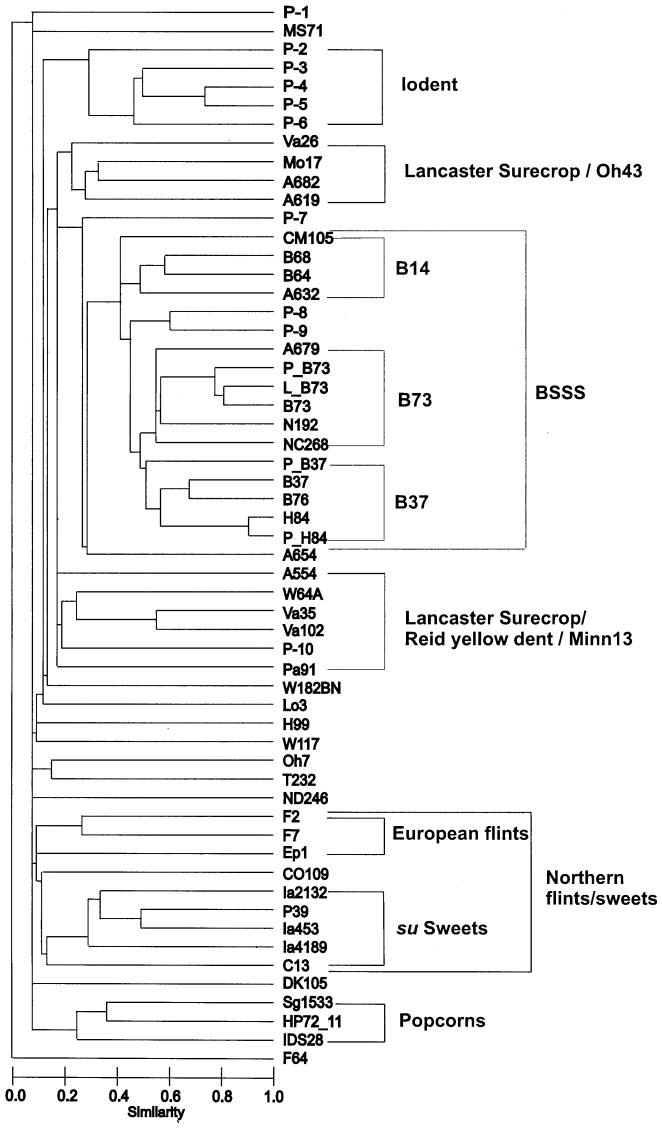

Fig. 2 Associations among maize inbred lines revealed by cluster analysis of SSR similarity data

Table 1 Comparison of SSR band sizes differences in different sources of B37 and B73. Comparisons are shown only for those SSR for which the band sizes for B37 and P_B37 were different

SSR	C	cM	B37	P_B37	B73	P_B73
bnlg149	1	3.5	183.0	186.8	186.9	186.8
phi056	1	6.1	256.8	248.7	248.1	248.1
bnlg1007	1	39.7	108.4	104.4	100.2	100.2
bnlg1092	2	13.2	162.0	116.3	116.3	116.3
bnlg1338	2	20	221.7	175.4	175.4	175.4
bnlg2277	2	42.1	196.3	194.4	194.4	194.3
bnlg1537	2	55.7	186.2	170.2	209.8	209.9
bnlg1036	2	88.7	134.0	165.6	165.9	165.7
bnlg1329	2	104	103.5	93.4	93.4	93.4
phi127	2	106	112.8	127.3	127.4	127.2
bnlg1662	2	111	134.2	160.9	160.9	160.9
bnlg1721	2	113	120.5	93.2	93.3	93.3
bnlg2144	2	118	175.0	127.9	127.9	127.9
bnlg1520	2	130	204.1	178.4	178.4	178.4
bnlg1638	3	46.3	195.7	107.9	108.1	108.1
bnlg1022	3	55.7	109.0	116.0	116.2	116.0
bnlg1257	3	105	229.2	231.2	229.2	229.1
NC130	5	3.5	150.2	148.7	148.7	148.7
bnlg105	5	50	92.4	94.5	94.5	1,879
bnlg1879	5	53.2	153.8	151.7	151.8	151.8
bnlg1046	5	55.6	237.8	216.0	216.3	216.0
bnlg1237	5	93.8	203.5	161.6	161.7	161.6
bnlg2305	5	118	218.0	190.3	190.2	190.2
bnlg1118	5	122	234.0	240.2	240.2	240.2
bnlg1346	5	125	181.5	183.4	183.5	183.4
bnlg1740	6	90.8	159.0	120.0	120.0	120.0
bnlg2132	7	13.1	254.2	223.5	223.5	223.5
bnlg1695	7	78.7	163.7	156.9	156.9	156.9
bnlg2259	7	86.8	203.3	173.5	173.3	173.4
phi051	7	110	146.8	148.8	148.8	148.9
phi116	7	114	181.6	177.5	177.5	177.4
bnlg2082	8	34.2	237.0	219.2	219.1	219.1
bnlg1834	8	35.9	210.2	216.2	216.2	216.2
bnlg1812	8	50.2	189.7	176.6	176.6	176.6
bnlg2181	8	53.7	200.3	188.3	188.4	188.3
bnlg244	9	33.3	144.8	190.2	190.2	190.2
phi061	9	40.8	92.9	84.9	84.9	84.8
phi032	9	58.4	240.9	234.0	234.0	234.0
bnlg1191	9	86	240.4	206.2	206.1	206.1
bnlg1839	10	83.9	192.0	184.0	183.9	183.9

Flints of the Northeastern United States (Galinat 1971; Revilla and Tracy 1995). The popcorns grouped outside of the flint/sweet cluster. Lo3, extracted from the Italian variety Nostrano dell'Isola, falls outside of the BSSS and both of the Lancaster groups, but is more associated with them than to H99 or W117. H99, one of the few maize inbreds that is regenerable in tissue culture, was selected out of the Illinois Synthetic 60C. MS71, out of A619×R168, and P-1 (Alberta flint, Midland yellow dent, Osterland Yellow Dent, Illinois long ear and Illinois two ear) may share lineage through the Illinois lines. ND246, an early maturing semident, lies equidistant from the major dent and flint groups, as expected. DK105, a German line selected out of Gelber Badischer Landmais, is clearly not from the same flint lineage as the Northern flints or the popcorns. F64, an Argentine line selected out of PI86223 in 1953, is distinct from all other entries.

Contrary to expectation, the entry P_B37 differed from B37 for 40 of 184 possible comparisons. Examination of the data for all entries showed that 37 of these 40 P_B37 SSR fragments have the same band size as that detected in B73 (Table 1). The close match with B73 and the absence of apparent heterozygosity in P_B37 suggest a B73 introgression event followed by selfing. While these data clearly indicate that B73 has contributed to P_B37, P_B37 has three SSR alleles that are present neither in B37 nor in B73. All of these occur in SSRs that have dinucleotide repeats. That these are simple dinucleotide repeats in B37 is reasonably certain because the DNA sequences within which these SSRs were originally detected came from a B37 source from the Plant Introduction Station at Ames, Iowa, as did the B37 source used in this study. The P_B37 allele for SSR 1257 is 2 bp larger than the allele for B37 and two sources of B73. This allele size, 231 bp, occurs only in P_B37, H84 and P_H84. The GE440 allele size for SSR 1257 is 183 bp. P_H84 descends directly from a cross made in Indiana between P_B37 and GE440. H84, the inbred conserved at the Plant Introduction Station, descends from P_H84. These data are consistent with the hypothesis that the P_B37 allele arose de novo after Purdue's acquisition of the original release of B37 in 1958 (Gracen 1986).

Table 2 Pedigree information for selected groups of maize inbreds. The date is the date of release unless otherwise indicated

Group	Name	Date	Pedigree
Iodent	P-2	1970s[a]	Iodent (29%), Midland Yellow Dent (13%), Minnesota 13 (11%), Southern US Landrace Synthetic (9%), Long Ear (9%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two Ear (6%)
	P-3	1970s[a]	Iodent (35%), Long Ear (12%), Minnesota 13 (12%), Osterland Yellow Dent (7%), SRS 303 (6%), Reid (6%)
	P-4	1970s[a]	Iodent (59%), Long Ear (20%), Minnesota 13 (13%), Troyer Reid (5%)
	P-5	1970s[a]	Iodent (59%), Long Ear (20%), Minnesota 13 (II%), Troyer Reid (5%)
	P-6	1970s[a]	Iodent (30%), Lancaster Low Breakage (13%), Long Ear (10%), Southern U.S. Landrace Synthetic (9%), Osterland Yellow Dent (9%), Minnesota 13 (7%), Funks G 4949 (6%)
B73	A679		[(A662/B73)B73(3)]
	B73	1972	(C5) of Iowa Stiff Stalk Synthetic (BSSS)
	N192		S5 line selected from (CM105/B73)
	NC268		(B73/NC250)B73
European Flint	F2	1950s	Lacaune
	F7	1950s	Lacaune
	EP1	1950s	Lizargarote
Popcorn	Sg1533	1960	Supergold
	HP72-11	1982	C103/(South American, Early Yellow)
	IDS28	1964	Amber Pearl
Sweet corn (*su1*)	IA2132	1953	(TSR/45)/4329, 45 from Bantam Evergreen
	P39		Purdue Bantam, from Bantam Evergreen
	IA453		[(P39/45)45(2)]
	IA4189	1890	Country Gentleman
	C13		Golden Early Market

[a] Year the inbred finished the breeding process

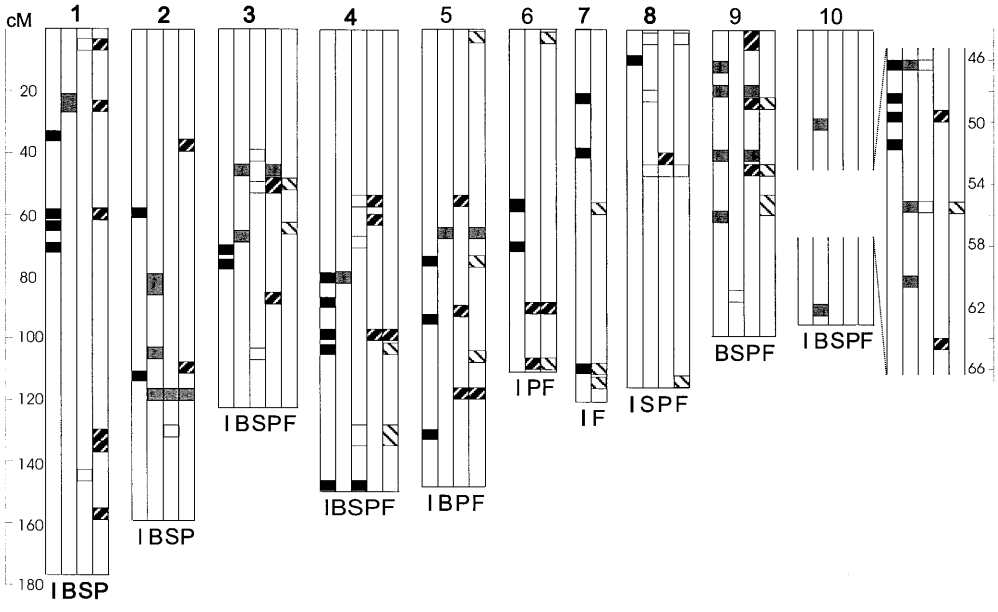

Fig. 3 Map positions of shared haplotypes and unlinked monomorphic SSR loci within groups of iodents (I, *black*), the B73 group (B, *gray*), sweet corns (S, *open*), popcorns (P, *white stripe on black*) and three European flints (F, *black stripe on white*). Filled boxes that cross groups at the same map position indicate a common allele for those groups (Table 3)

Shared haplotypes within related groups

Linked markers might offer a more rigorous test of line identity because segments of the haplotypes of the original progenitor population will persist in the inbreds extracted from it. Although Mendelian genetics predicts this outcome, a high rate of de novo allele generation might obscure the line of descent. The practice of using very small population sizes, often as few as 20 plants for seed increase, increases the probability that new alleles might be fixed by genetic drift. Only those SSRs for which data existed for all of the entries within a group are shown. The pedigrees of the B73 group suggest close relationships (Table 2). The pedigrees of the remaining groups do not necessarily indicate a close relationship among all group members, although the popcorns are

Table 3 Inicidence of shared alleles and shared haplotypes within the iodent (I), B73 (B), sweet corn (S), popcorn (P), flint corn (F) and stiff stalk (s) groups of maize inbreds. The columns show SSR name, chromosome (C), centimorgan distance (cM) , number of alleles detected (N), polymorphic index content (PIC), binned allele sizes (B) and group (G)

SSR	C	cM	N*	PIC*	B	G	SSR	C	cM	N	PIC	B	G	SSR	C	cM	N	PIC	B	G
phi056	1	6.1	9	0.74	248	P	*252*	4	68.3	12	0.68	154	S	*1252*	8	3.2	7	0.51	130	F
					251	S	*1784*	4	79.9	10	0.81	250	B						130	S
1179	1	23.7	9	0.69	212	B						254	I	*1194*	8	9.7	20	0.84	144	I
1014	1	24.5	10	0.79	169	B	*1189*	4	87.1	10	0.81	227	I	*phi119*	8	22.9	7	0.79	172	S
					153	P	*2162*	4	98.0	14	0.82	152	F	*1863*	8	42.9	16	0.84	123	P
1429	1	35.5	14	0.85	191	I	*DUP28*	4	98.0	14	0.82	131	I	*phi014*	8	45.8	3	0.56	298	F
2180	1	60.0	10	0.81	107	I	*2162*	4	98.0	14	0.82	152	P						298	P
					95	P	*phi093*	4	103.0	8	0.79	298	F						298	S
2238	1	64.4	18	0.89	196	I						292	I	*1131*	8	112.1	15	0.89	115	F
1811	1	70.7	14	0.87	197	I	*1917*	4	134.9	9	0.74	116	F	*1724*	9	0	13	0.85	103	P
1720	1	133.9	12	0.77	241	P						110	S	*1288*	9	4.8	9	0.75	118	P
1502	1	136.4	7	0.80	190	P	*1890*	4	137.8	10	0.76	142	F	*2122*	9	12.3	15	0.89	254	B
1347	1	144.1	11	0.78	87	S						136	S	*phi033*	9	19.4	7	0.32	258	s
1055	1	158.3	23	0.93	242	P	*589*	4	148.0	7	0.62	176	I						258	P
1302	2	36.6	13	0.81	147	P						156	S	*phi017*	9	23.7	4	0.68	106	F
2248	2	59.0	12	0.78	204	I	*NC130*	5	3.5	8	0.80	149	F						114	P
1018	2	81.6	15	0.85	130	B	*1046*	5	55.6	20	0.90	194	P	*phi061*	9	40.8	4	0.53	85	B
1831	2	84.3	10	0.76	186	B	*1208*	5	66.1	11	0.73	128	B						85	P
1329	2	103.6	14	0.85	93	B						128	F	*phi065*	9	45.6	5	0.67	158	F
phi090	2	109.5	8	0.51	140	P	*1287*	5	74.1	4	0.65	158	F						138	P
1746	2	112.5	15	0.85	137	I						160	I	*1714*	9	55.5	13	0.81	205	F
2144	2	118.2	7	0.62	128	B	*1885*	5	91.1	17	0.88	256	P	*1884*	9	58.4	14	0.82	195	F
					128	P	*1237*	5	93.8	11	0.65	160	I	*phi032*	9	58.4	4	0.60	234	F
					128	S	*phi101*	5	105.8	6	0.69	99	F	*1209*	9	60.1	6	0.64	184	s
1520	2	130.0	11	0.75	187	S	*phi085*	5	117.2	12	0.73	261	F	*1191*	9	86.0	17	0.90	179	S
1447	3	41.7	13	0.83	110	S						261	P	*1451*	10	30.3	5	0.61	114	B
1638	3	46.3	11	0.76	108	B	*118*	5	131.0	6	0.70	111	I	*1037*	10	46.2	13	0.79	80	B
					108	P	*1371*	6	3.9	18	0.82	87	F	*1079*	10	46.2	12	0.84	174	I
2047	3	50.8	6	0.65	148	F	*1702*	6	57.5	19	0.77	171	I	*1037*	10	46.2	13	0.79	160	S
			6	0.65	144	P	*phi078*	6	58.5	7	0.68	133	I	*1712*	10	46.2	7	0.81	89	S
phi053	3	51.4	5	0.64	169	P	*1732*	6	70.2	8	0.79	100	I	*1655*	10	48.6	12	0.81	150	I
					194	S	*1740*	6	90.8	11	0.85	175	F	*phi050*	10	49.8	5	0.60	93	B
1449	3	63.9	8	0.78	156	F						175	P						93	I
1796	3	67.2	8	0.69	148	B	*phi089*	6	108.3	6	0.70	100	F						91	P
1601	3	68.1	16	0.88	214	I						93	P	*1526*	10	51.9	12	0.77	125	I
1931	3	76.1	8	0.62	174	I	*1292*	7	22.7	5	0.61	268	I	*1074*	10	55.8	6	0.74	186	B
1108	3	88.1	13	0.81	120	P	*1094*	7	39.3	17	0.88	172	I						180	F
1257	3	105.1	13	0.88	183	S	*1305*	7	58.0	10	0.58	160	F						180	S
1265	4	54.7	18	0.89	219	P	*phi051*	7	109.6	6	0.63	151	F	*1185*	10	60.6	6	0.50	142	B
					205	S						149	I	*1028*	10	64.6	6	0.77	161	P
1937	4	62.8	9	0.78	173	P	*phi116*	7	113.8	8	0.79	171	F	*1380*	10	90.4	5	0.67	138	B

[a] N=total number of alleles for all 57 entries. PIC value includes data for all entries

thought to have a narrow genetic base (Kantety et al. 1995).

The popcorns had the highest incidence of monomorphic alleles (31), followed by Iodents (26), northern flints (26), the B73 group (19), the sweet corns (19) and the Lancaster Surecrop/Oh43 group (1). The incidence of monomorphic SSRs within groups occurred regardless of the PIC value of the SSRs (Table 3). PIC values take into account the number of alleles and the relative frequencies of those alleles. Low PIC values indicate a high frequency of one or two alleles. Thus SSRs with low PIC values might be monomorphic within a cluster due to chance. The markers bnlg1055 and bnlg1046 detected 23 and 20 alleles, respectively, but were monoallelic within the popcorn group. The markers phi056 and phi053 have low PIC values, as might be expected for SSRs occurring within coding sequences. However, for these two markers, the popcorn and sweet corn groups are monoallelic for different alleles. Monoallelism for different alleles across groups also occurred in four other SSRs within coding sequences: phi089, phi051, phi033, phi065 and phi050. We also found that the entire stiff stalk group was monoallelic for phi033 and bnlg1209 on chromosome 9. The sweet, pop and flint corns all showed the same band size for phi014, but the entire data set showed only three band sizes (291, 292 and 298) in frequencies of 0.42, 0.07 and 0.51, respectively.

The Lancaster Surecrop/Oh43 group (Va26, Mo17, A682 and A619) was monomorphic only for bnlg1292 on chromosome 7. Thus no shared haplotypes were detected. Shared haplotypes occurred frequently in the other groups (Fig. 3). The Iodents showed shared haplotypes on chromosomes 1, 3, 4 and 10. The B73 group, popcorns and flint corns showed extensive haplotype-sharing on chromosome 9 while the Iodents showed none at all for this chromosome. There was a high inci-

dence of shared haplotypes within a 20-cM region on chromosomes 3 and 10. Only the Iodents and the flints had shared haplotypes on chromosome 7. The B73 group forms a tight cluster but does not show haplotype sharing on chromosomes 6, 7 and 8. Pedigree notation does not include descriptons of the actual breeding process. Random mating in the F_2 generation, for example, tends to break up haplotypes by exchanging alleles through recombination, without loss of parental alleles in the population. If selection favors the new combinations, a derived inbred may inherit many alleles from a superior parent, without retaining many of superior parent's haplotypes.

Derivation of the European flints

Dubreuil and Charcosset (1999) found that the European heterotic group, which includes F2, F7 and EP1, is closer to 'Compton's Early', a Northern flint line, than to the Reid Yellow Dents, represented by B73, B37 and other US corn belt dents. In this study, F2, F7 and EP1 grouped next to CO109. CO109 was extracted from Early Butler, another Northern flint. Even though EP1 derives from a different population, the cluster analysis and the shared haplotypes with F2 and F7 suggest that the founder populations (Lacaune and Lizargarote) may share common ancestors. DK105, already shown to be distinct from Lacaune derivatives in RFLP studies (Messmer et al. 1992a, b), is clearly distinct from EP1 as well. The US sweet corn lines, representing the traditional *sugary1* sweet corns, are more closely related to F23, F7 and EP1 than is DK105. Evidence of distinct lineages within the European flints is consistent with documented multiple introductions of flint corn from North, Central and South America since the time of the European discovery of the New World (Gracen 1986).

Inbred identity, pedigree analysis, and essential derivation

In maize germplasm development, the pedigrees of interest involve recent ancestry rather than ancient relationships. The abundantly polymorphic SSR markers can easily distinguish closely related inbred lines and detect pedigree relationships that may not be evident either in the phenotype or in written records. However, the polymorphism that permits these distinctions may compromise the identity of an inbred line if identity is too strictly defined. The presence of a few SSR band size differences between different sources of the same inbred may be due to nongenetic variance (DNA extraction, PCR reaction or data acquistion problems). Alternatively, minor differences may arise from residual heterozygosity in the original release of the inbred, incidental pollination with a closely related sister line, or de novo mutations in the SSR allele that subsequently became fixed. Actual population sizes for inbred-increase may be small enough in some cases to permit fixation of de novo alleles due to genetic drift alone. Because minor differences are difficult to explain without an analysis of remnant seed from every seed increase ever made of the inbreds in question, the identity of a maize inbred should not be based on the expectation that different inbred seed sources will show identical PCR products for any specific SSR marker or for 100% of any group of SSR markers.

Similar selection indices may result in independent fixation of the same allele in different inbreds. For example, the B73, popcorn and sweet corn inbreds included in this study all show the same band size (128 bp) for bnlg2144. Pedigree analysis based on a large set of well-distributed SSR markers mitigates against this potential bias. All of the data are used to generate all possible pairwise distance-measures between all of the entries. Distance-measures ignore the effect of genetic linkage. Recombination erodes linkage disequilibrium, but does not affect identity by descent for a given allele. The BSSS inbreds included in this study are an excellent example of how distance-measures can reveal descent from common progentitors regardless of multiple generations of intermating and introgression. In contrast, shared haplotypes arise through the transmission of unrecombined DNA through the gametes of a recent common ancestor. Shared haplotypes can permit detection of essential derivation, a circumstance in which inbred lines are extracted directly from the population produced by selfing a single hybrid. The most-telling evidence of essential derivation, as defined above, is not the existance of a shared haplotypes per se, but the detection of a rare shared haplotype. If a protected inbred possesses a haplotype that is clearly different from the haplotypes existing in related public lines, then the appearance of that haplotype in a subsequently developed inbred is likley to be due to essential derivation.

For a given SSR allele, identity in state may not result from identity by descent. Size homoplasy (PCR products of the same size) can arise from independent mutations. In our next study, we will sequence SSR primer products from groups of related and unrelated inbreds to estimate the incidence of size homoplasy.

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