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Genetic relationships among Native American maize accessions of the Great Plains assessed by RAPDs

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Abstract Genetic variation among 15 accessions of Native American maize from the Great Plains was investigated using random amplified polymorphic DNA (RAPD). RAPDs revealed very high levels of polymorphism among accessions. Banding patterns ranged in percentage polymorphism from 46.7% to 86.2% with an overall mean of 70.7% for the primers analyzed. The construction of genetic relationships using cluster analysis and principal coordinates analysis revealed that RAPDs are successful in confirming hypothesized relationships and in identifying misclassified specimens. Furthermore, the phenogram fails to reveal a strong correspondence between genetic relationships and the geographical position of Native Americans prior to contact. This provides support for the hypothesis that multiple introductions of maize into the Great Plains via trade may have resulted in the great morphological variation found among accessions in the region. Based on these data, it is unlikely that a separate Great Plains race of maize can be distinguished. In general, we conclude that RAPDs are potentially very useful in organizing seed collections and understanding intraspecific genetic differentiation.

Key words Native American maize · RAPD · Genetic relationships · Reproducibility · Geography and evolution

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Introduction

The morphology of maize accessions (*Zea mays* L. ssp. *mays*) developed by native agricultural societies has been studied extensively in order to understand evolutionary diversification and patterns of migration throughout the Americas. The addition of results from genetic research on maize races (e.g. cytology: McClintock et al. 1981; isozymes: Doebley et al. 1986, Bretting et al. 1990) to the wealth of morphological data has significantly refined classification and theories of maize evolution. The distribution and extent of genetic variation have been of particular interest in assessing genetic relationships among maize races, conserving genetic resources for plant breeding, and classifying archaeological specimens to understand maize migration and development by early agriculturists.

Through artificial selection, native cultures independently developed maize accessions for desired traits such as yield, dietary function, and kernel color (Hernandez 1985; Adams 1994). Intense selection for these various functions has resulted in great morphological diversity rarely seen in a plant species (Doebley 1994). In particular, the variability in maize ear morphology is extensive, not only between regions but also within cultures, making classification difficult (Cutler and Blake 1973; Adair 1988). Comparative analyses of ear characters has led to a number of hypotheses regarding the migration and evolution of maize varieties. Despite their genetic basis, these highly variable or plastic traits are not appropriate for the reconstruction of genetic relationships (Doebley and Iltis 1980).

The introduction of maize into the United States occurred about 3000 years before present (BP) from trade with cultures of Mesoamerica (see Adams 1994). Isozyme variation reveals a close relationship between the maize of Mesoamerica and maize of the Southwest U.S., confirming the direction of migration (Doebley et al. 1986;1988). Maize became important to Native Americans in the United States for both dietary and ceremonial purposes (Cushing 1920; Ford 1994). The dominant race

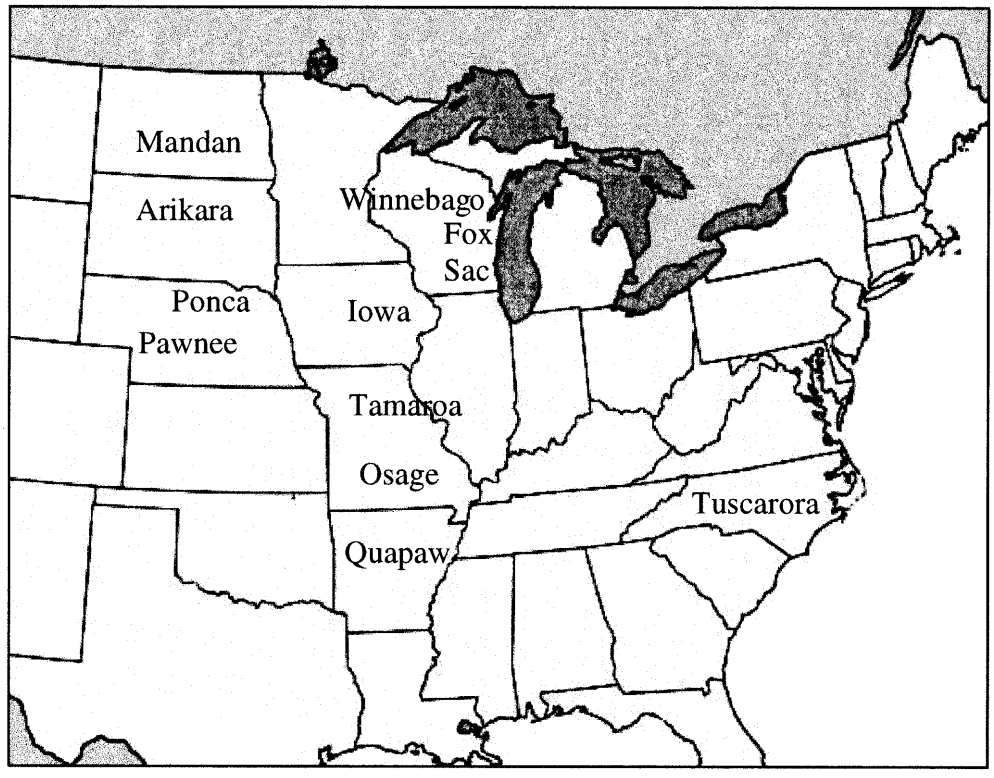

Fig. 1 Geographic distribution of tribes represented in this study. The position of the names approximates the location of tribes prior to contact with Europeans

of maize to appear in the archaeological record of the Southwest (often called Chapalote-like) is characterized by small cigar-shaped cobs with small triangular kernels in 12–14 rows (Anderson and Cutler 1942; Upham et al. 1987). Native Americans of the Southwest developed an intensified agricultural system between 3000–2000 BP based largely upon this new crop (Adams 1994).

Current theories suggest that through trade between cultures of the Southwest and cultures of the Great Plains and Eastern Woodlands, maize was introduced across the continental United States where it underwent evolutionary modification due to artificial selection and adaptation to new environments (Doebley et al. 1986; Fritz 1992). The passage of maize through the Great Plains probably did not initially result in the integration of maize into existing subsistence strategies. Rather, maize intensification was not widespread upon its introduction (Chapman and Watson 1993; Fritz 1993; Adair 1994). Maize became the dominant food crop of many native cultures of the Eastern Woodlands by the Late Woodland Period (1250 BP) (see Wagner 1994) and of the Great Plains by the Plains Village Period (after 1000 BP) (Adair 1994). These dates suggest that maize became a dominant crop in the Eastern Woodlands prior to achieving this status in the Great Plains. Therefore, maize intensification in the Great Plains may have followed intensification in both the Southwest and the Eastern Woodlands. Based on a great reduction in isozyme variation in the Northern Flint race compared with the southwestern races, Doebley et al. (1986) suggest that the Northern Flint race evolved under relative isolation and shows strong divergence, potentially due to genetic drift and changes in the selection regime. Their data indicate levels of divergence often found between species. In addition, these data indirectly support the hypothesis that maize was not initially an important crop in the Great Plains.

Great Plains specimens exhibit considerable variation in ear morphology for characters such as row number and have been called intermediate between the southwestern races and the Northern Flint race (Cutler and Blake 1973; Adair 1994). The grouping of midwestern accessions in classification efforts and in the conservation of genetic resources has recently been questioned as surveys of morphological data reveal a lack of regional cohesion, particularly when compared with maize from the Southwest or Eastern Woodlands (Fritz 1992; Adair 1994). Great Plains accessions have not been subjected to molecular genetic analysis which may provide a more efficient means for addressing many long-standing questions in maize evolution. In particular, little is known about the extent to which evolutionary relationships among accessions agree with their geographic distribution. This is of special interest in the Great Plains where many varieties may have been acquired via trade. The ability to determine relationships using genetic markers may additionally provide important information for organizing seed collections which are of both agricultural and historical significance.

Molecular techniques such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), and isozymes are commonly employed to study genetic relationships among closely related organisms. More recently, the polymerase

Table 1 Native American maize accession names and the tribe from which each was presumably obtained representing the Great Plains of the United States (*N/A* not available)

Number	Accession [a]	Tribe
1	Tamaroa red striped corn	Tamaroa
2	Osage sacred red corn	Osage
3	Pawnee-Arikara white flour corn	Pawnee-Arikara
4	Tamaroa-Tuscarora white flour corn	Tamaroa-Tuscarora
5	Tamaroa white flour corn	Tamaroa
6	Ponca grey flour corn	Ponca
7	Osage brown flour corn	Osage
8	Osage red flour corn	Osage
9	Fox blue corn	Fox
10	Winnebago blue speckled flour corn	Winnebago
11	Silver queen sweet corn	N/A
12	Oklahoma bronze	Arikara
13	Quapaw red	Quapaw
14	Sac blue	Sac
15	Wa-haa-ha-kow	Winnebago
16	Mandan red flour	Mandan

[a] For more information concerning the sources of these accessions, contact David Moeller

chain reaction (PCR)-based technique of random amplified polymorphic DNAs (RAPDs) has proven sensitive and efficient in detecting genetic variation (Welsh and McClelland 1990; Williams et al. 1990). RAPDs have gained attention for their ability to detect regions of DNA that apparently evolve more quickly than isozymes (Brauner et al. 1992; Baruffi et al. 1995). In addition, a great deal of data can be gathered relatively quickly without large costs. By contrast, RAPDs have been widely criticized because the origin of polymorphism is not understood, band homology cannot be assumed in phylogeny reconstruction (Rieseberg 1996), and banding patterns are not reliable and reproducible in many cases (Jones et al. 1997, Perez et al. 1998). Despite these criticisms, many investigators have demonstrated success in generating informative data consistent with other techniques, particularly within species (e.g. Lerceteau et al. 1997; Clerc et al. 1998).

In the study reported here, we employed RAPDs to determine genetic relationships among Native American maize accessions of the Great Plains region. In particular, we are interested in the extent to which genetic relationships mirror former geographic patterns of native tribes. In addition, we discuss the potential for RAPDs in identifying accessions and in organizing maize seed collections critical for plant breeding programs.

Materials and methods

Seed collection

Accessions of Native American maize representing cultures of the Great Plains region were selected for this study (Fig. 1). A total of 16 accessions were obtained from Native American individuals and several native seed collections including the USDA Experimental Station (Ames, Iowa) and Living History Farms (Urbandale, Iowa) (Table 1). Accessions were selected from these sources because they maintained reliable breeding programs and provided background information detailing whether cultural origin was known or equivocal. The majority of accessions in these collections were obtained directly from Native Americans as early as 1900 and were preserved by seed-saving organizations until the present. Accessions represent a large fraction of the Great Plains region, and several accessions represent cultures that prehistorically bordered this region (Fig. 1). In addition, commercially produced silver queen sweet corn was included for comparison as it is relatively recently developed in contrast to the native varieties.

DNA extraction, quantification, and amplification

Total cellular DNA was extracted directly from seeds. Seeds were partially dissected to remove the majority of endosperm before proceeding with the extraction. The remainder was ground using sterilized sand in an Eppendorf tube until a fine consistency was achieved. DNA was extracted from the ground material using the CTAB method (Doyle and Doyle 1987). The seed tissue and sand mixture was combined with 650 µl of CTAB buffer (100 m*M* TRIS-HCl, 1.4 *M* NaCl, 20 m*M* EDTA, 2% CTAB, 1% PVP-40, 0.2% β-mercaptoethanol). Samples were incubated at 67°C for 1.5 h with occasional agitation. An equal volume of chloroform-isoamylalcohol (24:1) was added and mixed until an emulsion formed. The mixtures were centrifuged at 12 500 rpm for 5 min. The aqueous layer was removed and the process repeated. Following the second addition, the aqueous supernatant was mixed with 2× volume (1300 µl) of cold 95% ethanol and placed on ice for 1 h in order to precipitate the extracted DNA. DNA was collected by centrifuging the samples at 9000 rpm for 10 min at 4°C until a pellet formed. Samples were then cleaned twice with 70% ethanol and subsequently dried. The resulting DNA was resuspended in 100 µl TE.

DNA was quantified using a TKO 100 DNA mini-fluorometer (Hoefer Scientific Instruments, San Francisco, Calif.). The fluorometer was calibrated to zero with 2 ml of a blank solution containing 240 µl TNE and 4 µl flurochrome Hoechst 33258. The fluorometer was then calibrated by adding 2 µl of lambda DNA at various concentrations (20 ng/µl–200 ng/µl) to 2 ml of blank solution. Calibrations were repeated to ensure replication. DNA concentrations of maize samples were then established with the same technique employed for lambda DNA. Maize DNA concentrations were also estimated by comparing band intensities of extracted DNA with known concentrations of lambda DNA on an agarose gel. These measurements were used to make final dilutions of 3 ng/µl for use in PCR reactions.

DNA was amplified in 30-µl reactions containing 2.5 m*M* $MgCl_2$, 0.1 m*M* each of dATP, dCTP, dGTP, and dTTP, 0.4 µ*M* of decamer oligonucleotide primers (Operon Technologies, Alameda, Calif.), 30 ng of genomic DNA, buffer (TRIS-HCl, pH 9.0, 50 m*M* KCl, and 2.5 m*M* $MgCl_2$), and *Taq* polymerase. Approximately 15 µl of mineral oil overlaid each reaction mixture to avoid evaporation during the thermocycling process. Reactions were carried out in an Omni Gene Thermocycler (Hybaid Instruments, Holbrook, N.Y.) with the following program: 1 cycle of 94°C for 2 min; 5 cycles of 94°C for 40 s, 35°C for 1 min, and 72°C for

Table 2 Decamer oligonucleotide primers producing banding patterns, the total number of polymorphic fragments for each primer, and the number of reproducible fragments included in the analysis

Primer	Primer sequence (5' to 3')	Total number of polymorphic fragments	Reliable polymorphic fragments used in the analysis
OPA-2	TGCCGAGCTG	9	6
OPA-3	AGTCAGCCAC	14	3
OPA-4	AATCGGGCTG	13	2
OPA-7	GAAACGGGTG	4	1
OPA-9	GGGTAACGCC	10	4
OPA-10	GTGATCGCAG	11	3
OPA-11	CAATCGCCGT	5	3
OPA-13	CAGCACCCAC	7	1
OPA-18	AGGTGACCGT	13	5
OPB-6	TGCTCTGCCC	8	2
OPB-7	GGTGACGCAG	16	3
Total	11	110	33

2 min; 35 cycles of 94°C for 20 s, 35°C for 1 min, and 72°C for 2 min; 1 cycle of 72°C for 5 min.

Following amplification, DNA products were mixed with 4 µl loading dye, and 10 µl of each sample was delivered into wells of a 2% agarose gel prepared with 1 × TBE buffer for analysis via gel electrophoresis. Gels were stained in ethidium bromide for 30 min, destained in dH_2O for approximately 30 min, and photographed under UV light. Polaroid photographs provided records of banding patterns and allowed for scoring of polymorphic DNA fragments.

Data analysis

RAPD products were scored by assigning presence (1) or absence (0) for each DNA fragment evaluated. Primers were chosen that produced robust amplified products for each accession and revealed polymorphisms as well as overall reproducibility (Table 2). Similarly, informative polymorphic bands were chosen based on the strength of the bands and the ability to assign presence or absence without ambiguity. Apparent polymorphisms appearing at the upper and lower limits of the banding patterns were rejected due to the inconsistency of amplification and weakness of bands. In addition, this analysis was based only upon polymorphic fragments so numerous monomorphic fragments were present but uninformative and therefore not scored.

Data were analyzed using Nei and Li's index of similarity (Nei and Li 1979):

$$Similarity = 2N_{ab} / N_a + N_b$$

where N_{ab} represents the number of fragments shared by accession a and b, N_a represents amplified fragments in sample a, and N_b represents amplified fragments in sample b. For comparison, data were analyzed using the coefficient of Jaccard (Jaccard 1908), which does not consider common negative data. A similarity matrix was generated for all pairwise comparisons among the 16 maize accessions for each similarity coefficient.

Unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal 1973) cluster analysis was performed on the similarity matrices in order to generate phenograms. Principal coordinate analysis (Gower 1966) was also conducted to graphically display relationships. All procedures were performed using NTSYS-pc (Exeter Software, Setauket, N.Y.).

Results and discussion

RAPD variation

After screening 30 primers for DNA amplification and the presence of polymorphic fragments, 16 primers produced informative banding patterns and of these, 11 proved to generate reliable and reproducible polymorphic fragments (Table 2). Amplified fragments ranged in size from 220 bp to 3000 bp for all primers included in the analysis. The widest range for a single primer was 1816 bp (OPB-7), while the narrowest was 660 bp (OPA-11).

The number of bands per primer ranged from approximately 7 bands (OPA-7) to 21 bands (OPA-3). These figures include both polymorphic and monomorphic bands and are approximate due to the high number of bands produced that appear very weak and unreliable. The total number of bands scored was 154 with an average of 14 bands per primer. The total number of monomorphic bands was 45 (29.2%) with an average of 4.1 monomorphic bands per primer. Banding patterns ranged in percentage polymorphism from 46.7% to 86.2% per primer with a total of 109 polymorphic bands (70.8%). Of the polymorphic bands scored, 33 bands were considered to be reliable and reproducible. Several primers that resulted in successful amplification revealed no polymorphisms and were excluded from the analysis.

It is important to note that the number of amplified fragments per primer and fragment strength may depend upon the degree to which the RAPD assay was optimized. Williams et al. (1990) found that even minor alterations in reaction conditions can affect banding patterns. This study revealed considerable variability in the number of amplified fragments and their intensity with any slight variation in reaction conditions.

Due to the variation in RAPD profiles and difficulties with reproducibility, the scoring of RAPD fragments was done very conservatively. In this analysis, weak and unreliable fragments along with all fragments at the upper and lower margins of each RAPD profile were not scored. In addition, a minimum of two separate reactions was run for each primer to check for reproducibility. Approximately 7 bands per primer (49.3%) were excluded from analysis because they were not reproducible or the assignment of presence/absence was ambiguous for several accessions. While our results are certainly upper estimates of the number of unreliable bands per primer, these data indicate considerable reproducibility prob-

Table 3 Similarity matrix for 16 maize accessions numbered according to Table 1. Similarity was calculated using Nei and Li's index of similarity

Accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	–															
2	0.467	–														
3	0.580	0.580	–													
4	0.705	0.470	0.514	–												
5	0.581	0.581	0.625	0.800	–											
6	0.533	0.467	0.710	0.588	0.581	–										
7	0.483	0.483	0.533	0.606	0.667	0.345	–									
8	0.500	0.642	0.551	0.437	0.552	0.428	0.518	–								
9	0.580	0.452	0.562	0.571	0.688	0.581	0.533	0.621	–							
10	0.308	0.385	0.592	0.467	0.592	0.692	0.400	0.333	0.518	–						
11	0.571	0.571	0.621	0.500	0.552	0.643	0.444	0.538	0.621	0.500	–					
12	0.480	0.240	0.444	0.621	0.518	0.385	0.417	0.348	0.444	0.364	0.333	–				
13	0.538	0.462	0.500	0.667	0.714	0.444	0.560	0.500	0.500	0.522	0.560	0.522	–			
14	0.518	0.592	0.483	0.710	0.759	0.571	0.462	0.400	0.552	0.500	0.615	0.667	0.560	–		
15	0.625	0.562	0.588	0.667	0.706	0.545	0.581	0.667	0.529	0.483	0.581	0.621	0.600	0.710	–	
16	0.462	0.308	0.500	0.533	0.500	0.518	0.320	0.083	0.357	0.522	0.320	0.609	0.500	0.560	0.533	–

lems. A consequence of these conservative measures is a smaller data set and the elimination of potentially informative polymorphisms. However, this approach avoids problems associated with primer mismatches resulting in weak bands and greatly increases confidence in bands included in the analysis.

Overall, these results indicate a very large number of polymorphic bands detected per primer and suggest very high genetic variation among Native American accessions from the Great Plains. Preliminary data on RAPD variation within 1 accession (Osage red corn) indicates that mean polymorphism is 57.8%, 14.5% less than among accessions but unexpectedly high (Moeller and Schaal, unpublished data). Other studies of genetic variation in maize have revealed similar results. Heun and Helentjaris (1993) similarly found 20.7% of fragments to be monomorphic in a RAPD analysis of maize hybrids, indicating high levels of polymorphism for this species. DNA sequence and RFLP analyses of maize have also indicated high levels of variation (Shattuck-Eidens et al. 1990).

Genetic relationships among maize accessions

A similarity matrix based on Nei and Li's index of similarity was used to conduct UPGMA analysis in order to generate the phenogram (Fig. 2). When the same analysis was conducted employing Jaccard's coefficient, similarity indices were nearly equivalent, and an identical phenogram was produced. Only the similarity indices based on Nei and Li's index are reported (Table 3).

Principal coordinates analysis performed on these data support the major clustering patterns. The first and second principal coordinates sufficiently explain the major clusters indicated by numbers on the phenogram (Fig. 3). The first and second principal coordinate explain 19% and 15% of the total variation, respectively. Within each of these major clusters, the third principal coordinate (not shown) corroborated some tertiary relationships indicated by the phenogram but was inadequate in confirming others. A larger data set would improve the resolution of relationships within the major clusters but was beyond the scope of this study.

The phenogram reveals close relationships between accession identities and accessions obtained from the same tribe. For example, the Osage red and Osage sacred red accessions clustered together (cluster 3, Fig. 2; similarity = 0.642). It appears from the RAPD data that these varieties may be derived from the same source and are closely related. All Tamaroa varieties also revealed close genetic affinities (cluster 1, Fig. 2). The similarity index for Tamaroa white and Tamaroa/Tuscarora white was quite high (Fig. 2; similarity = 0.8), showing that RAPDs are capable of grouping Tamaroa white, a pure strain, with F_1 hybrids of the cross between Tamaroa white and Tuscarora white.

Mandan red and Arikara bronze (both "red" corns) show close genetic affinity, which confirms historical records (cluster 4, Fig. 2). Despite incongruent names, the Mandans were very likely the source of the Arikara accession via trade (Will and Hyde 1917). In fact, Will and Hyde (1917) assert that many Arikara maize varieties are probably the same as corresponding Mandan varieties but were given new names following trade. Other notable patterns include the blue corns from the Great Lakes region (Fox blue, Winnebago blue-speckled, and Ponca grey), which all reside in the same major cluster (cluster 2, Fig. 2). An interesting exception is Sac blue which is considered to be identical to Fox blue in historical accounts (Will and Hyde 1917) but clusters instead with the Tamaroa white flour corns. While the name Sac blue implies that this accession is a blue corn, the endosperm is conspicuously white and the ear morphology is quite different from Fox blue. These two lines of evidence seem to indicate that Sac blue is a misidentified specimen or has experienced significant introgression.

The Osage brown accession may be a similar example of misidentification. Information obtained along with

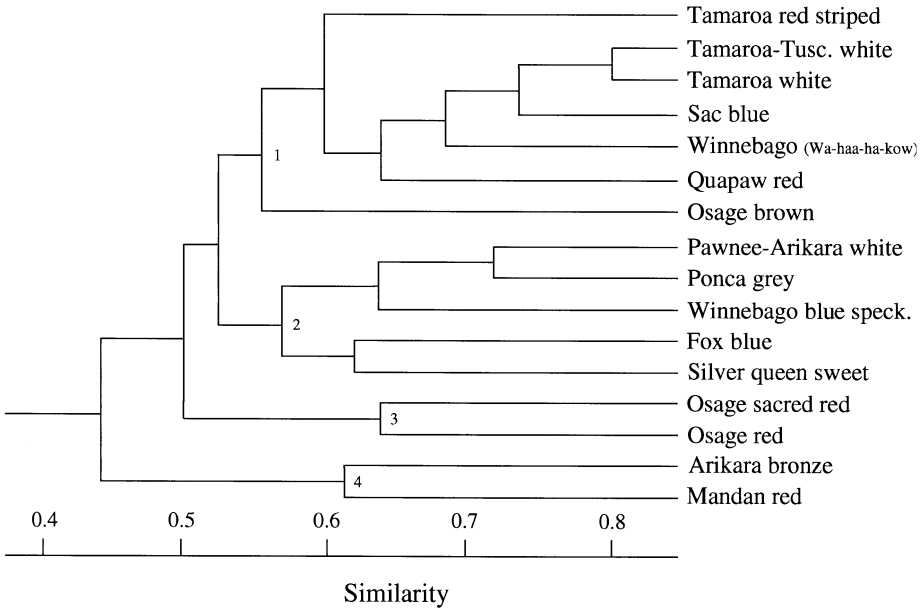

Fig. 2 Phenogram of 16 maize accessions produced using UPGMA cluster analysis based on the similarity matrix shown in Table 3. Similarity values were calculated using Nei and Li's index of similarity. *Numbers* on the phenogram label the four major clusters referred to in Fig. 3 and the Discussion.

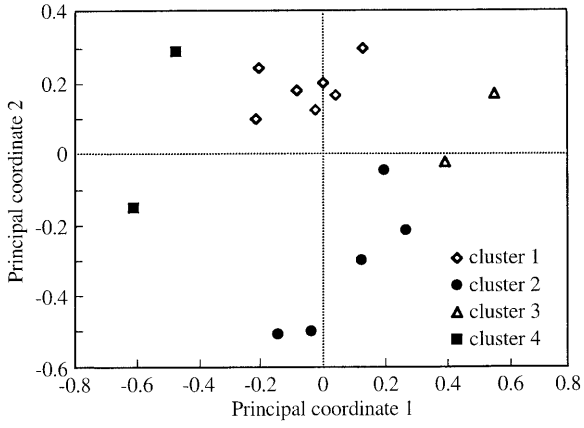

Fig. 3 Principal coordinates analysis of the 16 maize accessions included in this analysis. The accessions are marked according to the major cluster to which each belongs (see Fig. 2). The first principal coordinate explains 19% of the variation, while the second principal coordinate explains 15% of the variation.

this sample indicates that this accession is of uncertain origin and may have originally belonged to the Iowa. The phenogram reveals no affinity between Osage brown and the other members of the Osage collection. In examining historical accounts of plant use by these groups, we were unable to find records which indicate the maintenance of a brown variety by the Osage. However, there are records indicating that the Iowa possessed a sacred brown variety that was handled very carefully and is "evidently a pure strain" (Will and Hyde 1917). Based on both RAPD data and historical accounts, it is seems more likely that Osage brown is synonymous with Iowa brown and was either misidentified or traded from the Iowa to the Osage prior to collection.

Each of these examples demonstrates that with stringent band scoring criteria, RAPDs can be successfully used for organizing seed collections and preserving important genetic resources. This technique may also provide a means for determining the relationships between archaeological specimens and Native American maize accessions that have been maintained. However, there are potential problems with determining genetic relationships among closely related crop accessions. First, these analyses assume that strain "purity" was maintained by preventing gene flow between accessions. This assumption may not always be reasonable since maize is wind-pollinated and pollen flow may occur among adjacent fields. Second, strain identification may be inaccurate due to poor records or improper reorganization of accessions based on convergent morphological characters. Despite our efforts to minimize this uncertainty by carefully excluding unreliable seed sources, the problem still exists and must be taken into consideration when interpreting the results.

Geography and the evolution of maize accessions

In addition to specific comparisons, we were interested in asking whether genetic relationships among Great Plains accessions mirror the geographic position of tribes within the region prior to European influence. This topic is of central importance in understanding the development of maize agriculture and the extent to which diversification took place within the region. In the maize of the Great Plains, we found no evidence to suggest that genetic relationships mirror geography. Instead, the relationships suggest that many maize accessions may have been traded into the region or migrated with tribes who had begun maize agriculture in previous locations. This conclusion does not exclude the possibility that a number of accessions were developed in the region but does suggest that the isolated development of a Great Plains race is unlikely.

The very high levels of polymorphism and low levels of genetic similarity among accessions of the Great Plains similarly support this hypothesis. In addition, archaeological specimens from the Midwest show great variation in ear morphology with respect to varieties found in the Southwest and Eastern Woodlands (Cutler and Blake 1973; Adair 1988). In general, these data support the hypothesis that multiple introductions into the Great Plains from adjacent regions may explain the great morphological and genetic diversity characterizing maize found in this region and do not support classifying maize accessions of the Great Plains into a distinct race.

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