Archaeological and contemporary native breeds of corn (*Zea mays*) from North Peru: phylogeny by microsatellite (STR) fingerprinting

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Abstract

Microsatellites were used to evaluate and compare the lineage relationships and genetic diversity of two improved cultivars of corn (*Zea mays* L.), seven modern native races, and eight archaeological Chimú samples (1.100-1.500 AD) of the North Peruvian coast. Electrophoresis was required to remove the PCR inhibitors from ancient DNA. Eight STR primer pairs generated amplicons from the genome of modern corn, but only five amplified ancient corn DNA. To establish the lineage relationships among samples, the coefficient of similarity of Jaccard and the technique of analysis of conglomerate with binding average were used. Thus, the pattern of genealogical and genetic relationships of the genotypes were determined. Seven out of the eight pairs of SSR primers revealed polymorphism, being 2.47 (range 2-6) the average of polymorphic alleles. The total alleles tested were 23, with 97 polymorphic bands for the modern corn, yet being monomorphic for the Chimú corn, generating an average of genetic similarity of 0.44 and 1, respectively. These results suggest the existence of inbreeding in some populations of Chimú corn of the north coast of Peru 500 to 900 years ago. Modern races exhibited higher polymorphism, due to hybridization and better assisted selection, in agreement with cytogenetic data.

***Key words**:* dendrogram, molecular markers, short tandem repeats, single sequence repeats, taphonomic history, UPGMA

Resumen

Se utilizaron microsatélites para evaluar y comparar las relaciones de linaje y la diversidad genética de dos cultivares mejorados de maíz (Zea mays L.), siete razas nativas modernas y ocho muestras arqueológicas Chimú (1100-1500 d.C.) de la costa norte peruana. Se requirió electroforesis para eliminar los inhibidores de la PCR del ADN antiguo. Ocho pares de cebadores STR generaron amplicones del genoma del maíz moderno, pero sólo cinco amplificaron el ADN del maíz antiguo. Para establecer las relaciones de linaje entre muestras se utilizó el coeficiente de similitud de Jaccard y la técnica de análisis de conglomerado con promedio de unión. Así, se determinó el patrón de relaciones genealógicas y genéticas de los genotipos. Siete de los ocho pares de cebadores SSR revelaron polimorfismo, siendo 2,47 (rango 2-6) el promedio de alelos polimórficos. El total de alelos probados fue 23, con 97 bandas polimórficas para el maíz moderno, pero monomórficas para el maíz Chimú, generando un promedio de similitud genética de 0,44 y 1, respectivamente. Estos resultados sugieren la existencia de endogamia en algunas poblaciones de maíz Chimú de la costa norte del Perú hace 500 a 900 años. Las razas modernas exhibieron un mayor polimorfismo, debido a la hibridación y a una mejor selección asistida, de acuerdo con los datos citogenéticos.

Palabras clave: dendrograma, marcadores moleculares, repeticiones cortas en tándem, repeticiones de secuencia única, historia tafonómica, UPGMA

Introduction

The corn (*Zea mays* L.) is the third world crop in economic importance after the wheat (*Triticum aestivum* L.) and the rice (*Oryza sativa* L.). Archaeological records of maize date back 7.000 years. The corn originated in the American continent from wild species ("teocintles" or "teosintes"), which had a very different appearance than modern corn, propagating into South America before the Spanish conquest. It is currently accepted that the maize domestication was accomplished through the selection of regulatory elements of the gene *teosinte brancheli* (*tbi*). Actually, the variation in the coding region of the gene is similar in modern maize and teosinte (Wang et al, 1999). The process generated new maize breeds with short branches tipped with ears rather than long branches with tassels at the tip. It is believed that such traits became fixed during hundreds of years of human selection together with introgressions after accidental back crossings of cultivated breeds with wild teosinte specimens (Henry, 2001).

Several archaeological evidences suggest that the corn was grown independently in Peru, which shows a large number of breeds. This fact has been considered to propose Peru as an independent domestication center (Grobman et al, 1961; Bonavía and Grobman, 1978). Yet, the absence of the wild prototypes *Zea mexicana*, *Z. diploperennis* and *Z. perennis* (the teosintes or wild relatives of corn), which are only found in Meso America and Central America, spell doubts on the independent domestication of corn in South America. Therefore, it is currently accepted that Peru was instead a secondary diversity center (Hawkes, 1991).

So far, 132 breeds of corn have been described in the Andean region. Up to 55 of them have been considered typical Peruvian, defined after morphological and cytogenetic characters (Grobman et al, 1961). The description of such breeds is rather complicated due to the lack of a clear differentiation between some of them. This is a consequence of the continuous genetic recombination, together with mutations, hybridations and lack of geographical isolation which prevent genotype fixation. These factors have contributed to the large genetic variation found in this crop, which has been analyzed at two levels: archaeobotanical (Yacovleff and Herrera, 1934; Grobman et al, 1961; Towle, 1961; Galinat, 1970; Bird, 1978, 1980, 1985, 1989, 1990, 1994; Dunn, 1979; Bird and Bird, 1980; Bonavía, 1982) and evolutive (Harlan, 1971; Bonavía and Grobman, 1978; Bird, 1979, 1980, 1990; Pearsall, 1986; Engel, 1987).

The climatic conditions of the Peruvian coast (one of the driest deserts in the world) has favored the preservation of large quantities of archaeological remains of corn (shoots, dry leaves, seeds and even full cobs). This has allowed the development of a large number of studies about the early farming of this crop in the prehistory of the Peruvian coast. Nevertheless, the systematic studies to know the evolutive and genetic-molecular aspects of

ancient corn are scarce. Such kind of knowledge is very interesting, since it would allow to determine the molecular diversity fingerprints and patterns in ancient corn. Furthermore, the comparison of molecular data between ancient and modern breeds will shed new light to explain the genetic relationships and evolution patterns along the corn domestication and breeding history.

A particularly important aspect of the analysis of the corn archaeological specimens is the establishment of their racial identity. This has been accomplished in the past based only on morphological aspects of both the corncobs (size, shape, number of strings, seeds per string, etc.) and the seeds (shape, color, etc.). Yet, these traits can be modified by the prevalent environment conditions during plant farming and preservation, including ambient humidity, compression and fragmentation. All these factors, together with the human handling may hinder a proper identification.

Fortunately, the DNA fingerprinting technologies can be exploited to properly identify and classify the archaeological corn samples. DNA-based genotyping is in fact a valuable new approach to determine the identity, evolution and phylogeny (Doebley, 1994). Thus, high molecular weight DNA (1.5 - 2.0 kbp) isolation from deseeded corncobs 4700 ± 500 years old from Peru and Chile has been reported. This allowed allelic comparisons for a fragment of the *adh*$_2$ gene in three archaeological specimens, four *teocintle* types and four modern corn breeds. Some old alleles of the *adh*$_2$ gene were identical or closely related with modern corn alleles. These findings pointed three issues: that the corn genome is very old indeed, that there was a domestication process previous to the South American introduction, and finally the development of the modern corn breeds from wild populations (Goloubinoff et al, 1993).

Another DNA-based study on archaeological corn from Peru was carried out with nine well-preserved cobs retrieved from a burial of the Wari culture (600-850 years AD). They were tentatively classified as 'Proto-Pagaladroga' since the radio-carbon dating assigned them 980 ± 95 years (with reference to year 1.950). The nucleic acids were extracted from the seeds using phenol and segregated by on 2.5% (w/v) agarose gel electrophoresis fractioning. The isolated material showed a low molecular weight. These results were further confirmed by radioactive ATP labeling and 8% (w/v) polyacrylamide gel electrophoresis. The sequencing of the amplified DNA showed differences when compared to modern corn. This represents the first evidence clearly demonstrating that the seeds of Wari corn contain endogenous DNA (Rollo et al, 1994a).

It has also been reported the isolation of high molecular weight DNA (up to 20 kbp) from the 'Anazasi' North American native archaeological corn from the XII century. This allowed the application of the Restriction Fragment Length Polymorphism (RFLP) methodology and thus allelic comparisons between archaeological and modern corns (Helentjaris, 1988). Yet, although the author demonstrated that enough quantity of good quality DNA can be extracted

from archaeological cobs for RFLP analysis, the small number of probes tested did not allow a phylogenetic analysis.

Previous work has reported comparisons of Chimú corn with modern breeds from the north coast of Peru using molecular tools like Random Amplified Polymorphic DNA (RAPD) at the "Centro Internacional de Mejoramiento de Maíz y Trigo" (CIMMYT, Mexico). The DNA was extracted from a total of 22 archaeological samples. The RAPD CIMMYT procedure allowed the amplification of four native archaeological samples, three modern highlander breeds and one Panamanian cultivar. The primers used generated small DNA amplicons (usually about 70 to 220 bp), yet no phylogenetic relationships could be established due to the small number of alleles compared (Bird et al, 1995b)

On the other hand, the archaeological patterns predict that the 'Cuzco' breed could be more related to the Chimú corn than to the other breeds which are more geographically proximate (Bird et al, 1995b).

The purpose of the present work was to overcome the classification methods based on morphological traits to establish the racial identity and diversity of the archaeological corn remains and their kinship links. To that goal we have used the DNA-based methodology of microsatellites, also known as Short Tandem Repeats (STR) or Single Sequence Repeats (SSRs). This procedure will allow to decipher the genetic relationships between the Chimú corn and the modern breeds of the North Peruvian coast. Additionally, we wanted to evaluate the hypothesis of a hybridization between Northern ('Alazán', 'Pagaladroga', 'Mochero' and 'Arizona') and Southern ('Huayleño', 'Paro" and 'Cuzco') corn breeds when the Chimú culture was conquered by the Incas (Southern Mountain range), which likely produced a corn germplasm interchange.

Materials and methods

Archaeological corn collections

The archaeobotanical samples of corn (*Zea mays* L.) were discovered in two archaeological projects currently running in the North Peruvian coast. An important quantity of ancient corn remains related to the Chimú culture (1.100–1.500 AD) was recovered from them. Sampled sites included the Archaeological Complexes of 'El Brujo' (Department of La Libertad), particularly for houses; and 'Puerto Pobre' (Department of Ancash) (Fig. 1), for both houses and garbage dumps. The 'El Brujo' collection was made of two well-preserved samples: T1 and T4, including 45 and 28 isolated seeds, respectively. The 'Puerto Pobre' collection was made of six full-cob samples (Fig. 2): 3-513-PP, 4-518-PP, 5-519-PP, 6-520-PP, 7-525-PP and 8-526-PP. An average sample of 40 seeds were chosen from the central portion of the

cob, where best preserved alleles are expected. Appropriate authorizations were obtained from the “Instituto Nacional de Cultura” (Peru) for the corresponding exportations and studies of DNA markers (Mexico), Scanning Electronic Microscopy (SEM) (Spain) and radiocarbon dating (Denmark and USA), taking into account the original cultural scenarios.

Figure 1. **Archaeological sites**. Geographical situation of the main archaeological corn (*Zea mays*) remains in the Peruvian coast.

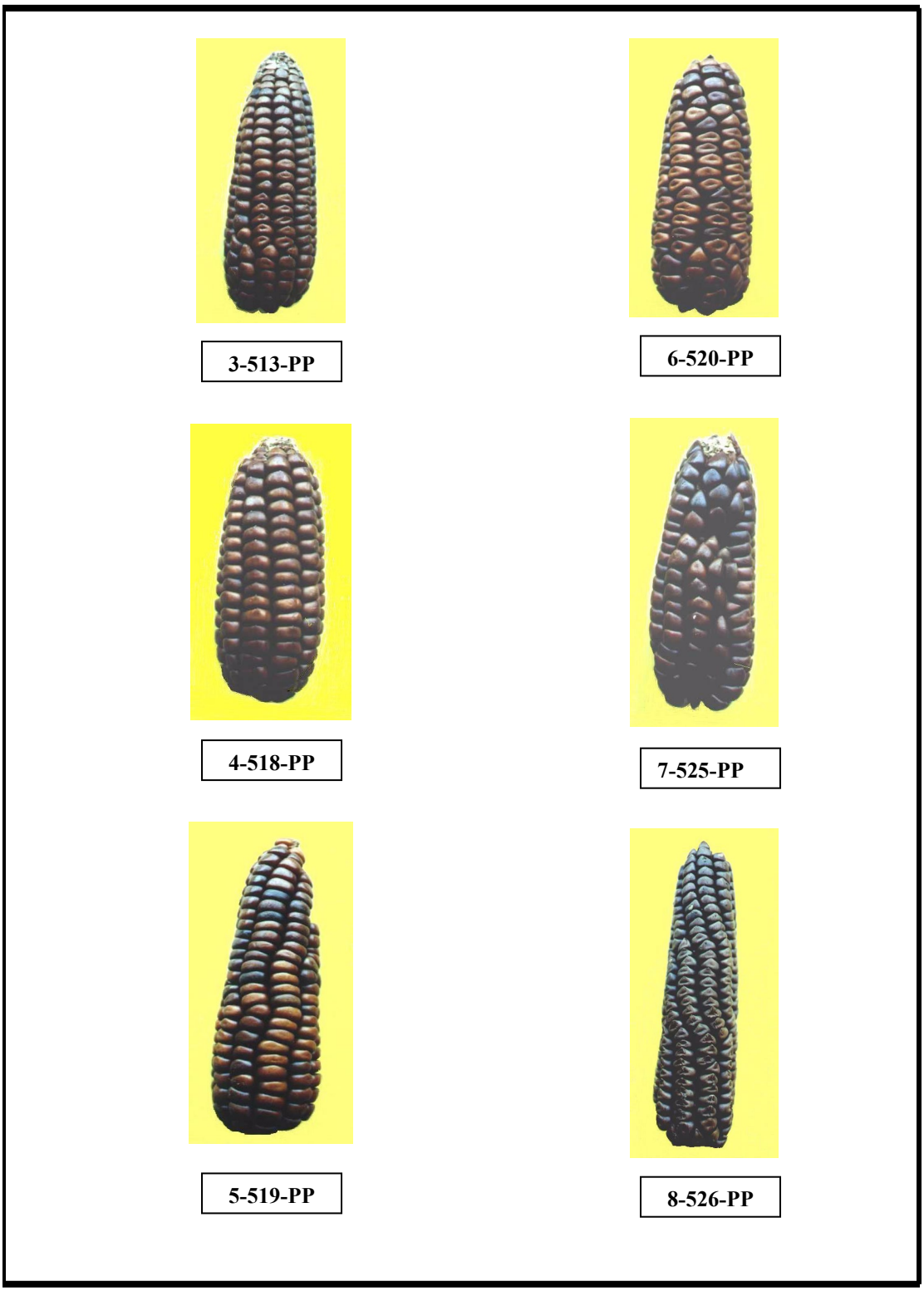

Figure 2. Collections of archaeological Chimú corn (*Zea mays*).

Modern samples: selection and sowing of corn breeds

We have used seven native breeds of Peruvian corn (Table 1) and two improved cultivars generated at the CIMMYT. The samples from Peru included 'Alazán', 'Pagaladroga', 'Mochero' (primary breeds) and 'Arizona' (secondary breed), cultivated in the Northern coast; 'Huayleño' (primary breed) from the Ancash Mountain range; and 'Paro" and 'Cuzco' (primary breeds) from the Southern range. Finally, the CIMMYT samples (CML123 and K64R) had known genealogies and were thus used as positive controls.

Table 1. Native modern breeds of corn (*Zea mays* L.) from Peru analyzed in this work.

Sorting number	Accession number	Pedigree	Breed (acronym)	Altitude[1] (m.o.s.l.)	Place of origin (reference)
1	8955	Perú 585	Alazán (ALA)	55	Lambayeque (74-440-0)
2	15454	Perú 752	Huayleño (HUA)	2600	Chiclayo (Q95-87)
3	8917	Perú 382	Paladroga (PAG)	55	Lambayeque (76-80-0)
4	13526	Perú 526	Cuzco (CUZ)	_	Chiclayo (Q94)
5	_	_	Paro (PAR)	_	Chiclayo (Q98)
6	_	_	Mochero (MO)	50	Macabi
7	_	_	Arizona (ARI)	50	Trujillo

[1]Meters over the sea level (m.o.s.l.)

The modern seeds were obtained from the Corn Germplasm Bank of the CIMMYT and sown in 50 cm tall flowerpots containing a mixture substrate decontaminated with methyl bromide and made of equal amounts of sand, soil and manure. Three weeks after the germination of the seeds in the greenhouse, 4 g of young and healthy leaf tissue were excised from each plant. To increase the variability of the samples, three plants were analyzed for each breed. The leaves were chopped with sterile scissors and washed with 70% (v/v) ethanol to remove any remains of dirt or microorganisms like bacteria and fungi.

Laboratory tests

The radiocarbon dating was carried out at the Geology Laboratory of Copenhagen (Denmark) and the Beta Analytic Radiocarbon laboratories at Florida (USA) for the samples from 'El Brujo' and 'Puerto Pobre', respectively. The scanning electronic microscope inspections of the archaeological corn seeds were performed at the Laboratories of Electronic Microscopy of the "Universidad Autónoma de Madrid" (Spain). The genomic DNA extraction was carried out in the Molecular Biology Laboratory of the "Universidad Autónoma Chapingo" (Mexico). The STR tests were performed in the Applied Molecular Genetics Laboratory of the CIMMYT (Mexico), following the procedures described in the Laboratory Protocol Manuals (Hoisington et al, 1998).

Genomic DNA isolation from archaeological and modern corn

The extraction of the genomic DNA from the modern breed leaves (2.5 g) and from the archaeological seeds (5 g) was accomplished after the Dellaporta method (Dellaporta *et al*., 1983) with some modifications. Briefly, it included four steps: i) extraction with phenol to obtain high purity DNA; ii) electrophoresis in low melting point agarose (archaeological samples) from BDH Laboratory Supplies (Poole, UK; www.bdh.com) to discard PCR inhibitors after Sambrook and Russell (2001); iii) precipitation with cold (4º C) absolute ethanol in the case of modern breeds or after recovery from agarose plugs for archaeological samples (melting at 65º C, addition of one volume of TE buffer, pH 8.0 and incubation at 65º C, addition of one volume of equilibrated phenol, centrifugation, recovery of the aqueous phase, and precipitation with absolute ethanol); and iv) three 70% (v/v) ethanol washes (archaeological samples) to further clean the isolated DNA. The DNA was quantitated reading the optical density at 260 nm in a DU-65 spectrophotometer from Beckman Coulter (Fullerton, CA, USA; www.beckmancoulter.com). The DNA samples were stored at 4º C and diluted to 100 ng/µl or 125 ng/µl before their use.

PCR amplification of the STR molecular markers

The STR typing was carried out after PCR amplification, size fractioning and staining (Hoisington *et al*., 1998), as described below. Eight pairs of PCR primers were chosen, based on their higher polymorphism and location in the corn genome (Table 2).

Table 2. Microsatellite markers tested on seven native Peruvian breeds, two improved cultivars and eight archaeological samples of corn (*Zea mays* L.).

ırting mber	Loci name	Allele si (bp)	PCI (°C	Repetition patter (5' => 3')	Forward and reverse primers (5' => 3')
	bnlg1	< 118	5	$(AG)_{17}$	CTCTGCGCTACCTTTCTGAGTC
					GCGGAATCCTTGTGTTCTTG
	dup0	118-19	5	$(CT)_3$ T $(CT)_6$ (CA	AGCAGGTACCACAATGGAG
					GTGTACATCAAGGTCCAGATTT
	phi01	110-12	5	GGA	GAGCTTCAGCAAGAGCATCCAG
					CAACGCGATCGATGTGAGCACA
	phi04	62-66	5	ACGC	ATCTCGCGAACGTGTGCAGATTCT
					TCGATCTTTCCCGGAACTCTGAC
	phi07	156-16	5	GAGCGG	TTCTTCCGCGGCTTCAATTTGACC
					GCATCAGGACCCGCAGAGTC
	phi11	120-28	5	GTCT	GCTCCAGGTCGGAGATGTGA
					CACAACACATCCAGTGACCAGAGT
	phi11	93-11	5	TA-ATAC	CTAGTGGGCGAACAACTGGTAAG
					AAAGAGACCGTGTCAGGATTGCC
	phi12	100-12	5	AAGCG	TTGCTCGGTATGAAGAAAATAGTCT
					ATCTTGCAACTAGACTGAGGCAACC

The PCR mixture used to amplify the archaeological/modern (respectively) corn DNA contained the following components in a total of 20 µl reaction volume per tube: 125/50 ng of DNA, 1X PCR buffer solution for the *Taq* DNA polymerase enzyme, 5.0/3.5 mM $MgCl_2$, 150 µM of each deoxyribonucleotide triphosphate (dATP, dCTP, dGTP and dTTP), 2.0/1.5 U of the *Taq* DNA polymerase enzyme from Invitrogen (Carlsbad, CA, USA; www.invitrogen.com), 10% (v/v) glycerol and 0.25 µM of each (forward and reverse) corn-specific primer from Primer Research Genetics (Huntsville, AL, USA; www.primerresearch.com). The amplification was accomplished in a Thermal Cycler 480 from Applied Biosystem (Foster City, CA, USA; www.appliedbiosystems.com) with the following cycling profile: one first denaturing step (1 min at 93° C); 35 cycles (1 min denaturing at 93° C, 2 min annealing at "X" ºC, and 2 min extension at 72° C); and one final step (7 min at 72° C) to help finish incomplete amplicons. The "X" represents the specific hybridization temperature used for each pair of STR primers, in a 54° C to 58° C range (see Table 2), being selected with the thermal intervals of the Maize Data Base (MaizeDB) at <http://www.agron.missouri.edu/ssr.html>.

The amplicon segregation was accomplished in 3% (w/v) Seakem agarose gels from BMA (Rockland, MD, USA; www.bmaproducts.com) for the preliminary tests and then in 12% (w/v) denaturing polyacrylamide gels (13 cm tall x 14 cm wide x 1 mm thick). Independent gels were used for each STR marker. Aliquots of 4.5 µl of each PCR product were placed on the gel wells made with 28-tooth combs. Three wells per gel were reserved for molecular weight

standards: '*phi*X174/*Hae*III' for the first and last wells, and '100 bp DNA ladder' from Invitrogen between the archaeological (left side samples: T1, T4, 513, 518, 519, 520, 525 and 526) and modern (right side samples: Alazán, Arizona, Mochero, Pagaladroga, Huayleño, Paro, Cuzco, CML131 and K64R) corns. The separation was performed in AE-6220 vertical electrophoresis systems from Atto Corporation (Tokyo, Japan; www.atto.com). The gel electrophoresis was run at 300 V and 95 mA (28.5 W) for 3 h.

The DNA amplicons were visualized after 0.2% (w/v) silver nitrate staining. The STR alleles were visually scored based on their molecular sizes. The developed gels were documented with a Digital Science 1D camera from Kodak (Rochester, NY, USA; www.kodak.com) under visible light.

Statistical procedures

Data analysis was based on the DNA band (allele) scoring on the gels (presence or absence of particular bands in the gel photographs). The alleles obtained in the different amplification replicas were compared, confirming their expected sizes with the molecular weight standards included in the gels. The analyses were very restrictive. Therefore, only clear and consistent bands showing the expected sizes (electrophoretic mobility) through all repetitions and gels were considered as equivalent and thus scored. The gel bands corresponding to the STR amplicons were input in a Basic binary Data Matrix (BDM) for both archaeological and modern corn samples. This was accomplished using the spreadsheet Excel from Microsoft (Redmond, WA, USA; www.microsoft.com), scoring zero ("0") when the band was absent, one ("1") when it was present, and a question mark ("?") when it was doubtful. The BDMs were then transformed in similarity matrices applying the Jaccard's Similarity Coefficient: $a/(n–d)$; being "a" the number of DNA fragments (gel bands) shared between two genotypes; "n" the total number of polymorphic bands found after the gel screening, and "d" the number of missing bands in both genotypes (Jaccard, 1908). This generated a matrix made of zeros and ones for each primer pair used in the PCR amplifications. The columns represent the samples analyzed and the rows correspond to the bands (alleles) selected in the molecular weight range expected for the selected STR primers. The different matrices obtained for each primer pair were joined to build a single one, which was then analyzed using the application NTedit version 1.1b (1998) from Applied Biostatistics (Foster City, CA, USA; www.appliedbiostatistics.com). The matrix of zeros and ones was exported to the application NTSYSpc version 2.02i (1998) from the same manufacturer and further transformed in a genetic similarity matrix. The pattern of genotype relationships for the molecular marker matrix was obtained applying the procedure of conglomerate analysis based on the Unweighted Pair Group Method Arithmetic Average (UPGMA). Finally, the dendrogram showing the phylogenetic relationships was built. The data were processed with the applications NTSYSpc (Rohlf 1997) and Excel.

Results

Radiocarbon dating of archaeological collections

The antiquity (authenticity) of the ancient Chimú corn collections and therefore the DNA isolated from them was confirmed with radiocarbon dating of representative samples from each emplacement. Thus, a sample corresponding to the 'El Brujo' (from 'Huaca Cao Viejo') was analyzed in the Geology Laboratories of the National Museum of Denmark. The expected age was 590 ± 70 years (relative to the year 1950). The dating showed figures between 1.300 to 1.420 years AD; that is 640 years ago, considering the year 1.360 AD as average.

Another sample from 'Puerto Pobre' was dated at the Beta Analytic Radiocarbon Laboratories of Miami. The expected age was 440 ± 50 years (relative to the year 1950). The calibration with 2 sigma showed dates between 1415 to 1520 years AD with 95% probability; that is, about 530 years ago, considering the year 1470 AD as average.

Scanning electronic microscope topography of ancient Chimú corn seeds

The photographs corresponding to sample 518 from 'Puerto Pobre' show a clean reticulated look of the seed head. There is also a large quantity of starch grains from the endosperm, with some proteic bodies typical of this species (Fig. 3). The magnification is 1000X, showing a good preservation and the absence of microorganisms, which supports the endogenous nature of the isolated DNA.

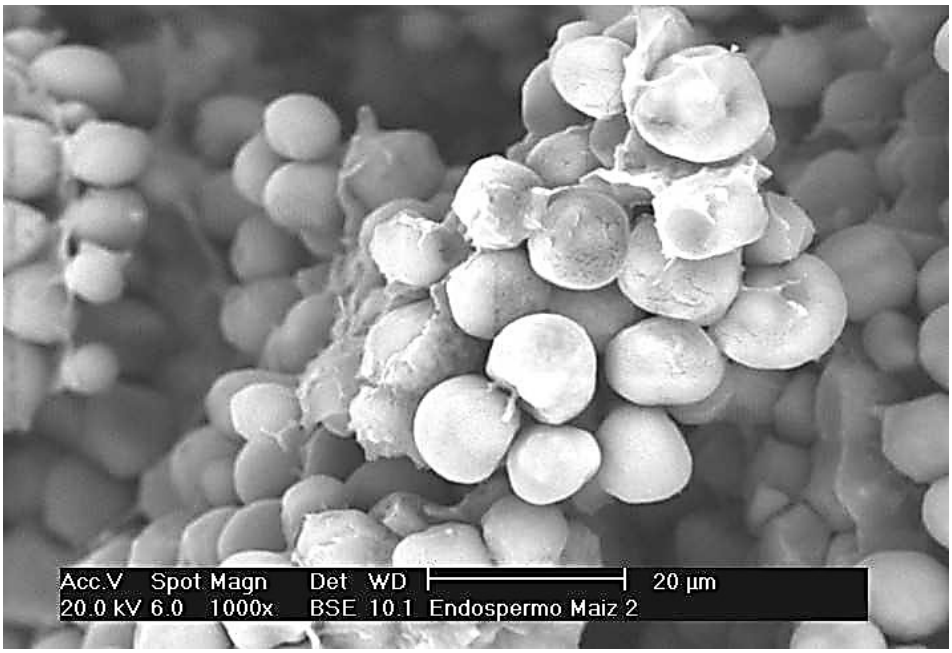

Figure 3. **Scanning electron microscopy**. Endosperm topography of archaeological Chimú corn (*Zea mays*) seeds. Samples were vacuum–stained with gold and amplified 1,000X.

Isolation of genomic DNA from the archaeological corn samples

The DNA was isolated from leaves (modern breeds) or from seeds (archaeological corn). The latter showed good band signals of 70 to 500 bp on 2% (w/v) agarose gels stained with ethidium bromide after UV illumination (Fig. 4).

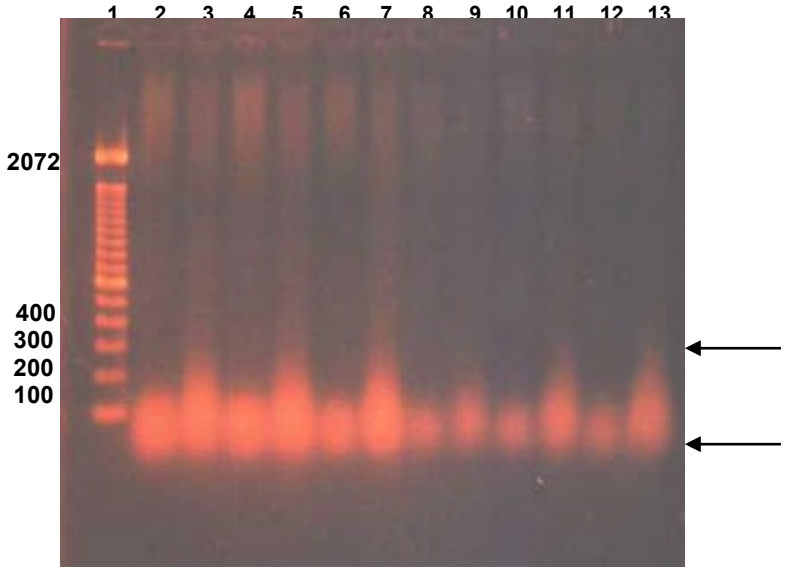

Figure 4. **Total nucleic acids from archaeological Chimú corn (*Zea mays*) collections**. Samples were loaded on 2% (w/v) agarose gels and photographed under UV. light after ethidium bromide staining. Lane 1: "1,000 bp ladder" molecular weight marker; lanes 2 to 7: 'Puerto Pobre' corn samples; Lanes 8 to 13: 'El Brujo' samples.

Yet, old corn DNA contained many impurities (salts, tannins, humic acids, etc.). Contaminants were effectively removed segregating the PCR reaction product by electrophoresis (like the low melting point agarose approach described in this work) and excising the amplified DNA band of interest. The brownish contaminants (tannins and humic acids) exhibited a slower migration than the partially degraded DNA and RNA molecules. After ultra violet (UV) light irradiation, such contaminants produced a green-bluish fluorescent blotch, clearly different and segregated from the orange-reddish fluorescence of the DNA. The latter was selected, excised, melted and subjected to DNA re-extraction. The newly purified and quantified material was diluted and used in the subsequent PCR reactions, which amplified the STR targets. Not surprisingly, the concentration data from the re-extracted DNA (old genotypes) revealed that the quantity and quality of ancient DNA that could be isolated from each sample was directly associated with the preservation grade of the seeds (the better preservation of the seeds, the more and better DNA that could be retrieved from them).

STR typing

Different experimental conditions were tested to optimize the STR amplifications by PCR. For instance, it was found that the amplification of ancient corn DNA required higher concentrations of $MgCl_2$ and *Taq* DNA polymerase than the ones needed for modern samples. Interestingly, such concentrations were also somewhat higher than the ones previously reported for modern corn DNA by Hoisington et al, (1998). On the other hand, the optimal annealing temperatures found in this work (54° C and 58° C) for the eight pairs of primers are in agreement with the published data in 'MaizeDB', except for the primer pair *bnlg*131. The latter amplified Alazán, CML and K64R, yet failed to properly amplify the other modern genotypes with a clear and consistent pattern, being thus scored as a question mark ("?") in the matrix data (BDM). It must be pointed out that we did not test lower temperatures, which might have generated positive results for such primer pair.

Five (*dup*014, *phi*046, *phi*128, *phi*113 and *phi*115) out of the eight pairs of STR primer pairs tested usually generated DNA fragments (alleles) with good resolution in both old and modern samples. The only exceptions were *dup*014 (which amplified Alazán but did not generate unambiguous bands with the other modern genotypes), and *bnlg*131 (as previously indicated), *phi*011 and *phi*076 (which did not produce any amplified DNA from archaeological samples). The eight STR markers generated 23 total alleles, being nine of them present in the archaeological samples. The average number of alleles was 2.9 per STR: from two alleles for four primer pairs (*phi*046, *phi*011, *phi*128 and *phi*115) to six alleles for the *bnlg*131 primer pair. As an example, the Fig. 5 shows the results obtained with primers *phi*046 and *phi*115 on both archaeological and modern samples. The archaeological samples showed a value of zero (that is, where monomorphic), whereas the modern samples had a score of 2.71 (Table 3). The genetic similarity average of both samples was calculated with the application NTSYSpc.

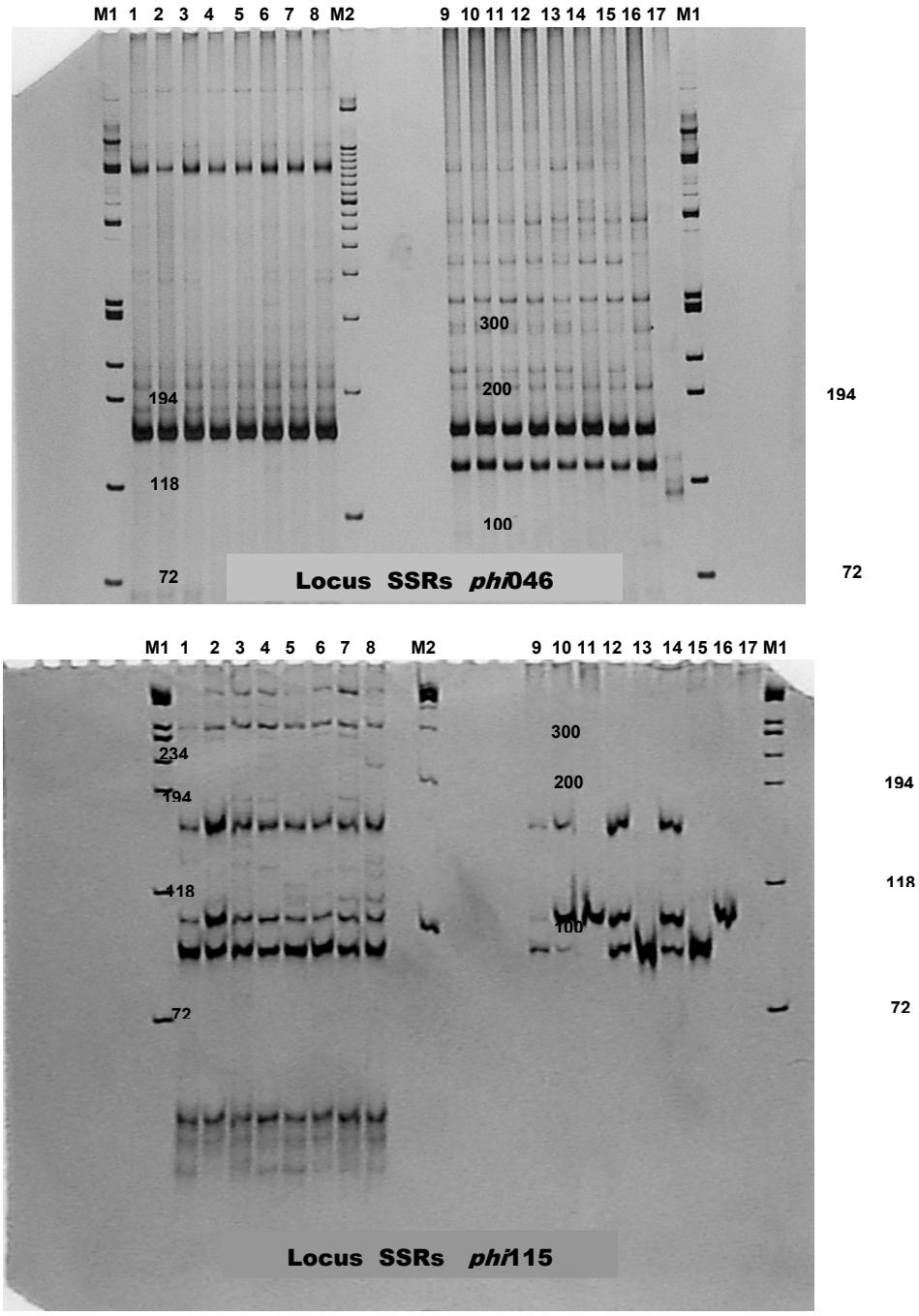

Figure 5. **PCR amplification products of both archaeological and modern corn (*Zea mays*)**. Genomic DNA was amplified using the STR primer pairs *phi*046 and *phi*115, segregated on 12% (w/v) polyacrylamide gels and stained with silver nitrate. Lanes M1 & M2: "*phi*X174/*Hae*III" & "100 bp ladder" molecular weight markers, respectively; lanes 1 to 8: archaeological samples (genotypes T1, T4, 513, 518, 519, 520, 525 & 526, respectively); lanes 9 to 17: modern samples (genotypes Alazán or ALA, Arizona or ARI, Mochero or MO, Pagaladroga or PAG, Huayleño or HUA, Paro or PAR, Cuzco or CUZ, CML131 or CML & K64R, respectively).

Table 3. Analysis of eight STR markers in eight archaeological samples, seven modern native breeds and two improved cultivars of corn (*Zea mays* L.).

Sample	Total bands (alleles)	Total polymorphic Bands	Average of polymorphic alleles	Average of genetic similarity
Archaeological	9	0	0	1
Modern	19	97	2.71	0.43
Total	23	21	2.90 (2 - 6)	0.44

The amplicons generated by each corn STR primer pair used in this work have a defined and known size range (Table 2), which reduces the risk of false assignments. Besides, the amplicon band images corresponding to them are available in the MaizeDB at <http://www.agron.missouri.edu/ssr.html>.

Genetic similarity matrix

The distribution range of the 153 figures of the genetic similarity matrix for the STR markers (Table 4) encompassed values from 0.08 to 1.00, with an average of 0.44 for the whole sample. The distribution range of modern samples varied from 0.08 to 0.69, with an average of 0.43 and showing 60% of values smaller than 0.50. On the other hand, as previously indicated, the archaeological samples were all alike; that is, showed the same genetic similarity value (1).

Table 4. Genetic similarity matrix (Jaccard's coefficient) between the eight archaeological samples, seven modern native breeds and two improved cultivars of corn (*Zea mays* L.).

		Archaeological corn								**Modern corn[1]**								
		T1	**T4**	**513**	**518**	**519**	**520**	**525**	**226**	**ALA**	**ARI**	**MO**	**PAG**	**HUA**	**PAR**	**CUZ**	**CML**	**K64R**
Archaeological corn	**T1**	1																
	T4	1	1															
	513	1	1	1														
	518	1	1	1	1													
	519	1	1	1	1	1												
	520	1	1	1	1	1	1											
	525	1	1	1	1	1	1	1										
	526	1	1	1	1	1	1	1	1									
Modern corn	**ALA**	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	1								
	ARI	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.33	1							
	MO	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.55	0.60	1						
	PAG	0.29	0.290.29	0.29	0.29	0.29	0.29	0.29	0.57	0.54	0.69	1						
	HUA	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.30	0.50	0.27	0.45	1				
	PAR	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.60	0.40	0.60	0.64	0.50	1			
	CUZ	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.33	0.33	0.27	0.45	0.67	0.57	1		
	CML	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.53	0.67	0.60	0.50	0.30	0.50	0.20	1	
	K64R	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.46	0.44	0.67	0.46	0.22	0.63	0.38	0.50	1

[1]ALA: 'Alazán'; ARI: 'Arizona'; MO: 'Mochero'; PAG: 'Pagaladroga'; HUA: 'Huayleño'; PAR: 'Paro'; CUZ: 'Cuzco'; CML: CIMMYT improved cultivar; K64R: CIMMYT improved cultivar.

Dendrogram and clustering pattern

The Fig. 6 shows the pattern of genetic relationships of the full sample with eight STR polymorphic markers. The clustering analysis was performed at 0.45 from the interval of the Jaccard's similarity coefficient. The two samples (archaeological and modern) were clearly separated in two groups. As indicated above, all archaeological genotypes exhibited the same genetic similarity value. On the other hand, the modern sample genotypes clustered in three subgroups (A, B and C), which contained between two and five genotypes each. Thus, A was made of five genotypes: 'Alazán' (ALA), 'Mochero' (MO), 'Pagaladroga' (PAG), 'Paro' (PAR) and one improved line (K64R).

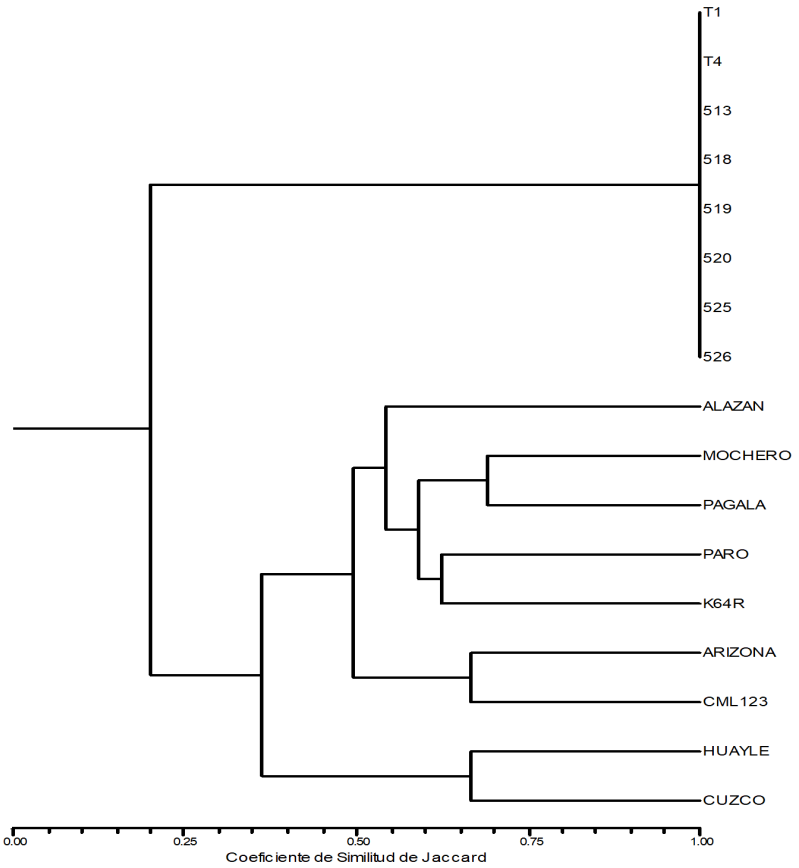

Figure 6. **Phylogenetic tree**. The dendrogram was built with STR markers using the UPGMA algorithm. It shows the genetic diversity kinships for eight archaeological Chimú samples, seven modern native breeds and two improved cultivars of corn (*Zea mays*).

The north-coastal breeds ('Mochero' and 'Pagaladroga') shared a genetic similarity average of 0.68. The B subgroup included two genotypes: 'Arizona' (ARI) and CML123 (CML), which was the other improved line. They exhibited a genetic similarity average of 0.66. Finally, the C subgroup was constituted by two genotypes: 'Huayleño' (HUA) and 'Cuzco' (CUZ), with a genetic similarity average of 0.66. This dendrogram clearly shows that the three primary north-coastal breeds ('Alazán', 'Pagaladroga' and 'Mochero') share the same cluster. This is in agreement with their geographical proximity and supports their genetic similarity. Likewise, 'Alazán' (ALA) is the breed more closely (genetically) related to the archaeological genotypes of corn.

Discussion

Archaeobotanical description of the Chimú corn collections

The data obtained in this work have allowed to affiliate some archaeological specimens of corn to proto-breeds that were present in the pre-Hispanic northern coast of Peru; particularly those that were grown during the Chimú period, both at the Chicama and the Casma valleys. The cobs from 'Puerto Pobre' showed traits typical of both north-coastal proto-breeds, as well as north-coastal intermediate proto-breeds with introgressions from highlander breeds. The isolated corn seeds from the 'El Brujo' samples would belong to seaside breeds; possibly 'Proto-Alazán' and 'Proto-Pagaladroga', which is in agreement with Grobman et al, (1961) and Sevilla (1994). The corn cultivars of the North-Peruvian coast 1.200 years ago are known as proto-forms of the 'Alazán' breed, with cylindrical and short cobs showing 10 grain strings. There is still doubt about the archaeological 'Proto-Alazán' in relation to the other two breeds sympatrically distributed ('Proto-Mochero' and 'Proto-Pagaladroga'), due to the intermediate cob traits.

Isolation, preservation degree and authenticity of the Chimú corn DNA

The isolation of nucleic acids with high molecular weight from old seed samples does not guarantee the absence of microbial contamination. Actually, it may rather be an indicative of such contamination. Thus, the isolation of partially degraded nucleic acids from the Chimú corn collections is evidence that at least part of the original genetic material of the archaeological seed has been preserved.

Other studies have also reported the isolation of partially depolymerized DNA from archaeological corn found in the north coast of Peru. One report was about poorly preserved seeds from Sipán (Moche culture; approximately 500-year AD) (Rollo et al, 1991). Another study found very low-molecular weight nucleic acids with highly degraded DNA in individual embryos of archaeological corn samples from Casma (north coast of the Department of Ancash). In yet a few more instances, some small embryos were combined in a mixed sample

to have a larger weight and thus generate a higher DNA concentration, which ranged from 0.5 to 1.0 g (Bird et al, 1995b). It must be mentioned that Sipán is about 300 km away from the sites where the Chimú corn samples (used in our work) were collected. Hard tissues and structures (like those in seeds) usually are better preserved than parenchymatic tissues. This is due to the scarcity of water and enzymes in the former, as well as their higher mechanic protection (Hermann and Hummel, 1994).

On the other hand, the abiotic conditions that may contribute to extend the preservation have been less studied. Nevertheless, it is probable that the aridity of the north coast of Peru has played a favorable role to preserve its archaeobotanical material. The collections of Chimú corn belong to coastal sites (Fig. 1) with extreme aridity, being sometimes preserved as full cobs. Such is the case for the 'Puerto Pobre' sample (Fig. 2). This likely favors the embryo protection, increasing the probabilities of extracting less degraded DNA. Pääbo (1985, 1989) has suggested that a quick dehydration is required to enhance the DNA preservation, due to the susceptibility of such molecules to the enzymatic hydrolysis. Besides, the dehydration may contribute to the differential conservation of some organelles in plant tissues. Additionally, it is expected that the dehydration would reduce the endogenous protease activities in relation to the senescence properties of the leaves. Yet, some old tissues containing DNA were preserved fully hydrated (Golenberg et al, 1990; Hagelberg and Clegg, 1991).

Another important issue affecting the preservation and authenticity of nucleic acids isolated from old samples is the possible microbial contamination and activity. Microorganisms may degrade organic material in general and nucleic acids in particular. Thus, a scanning electronic microscopy (SEM) study is recommended to evaluate the potential preservation of the samples and thus their expected proportion of old DNA (Hermann and Hummel, 1994). The samples of Chimú corn analyzed in the present work showed a good conservation and were further subjected to a topographic exploration of the head surface and endosperm by SEM. As shown in Fig. 3, no microorganisms like bacteria or fungus were detected. Besides, the starch grains and proteic bodies exhibited a good preservation and morphology. This is probably due to the quick dehydration to which the archaeological samples of the north coast of Peru are subjected along their taphonomic history, as previously indicated. The cause for dryness would be the abundant salts found in the archaeological environments of 'El Brujo' and 'Puerto Pobre', together with the limited rainfall rate, which should limit the microbial proliferation. Therefore, the microelectronic study supports the endogenous nature of the DNA isolated from the corn seeds used in our work. Similar analyses were carried out with corn seeds from a burial of the Wari culture (800 years AD), showing a well-preserved endosperm with starch grains, without evidence of contamination by bacteria or fungus (Rollo et al, 1994a). Therefore, this asepsis seems a common feature of the archaeobotanical corn material found at the north coast of Peru.

Yet another factor considered to support the authenticity of the isolated DNA is the inclusion of independent replicas for each sample. The accordance and consistency of the results generated in this work with the different extracts further supported the authenticity of the material used and the reliability of the experimental approach.

Last, but not least, perhaps the most important criterion of authenticity for the DNA isolated from the archaeological corn collections used in this work was the radiocarbon dating, which confirmed the seniority of the corn seeds and thus the old age of their DNA, as previously reported.

Isolation and amplification of archaeological and modern corn DNA

The total nucleic acids isolated from the ancient corn collections are shown on Fig. 4. Besides the contamination with exogenous DNAs, another potential problem when dealing with ancient DNA is the possible presence of unwanted coprecipitated compounds such as polysaccharides, tannins and humic acids. The latter are substances that originate in the soil and that may bind to the seed during its taphonomic history. They can be recognized by the brownish color of the DNA isolated from archaeological corn and because they generate a green-bluish fluorescence in agarose gels under UV light. Such fluorescent material may also contain sulfides and has an electrophoretic mobility on agarose gels equivalent to dsDNA fragments of about 600 bp. Those chemicals may copurify with the DNA and thus inhibit the enzymatic amplification of DNA by PCR.

The first PCR amplification trials carried out in this work failed to amplify the archaeological DNA samples. This was likely due to the presence of humic acids (known inhibitors of the PCR reaction). We performed 10 PCR trials with such ancient DNA (before removing the putative PCR inhibitors) using specific STR primers for modern corn. These trials were carried out with DNA diluted 1:50 and 1:100 fold, to try and abolish or reduce the putative inhibitory effect on the PCR as indicated by Golenberg (1991). In all cases, the result was negative; that is, no amplicon was generated. Therefore, we further purified the previously isolated DNA (ranging from 70 to 500 pb), by segregation in low melting point agarose gel electrophoresis. We detected better DNA signals in the ‘Puerto Pobre’ samples (possibly because they came from full cobs) and less intense bands or signals in the ‘El Brujo’ ones (from isolated seed grains). The gel plugs containing the orange-reddish fluorescence (that is, the DNA) for each ancient sample were excised, melted and subjected to phenol extraction to try and recover the inhibitor-free nucleic acids. Samples were not further dialyzed, since the above approach generated satisfactory amplification results.

Thus further experiments were carried out in which extra-purified samples (free of inhibitors) were used in PCR amplification reactions with the *dup*014 STR primer pair of corn. Besides, we increased the concentrations of both $MgCl_2$ and *Taq* DNA polymerase recommended by

Hoisington *et al. (*1998) for modern DNA, obtaining the best amplifications with 5.0 mM $MgCl_2$ and 2.0 U *Taq*. Concentrations were also modified for the modern corn samples: 3.5 mM $MgCl_2$ and 1.5 U *Taq*. The optimal DNA concentrations in the STR amplification with *dup*014 primers were 125 ng (ancient DNA) or 50 ng (modern DNA). Our DNA cycling amplification protocol by PCR generated positive results both in modern and ancient DNA. Other authors like Bird *et al*. (1995b) have used different profiles to amplify archaeological corn DNA: 30 short cycles (94° C/30 s, 61° C/50 s, and 72° C/2 min). A similar profile of 40 short cycles (93° C/40 s, 55° C/1 min, and 72° C/1.5 min) was used by Goloubinoff *et al*. (1993) to amplify archaeological corn from Peru and Chile. Therefore, the removal of PCR inhibitors and the presence of appropriate concentrations of $MgCl_2$ and *Taq* DNA polymerase are critical parameters to accomplish the enzymatic amplification of ancient DNA by PCR.

The design of the primers is also of paramount importance. As an example, the primer pairs *bnlg*131 and *dup*014 amplified well some modern genotypes, yet failed to amplify others, as previously indicated. This may indicate a suboptimal primer design likely linked with polymorphisms (like SNPs) in ancient corn STRs flanking sites, particularly at the 3'–end of the annealing targets. This problem could be alleviated or overcome lowering the annealing temperature and/or redesigning the primer(s) involved.

The above PCR conditions to amplify ancient DNA (particularly the high concentrations of $MgCl_2$ and *Taq*) may reduce the *Taq* DNA polymerase fidelity. In fact, it has been described that, depending on the experimental conditions, such an enzyme may generate a mutation for each 250-b polymerized (Innis et al, 1988). This error rate could even increase for ancient and damaged DNA (Pääbo et al, 1990). Yet, these problems are only important if the amplicons are further cloned and sequenced (since in such a case, a single molecule is usually picked up and amplified in the bacterial host). Therefore, should the amplicons be sequenced, it is advised to sequence them in both the forward and reverse senses from three independent clones or, alternatively, direct-sequence in both directions the amplicon products without cloning, thus effectively diluting the present mutations and generating a true consensus reading (Pääbo et al, 1989).

Analyses of the STR polymorphism

The amplification products generated using the eight corn-specific STR primer pairs with the eight samples of archeological corn and the nine samples of modern breeds were visualized in 12% (w/v) polyacrylamide gels after electrophoresis and silver nitrate staining. This allowed a better allele separation and thus a more precise scoring of the polymorphic bands. All the eight tested primer pairs amplified the modern samples, yet only five of them amplified STR loci from ancient corn DNA. The analysis was performed under very restrictive conditions. Thus, the bands not well defined were not considered for the final analysis.

Unspecific bands of higher molecular weight may arise from ancient DNA due to the less astringent conditions commonly used in such amplifications, including higher reagent concentrations (like $MgCl_2$ and *Taq* DNA polymerase) and lower annealing temperatures. But they may also arise due to differences in the target genomic DNA. Thus, different amplicon sizes may be generated from different genotypes; particularly from archaeological ones, which may complicate their assignment and analysis. For such a reason, a range of amplicon sizes is usually associated with each primer pair (Table 2). As we have found, such a range from the Maize DB (modern corn breeds and native Mexican genotypes) does not always correspond with the amplicons obtained with the ancient corn from Peru analyzed in the present work. The sequencing of the different amplicons generated in all modern, native and ancient samples could further confirm these divergences.

The average polymorphic alleles found by the STR genotyping was rather low (2.9). The reason for it is twofold: first, the band scoring was very conservative and restrictive as previously indicated; and second, all archaeological samples were monomorphic.
Other authors have compared the STR typing of both ancient and modern corn samples. They found that whereas some specimens generated clear bands for many STR primer pairs, other samples did not produce positive results. Additionally, such authors warned that the variability found in the ancient specimens was not consistent or repetitive. As a consequence, they could not properly define the genetic structure of old corn populations at the north coast of Peru (Bird et al, 1995a, b). Similar results have been published for seed DNA from archaeological corn; although they were genetically informative, they did not show polymorphism between ancient and modern samples (Rollo et al, 1994a,b). It has been proposed that, in general, the polymorphisms based in both the copy number and sequence variation at nuclear DNA regions might be a better approach than the polymorphisms based only on the sequence variation for diachronic genetic studies of ancient seeds (Rollo et al, 1994b).

Kinship among the analyzed samples

The total number of STR sequences in the corn and other plant species is surprisingly high (Senior and Heun, 1993). Thus, it has been considered that the corn genome may hold about 10^4 – 10^5 copies of the microsatellites $(GT)_n$ and $(AG)_n$ (Condit and Hubbel, 1991); having about 5 x 10^3 and 3 x 10^5 copies for $(AC)_n$ and $(AG)_n$ microsatellites, respectively (Cregan, 1992).

The inherent instability of the STR sequences make them extremely useful for both evolutive and genetic studies. Such a high mutation rate is mostly due to changes in the copy number of the basic structural unit or pattern of bases that does repeat in a tandem fashion, building the microsatellite (e.g., GT). Yet, not all STRs are equally instable, simply because not all are equally prone to the mutation processes (Eisen, 1999).

We have analyzed eight corn STR sequences based on their genome location and high polymorphism level as described in the Maize DB. Theoretical considerations indicate that it is necessary to study several loci to establish reliable phylogenetic relationships. Additionally, it is recommended to analyze 30-50 STRs for evolutive studies. This would allow to classify the archaeological corn samples in clusters with higher accuracy and precision, thus generating better phylogenetic relationships (Smith and Helentjaris, 1994).

The genetic similarity matrix generated in this work (Table 4) demonstrates that the STR markers used span from 0.08 to 1, with an average of 0.44 for the full sample. As deduced from such similarity value of 1, and as shown in the clustering pattern of the dendrogram, the archaeological samples were monomorphic (Fig. 6). It is plausible that the ancient corn population from which the samples were obtained was rather homozygous. This is not surprising, since although the corn is an allogamous plant, it is possible to fix all the evaluated loci by means of genetic drift events, which might be further emphasized in small populations. Thus, the analyzed populations could have been a food source for just a few families. More importantly, they likely maintained their corn breed saving just a few cobs for the next sowing, instead of flailing all the cobs of the crop and then choosing the best grains as the next generation seeds. This latter approach would be the best one to maintain and even increase the genetic variability of the population along generations. On the contrary, the first method would favor the consanguinity and would drastically reduce the genetic variability and heterosis of the corn population after a few generations of practice. Curiously, such “old and inbreeding” procedure can be observed even nowadays among the farmers of the villages nearby the sampled archaeological sites. In fact, they store their cobs inside river sand to protect them from insects and other pests, optimizing the seed viability. Obviously, they are not aware of the endogamy and lack of genetic variability that such procedure represents.

The previously described endogamic practice would imply that very few individuals indeed would contribute to each new corn generation. Therefore, the true (genetic) size of the population would be reduced with every corn generation in which such human groups of farmers would remain isolated (i.e., with no corn germplasm interchange with other humans). In relation with that, it must be stressed that the distance between the two archaeological sites (‘El Brujo’ and ‘Puerto Pobre’) from which the ancient corn samples evaluated in this work came from is just 220 km. Besides, the radiocarbon dating assigned 440 ± 50 years to the ‘Puerto Pobre’ samples; that is, between 1.415- and 1.520-years AD. On the other hand, the ‘El Brujo’ samples have an age of 590 ± 70 years; that is, between 1.300- and 1.420-years AD. Consequently, these facts suggest that at some time in their history there had been contacts between the different valleys, which were incorporated into the Chimu state after different stages of expansion of this empire (Keatinge, 1974). This can explain such corn germplasm interchange, as demonstrated by Bird (1990). To support this rationale, the written records of the Spanish chronicler’s report that the corn was harvested in the Chicama valley (‘El Brujo’) and was then carried to ‘Huanchaco’ (Moche valley). From there it was shipped

by sea vessels to a small fisherman bay known as 'Los Chimos' (near Casma and 'Puerto Pobre'), where it was a very valued commodity (Cristóbal Campana, 2001 personal communication). This historic interchange of corn germplasm between both archaeological sites would reinforce out hypothesis that both ancient DNA samples had had a common stabilized pool of genes, which could be put to the test analyzing more STR markers.

On the other hand, there are evidences that the corn breed known as 'Cuzco' has had significant influence in the Andes (including several cultivars from the coast and mountain range exhibiting a large grain and a low number of narrow rows). Additionally, it has been demonstrated the presence of the 'Cuzco' breed in the Peruvian coast, both as pictorial representations in the ceramic of the Mochica and Chimú cultures, as well as by the Spanish chroniclers which frequently wrote about such a corn breed (Sevilla, 1994). Yet, we have not detected such evidences in our STR study.

The UPGMA-based dendrogram (Fig. 6) also demonstrates that the 'Cuzco' breed is clustering (group 'C') with the 'Huayleño' breed, segregated from the group 'A' made of the three north-coastal breeds ('Alazán', 'Mochero' and 'Pagaladroga'). Similar results were also found in a study of molecular characterization of the modern breeds of Peruvian corn using 90 Amplified Fragment Length Polymorphism (AFLP) markers; the 'Cuzco' breed did not cluster together with any other north-coastal breed (Blas et al, 1998). In relation to the group 'A' (Fig. 6), and based on morphological and cytogenetic data, Grobman et al, (1961) proposed that the 'Proto-Alazán' was an evolutionary product of early hybridizations between 'Proto-Mochero' and 'Proto-Pagaladroga'. Nevertheless, it has been also proposed that the 'Proto-Mochero' is related to both the 'Huachano' and 'Pardo', which are south-coastal breeds and that very likely could have relationships with the 'Puerto Pobre' corn, based on its intermediate allocation in the Peruvian coast.

Currently, the north-coastal breeds like 'Mochero', 'Alazán' and 'Pagaladroga' have a limited distribution in the Departments of the north coast of 'La Libertad', Lambayeque and Piura, at elevations lower than 50 m over the sea level (m.o.s.l.). In the north coast, the corn breed 'Mochero' is secondarily used (after 'Alazán') as raw material for the manufacture of the *chicha*, a local fermented drink (Manrique, 1997).

There are evidences about the existence of 'Mochero' as a different breed ('Proto-Mochero) in prehistoric ages and in the same area were it is found today. Undoubtedly, 'Mochero' is a direct descendant of the early popcorn named 'Confite Iqueño', which grew in the coast with very limited hybridizations preceding the formation of this kind of maize. The hybridizations between 'Mochero' and 'Pagaladroga' (another coastal breed) are also very probable. Thus, a few endogamous lines derived from 'Mochero' exhibit typical 'Pagaladroga'-like cobs. 'Mochero' is also related to a complex of similar floury breeds, widely distributed along the full Peruvian coast (Grobman et al, 1961).

As indicated above, the other north-coastal breed is 'Pagaladroga', distributed in valleys from Trujillo to Piura. It is almost extinct nowadays, yet it can still be found at a few farms, growing in mixed batches with 'Alazán'; particularly in the small area of Pueblos on the extremely arid riverside of the 'La Leche' valley, on the north side of the Department of Lambayeque. Probably, this breed was widely distributed (as 'Proto-Pagaladroga') in the north and central coast of Peru, during the Formative (Cupisnique and Salinar) and Classic periods (Moche and Chimú). 'Pagaladroga' derived in the north Peruvian coast from an ancestor similar to the current 'Confite Puntiagudo'. Very likely it had a red pericarp, as exhibited by the pre-Hispanic corn specimens. This breed also has very close phylogenetic relationships with 'Confite Iqueño', ancestor of the 'Mochero' breed. On the other hand, it is possible that 'Confite Puntiagudo' hybridized with the ancestor of 'Mochero', and that the resulting offspring was 'Pagaladroga', the other north-coastal breed (Grobman *et al*., 1961). We could not evaluate the 'Confite Puntiagudo', since the active collections of such a breed were depleted at the corn germplasm of the CIMMYT.

These genetic relationships between the two north-coastal proto-breeds ('Mochero' and 'Pagaladroga') with 'Confite Puntiagudo', could represent an efficient strategy to exhibit more quickly the phenotype of the coastal Chimú cobs, when crossed with 'Puerto Pobre' specimens (Fig. 2, 7-525-PP), which show imbricated and acuminated grains. Nevertheless, there is also the possibility of an interbreed with another highlander corn like 'Paro' (Department of Apurimac), which exhibits higher genetic similarity with the coastal breeds (Grobman et al, 1961). In fact, there are morphological evidences of a strong introgression of highlander breeds in the 'Puerto Pobre' sample, although the chronology is not clear. Nevertheless, those morphological evidences have not been confirmed with molecular studies. Thus, the coastal proto-forms previously indicated keep on maintaining their genetic uniformity, although their genome had experienced some kind of introgression from germplasms of other breeds at different moments in their evolutive history.

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