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Phylogenetic Relationships among North American Popcorns and Their Evolutionary Links to Mexican and South American Popcorns

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Abstract
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Phylogenetic Relationships among North American Popcorns and Their Evolutionary Links to Mexican and South American Popcorns

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ABSTRACT

To determine genetic relationships among representative popcorns (*Zea mays* L.) of the New World, 56 maize populations from the USA and nine Latin American countries were characterized for 29 morphological traits, 18 isozyme loci, and 31 SSR loci. Cluster and principal component analyses were performed upon standardized morphological data and allelic frequencies from isozyme and SSR loci to elucidate relationships among populations within a geographical and historical context. Three groups of popcorn, with distinctive morphological characteristics and genetic profiles, were identified in the North American populations. The first group includes the North American Yellow Pearl Popcorns, which are currently the most important for U.S. commercial production. This group could be derived from introductions of the race Curagua from Chile into New England in the 19th Century. The second group includes the North American Pointed Rice Popcorns, which probably originated from the complex of traditional races of pointed popcorns from Latin America, such as Palomero Toluqueño, Confite Puntiagudo, Canguil, and Pisankalla, which diffused from the highlands of central Mexico into northern Mexico and then into southwestern USA. The third group includes the North American Early Popcorns, which show a marked influence of Northern Flint maize, from which they probably acquired the trait of early maturity. This third group also shows genetic influences of maize from northwestern Mexico and even from early European varieties of popcorn introduced late in the 19th Century. We propose that the three groups of North American popcorn identified in this study be recognized taxonomically as distinct races, and we provide characteristic traits as well as isozyme and SSR alleles to define the new races.

Popcorn is an important commercial crop in the USA, and its importance has been steadily increasing for decades (Ziegler, 2001). The Corn Belt states of Indiana, Nebraska, Illinois, Ohio, and Iowa account for nearly 90% of the area planted to popcorn in the USA. By the time Columbus arrived in the New World, many types of corn, including popcorn, had been incorporated into agricultural systems by several Native American tribes in the lands now comprising the USA. Smith (1999) documented the use of parched and popped corn by Native Americans in Canada and the USA, but noted the almost total absence of evidence of popcorn in eastern North America, in contrast to the southwestern USA, where popcorn was clearly cultivated in ancient times. Recent studies by Matsuoka et al. (2002) strongly support the hypothesis that popcorn is indeed ancient and basal in the domestication history of maize, as some Mexican popcorn races, such as Cristalino de Chihuahua, Palomero de Chihuahua, and Palomera Toluqueño, grouped immediately after teosinte populations in a phylogram constructed from microsatellite data.

Popcorn increased in importance in the USA during and after colonial times, such that, by the mid-19th Century it was a popular snack food (Pollak and White, 1995). Incipient identification of popcorn varieties began by that time, when varieties, such as Blue Pop, Yellow Pop, White Pop, and Ladyfinger, were first mentioned, and Smith (1999) citing the 1875 American Cyclopedia reported that "several varieties are known as pop-corn, of which there are white and yellow kinds, those with kernels pointed at the ends and others with the grain of the ordinary shape." Late in the 19th Century, Sturtevant (1899) described a group of 25 popcorn varieties in the USA. During the last two decades of the 19th Century, popcorn became a prominent commercial crop in the USA. It was no longer grown only in gardens, and its cultivation became an authentic industry. In 1912, there were 7700 ha under commercial production (Pollak and White, 1995). By 1925, the pointed type White Rice was the most important popcorn variety, although others such as the rounded or pearl type Queen's Golden and the pointed kernel Japanese Hulless were also used in commercial production. A few years later, Queen's Golden was replaced by the pearl types South American, Supergold, and Yellow Pearl (Ziegler, 2001). The first commercial hybrid of popcorn was released in 1934 in Minnesota and consisted of a single cross of two closely related inbred lines derived from Japanese Hulless. The first popcorn hybrids for the central U.S. Corn Belt were developed early in the 1940s, and, by the late 1940s, open-pollinated varieties had been replaced by those hybrids (Ziegler, 2001).

Despite its inherent genetic diversity, the systematics of popcorns grown in the USA has received little attention (Goodman and Brown, 1988). Thus, the objectives of this study were (i) to categorize popcorn populations into groups characterized by distinctive morphological patterns and allelic frequencies of selected molecular markers and (ii) to investigate possible associations among popcorn populations, or groups of populations, from the USA and those from Mexico and South Amer-

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Abbreviations: NCRPIS, North Central Regional Plant Introduction Station; PCR, polymerase chain reaction; SSR, simple sequence repeat.

ica through analyses of variability in morphology, isozymes, and DNA polymorphisms.

MATERIALS AND METHODS

In 1998 and 1999, morphological characterization was conducted on a group of popcorn populations grown at the NCRPIS at Ames, IA. In 1998, 144 populations of popcorn from the USA, Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Mexico, and Paraguay were characterized. These populations were supplied by the NCRPIS, the Popcorn Breeding Program of Iowa State University through Kenneth Ziegler, the Committee for Agricultural Development of Iowa State University, and the McHone Popcorn Seed Company, Ames, IA. Populations were planted in a 12 by 12 square lattice experimental design with two replications. For the second year of morphological data collection, a subset of 56 populations from those evaluated in 1998 was cultivated again for further study (Table 1). The subset was selected to eliminate redundant entries, contaminated seed lots, and populations initially misidentified as popcorn, while at the same time incorporating the most representative groups of popcorn from both the USA and Latin America. The experimental design used was a randomized complete block design with two replications, following the same methodology as in 1998.

Table 2 shows the morphological characters measured on 10 competitive plants per population each year. Five plants from each replication were chosen and marked early in the growing season, avoiding those at row ends and those without a competitive neighbor. The morphological characters measured were selected on the basis of high heritability and repeatability, as reported by Sánchez González et al. (1993) and Ortiz and Sevilla (1997). For measurement of popping characteristics, kernels were put into cotton bags, then placed for 6 wk into a conditioning chamber where temperature was constantly maintained at 21°C and relative humidity at 70%, reaching an equilibrium moisture content of approximately 135 g kg^{-1}, which

Table 1. Populations included in this study (ordered in correspondence with their placement in Fig. 1) and their country of origin.

Name	PI Number	Country	Race
Maiz Chapalote	PI 217409	Mexico	Chapalote
Chapalote Acc. 9	PI 420245	USA	Chapalote
PG No. 10	PI 303850	Chile	Possibly Curagua
Unselected Little Red Flint		Uruguay	Latin
Little Red Flint		Uruguay	Latin
Uruguay 633	PI 511635	Uruguay	Cateto Sulino
Chihuahua 129	PI 484404	Mexico	Cristalino de Chihuahua
Chapalote 35 Acc. 35	NSL 2833	Mexico	Chapalote
Maiz Reventador	PI 420244	Mexico	Reventador
B73 × Mo17	Ames 19097	USA	Corn Belt Dent
ARZM 13 073	PI 492781	Argentina	Perla Mediano
Argentine Pop	PI 217404	USA	Latin
Brazil 2785	PI 490813	Brazil	Avatí Pichinga
Brazil 2823	PI 503889	Brazil	Avatí Pichinga Ihu
ARZM 04 014	PI 491812	Argentina	Perlita
Pichinga Redondo	PI 472117	Paraguay	Pichinga
CHZM 07 097	PI 477471	Chile	Curagua
Nebraska Supergold × Tom Thumb	Ames 14285	USA	North American Yellow Pearl Popcorn
Ladyfinger	PI 217407	USA	Undefined
Nebraska Yellow Pearl	Ames 14287	USA	North American Yellow Pearl Popcorn
Yellow Pearl Pop	PI 311254	USA	North American Yellow Pearl Popcorn
Amber Pearl	Ames 14274	USA	North American Yellow Pearl Popcorn
Sg 1533	PI 587132	USA	North American Yellow Pearl Popcorn
Supergold	Ames 14283	USA	North American Yellow Pearl Popcorn
Supergold Popcorn	Ames 21962	USA	North American Yellow Pearl Popcorn
Ohio Yellow	Ames 14279	USA	North American Yellow Pearl Popcorn
South American Pop	Ames 14281	USA	North American Yellow Pearl Popcorn
Iopop 12	Ames 24574	USA	North American Yellow Pearl Popcorn
IDS28	Ames 24575	USA	North American Yellow Pearl Popcorn
Red Pop	Ames 14280	USA	North American Yellow Pearl Popcorn
HP301	PI 587131	USA	North American Yellow Pearl Popcorn
IDS91	Ames 24577	USA	North American Yellow Pearl Popcorn
IDS69	Ames 24576	USA	North American Yellow Pearl Popcorn
Fairfax Brown	PI 213756	USA	Southwestern U.S. Group
Tama Flint	PI 217411	USA	Northern Flint
Spanish Pop	PI 311249	USA	North American Early Popcorn
Black Beauty Acc. 50	PI 317680	USA	North American Early Popcorn
Black Beauty Acc. 52	PI 452061	USA	North American Early Popcorn
Carnival	PI 219873	USA	North American Early Popcorn
North Dakota Tom Thumb	PI 269757	USA	North American Early Popcorn
Tom Thumb Pop	PI 213701	USA	North American Early Popcorn
Pinky Popcorn	PI 452064	USA	North American Pointed Rice Popcorn
White Rice	Ames 14284	USA	North American Pointed Rice Popcorn
Japanese Hulless	Ames 14277	USA	North American Pointed Rice Popcorn
Illinois Hulless	NSL 5780	USA	North American Pointed Rice Popcorn
Golden Australian Hulless	PI 452063	USA	North American Pointed Rice Popcorn
Bearclaw	Ames 7998	USA	North American Pointed Rice Popcorn
Black Beauty Acc. 46	Ames 14276	USA	North American Pointed Rice Popcorn
R-Strawberry Open Pollinated	PI 340840	USA	North American Pointed Rice Popcorn
White Rice Pop	PI 311250	USA	LatPointed
ARZM 06 073	PI 492073	Argentina	Pisingallo
Acoma Pueblo	PI 218140	USA	LatPointed
Chihuahua 150	PI 484424	Mexico	Palomero Toluqueño
W-C 990	PI 390552	Ecuador	Canguil
No. 1	PI 240318	Bolivia	Pisankalla
Cuzco 31	PI 571899	Peru	Confite Puntiagudo

Table 2. Morphological characters evaluated.

Traits
Vegetative and phenological traits: plant height (cm), ear height (cm), number of leaves, number of tillers per plant, leaf length (cm), leaf width (cm), days to pollen shed, days to silk, anthesis-silking interval (days).
Tassel traits: tassel length (cm), peduncle length (cm), branched part length (cm), central spike length (cm), number of primary branches, peduncle length/tassel length, central spike length/tassel length.
Ear traits: number of ears per plant, ear length (cm), ear diameter (cm), number of kernel rows, ear length/ear diameter.
Kernel traits: length (mm), width (mm), thickness (mm), number of kernels per 10 g, length/width, width/thickness.
Popping quality: popping expansion (cm^3/30 g), type of flake (numerical rating 1–5).†

† 1 = Very small flake: kernels just split with a little puffed endosperm; 2.5 = Mushroom: round ball type flake; 5 = 100% butterfly flake.

has been determined to be optimal for maximizing popping expansion (Metzger et al., 1989). A sample of 30 g of kernels from each replication was popped in a microwave oven and the volume of product measured in a 2000 mL graduated cylinder, 8.89 cm in diameter.

The subset of 56 populations characterized for morphological traits in 1999 was evaluated for isozyme polymorphisms, using the inbred lines B73, Mo17, Tx303, and Mo24W as checks. An array of 18 well-characterized isozyme loci (Table 3), distributed among eight of the 10 maize chromosomes, was selected for evaluation. Enzymes from 10 plants of each population were extracted from the coleoptiles of 9-d-old seedlings, subjected to starch gel electrophoresis and stained according to the protocols described in detail by Stuber et al. (1988), except for *Acp4*, where scoring was based on guidelines provided by Kahler (1983).

DNA from 10 additional plants of each of the 56 populations sampled for isozyme analysis was used for the amplification of 31 SSR loci (microsatellites) distributed throughout all 10 chromosomes of maize. These loci are documented on the Internet (Lawrence et al., 2004; Maize Genetics and Genomics Database, 2004) at http://www.maizegdb.org/ssr.php#. A list of the loci evaluated, chromosomal location (Bin number) and associated primers flanking the repeats is shown in Table 4. This group of primers is used by the Maize Breeding Program at Iowa State University and was provided by Dr. Kendall Lamkey, Iowa State University Agronomy Department, through Dr. Joanne Labate from the Institute for Genomic Diversity at Cornell University. Genomic DNA was extracted from 30 mg of fresh, young leaf tissue with a PUREGENE DNA Isolation Kit (Gentra Systems, 1998). For quantification of DNA concentration, readings of the absorbance at 260 and 280 nm were performed with a Power Wave$_X$ microplate scanning spectrophotometer (Bio-Tek Instruments, Winoosi, VT).

Table 3. Isozyme loci evaluated, gel systems and chromosomal location.

Enzyme	Bin†	Symbol	Optimal gel system‡
Acid phosphatase-1	9.03	*Acp1*	B
Acid phosphatase-4	1.11-1.12	*Acp4*	B
Alcohol dehydrogenase-1	1.10	*Adh1*	C
Catalase-3	4.19	*Cat3*	C
β-glucosidase-1	10.03	*Glu1*	B
Glutamic-oxaloacetic transaminase-1	3.08	*Got1*	C
Glutamic-oxaloacetic transaminase-2	5.08	*Got2*	C
Glutamic-oxaloacetic transaminase-3	5.03-5.04	*Got3*	C
Isocitrate dehydrogenase-1	8.05-8.06	*Idh1*	D
Isocitrate dehydrogenase-2	6.07	*Idh2*	D
Malate dehydrogenase-1	8.03	*Mdh1*	A,B
Malate dehydrogenase-2	6.07	*Mdh2*	A,B
Malate dehydrogenase-3	3.08	*Mdh3*	A,B
Malate dehydrogenase-4	1.07-1.08	*Mdh4*	A,B
Malate dehydrogenase-5	5.03	*Mdh5*	A,B
Phosphohexose isomerase-1	1.11	*Phi1*	B
6-Phosphogluconate dehydrogenase-1	6.01	*Pgd1*	D
6-Phosphogluconate dehydrogenase-2	3.05	*Pgd2*	D

† The Bin number is an interval between two fixed core maize-genomic marker loci, and includes the top marker on the map bins (http://www.agron.missouri.edu/).
‡ According to Stuber et al. (1988).

PCR amplifications were performed in a PTC-100 (MJ Research, Watertown, MA) thermocycler. Primers were amplified in multiplex groups, as indicated in Table 4. Multiplexing was designed to create combinations in a way that the sizes of the PCR products and the labeling of primers allowed the differentiation of banding patterns without interference among markers. Forward primers were 5′ fluorescently labeled with one of the ABI Prism dyes (PE Applied Biosystems, Foster City, CA): HEX, 6-FAM, or NED.

The protocol for the PCR amplification consisted of an initial denaturation time of 4 min at 95°C, followed by 25 cycles of 1 min at 95°C (denaturation), 2 min at 55°C (annealing) and 2 min at 72°C (extension), and a final step of 1 h of further extension at 72°C. Each individual PCR amplification reaction consisted of 2 μL of 10× PCR buffer (500 m*M* KCl, 100 m*M* Tris-HCl, pH 9.0 at 25°C), 0.4 μL of 10 m*M* dNTPs (2.5 m*M* each dNTP), 1.2 μL of 25 m*M* $MgCl_2$, 0.2 μL of Taq DNA polymerase (1 unit total), 2.5 μL of template DNA (10 ng μL^{-1}), 2 μL of 4 p*M* of each primer pair (1 μL each, forward and reverse), and 11.7 μL of ddH_2O.

PCR products were electrophoresed in polyacrylamide gels with ROX-500 internal size standards at the DNA Sequencing and Synthesis Facility of Iowa State University on an ABI Prism 377 DNA sequencer. Data files were generated by ABI GeneScan software, and scored for allelic variability by using STRand software (Locke et al., 2000).

Standard analyses of variance were performed for the morphological traits by SAS (SAS Institute, 1985). Allelic frequencies were calculated for each popcorn population for both isozyme and SSR loci through POPGENE (Population Genetic Analysis) software, Version 1.32 (Yeh et al., 1999). Matrices of isozyme and SSR allelic frequencies were standardized by subtracting the mean and dividing by the standard deviation; then they were combined with the matrix of standardized morphological characters to construct a pooled dataset. This single dataset was used to calculate pairwise Gower distances between popcorn populations and to perform cluster analysis by the Neighbor-Joining method (Saitou and Nei, 1987), utilizing Genetic Data Analysis (GDA) software, Version 1.0 (Lewis and Zaykin, 2001). A phylogenetic tree was rendered with TREEVIEW software, Version 1.6.1 (Page, 1996) with Chapalote selected as an outgroup, because Chapalote is considered one of the most ancient races of maize, as indicated by the archaeological record (Mangelsdorf, 1974).

Allelic frequencies, in combination with morphological information, were used to construct a combined intercharacter correlation matrix. The correlation matrix, with each morphological character and each allele considered as an independent variable, was subjected to a principal component analysis so as to produce standardized principal component scores for each population with SAS software (SAS Institute, 1985).

Table 4. Primers used for the amplification of simple sequence repeat loci.

Multiplexing group	Locus	Bin number	Repeat	Allelic range (base pairs)	Fluorescent label-forward primer//reverse primer
1	phi127	2.07	GTGC	105–126	NED-ATATGCATTGCCTGGAACTGGAAGGA//AATTCAAACACGCCTCCCGAGTGT
	phi051	7.06	AGG	136–154	6-FAM-GCGAAAGCGAACGACAACAATCTT//ACATCGTCAGATTATATTGCAGACCA
	phi115	8.03	ATAC	292–312	HEX-GCTCCGTGTTTCGCCTGAA//ACCATCACCTGAATCCATCACA
	phi015	8.08	TTTG	76–113	HEX-GCAACGTACCGTACCTTTCCGA//ACGCTGCATTCAATTACCGGGAAG
	phi033	9.02	CCT	224–270	6-FAM-ATCGAAATGCAGGCGATGGTTCTC//ATCGAGATGTTCTACGCCCTGAAGT
2	phi053	3.05	ATGT	169–213	NED-CTGCCTCTCAGATTCAGAGATTGAC//AACCCAACGTACTCCGGCAG
	phi072	4.01	CAAA	134–163	6-FAM-GTGCATGATTAATTTCTCCAGCCTT//GACAGCGCGCAAATGGATTGAACT
	phi093	4.08	CTAG	272–296	NED-GTGCGTCAGCTTCATCGCCTACAAG//CCATGCATGCTTGCAACAATGGATACA
	phi024	5.00	CCT	354–376	HEX-CTCCGCTTCCACTGTTCCA//TGTCCGCTGCTTCTACCCA
	phi085	5.06	GCGTT	233–266	6-FAM-AGCAGAACGGCAAGGGCTACT//TTTGGCACACCACGACGA
	phi034	7.02	CCT	123–160	HEX-TAGCGACAGGATGGCCTCTTCT//GGGGAGCACGCCTTCGTTCT
	phi121	8.04	CCG	93–105	6-FAM-AGGAAAATGGAGCCGGTGAACCA//TTGGTCTGGACCAAGCACATACAC
3	phi056	1.01	GCC	231–278	NED-ACTTGCTTGCCTGCCGTTAC//CGCACACCACTTCCCAGAA
	phi064	1.11	ATCC	75–121	HEX-CGAATTGAAATAGCTGCGAGAACCT//ACAATGAACGGTGGTTATCAACACGC
	phi050	10.03	AAGC	77–87	NED-AACATGCCAGACACATACGGACAG//ATGGCTCTAGCGAAGCGTAGAG
4	phi96100	2.00-2.01	ACCT	218–300	6-FAM-AGGAGGACCCCAACTCCTG//TTGCACGAGCCATCGTAT
	phi101249	?	AGAT	114–161	NED-TTCCTCCTCCACTGCCTC//AAGAACAGCGAAGCAGAGAAGG
	Phi109188	?	AAAG	148–172	HEX-AAGCTCAGAAGCCGGAGC//GGTCATCAAGCTCTCTGATCG
5	phi029	3.04	AG-AGGG	139–176	NED-TCTTTCTTCCTCCACAAGCAGCGAA//TTTCCAGTTGCCACCGACGAAGAACTT
	phi073	3.05	AGC	186–203	HEX-GTGCGAGAGGCTTGACCAA//AAGGGTTGAGGGCGAGGAA
	phi96342	10.XX	ATCC	223–256	6-FAM-GTAATCCCACGTCCTATCAGCC//TCCAACTTGAACGAACTCCTC
	Phi109275	?	AGCT	121–144	6-FAM-CGGTTCATGCTAGCTCTGC//GTTGTGGCTGTGGTGGTG
6	Phi427913	1.XX	ACG	117–207	NED-CAAAAGCTAGTCGGGGTCA//ATTGTTCGATGACACACTACGC
	Phi265454	1.10-1.11	AGG	190–240	6-FAM-CAAGCACCTCAACCTCTTCG//TCCACGCTGCTCACCTTC
	Phi402893	2.00	AGC	205–243	HEX-GCCAAGCTCAGGGTCAAG//CACGAGCGTTATTCGCTGT
7	Phi346482	1.XX	AGG	103–141	HEX-GCATCACACTTCACACAACAA//GTGGAATAGGAGGCGAGAGAGG
	Phi308090	4.04-4.05	AGC	190–226	6-FAM-CAGTCTGCCACGAAGCAA//CTGTCGGTTTCGGTCTTCTT
	Phi330507	5.02-5.06	CCG	128–161	NED-GTAAAGTACGATGCGCCTCCC//CGGGGTAGAGGAGAGTTGTG
8	Phi213398	4.01-4.04	ACC	287–320	6-FAM-GTGACCTAAACTTGGCAGACCC//CAAGAGGTACCTGCATGGC
	Phi339017	5.XX	AGG	138–159	HEX-ACTGCTGTTGGGGTAGGG//GCAGCTTGAGCAGGAAGC
	Phi159819	6.00-6.08	CCG	119–139	6-FAM-GATGGGCCCTAGACCAGCTT//GCCTCTCCCATCTCTCGGT

Discriminant analysis was applied using the pooled covariance matrix from the first 12 principal components derived from the combined dataset with SAS software. This analysis generated a discriminant function from the populations preliminarily assigned into groups through cluster analysis based on results of the morphological characterization. Afterwards, the function was applied to corroborate group membership or to assign the population into a new group. Each population was placed in the group from which it had the smallest generalized squared distance, so that the preliminary assignment did not guarantee the subsequent membership into a certain group (SAS Institute, 1985).

RESULTS AND DISCUSSION

There were significant differences among accessions for each of the 29 morphological characters measured, as indicated by analyses of variance (Table 5). In fact, the source of variation corresponding to accessions was by far the most important in 23 of the 29 traits evaluated, whereas differences attributable to years were the second most important source of variation, predominating in the remaining six traits (days to pollen shed, days to silk, kernel thickness and the ratios kernel width/thickness, tassel peduncle length/tassel length, and central spike length/tassel length).

In situations where data from different sources are assembled for comprehensive analysis, a method to integrate diverse lines of evidence must be defined. One of the most accepted approaches is to analyze simultaneously all available character data, which is often referred to as the total evidence or character congruence approach (Kluge, 1989). There has been, however, some disagreement about the suitability of this approach; Bull et al. (1993) and de Queiroz (1993) argue that it is not completely appropriate to perform a combined analysis in datasets where the patterns of classifications from individual datasets are significantly different from one another because different groups of characters may have evolved following different evolutionary rates and constraints. In contrast, other authors, including Chippindale and Wiens (1994), strongly support this type of analysis, claiming that differences in the modes and rates of evolution in different characters could be more easily accommodated in the context of a joint data analysis. Moreover, when molecular and morphological data are involved, the combination of those datasets provides a truly comprehensive view for the classification of populations (Hillis, 1987; Kluge, 1989). Additionally, supporters of the combined approach do not discourage the use of separate analyses for each data set. In this paper, results of the analysis of a combined dataset from morphological characters, isozymes and SSRs are reported, but initial analyses of the three separate character sets were performed and discussed in Santacruz-Varela (2001).

Figure 1 shows a phylogenetic tree resulting from the application of the Neighbor-Joining method to the matrix of interpopulational, pairwise Gower distances based on 29 morphological characters, 58 isozyme alleles, and 191 SSR alleles. Figure 1 broadly defines five groups of populations, with subdivisions within one of the clusters. Group I includes populations from Chile, Uruguay, and north and northwestern Mexico belonging to typical races of popcorn as well as flint-type populations with low popping capacity. Group II contains popcorn populations from Argentina, Brazil, and Paraguay, and it includes Argentine Pop, a population introduced from Argentina into the USA by Edgar Anderson some 50 yr

Table 5. Mean squares from the analysis of variance for 29 morphological traits measured over the maize populations involved in this study†.

Trait	Sources of variation				
	Years	Reps within years	Accessions	Accessions × years	Error
Plant height	239.62 NS	1 590.87**	16 967.31**	340.11 NS	247.53
Ear height	418.96 NS	927.34**	9 818.68**	179.37 NS	138.75
Number of leaves	5.98**	2.92**	79.65**	0.78**	0.30
Tillers per plant	0.49 NS	0.36 NS	7.49**	0.47 NS	0.37
Leaf length	229.70**	18.61 NS	860.78**	23.62*	14.77
Leaf width	0.01 NS	0.25 NS	9.83**	0.26 NS	0.21
Days to pollen shed	5 460.07**	50.00**	564.38**	12.81**	4.73
Days to silk	4 963.75**	61.87**	737.34**	12.57**	6.55
Anthesis-silking interval	11.82**	0.93 NS	46.86**	3.20**	1.21
Tassel length	10.78 NS	9.91 NS	385.74**	15.84**	9.03
Peduncle length	85.56**	12.98 NS	100.39**	8.71**	5.10
Branched part length	0.90 NS	2.13 NS	75.36**	1.43 NS	1.81
Central spike length	25.70**	1.50 NS	106.92**	5.74**	3.10
Number of primary branches	0.01 NS	12.51 NS	263.75**	6.85 NS	5.87
Peduncle length/tassel length	2.7×10^{-3}**	1.0×10^{-3}**	2.5×10^{-3}**	1.0×10^{-3}**	7.0×10^{-4}
Central spike length/tassel length	13.9×10^{-3}**	1.0×10^{-3} NS	7.0×10^{-3}**	1.0×10^{-3}**	7.0×10^{-4}
Ears per plant	0.09 NS	1.14 NS	7.65**	0.66*	0.45
Ear length	10.73**	0.22 NS	61.66**	1.84**	0.81
Ear diameter	0.15**	0.06*	1.14**	0.03**	1.7×10^{-3}
Number of kernel rows	0.36 NS	1.59 NS	63.17**	1.48*	0.89
Ear length/ear diameter	2.45**	0.10 NS	5.49**	0.19**	0.10
Kernel length	0.08 NS	0.24*	6.94**	0.15**	0.08
Kernel width	0.12 NS	0.13 NS	6.21**	0.09*	0.06
Kernel thickness	3.56**	0.06 NS	0.80**	0.09**	0.04
Number of kernels per 10 g	303.03*	205.36 NS	7 358.59**	669.23**	68.00
Kernel length/width	6.0×10^{-4} NS	3.3×10^{-3} NS	0.31**	7.9×10^{-3}**	3.6×10^{-3}
Kernel width/thickness	0.37**	1.0×10^{-3} NS	0.30**	0.01**	6.6×10^{-3}
Popping expansion	99 556.36**	20 196.36 NS	518 210.97**	33 915.62**	9 003.77
Type of flake	0.77*	0.27 NS	1.54**	0.35**	0.19
Degrees of freedom	1	2	54	54	108

NS, Nonsignificant.
* Significant at the 0.05 level.
** Significant at the 0.01 level.
† Accession Unselected Little Red Flint was evaluated only in the second year, so it was not included in the combined analysis of variance.

ago (Galinat, 1954). Notably, none of the pointed popcorns from Latin America is included in those groups, having fallen instead in subgroup V-B, close to the pointed popcorns from the USA.

Group III includes a well-defined cluster of pearl popcorns from North America with predominantly yellow kernels and favorable agronomic characteristics, a group that we will refer to as the North American Yellow Pearl Popcorns, and also includes CHZM 07 097 from Chile and Ladyfinger, which differs morphologically from the rest of the members of this group but displays isozyme and SSR allelic profiles that resemble those of the North American Yellow Pearl Popcorns. It also includes Nebraska Supergold × Tom Thumb, a hybrid population involving Tom Thumb, a population with a different genetic background.

A cluster of the earliest North American popcorn accessions, which we will refer to as the North American Early Popcorns, were allied in group IV, not too distantly separated from the pointed, rice types in group V. Interestingly, Tama Flint, a population belonging to the Northern Flint and Flour race (González-Ugalde, 1997), and Fairfax Brown, which is best assigned to the South Western racial group of maize (González-Ugalde, 1997), are included in this group. These two populations do not morphologically resemble the North American Early Popcorns and have only an incipient popping-expansion volume, but patterns of their isozyme and SSR allelic frequencies resemble the popcorns in group IV. It is possible that the Northern Flints and Flours are related genetically to the North American Early Popcorns, which may also be related genetically to some European popcorns introduced to the USA in the 19th Century from France and Italy (Sturtevant, 1899).

Group V includes the pointed popcorns, and can be unambiguously divided into two subgroups, with subgroup V-A comprising all the typical pointed-kernel popcorns from North America, which we will refer to as North American Pointed Rice Popcorns ("Pointed" in Fig. 2 and 3) and subgroup V-B including Latin American pointed popcorns and two pointed popcorns from southwestern USA, Acoma Pueblo and White Rice Pop, which resemble their Latin American counterparts ("Lat-pointed" in Fig. 2 and 3). The establishment of these six groups of populations delineated by the Neighbor-Joining method was generally corroborated by the discriminant function, a technique used to build a predictive model of group membership on the basis of observed characteristics of each population (data not shown).

Principal component analysis was applied to 29 morphological traits, 58 isozyme alleles, and 191 SSR alleles. The first three principal components presented large eigenvalues (25.6, 21.0, and 13.5) explaining 9.28, 7.61, and 4.89% of the total variance, respectively, for a cumulative percentage of 21.78.

The first and second principal components are plotted

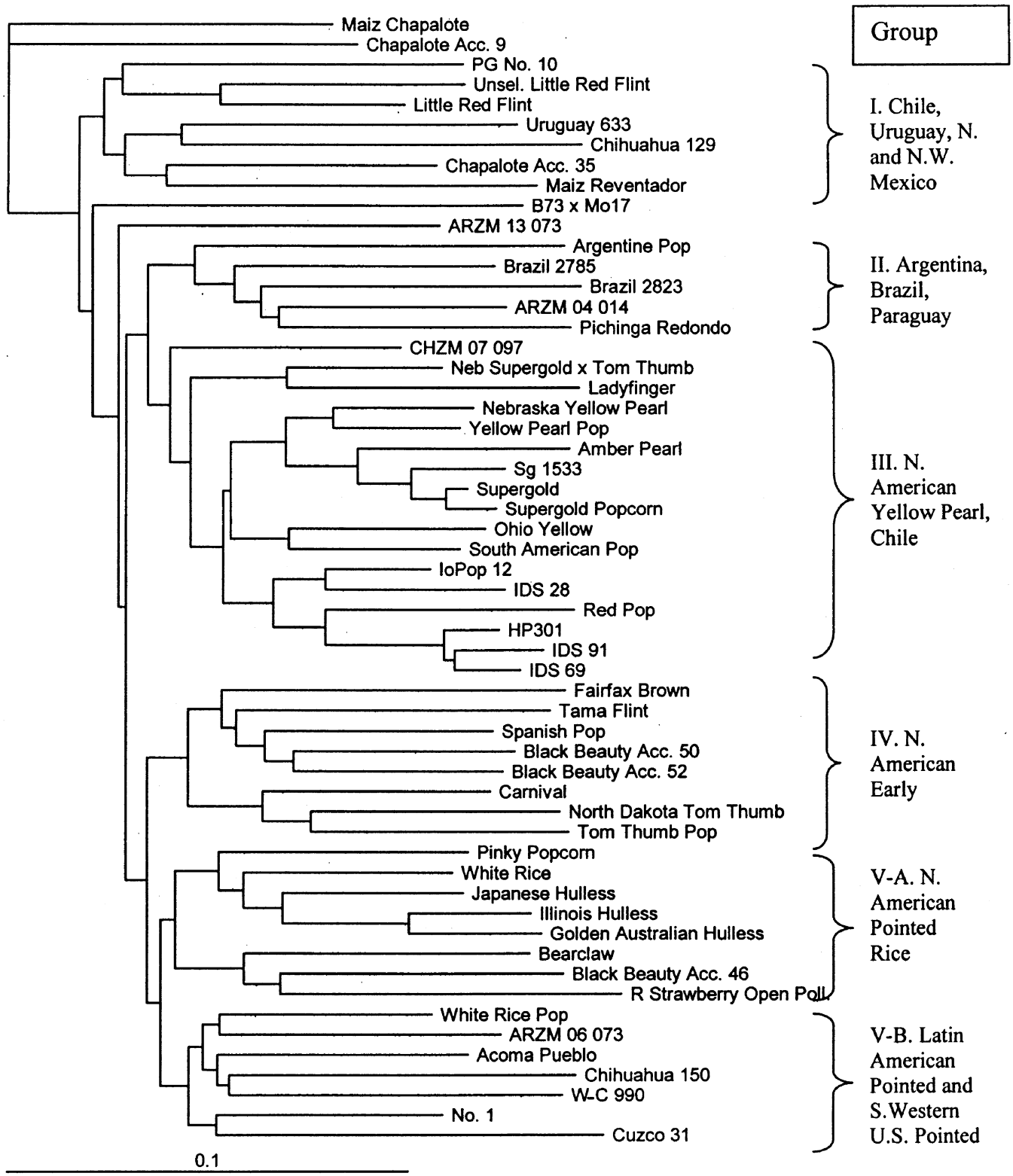

Fig. 1. Phylogenetic tree produced by a Neighbor-Joining analysis for 56 maize populations based on a matrix of Gower distances derived from 29 morphological traits, 58 isozyme alleles, and 191 SSR alleles.

together in Fig. 2, where the North American Yellow Pearl Popcorns, including Ladyfinger and CHZM 07 097, form a distinct, compact group in the negative part of both axes. The North American Pointed Rice Popcorns and North American Early Popcorns can be found loosely aggregated, with a few populations, such as Spanish Pop, Pinky Popcorn, and Black Beauty Acc. 50, placed between the two groups. Just above this loose aggregation lies a group including White Rice Pop, Acoma Pueblo, the non-popcorn Tama Flint from the USA, and various pointed popcorns from Latin America. The Latin American pointed popcorn positioned

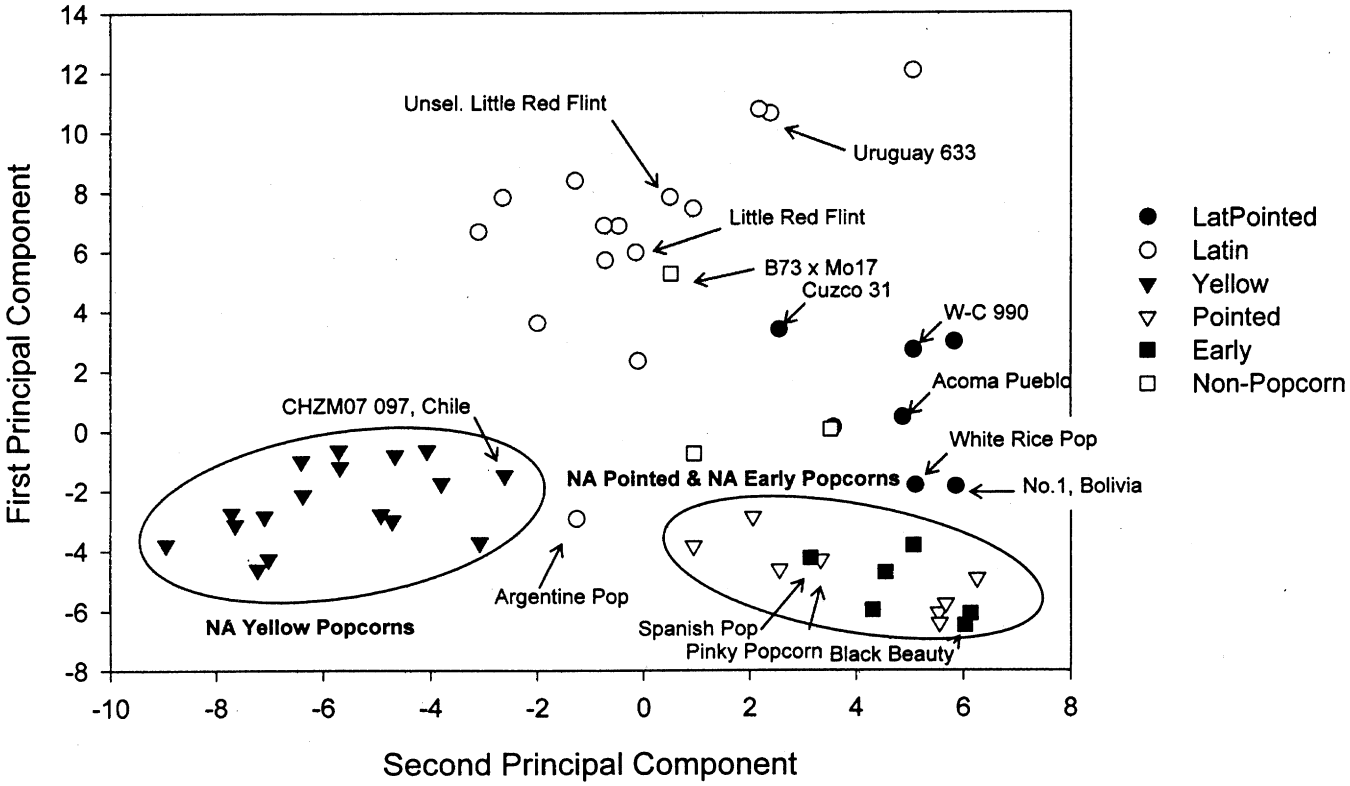

Fig. 2. Representation of the first and second principal components based on the correlations matrix among 29 morphological traits, 58 isozyme alleles, and 191 SSR alleles scored for 56 maize populations.

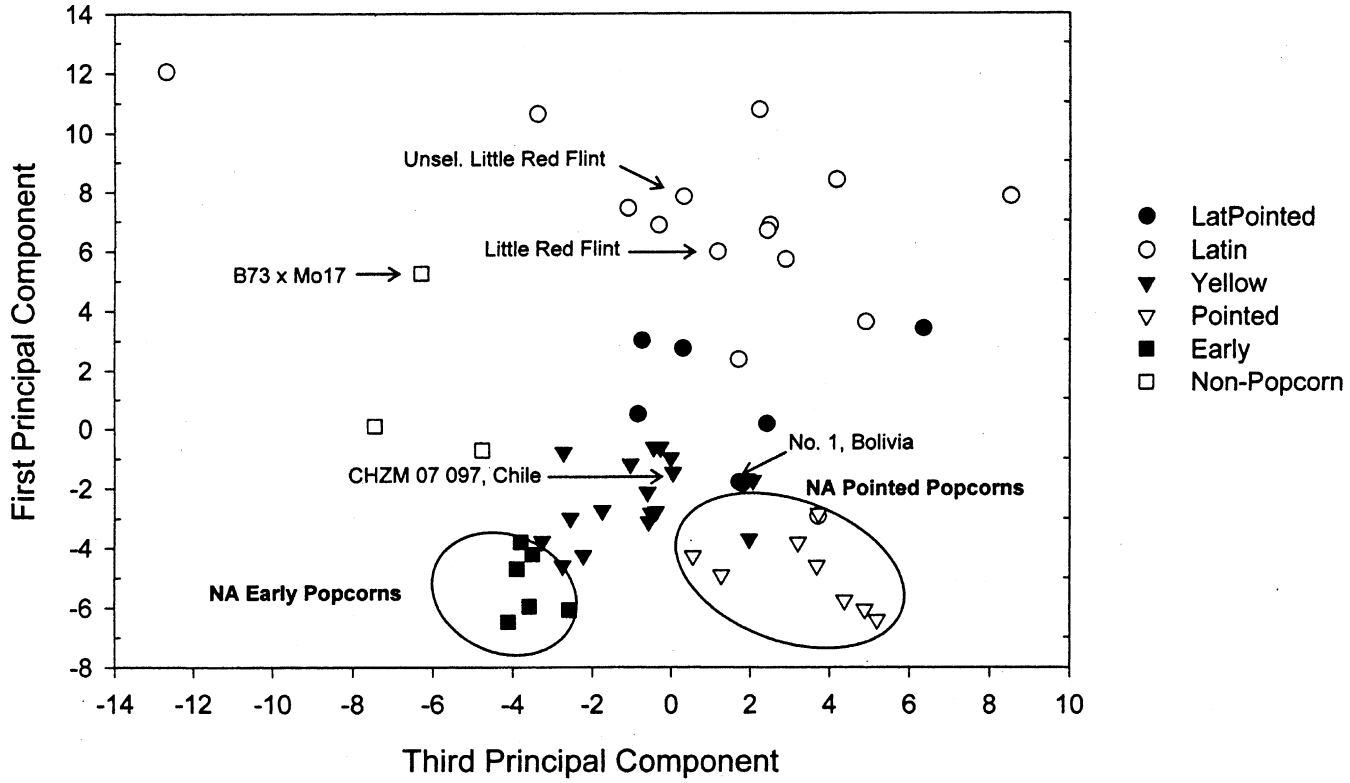

Fig. 3. Representation of the first and third principal components based on the correlations matrix among 29 morphological traits, 58 isozyme alleles, and 191 SSR alleles scored for 56 maize populations.

most closely to the groups of early and North American Pointed Rice Popcorns is "No. 1" from Bolivia, a population of the race Pisankalla. The remaining populations correspond to the non-pointed Latin American popcorns and certain non-popcorns, including B73 × Mo17 and the flint types Uruguay 633 and Little Red Flint, both in its "original" version and a "selected" version after seven cycles of selection for improved popping expansion.

The first principal component separated populations according to their geographic provenance. In Fig. 2, the first principal component separated populations of the USA from those of Latin America, with two notable exceptions. The first is CHZM 07 097 from Chile, with principal component scores that ally it with North American Yellow Pearl Popcorns. The second exception is the Bolivian population "No. 1," which belongs to the race Pisankalla. According to Sánchez-González (1994), the race Pisankalla is likely related to Palomero Toluqueño and other Latin American pointed popcorns, such as Imbricado from Colombia, Confite Puntiagudo from Peru ("Cuzco 31" in this study), and Canguil from Ecuador ("W-C 990" in this study). In fact, the principal component analysis of Fig. 1 suggests that this group of Latin American pointed popcorns may be a transitional group between the typical North American Pointed Rice Popcorns and other popcorns from Latin America.

Figure 3 depicts these populations' scores for the first and third principal components. As explained above, the first principal component separated popcorn populations of the USA from the Latin American popcorns; but, in this case the third principal component separated the North American Pointed Rice Popcorns from the North American Early Popcorns. The North American Yellow Pearl Popcorns are positioned as an intermediate group on the third principal component, and the Latin American popcorns are scattered across the upper part of Fig. 3.

Isozyme and SSR allelic frequencies associated the flint-type, non-popcorns Tama Flint and Fairfax Brown with the group of North American Early Popcorns. But those populations exhibit only a slight popping capacity and are not used as popcorns. That they share many diagnostic alleles with the North American Early Popcorns could imply that both groups, flint corns and North American Early Popcorns, share common progenitors, perhaps maize from northwestern Mexico, as proposed by Galinat and Gunnerson (1963) and supported by González-Ugalde (1997) for Northern Flints, or that gene flow between North American Early Popcorns and Northern Flints occurred after a recent (post-1800) introduction of early popcorns to the northeastern USA from South America (Sánchez-González, 1994) and/or Europe (Sturtevant, 1899).

Origin of the Popcorn Groups

These analyses strongly suggest that the different groups of popcorn in the USA have different evolutionary histories and do not share a common, recent origin. In group III of Fig. 1, given the close genetic resemblance between the North American Yellow Pearl Popcorns and the Chilean population CHZM 07 097, a representative of the race Curagua, with respect to allelic frequencies for both isozymes and SSRs, as well as the remarkably strong morphological similarity among them (including popping expansion), the race Curagua from Chile may be the most likely progenitor of the North American Yellow Pearl Popcorns. Smith (1999) noted that, in the 19th Century, American traders, sailors, and whalers may have introduced it from Chile into New England, where, at that time, a popular popcorn variety was called "Valparaiso," a name most likely associated with that city in Chile where sailors likely first collected it. Furthermore, Timothy et al. (1961) described the ecogeographical distribution of the race Curagua as occurring in central Chile, around the region of Valparaiso, Talca, and Rancagua. According to the passport data, population CHZM 07 097 was collected in Maule, Talca Province, in 1982 (USDA-ARS Germplasm Resources Information Network-GRIN, http://www.ars-grin.gov/cgi-bin/npgs/html/acchtml.pl?1372407; verified 19 March 2004). Notably, the popcorns of temperate Chile and the other nations of southern South America are photoperiod insensitive, so they could immediately be grown in the high latitudes of the USA, in contrast to popcorns from Central America or other more equatorial locations (Goodman and Brown, 1988). Additionally, allelic diversity of the Chilean population is richer than that of the North American Yellow Pearl Popcorns, collectively, (data not shown), and this single representative of race Curagua includes the predominant allele of the North American Yellow Pearl Popcorns at 48 of 49 SSR and enzyme loci evaluated. The phylogenetic tree (Fig. 1) also supports the hypothesis of Curagua as the progenitor of North American Yellow Pearl Popcorns by placing CHZM 07 097 as the most basal member of group III.

Regarding the pointed group of popcorns (group V), two subgroups are clearly defined. The first subgroup is more genetically homogeneous, and includes typical North American Pointed Rice Popcorns, such as Japanese Hulless, Illinois Hulless, and Golden Australian Hulless. The second subgroup mainly includes populations representative of the major pointed popcorn races from Latin America, such as Palomero Toluqueño from Mexico, Confite Puntiagudo from Peru, Canguil from Ecuador, Pisankalla from Bolivia and Pisingallo from Argentina (Wellhausen et al., 1951; Ramírez et al., 1960; Grobman et al., 1961; Timothy et al., 1963). Interestingly, there are also a few populations within this second subgroup, such as White Rice Pop and Acoma Pueblo, that are from the USA. These two populations resemble Latin American pointed popcorns more than they do other pointed populations from the USA, as manifest by more luxurious vegetative development, more slender ears, and kernels with less pronounced points and lower popping expansion. Notably, both subgroups are allied morphologically and by patterns of isozyme and SSR loci. White Rice Pop and Acoma Pueblo could be viewed as genetically intermediate between pointed populations from Latin America and those elsewhere in the USA. Doebley et al. (1983) studied the Acoma Pueblo popula-

tion included in this study (PI 218140) and described it as a pointed popcorn that may have been acquired by the Acoma from either the Anglo or Hispanic cultures during colonial times. As noted by Sánchez-González (1994), some pointed popcorns of the central highlands of Mexico diffused through the northern plains of Mexico and then into the USA.

Morphological and possible genetic similarities among the different pointed popcorns from Latin America have already been noted by several authors (Mangelsdorf, 1974; Sánchez-González, 1994; Sevilla, 1994). Pre-Columbian communication and exchange of germplasm between indigenous groups from Mexico and South America have been documented (Zeven and de Wet, 1982; Melgar-Tisoc, 2003), so these popcorn types may have been diffused widely before European colonization. Other authors (Anderson, 1947; Mangelsdorf, 1974) noted similarities and likely relationships between pointed popcorns, such as Japanese Hulless from the USA and Latin American pointed popcorns, such as Palomero Toluqueño. The present study supports the preceding hypothesis as it demonstrates genetic similarities between North American and Latin American subgroups. Selsam and Wexler (1976) mentioned that some of the oldest evidence for the presence of popcorn in the USA can be traced to New Mexico, which is compatible with a proposed overland route of dispersion of maize, including pointed popcorns, from the highlands of central Mexico, initially into the northern plains of Mexico and then into the southwestern USA (Sánchez-González, 1994).

The group North American Early Popcorns in group IV of Fig. 1 evinces a different and potentially complex evolutionary history. In certain dimensions generated by the principal component analysis, this group resembles the North American Pointed Rice Popcorns (Fig. 2), but the North American Early Popcorns do not seem to be closely related to other New World popcorns. However, the North American Early Popcorns and the non-popcorn, flint-type populations Fairfax Brown and Tama Flint were remarkably similar genetically (Fig. 2). Tama Flint is typical of the Northern Flint and Flour North American race, whereas Fairfax Brown is allied with maize of the southwestern USA (González-Ugalde, 1997). As proposed by Galinat and Gunnerson (1963) and supported by results from Doebley et al. (1986) and González-Ugalde (1997), the Northern Flints originated from northwestern Mexican maize that diffused into the southwestern USA and then to the northeastern USA, through the Pueblo region of New Mexico. It seems plausible that the North American Early Popcorns could be derived from the Northern Flints, through the selection of populations with smaller, denser kernels, or at least that gene flow between Northern Flints and the progenitors of the North American Early Popcorns was likely a key factor in conferring early maturity to populations that might not otherwise mature seeds in the northern U.S.

The Northern Flints, among the earliest maturing races in the world, have been of prime importance in the generation of other important corn varieties. Besides their relationship with the early popcorns, Northern Flints are closely related to many sweet corns, as noted by Revilla and Tracy (1995), who proposed that, as with modern U.S. popcorns, some of the modern sweet corns in the USA were derived either from intercrossing between Mexican germplasm and Northern Flints or directly from mutations in Northern Flint. Also, Northern Flints are one parent of Corn Belt Dents and contributed key traits

Table 6. Diagnostic morphological features of the three proposed new races of popcorn from North America.

	North American Yellow Pearl Popcorns		North American Pointed Rice Popcorns		North American Early Popcorns	
Trait	**Mean†**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Plant height (cm)	210 a	114–315	183 b	103–270	117 c	34–181
Ear height (cm)	100 a	41–188	76 b	9–146	20 c	2–60
Number of leaves	21 a	17–24	17 b	12–23	12 c	8–15
Number of tillers per plant	0.6 b	0–4	3.2 a	0–6	3.5 a	2–6
Leaf length (cm)	78 a	56–100	71 b	46–99	53 c	21–79
Leaf width (cm)	8.7 a	5.2–12.5	7.5 b	4.9–10.7	5.3 c	2.6–8.6
Days to pollen shed	74 a	61–89	69 b	54–88	51 c	40–60
Days to silk	78 a	64–95	73 b	55–92	52 c	41–63
Anthesis-silking interval (days)	3.6 a	1–9	4.0 a	0–11	1.2 b	−2–3
Tassel peduncle length (cm)	16 b	6–28	23 a	8–38	23 a	12–33
Tassel branched part length (cm)	14 a	8–24	8 b	2–15	6 c	1–15
Central spike length (cm)	23 a	15–32	24 a	13–35	19 b	3–37
Number of primary branches	21 a	10–42	11 b	1–32	8 c	1–21
Tassel peduncle length/tassel length	0.31 c	0.12–0.41	0.41 b	0.21–0.55	0.49 a	0.25–0.81
Central spike length/tassel length	0.43 a	0.27–0.56	0.44 a	0.31–0.60	0.38 b	0.11–0.55
Number of ears per plant	2.1 b	1–6	3.0 a	1–11	3.7 a	1–6
Ear length (cm)	15 a	8–22	10 c	3–15	12 b	5–20
Ear diameter (cm)	3.0 b	2.0–3.7	3.6 a	2.8–4.6	2.6 c	1.7–3.2
Number of kernel rows	15.0 b	8–24	22.0 a	14–32	11.5 c	8–16
Ear length/ear diameter	5.1 a	2.7–7.9	2.8 c	0.7–4.9	4.5 b	2.7–6.9
Kernel length (mm)	7.6 b	5.1–9.6	8.1 a	6.3–10.6	6.7 c	4.7–8.3
Kernel width (mm)	5.6 b	4.2–7.2	4.7 c	3.2–6.5	6.4 a	4.7–9.1
Kernel thickness (mm)	4.1 a	3.0–6.1	3.7 b	2.3–5.2	3.7 b	2.9–4.9
Number of kernels per 10 g	84 b	55–148	116 a	83–215	102 a	57–175
Kernel length/width	1.3 b	1.0–2.4	1.8 a	1.2–2.5	1.1 c	0.8–1.3
Kernel width/thickness	1.4 b	0.7–2.0	1.2 c	0.9–1.9	1.7 a	1.3–2.5
Popping expansion (cm^3/30 g)	1166 a	840–1600	954 b	600–1280	739 c	500–1040
Type of flake (numerical rating 1–5)	4.0 b	1.5–5.0	4.3 a	3.5–5.0	3.9 b	3.0–5.0

† Within rows, means with the same letter are not statistically different according to Tukey's test (0.05).

to this globally important race (Goodman and Brown, 1988).

Another likely contributor of germplasm to the North American Early Popcorns is the group of popcorns introduced from Europe in the 19th Century. As described by Sturtevant (1899), the kernel characteristics, plant dimensions, and phenology of 19th Century European popcorns closely resemble those of current Early Popcorns. Additionally, as noticed by Mangelsdorf (1974) and confirmed in this study, to a certain degree, the North American Early Popcorns resemble the pointed popcorns, perhaps the result of earlier derivation from maize from the highlands of central Mexico, related to maize of the southwestern USA (Goodman and Brown, 1988). Alternatively, perhaps the resemblance is the result of introgression from the North American Pointed Rice Popcorns. More detailed data and a fuller discussion of this topic are presented in Santacruz-Varela (2001).

Goodman and Brown (1988) noted the dearth of studies bearing on the systematic relationships of popcorns. The results of the present study provide a preliminary classification of the main popcorn populations of the USA, defining three groups: North American Yellow Pearl Popcorns, North American Pointed Rice Popcorns, and North American Early Popcorns. These groups can be regarded as races, as they fit the definition of race given by Anderson and Cutler (1942), "a group of related individuals with enough characteristics in common to permit their recognition as a group" and from the genetic perspective "a group of individuals with a significant number of genes in common." Tables 6 and 7 sum-

Table 7. Diagnostic isozyme and SSR alleles of the three proposed new races of popcorn from North America.

Locus	North American Yellow Pearl Popcorns		North American Pointed Rice Popcorns		North American Early Popcorns	
	Most common alleles† (freq.)					
Isozyme locus						
Mdh1	6 (0.94)	1 (0.04)	6 (0.98)	Null (0.02)	6 (0.90)	2.5 (0.10)
Mdh2	6 (0.60)	3.5 (0.38)	6 (0.66)	3 (0.32)	6 (0.98)	3.5 (0.02)
Mdh3	16 (1.00)	–‡	16 (1.00)	–	16 (1.00)	–
Mdh4	12 (1.00)	–	12 (1.00)	–	12 (1.00)	–
Mdh5	12 (1.00)	–	12 (1.00)	–	12 (1.00)	–
Acp1	2 (0.77)	3 (0.20)	4 (0.69)	2 (0.31)	4 (0.47)	2 (0.46)
Glu1	7 (0.82)	r (0.100)	7 (0.38)	6 (0.27)	7 (0.53)	2 (0.21)
Acp4	2 (0.68)	6 (0.13)	2 (0.82)	3 (0.17)	3 (0.53)	5 (0.28)
Adh1	4 (1.00)	–	4 (0.56)	6 (0.44)	4 (0.82)	6 (0.18)
Got1	4 (1.00)	–	6 (0.76)	4 (0.24)	4 (0.67)	6 (0.33)
Got2	4 (1.00)	–	4 (0.88)	2 (0.12)	4 (0.98)	2 (0.02)
Got3	4 (1.00)	–	4 (1.00)	–	4 (1.00)	–
Cat3	9 (0.87)	Null (0.07)	Null (0.51)	9 (0.48)	9 (0.43)	7 (0.29)
Phi1	4 (0.99)	5 (0.01)	4 (1.00)	–	4 (1.00)	–
Pgd1	3.8 (0.81)	2 (0.15)	3.8 (0.90)	2 (0.08)	3.8 (0.73)	2 (0.27)
Pgd2	5 (1.00)	–	5 (1.00)	–	5 (1.00)	–
Idh1	4 (0.99)	6 (0.01)	4 (0.97)	6 (0.03)	4 (1.00)	–
Idh2	4 (0.98)	6 (0.02)	4 (0.53)	6 (0.47)	6 (0.99)	4 (0.01)
SSR locus						
phi127	124 (0.46)	126 (0.43)	112 (0.65)	124 (0.19)	126 (0.70)	112 (0.28)
phi051	144 (0.77)	142 (0.20)	144 (0.81)	140 (0.10)	144 (0.53)	142 (0.36)
phi115	292 (0.54)	304 (0.46)	304 (0.83)	292 (0.17)	304 (0.72)	292 (0.28)
phi015	98 (0.79)	104 (0.20)	98 (0.48)	104 (0.38)	98 (0.50)	86 (0.48)
phi033	252 (0.92)	258 (0.07)	252 (0.73)	258 (0.27)	252 (0.95)	243 (0.04)
phi053	169 (0.84)	194 (0.12)	194 (0.72)	183 (0.15)	194 (0.76)	169 (0.15)
phi072	151 (0.78)	143 (0.20)	143 (0.63)	154 (0.24)	151 (0.36)	163 (0.33)
phi093	290 (0.72)	285 (0.26)	290 (0.56)	288 (0.17)	285 (0.38)	290 (0.36)
phi024	360 (0.59)	363 (0.22)	360 (0.52)	366 (0.37)	360 (0.58)	363 (0.18)
phi085	261 (0.99)	248 (0.01)	261 (0.58)	235 (0.28)	237 (0.87)	261 (0.08)
phi034	138 (0.78)	142 (0.17)	135 (0.63)	142 (0.20)	142 (0.66)	123 (0.28)
phi121	98 (0.99)	101 (0.01)	98 (1.00)	–	98 (1.00)	–
phi056	257 (0.89)	265 (0.08)	260 (0.57)	262 (0.23)	260 (0.64)	257 (0.23)
phi064	99 (0.75)	95 (0.10)	86 (0.27)	78 (0.26)	75 (0.51)	78 (0.35)
phi050	84 (0.92)	86 (0.08)	84 (0.88)	86 (0.12)	84 (0.73)	86 (0.19)
phi96100	296 (0.49)	289 (0.41)	296 (0.75)	289 (0.25)	296 (0.73)	284 (0.22)
phi101249	122 (0.44)	126 (0.43)	126 (0.29)	145 (0.29)	145 (0.59)	122 (0.21)
phi109188	164 (0.67)	168 (0.32)	168 (0.39)	164 (0.36)	164 (0.85)	168 (0.09)
phi029	154 (0.87)	150 (0.09)	161 (0.46)	150 (0.26)	161 (0.42)	150 (0.36)
phi073	189 (0.61)	186 (0.30)	192 (0.47)	194 (0.28)	194 (0.49)	192 (0.32)
phi96342	250 (0.58)	243 (0.41)	250 (0.57)	243 (0.43)	250 (0.72)	243 (0.28)
phi109275	129 (0.86)	135 (0.07)	135 (0.80)	129 (0.20)	129 (0.47)	122 (0.32)
phi427913	131 (0.54)	123 (0.45)	131 (0.90)	125 (0.09)	131 (0.56)	134 (0.33)
phi265454	220 (0.60)	228 (0.33)	220 (0.33)	228 (0.30)	220 (0.93)	232 (0.07)
phi402893	215 (0.79)	230 (0.20)	215 (0.72)	237 (0.12)	215 (0.93)	209 (0.06)
phi346482	129 (0.66)	123 (0.31)	129 (0.86)	123 (0.14)	129 (1.00)	–
phi308090	212 (0.58)	221 (0.28)	221 (1.00)	–	221 (0.81)	212 (0.16)
phi330507	136 (0.99)	133 (0.01)	136 (1.00)	–	136 (1.00)	–
phi213984	305 (0.89)	288 (0.11)	305 (1.00)	–	305 (1.00)	–
phi339017	148 (0.86)	151 (0.14)	148 (0.98)	151 (0.02)	148 (0.78)	154 (0.21)
phi159819	126 (0.63)	123 (0.26)	138 (0.53)	126 (0.35)	126 (0.57)	138 (0.20)

† Alleles with the highest frequency (mean frequency in parentheses) were determined on the basis of the average of all populations evaluated in each race, with isozyme alleles named according to the nomenclature used by Stuber et al. (1988) and SSR alleles named according to their fragment size (in base pairs).

‡ Locus was monomorphic for this set of populations.

marize the diagnostic morphological features, isozyme, and SSR alleles for the three proposed new maize races, and can serve as an analytical framework for analyzing other poorly known popcorns, *e.g.* those from Europe, Turkey, India, the Himalayan region, and Latin America.

In addition to a comprehensive characterization of these valuable genetic resources, an important function of this study is that it can help identify new heterotic patterns and a potentially more efficient exploitation of the phenomenon of hybrid vigor by popcorn breeders. Moreover, during the process of studying patterns of genetic diversity, important hints about underrepresented groups of populations can become evident, so that future programs of germplasm acquisition can be focused directly on those neglected groups to broaden the genetic base of ex situ germplasm collections and, ultimately, the diversity employed in plant breeding.

CONCLUSIONS

Three groups of popcorn from the USA that can be considered as races were defined, each with its own distinctive characteristics and evolutionary history: (i) North American Yellow Pearl Popcorns, a well-defined group embracing the popcorns most commonly grown for commercial production in the USA today; (ii) North American Pointed Rice Popcorns, a group commercially important during the first half of the 20th Century; and (iii) North American Early Popcorns, a group with marginal commercial importance as popcorn, but with potential for use as ornamental popcorn.

To augment this study and develop a more precise and comprehensive view of the historical background and genetic relationships among the North American popcorns, additional studies are vital for each of these three popcorn groups. Such studies should include much larger samples of races that may be ancestral to U.S. popcorns. For the North American Yellow Pearl Popcorns, extensive sampling of populations of the races Curagua and Curagua Grande from Chile is needed; for the North American Pointed Rice Popcorns, a better sampling of Palomero Toluqueño and other Latin American pointed popcorn races is required; whereas, for the North American Early Popcorns, a set of both popcorn and non-popcorn populations, encompassing a broad geographic range from northwestern Mexico to northeastern USA, along with additional Northern Flints and European popcorns, should be evaluated.

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