EVALUACIÓN DE LA DIVERSIDAD GENÉTICA Y FENOTÍPICA DE UN CONJUNTO DE MAÍCES (*ZEA MAYS*) ACTUALES Y ARQUEOLÓGICOS DE LA REGIÓN DE TARAPACÁ, NORTE DE CHILE.

EVALUATION OF THE GENETIC AND PHENOTYPIC DIVERSITY OF A SET OF CURRENT AND ARCHEOLOGICAL CORN SAMPLES FROM THE REGIÓN DE TARAPACÁ IN NORTHERN CHILE

Tesis
Entregada A La
Universidad De Chile
En Cumplimiento Parcial De Los Requisitos
Para Optar Al Grado De

Magíster en Ciencias Biológicas

Facultad De Ciencias

Por

Alejandra Isabel Vidal Elgueta

Junio, 2017

Director de Tesis Dr: Luis Felipe Hinojosa
Co-Directora de Tesis Dra: María Fernanda Pérez Trautmann

FACULTAD DE CIENCIAS

UNIVERSIDAD DE CHILE

INFORME DE APROBACION

TESIS DE MAGÍSTER

Se informa a la Escuela de Postgrado de la Facultad de Ciencias que la Tesis de Magíster presentada por la candidata.

Alejandra Isabel Vidal Elgueta

Ha sido aprobada por la comisión de Evaluación de la tesis como requisito para optar al grado de Magíster en Ciencias Biológicas, en el examen de Defensa Privada de Tesis rendido el día 20 de Marzo de 2017

Director de Tesis:
Dr. Luis Felipe Hinojosa ....

Co-Director de Tesis
Dra. Fernanda Pérez ....

Comisión de Evaluación de la Tesis

Dra. Alejandra González ....

Dra. Victoria Castro ....

"VI. El santo maíz sube en un ímpetu verde, y dormido se llena de tórtolas ardientes".

(Gabriela Mistral, El Maíz).

RESUMEN BIOGRÁFICO/BIOGRAPHY

Mi nombre es Ale Vidal Elgueta, nací en Santiago de Chile. Me crié en Tarapacá con Marginales de Altura. Arqueóloga de profesión y vocación, mis investigaciones han versado sobre el uso de las especies silvestres y agrícolas utilizadas por los grupos prehispánicos del Norte Grande de Chile. A partir de esta inquietud, me interesa abordar las perspectivas, desafíos y propuestas de la Ecología con miras a comprender la incidencia del factor humano en los recursos vegetales del presente y el pasado.

AGRADECIMIENTOS/ ACKNOWLEDGMENTS

A las mujeres y hombres agricultores de Tarapacá, Chusmiza, Sibaya, Limaxiña, Huaviña, Huarasiña, Camiña, Guatacondo y San Lorenzo de Tarapacá, por su generosidad en la entrega de sus conocimientos ancestrales y mantener viva las tradiciones de la tierra.

A mis profesores tutores Dr. Luis Felipe Hinojosa y Dra. María Fernanda Pérez por la confianza brindada, orientación y paciencia. Que la fuerza los acompañe.

A las evaluadoras Dra. Alejandra González y Maestra Victoria Castro, cuyos consejos han servido para mejorar este trabajo.

A mi familia, amigos(as) y compañeros(as) de trabajo por los buenos consejos, los buenos momentos y su pasión por la Ciencia: Mateo González Vidal, Rodrigo González, Susana Elgueta, Marcela Vidal, Guido Vidal, Mauricio Uribe, Pablo Méndez-Quirós, Magdalena García, Valentina Mandakovic, Gioconda Peralta, Paz Montenegro, Antonio Maldonado y a todos(as) los(as) que no menciono: infinitas gracias. A los proyectos FONDECYT 1150690, FONDECYT 1130279, Instituto de Ecología y Biodiversidad y a Becas de Magíster Nacional (CONICYT) por el financiamiento brindado.

AGRADECIMIENTOS/ ACKNOWLEDGMENTS

A las mujeres y hombres agricultores de Tarapacá, Chusmiza, Sibaya, Limaxiña, Huaviña, Huarasiña, Camiña, Guatacondo y San Lorenzo de Tarapacá, por su generosidad en la entrega de sus conocimientos ancestrales y mantener viva las tradiciones de la tierra.

A mis profesores tutores Dr. Luis Felipe Hinojosa y Dra. María Fernanda Pérez por la confianza brindada, orientación y paciencia. Que la fuerza los acompañe.

A las evaluadoras Dra. Alejandra González y Maestra Victoria Castro, cuyos consejos han servido para mejorar este trabajo.

A mi familia, amigos(as) y compañeros(as) de trabajo por los buenos consejos, los buenos momentos y su pasión por la Ciencia: Mateo González Vidal, Rodrigo González, Susana Elgueta, Marcela Vidal, Guido Vidal, Mauricio Uribe, Pablo Méndez-Quirós, Magdalena García, Valentina Mandakovic, Gioconda Peralta, Paz Montenegro, Antonio Maldonado y a todos(as) los(as) que no menciono: infinitas gracias. A los proyectos FONDECYT 1150690, FONDECYT 1130279, Instituto de Ecología y Biodiversidad y a Becas de Magíster Nacional (CONICYT) por el financiamiento brindado.

INDICE DE MATERIAS/INDEX

LISTA DE TABLAS/TABLE LIST

LISTA DE FIGURAS/FIGURE LIST

LISTA DE ABREVIATURAS/ABBREVIATIONS LIST

PCR: Polymerase Chain Reaction/Reacción en cadena de Polimerasa.

SSR: Short Sequence Repeats/Microsatélites

bp: base pairs/pares de base

DNA: Deoxyribonucleic acid/Ácido Desoxirribonucleico

µL: Microliters/Microlitros

ng: Nanograms/Nanogramos

UV: Ultraviolet light /luz ultravioleta

y BP:Years Before Present

PCA: Principal Component Analysis/Análisis de Componentes Principales

LDA:Linear Discriminant Analysis/Análisis Discriminante

Rpm: Revolutions per minute/Revoluciones por minuto.

PIC: Polymorphism index/índice de polimorfismo

LISTA DE ABREVIATURAS/ABBREVIATIONS LIST

PCR: Polymerase Chain Reaction/Reacción en cadena de Polimerasa.

SSR: Short Sequence Repeats/Microsatélites

bp: base pairs/pares de base

DNA: Deoxyribonucleic acid/Ácido Desoxirribonucleico

µL: Microliters/Microlitros

ng: Nanograms/Nanogramos

UV: Ultraviolet light /luz ultravioleta

y BP:Years Before Present

PCA: Principal Component Analysis/Análisis de Componentes Principales

LDA:Linear Discriminant Analysis/Análisis Discriminante

Rpm: Revolutions per minute/Revoluciones por minuto.

PIC: Polymorphism index/índice de polimorfismo

RESUMEN

El maíz de la región andina tiene una historia evolutiva y cultural compleja, que involucra interacción ecológica y humana. Hoy en día, mientras que para el maíz andino se reconoce una variada morfología y una baja diversidad genotípica, el proceso involucrado en su producción y selección es menos claro. En este trabajo nos preguntamos ¿cómo la diversidad morfológica y genética del maíz ha variado desde el Período Formativo hasta la actualidad en la Región de Tarapacá, en el norte de Chile?. Para responder a esto se analizaron treinta rasgos morfológicos y ocho marcadores microsatélites sobre cariopses y mazorcas arqueológicas (*ca.* 2500 a 500 A.P) y 95 ejemplares de maíces modernos derivados de razas primitivas. Nuestros resultados muestran importantes diferencias fenotípicas entre mazorcas y granos arqueológicos y modernos. En contraste, se reconoció una baja variabilidad genética para ambas muestras y distancia genética entre la muestra antigua y la actual. Para la muestra arqueológica, se postula una selección humana dirigida a aumentar el tamaño del maíz durante un corto período de tiempo. Esta manipulación se sumerge en una agricultura compleja y extensiva realizada por los agricultores de Tarapacá.

ABSTRACT

Maize from the Andean region has a recognized complex history, involving ecological and human interaction. Although Andean maize currently shows high morphological and low genotypic diversity, the processes involved in its production and selection are less clear. In this study we ask how the morphological and genetic diversity of maize has varied from the Formative Period to the present in the Tarapacá Region of northern Chile. To answer this we analyzed thirty morphological traits and eight microsatellite markers of archeological cobs and kernels (dated *circa* 500 B.C to 1400 A.D) and 95 modern ears derived from primitive races. Our results show significant phenotypic differences between archeological and modern cobs and kernels. By contrast, there was low genetic diversity in both samples and significant genetic distance between archeological and modern samples. For the ancient sample we postulate there was human selection directed to increase corn size during a short period of time. This manipulation is immersed in a complex and extended agriculture carried out by Tarapacá farmers.

ABSTRACT

Maize from the Andean region has a recognized complex history, involving ecological and human interaction. Although Andean maize currently shows high morphological and low genotypic diversity, the processes involved in its production and selection are less clear. In this study we ask how the morphological and genetic diversity of maize has varied from the Formative Period to the present in the Tarapacá Region of northern Chile. To answer this we analyzed thirty morphological traits and eight microsatellite markers of archeological cobs and kernels (dated *circa* 500 B.C to 1400 A.D) and 95 modern ears derived from primitive races. Our results show significant phenotypic differences between archeological and modern cobs and kernels. By contrast, there was low genetic diversity in both samples and significant genetic distance between archeological and modern samples. For the ancient sample we postulate there was human selection directed to increase corn size during a short period of time. This manipulation is immersed in a complex and extended agriculture carried out by Tarapacá farmers.

INTRODUCTION

The genetics and phenotypic diversity of *Zea mays* Lam. has been one of the main topics of study in the attempt to understand evolutionary history of maize (Matsuoka et al., 2002b; Vigouroux et al., 2008); both México and the Central Andes play a key role, with more than 200 native varieties (Goodman & Bird 1977). The Central Andes or Andean region has been considered as a primary center of maize diversification (Bonavia 2013) due to the existence of at least 52 current morphological varieties, of which at least 24 are primitive landraces which emerged after the introduction of the species to the Andes during pre-Hispanic times (Grobman 1982; Grobman 2013). Despite the high morphological variability reported for maize in America, its genetic variability is clustered in only seven groups (Vigouroux et al., 2008), in which maize from South America is grouped in a Low Land Cluster (varieties from the East Andes slope) and the Andean Cluster (varieties from the western Andes slope). The Andean cluster has shown a fewer allelic frequencies than Mexican or Caribbean clusters (Matsuoka et al., 2002a; Vigouroux et al., 2008).

INTRODUCTION

The genetics and phenotypic diversity of *Zea mays* Lam. has been one of the main topics of study in the attempt to understand evolutionary history of maize (Matsuoka et al., 2002b; Vigouroux et al., 2008); both México and the Central Andes play a key role, with more than 200 native varieties (Goodman & Bird 1977). The Central Andes or Andean region has been considered as a primary center of maize diversification (Bonavia 2013) due to the existence of at least 52 current morphological varieties, of which at least 24 are primitive landraces which emerged after the introduction of the species to the Andes during pre-Hispanic times (Grobman 1982; Grobman 2013). Despite the high morphological variability reported for maize in America, its genetic variability is clustered in only seven groups (Vigouroux et al., 2008), in which maize from South America is grouped in a Low Land Cluster (varieties from the East Andes slope) and the Andean Cluster (varieties from the western Andes slope). The Andean cluster has shown a fewer allelic frequencies than Mexican or Caribbean clusters (Matsuoka et al., 2002a; Vigouroux et al., 2008).

This low genetic variability has been explained as the result of a bottleneck and successive founder effects (Doebley et al., 2006; Grimaldo 2011; Lia et al., 2007), gene flow between distant geographical populations and directional selection by selective breeding (Pressoir & Berthaud 2004).

Examples of this low variability have been reported in corn samples from northwest Argentina, which showed variation in only one allele of three microsatellite markers (Lia et al., 2007). Similarly, STRUCTURE analysis applied to 19 microsatellites in archeological corn samples from Peru, Argentina and Brazil detected only two groups (K = 2) splitting Andean and non-Andean regions (Grimaldo 2011). The founder effect has been considered to be the main cause of these results. However, it could also be due to adaptation to local environmental conditions, seed exchange and artificial selection for breed improvements (Whitt et al., 2002). As a result of one or all these factors, both genetic and phenotypic variability tend to decrease in developed agricultural systems (Staller 2010; Whitt et al., 2002)

These studies focused on main areas of maize development, however, marginal areas of South Central Andes, such as Tarapacá (18-21 ºS) have received little attention, restricting our comprehension of how anthropic manipulation and ecological conditions influenced maize production through human history. For example, in Tarapacá nine primitive landraces have been reported (Paratori 1990; Timothy 1961), showing

This low genetic variability has been explained as the result of a bottleneck and successive founder effects (Doebley et al., 2006; Grimaldo 2011; Lia et al., 2007), gene flow between distant geographical populations and directional selection by selective breeding (Pressoir & Berthaud 2004).

Examples of this low variability have been reported in corn samples from northwest Argentina, which showed variation in only one allele of three microsatellite markers (Lia et al., 2007). Similarly, STRUCTURE analysis applied to 19 microsatellites in archeological corn samples from Peru, Argentina and Brazil detected only two groups (K = 2) splitting Andean and non-Andean regions (Grimaldo 2011). The founder effect has been considered to be the main cause of these results. However, it could also be due to adaptation to local environmental conditions, seed exchange and artificial selection for breed improvements (Whitt et al., 2002). As a result of one or all these factors, both genetic and phenotypic variability tend to decrease in developed agricultural systems (Staller 2010; Whitt et al., 2002)

These studies focused on main areas of maize development, however, marginal areas of South-Central Andes, such as Tarapacá (18-21 ºS) have received little attention, restricting our comprehension of how anthropic manipulation and ecological conditions influenced maize production through human history. For example, in Tarapacá nine primitive landraces have been reported (Paratori 1990; Timothy 1961), showing important morphological variation. Also, genetic diversity for pre-Hispanic samples has not been sufficiently studied. Indeed, one pre-Hispanic cob sample dated at 1500 ± 50 y BP accounts for an introduction of the GA$_4$TA allele from the eastern slope of the Andes (sequenced from the *ADH2* gene) (Freitas et al., 2003), suggesting gene flow between the slopes of the Andes range.

Exchange and reciprocity are two of the fundamental social and economic principles on which Tarapacá communities based their economies in pre-Hispanic times (Uribe 2006). Thus during the Formative Period (ca.2500-1000 BP) inhabitants of Pircas and Caserones villages exchanged several resources such as maize, *algarrobo* seeds, cotton and wood with coastal settlements (Uribe 2006; Uribe 2012). The social and economic system was partially sustained through the collection of *algarrobo* seeds and the development of an intensive agriculture of maize that emerged towards the Late Formative Period (García et al., 2014; Núñez 1966).

This agriculture allowed the founding of complex societies such as Aldea de Guatacondo (2290-1890 Cal. yBP), Ramaditas (2340-1870 Cal. yBP), Pircas (2320-1420 Cal. yBP) and Caserones (1880-1080 Cal. yBP) (Mostny 1971; Núñez 1966; Núñez 1981; Rivera & Dodd 2013; Uribe 2009; Uribe & Adán 2012).

The archeological record of *Zea mays* specimens recovered from the sites mentioned above includes kernels, cobs, husks and stalks of at least two phenotypes: a floury type similar to *Harinoso Tarapaqueño* and a popcorn type similar to the *Polulo* landrace (García et al., 2014; Meighan 1980; True 1980).

During the Late Intermediate Period (*ca.* 800-600 yBP), archeological sites situated in the Pampa del Tamarugal and the lower part of valleys were abandoned, and people moved to higher altitudes in the Camiña and Tarapacá valleys (García & Uribe 2012). These new settlements were the nucleated villages of Nama, Camiña, Jamajuga, Chusmiza, Mocha, Guaviña, Sibaya and Chiapa, (Adán et al., 2007). Only a few settlements endured in the lower parts of the valleys, such as Tarapacá 13 (930-490 Cal. yBP) and Tarapacá Viejo (662- 350 Cal. yBP). Agriculture techniques were modified and agricultural terraces were constructed in the upper portion of valleys (García & Uribe 2012). Maize was still the most important crop, but unlike the Formative Period, large amounts were not stored (García & Uribe 2012).

This intensive agriculture not only modified the dessert landscape with several agricultural fields (Rivera & Dodd 2013; Vidal et al., 2015), but probably modified the crops themselves. Thus it has been suggested that the increase in cob size observed in the Caserones archeological site was possibly due to human selection (Vidal-Elgueta et al., 2016). Human selection over several features in maize has been indicated as a common practice in Andean communities (Murra 2002). Although practices varied depending on types of agriculture, environmental conditions and even the personal preferences of farmers, the most usual were seed selection by size, color and taste and ear selection by size, colors and dispositions of rows (Murra 2002). There are also other indirect ways of maintaining certain features in maize. For example, Inca communities planted Maíz Colorado, used for *chicha* preparation, away from other landraces, thus avoiding cross pollination (Murra 1999).

Agronomic information indicates current differences in Tarapacá agriculture among valleys, determined by environmental conditions. Camiña valley is the most northern valley of Tarapacá, and today has the most important agriculture production of the region. Tarapacá valley, situated in the middle of Pampa del Tamarugal, currently has less agriculture production than Camiña. Despite these current differences, during the Formative Period these two valleys were closely related through cultural connections, sharing the same ceramic types and material culture (García & Uribe 2012).

According to the information outlined above we want to ask the following questions: a) Are there any phenotypic and genetic differences between current maize from the Camiña and Tarapacá valleys? b) Are there phenotype and genotype variations in

Tarapacá maize between pre-Hispanic and current times? c) Does the phenotypic and genetic diversity of Tarapacá maize indicate the introduction of new landraces since pre-Hispanic times?

The hypotheses are:

1. Given the cultural connections between Tarapacá farmers and the usual exchange of resources observed in Andean communities during the pre-Hispanic and current times there will be no genetic or phenotypic differences in Tarapacá maize.
2. Given the anthropic selection performed by Andean farmers on crops, pre-Hispanic maize will show greater genetic and phenotypic diversity compared to later and modem ones.
3. Given the particular environmental conditions and the geographical and cultural isolated situation of Tarapacá, low genetic and phenotypic variability is expected in this time span of 2000 years.

The main goal of this study is assess genetic and phenotypic diversity in *Zea mays* from the Formative period to current times in the Tarapacá region, Chile. Specifically, we will compare genetic and phenotypic diversity in a temporary gradient of 2000 years in archeological and current specimens, establish the morphological diversity of the sample and determine the affiliation between archeological and modern specimens.

MATERIAL AND METHODS

A total of 95 modern ears were collected from the Camiña and Tarapacá valleys, corresponding to five different locations and six populations, distributed in an altitudinal gradient ranging from 2400 to 3300 meters above sea level (Fig. 1 and Table 1, Fig. S1 to Fig. S6). Ears were collected randomly, with and without husk.

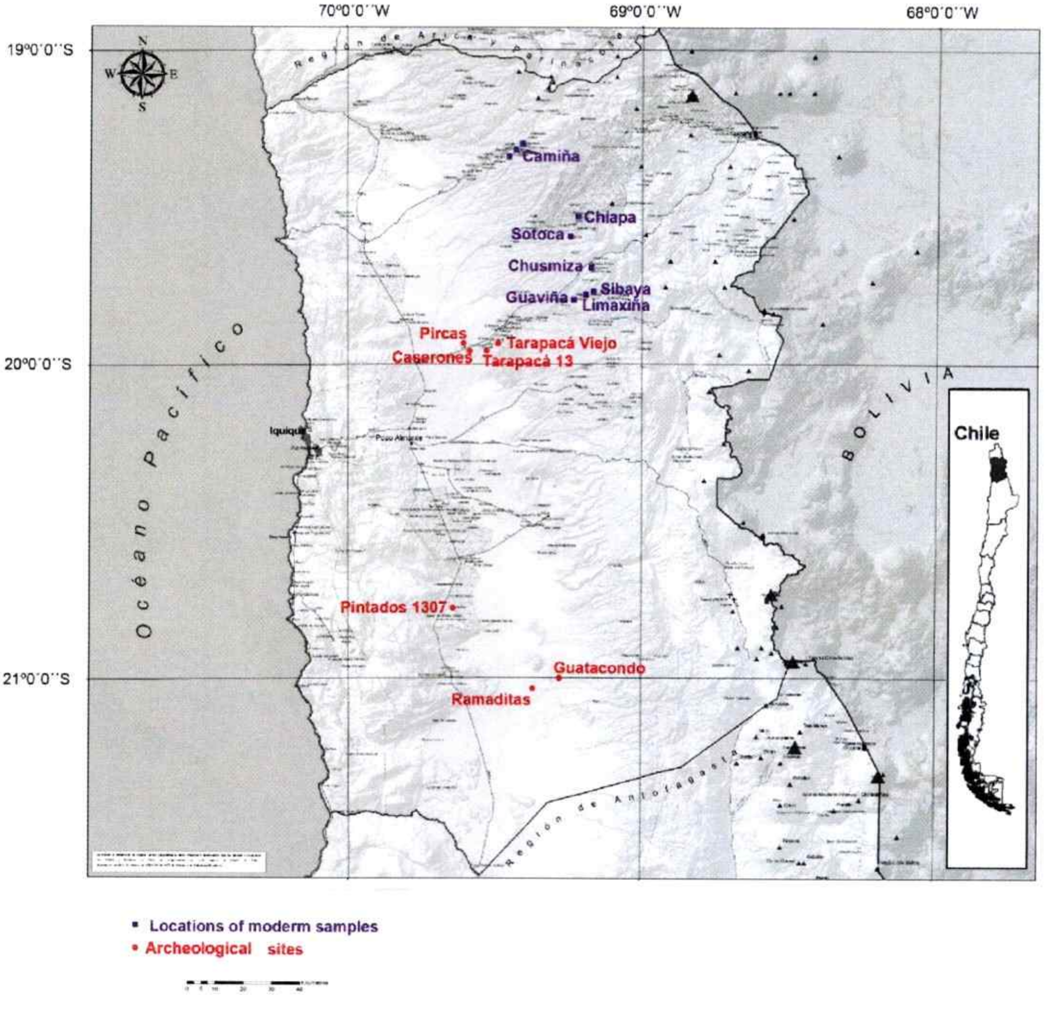


Figure 1. Locations of modern localities and archeological sites. Camiña, Chiapa, Sotoca, Guaviña, Chuzmisa, Sibaya and Limaxiña in purple dots, where modern maize specimens where collected. Archeological sites of Pircas, Tarapacá 13, Tarapacá Viejo, Pintados 1307, Guatacondo and Ramaditas in red dots.

Pop	Valley	Location	LAT/LONG.	Elevation	N	Type	PROVINCE
1	Tarapacá	Chusmisa	19° 41- 69° 11	3360	17	Ear and husk	Chiapa, Sotoca and Chusmiza
2	Tarapacá	Limaxiña	19° 47- 69° 10	3150	18	Ear and husk	Limaxiña
3	Tarapacá	Huaviña	19° 47- 69° 13	2470	10	Ear	Sibaya y Huaviña
4	Camiña	Camiña	19° 18-69° 25	2496	15	Ear and husk	Camiña
5	Camiña	Camiña alto	19° 18-69° 24	2615	20	Ear	Chiapa
6	Camiña	Camiña	19° 18-69° 25	2414	15	Ear and husk	Camiña

Table 1. Provenance and type of material studied. Pop, modern population; Valley, valleys of provenance of modern samples; Location, locations of modern samples; LAT/LONG, latitude and longitude coordinates of modern locations; Elevation, meters above sea level of each location; N, number of plants collected for each population; Type, vegetative part collected for each population and PROVINCE, provenance of maize seed for each population according to farmers' reports.

A total of 123 archeological cobs and 151 archeological kernels were analyzed (Figure 2, Figure 3 and Table 2). These samples were obtained from the Pircas (2320-1420 Cal. yBP), Pintados 1307 (1427-1568 Cal. yBP), Tarapacá 13 (930-490 Cal. yBP) and Tarapacá Viejo (662- 350 Cal. yBP) archeological sites during the excavation of their domestic contexts.

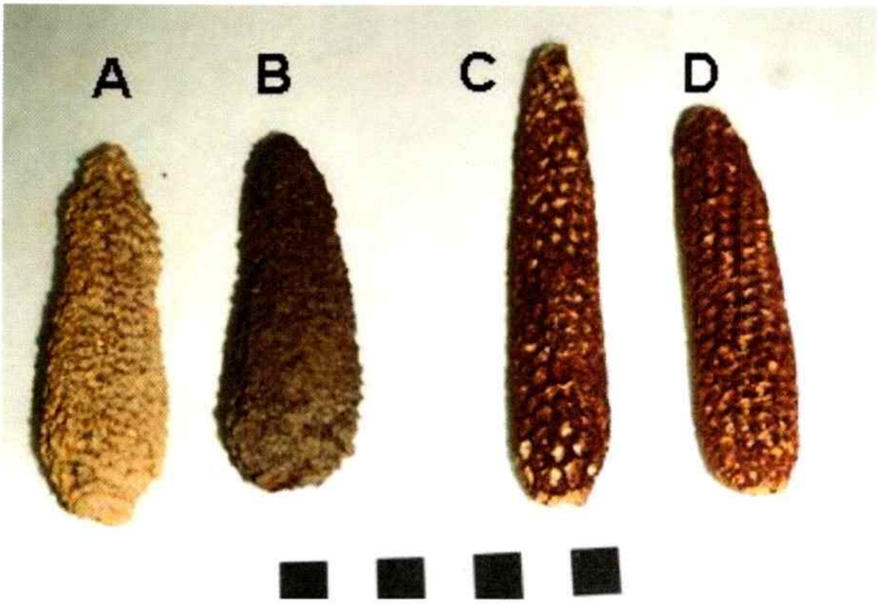


Figure 2. Some specimens of archeological cobs used in this study. Cobs A and B are from Tarapacá 13 and cobs C and D are from Tarapacá Viejo.

Figure 3. Archeological kernels recovered from Tarapacá Viejo, Area 8 Locus 12B. All the kernels in this image are pod kernels and were recovered from the same stratum and unit excavation.

Population	Location	Sites	Cultural Period	Lat./Long.	Elevation	Cb (N)	Kn(N)
7	Pampa del Tamarugal	Pintados 1307	Late Formative Period	20° 34- 69° 36	1012	0	6
8	Tarapacá	Pircas	Early Formative Period	19° 58- 69° 34	1312	15	1
9	Tarapacá	Tarapacá 13	Late Intemediate Period	19° 55- 69° 25	1310	26	32
10	Tarapacá	Tarapacá Viejo	Late Intemediate Period and Late Formative Period	19° 55- 69° 30	1425	82	112

Table 2. General information on archeological samples. Population, numbers 7, 8, 9 and 10 assigned to archeological populations; Location, locations of archeological sites; Sites, archeological sites of sample provenance (Pintados 1307, Pircas, Tarapacá 13 and Tarapacá Viejo), Cultural Period, cultural period associated according to the cultural sequence of the south-central Andes, LAT/LONG, latitude and longitude of each archeological site, Elevation, meters above sea level of sites; cb(N), number of cobs analyzed; kn(N) number of kernels analyzed for each archeological population.

2.1 Morphological Analysis

A total of 30 traits were measured on modern ears, cobs and kernels. 10 traits were measured and compared in archeological cobs and five traits in archeological kernels. Modern cobs and kernels were dehydrated, to replicate partially the hyperarid conditions found in archeological samples, allowing us to compare modern samples with archeological ones. Morphological traits considered were: Length, Inferior, Medium, Maximum and Upper Diameter on ears and cobs; Length, Width and Thickness on kernels; Number of Rows, Number of Kernels per Row and Total Number of Kernels in each ear, Perimeter and Area. These traits were chosen due to their use for previous similar analyses (Paratori 1990; Timothy 1961). To determine shape we measured the shape factor and Feret diameter using SIGMA SCAN PRO 5.0. In order to establish the variability of morphological traits we performed a Principal Component Analysis (PCA) performed in R and PAST 3.4 (Hammer 2016). To determine the probability of correctly attributing a given kernel and cob to the different clusters present, a Linear Discriminant Analysis (LDA) and ANOSIM test were performed. Also Permutational Multivariate Analysis of Variance (PERMANOVA) was performed to determine the statistical significance of morphological diversity between archeological and modern samples.

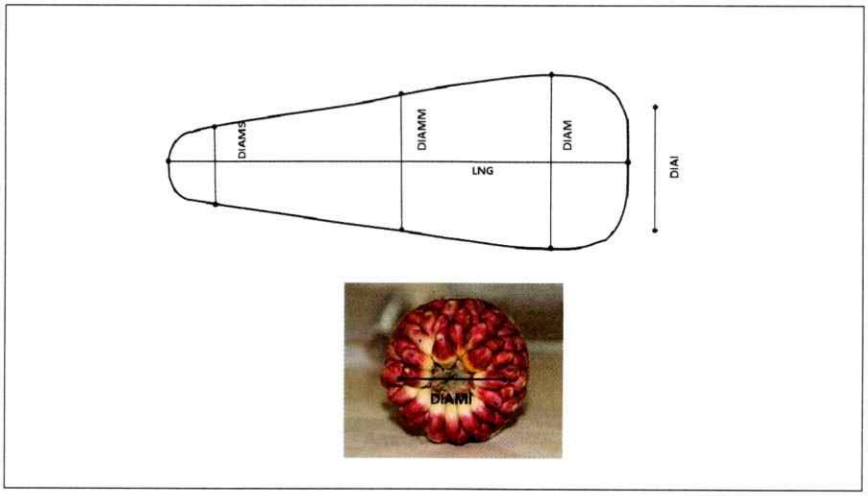


Figure 4. Measures on ears and cobs: DIAMS, upper diameter, DIAMM, medium diameter; DIAM, maximum diameter, DIAI, inferior diameter; LNG, length.

2.2 Genetic Analysis

Eight microsatellite markers or simple sequence repeats (SSR) were analyzed. SSR primers were selected according to their polymorphism index (PIC), their chromosomal locations in order to cover a wide range of chromosomal bin and by the small size of the amplification product previously reported (Grimaldo, 2011; Lia et al., 2007), thus allowing us to use them in archeological samples where DNA is presumably degraded. The microsatellites used were: phi029, phi034, Phi 056, Phi059, Phi063, Phi075, Phi127 and umc1332 (Table.S2). The SSR were obtained in MaizeGDB (www.maizegdb.org); an M13 (CACGACGTTGTAAAACGAC) tail was added to label amplicons for visualization on the capillary sequencer (Lorenz et al.,2001).

DNA extraction was carried out on husks and roots in modern samples and 21 archeological kernels, following a standard protocol based on CTAB buffer. To check the integrity of DNA in archeological samples we tested DNA extractions using QUBIT to establish the DNA double helix concentration. The SSR were analyzed by Peak Scanner Software 2.0 (Applied Biosystems 2012) and Genious v.5.0, and checked for null alleles in Micro Checker v.2.2.3.

Amplification of DNA samples was carried out by Polymerase Chain Reaction (PCR) under the following conditions: 0.1 µl Go Taq (Go Taq® Flexi DNA Polymerase Promega), 2 µl green buffer, 2 µl MgCl, 0.5 µl BSA, 0.1 µl forward primer, 0.,2 µl reverse primer, 1 µl each dNTP, 0.1 µl fluorescent dyes (VIC, NED, FAM, PET), 3 µl ADN (60 µl/ng) and 0.9 µl nuclease free water (Promega). PCR was performed under the following conditions: an initial denaturation cycle at 95 °C for 3 minutes, followed by a 30 denaturation cycles at 94 °C for 30 seconds, one annealing cycle with temperatures between 47 °C and 61 °C (Fig. S2) for 30 seconds, a cycle extension at 72 °C for 1.5 minutes, a final elongation at 72 °C for 10 minutes and a hold cycle to 10 °C ∞. The PCR products were checked in agarose gel (1.5% ammonium bromide) in 1X TAE buffer (0.04M Tris acetate, 0.001 M EDTA, pH 8.0) under electrophoresis and visualized under UV light. Fragment analysis by capillary electrophoresis was performed in the sequencing laboratory of the Pontificia Universidad Católica de Chile.

Twelve archeological samples amplified for six SSR (Table 3 and Table S2.). Phi 063 and Phi 029 partially amplified for archeological samples, therefore these were removed from the AMOVA and F_{ST} analyses for the archeological samples and subsequent comparisons between modern and archeological populations.

Genetic diversity was estimated by allelic frequency rates, average number of alleles per locus, observed heterozygosity (Ho) and expected heterozygosity (He). Differences between populations were estimated by fixation indexes F_{ST} and consanguinity by F_{IS} in

GenAlEx 6.5 (Peakall & Smouse 2012). Also to stablish the genetic distance between groups a hierarchical cluster analysis with F_{ST} values and STRUCTURE (Pritchard et al. 2000) analysis was performed. The number of cluster (K) was established using Structure Harvester software (Earl & von Holdt, 2012).

2.3 Agronomic and anthropological information

During the collection of modern specimens we interviewed nine local farmers to gather information about the following items: 1) provenance of seeds, 2) vernacular name of the variety, 3) age of maize sample collected, 4) sowing and harvesting times, 5) soil and water requirements, 6) use of fertilizer 7) hybridized or not hybridyzed 8) general characteristics of the maize varieties. An interview form was designed and questions followed an open structure interview (File S.1).

RESULTS

3.1 Measures

3.1.1 Modern samples

The average measures of traits in modern ears were: length 112.99 mm, upper diameter 24.5 mm, medium diameter 46.27, maximum diameter 48.65 mm, inferior diameter 37.13, number of rows 11.98 and number of kernels 18.94. Shape factors indicated: conic Index 11.6, perimeter 415.9, area 486.3, shape factor 3.77 and Feret diameter 77.44.

The average measures of traits in modern cobs were: length 109 mm, upper diameter 10.33 mm, medium diameter 19.69, maximum diameter 22.26 mm, inferior diameter 17.25, number of rows 11.98 and number of kernels 18.94. Shape factors: conic Index 6.48, perimeter 324.83, area 253.07, shape factor 3.22 and Feret diameter 56.17.

The average measures of traits in modern kernels were: length 14.78 mm, width 9.68 mm and thickness 5.88 mm. Shape factors: perimeter 45.11 mm, area 11.10 mm, shape factor 6.78 and Feret diameter 11.77 (Table 3).

3.1.2 Archeological samples:

The average measures of traits in archeological cobs were: length 5.86 mm, upper diameter 9.78 mm, maximum diameter 13.83 mm, inferior diameter 10.53 mm, number of rows 10.53 and number of kernels 11.66. Shape factors: conic Index 7.24, perimeter 18.92 mm, area 9.67 mm, shape factor 0.36 and Feret diameter 3.39.

The average measures of traits in archeological kernels were: length 10.64 mm, width 7.28 mm and thickness 5.96 mm. Shape factors: perimeter 3.07 mm, area 0.54 mm, shape factor 0.70 and Feret diameter 0.81 (Table 3).

Traits	Leng	U.Dia	Me.Dia	Ma.Dia	Inf. Dia	N Row	kern/Row	T/kern	Wid	Thick	CI	Per	Are	SF	DF
Spec.															
Ears	112.9	24.5	46.27	48.65	37.13	11.98	18.94	232.9			11.6	415.9	486.3	3.77	77.44
Cobs	109.5	10.33	19.69	22.26	17.25	11.98	18.94				6.48	32.35	253.07	3.22	56.17
Kern	14.78								9.68	5.88		45.11	1.11	6.78	11.77
a.Cob	58.6	9.78		17.65	13.83	10.53	11.66				7.24	18.92	9.67	0.36	3.39
a.Kern	10.64								7.28	5.96		3.07	0.54	0.70	0.81

Table 3. Measures in modern and archeological samples. Average over 13 traits in 94 modern specimens (ears, cobs and kernels) and 123 archeological cobs (a. Cob) and 151 archeological kernels (a.Kern): leng, length; U.Dia, upper diameter; Me.Dia, medium diameter; Ma.Dia, maximum diameter; Inf.Dia, inferior diameter; N Row, number of row in ears and cobs; kern/Row, number of kernel per row in ears and cobs; t/kern, total number of kernels; wid, width of kernel; Thick, thickness of kernel; Per, perimeter, Are, area; SF, Shape factor; DF, Feret diameter. All measures are expressed in mm.

3.1.3 PERMANOVA results

A one–way PERMANOVA revealed differences between archeological and modern maize in the measurements of the 10 variables analyzed (F=111.8, $p < 0.001$). The distance between the two groups (modern and archeological samples) is statistically significant; the size traits observed in the modern sample are larger than in the ancient sample. Also a one-way PERMANOVA of four groups of kernels (three archeological and one modern) indicated significant differences in the measurements of these five traits between groups (F=143, $p<0.001$).

3.2 Morphological diversity between valleys in current samples.

PCA of modern ears from Tarapacá and Camiña valleys showed no significant differences (Fig. 5). All six populations showed a homogeneous distribution and overlapped, thus the distribution of 95 specimens of the six population is homogenous. However, PCA over Camiña kernels alone indicated that population 5 and population 6 are segregated from each other, while population 4 is homogenously distributed (Fig. 6). Kernels from population 5 and population 6 are separated by size parameters; population 5 is larger than population 6.

The first two axes of the principal component analysis of modern ears accounted for 72.90% of the total variance; PC1 explained 48.48% and PC2 24.42% of the total variance.

The highest loadings for PC1 were number of kernels per row (0.519) and ear length (0.410). PC2 had high loadings for kernel area (0.552) and upper diameter of ear (0.480) (Fig. 5).

The first two axes of the principal components analysis of modern kernels from Camiña populations alone explained 74.26% of the total variance; PC1 explained 52.74% and PC2 explained 21.52%. PC1 had the highest loading for area of kernel (0.638) and PC2 thickness (loading = 0.989) (Fig. 6).

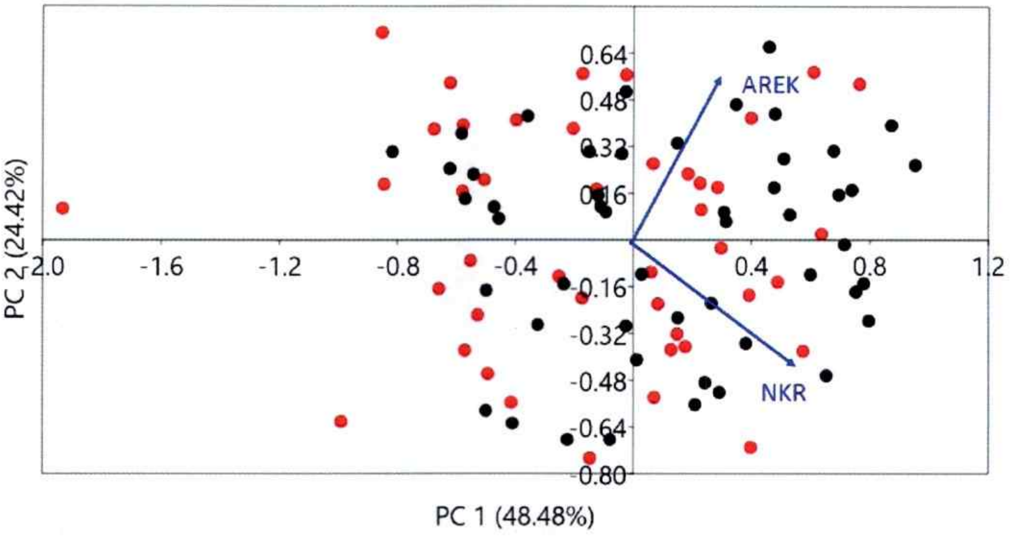


Figure 5. PCA on modern ears for 10 traits. Tarapacá modern populations 1, 2, and 3 represented by red dots, Camiña modern populations 4, 5 and 6 represented by black dots. The analysis shows no differences between the two valleys or populations. AREK=Area of kernel, NKR=Number of kernels per row.

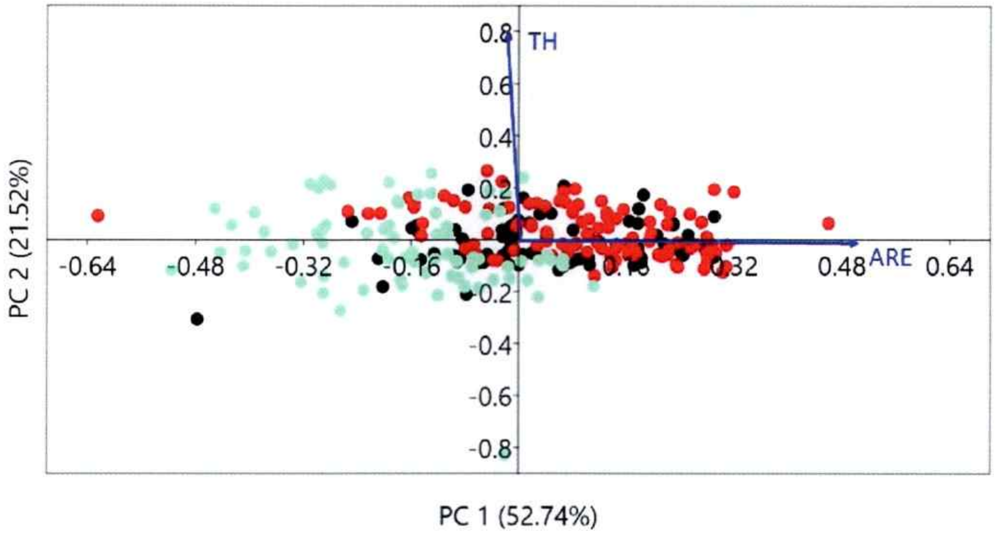


Figure 6. PCA for modern kernels of the Camiña population for nine traits. Population 4 represented by black dots, population 5 by red dots and population 6 by green dots. The Figure shows that population 5 and population 6 are segregated from each other, while population 4 is homogenously distributed. THIC=Thickness, ARE=Area of kernels.

3.3 Morphological diversity in archeological samples

The PCA analysis of cobs in archeological samples found no significant differences in size or shape. Some cobs from Pircas (blue dots in Figure 7) tend to group among the largest specimens. The first two axes of the PCA explained 84.47% of the total variance; PC1 explained 72.82% and PC2 11.65%. PC1 is mainly explained by area (loading = 0.558) and PC2 by maximum diameter (loading=0.682) (Fig. 7).

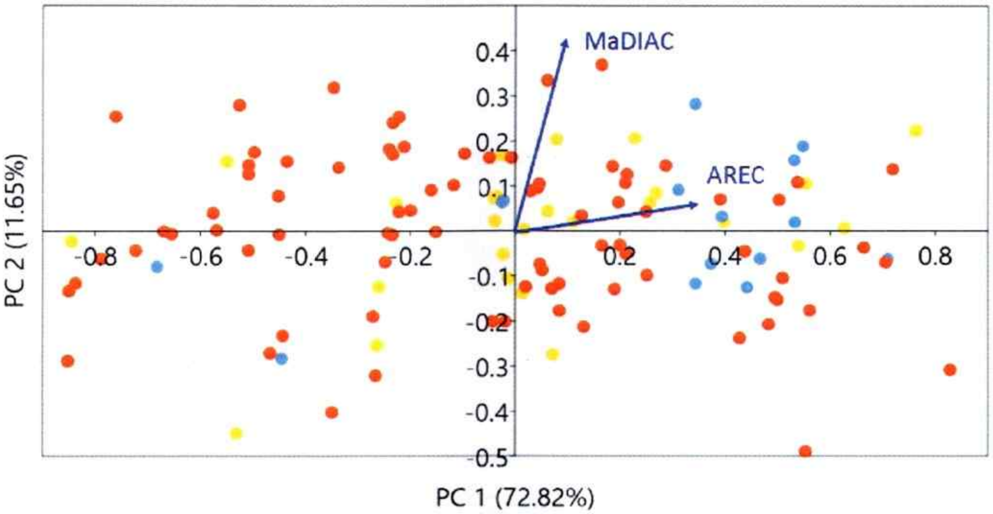


Figure 7. PCA of archeological cobs for 10 traits. Pircas cobs represented by blue dots, cobs of Tarapacá 13 by yellow dots and Tarapacá Viejo by orange dots. As shown, specimens have a homogenous distribution. AREC=Area of Cobs, MaDIAC=Maximum Diameter of cobs.

3.4 Morphological diversity between modern and archeological samples.

The results of the PCA indicate a clear segregation between pre-Hispanic and modern samples. Pre-Hispanic cobs are consistently smaller in perimeter and thus separated from modern cobs (Fig. 8). Only 14 cobs reached modern size; all these specimens belong to the Late Period.

The PCA of kernels showed a distinct situation: Pintados 1037 (Late Formative) kernels are segregated from the rest as they are round and smaller (blue dots). Kernels from Tarapacá 13 (yellow dots) are smaller than Tarapacá Viejo (orange dots), showing a progressive increase of size from the Late Intermediate Period to the Late Period. Kernels from Tarapacá Viejo are clearly the largest of the archeological sample, and some specimens even reached modern size. Finally, modern kernels are separated from all the rest, distributed in the right half of the biplot (Fig. 9).

The first two axes of the PCA of archeological and modern cobs accumulate 83.59 % of the total variance; PC1 explained 72.66% and PC2 10.93%. PC1 is mainly explained by perimeter (loading =0.658) and PC2 Feret diameter (loading=0.624) (Fig. 8).

The first two axes of the PCA of archeological and modern kernels accumulate 88.83% of the total variance; PC1 explained 73.98% and PC2 explained 14.85% PC1 is mainly explained by area of kernel (load=0.759) and PC2 mainly by thickness (loading=0.977) (Fig.9).

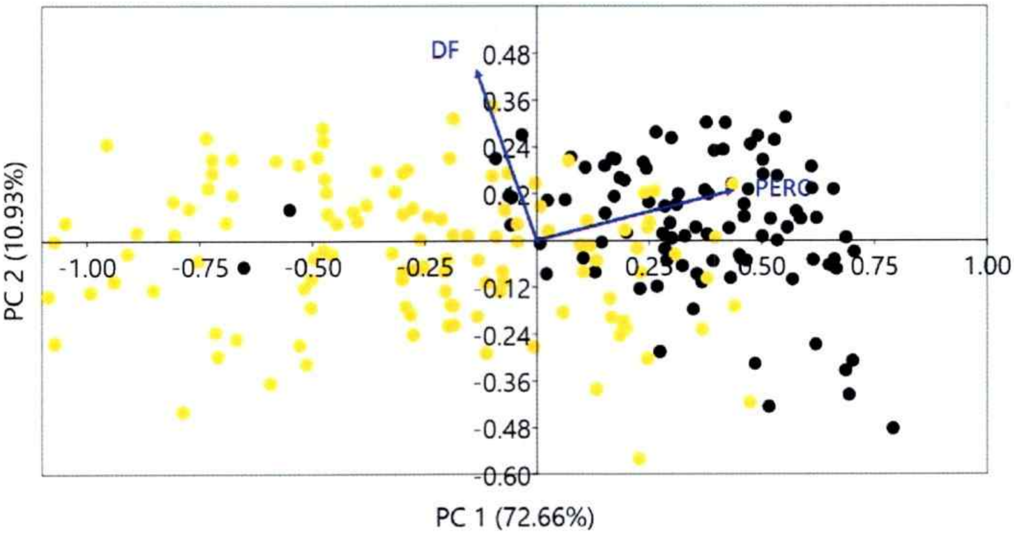


Figure 8 PCA of archeological and modern cobs for 10 traits. Archeological cobs are represented by yellow dots and modern cobs by black dots. As shown, modern and archeological cobs are separated in two distinct groups. PERC=Perimeter of cobs, DF=Feret diameter.

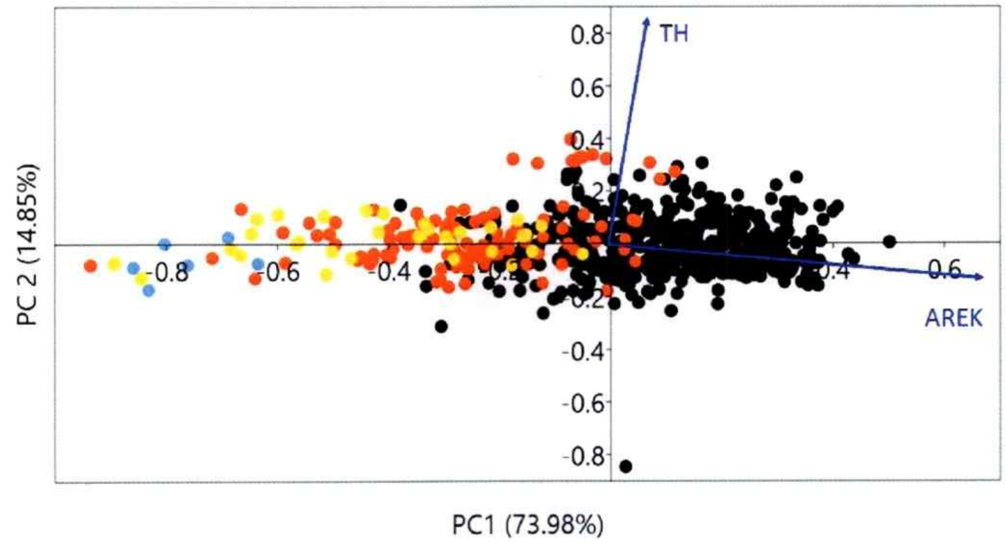


Figure 9. PCA of archeological and modern kernels for five traits. Archeological kernels from Pintados 1307 are represented by blue dots, archeological kernels from Tarapacá 13 are represented by yellow dots, archeological kernels from Tarapacá Viejo are represented by orange dots and modern kernels are represented by black dots. Kernels from Pintados 1307 (Formative Period) are grouped with Tarapacá 13 kernels (Late Intermediate Period-Late Period), while Tarapacá Viejo (Late Period) and modern kernel specimens are distant from the previous ones. AREK= Area of kernel, TH=Thickness.

According with LDA analysis, archaeological and modern cobs are discriminate into two groups (Group 1 modern; Group 2 archeological). In the Group 1, 96 % of individuals were correctly assigned and Group 2, 93 % of individuals were correctly assigned. LD 1 is explains by Area (loading=-0.15804) (Fig. 10). ANOSIM test performed over this two groups indicates a statistic R= 0.4393 ($p<0.001$).

LDA on kernels discriminates two groups (Group 1: modern; Group 2 archeological samples). The correct allocation percentage for Group 1 is 87%, meanwhile the correct allocation percentage for group 2 is 88% (Figure 11). The main variable for LD1 is Area (loading =0.1303). ANOSIM test performed over this two groups indicates a statistic R= 0.5593 ($p<0.001$).

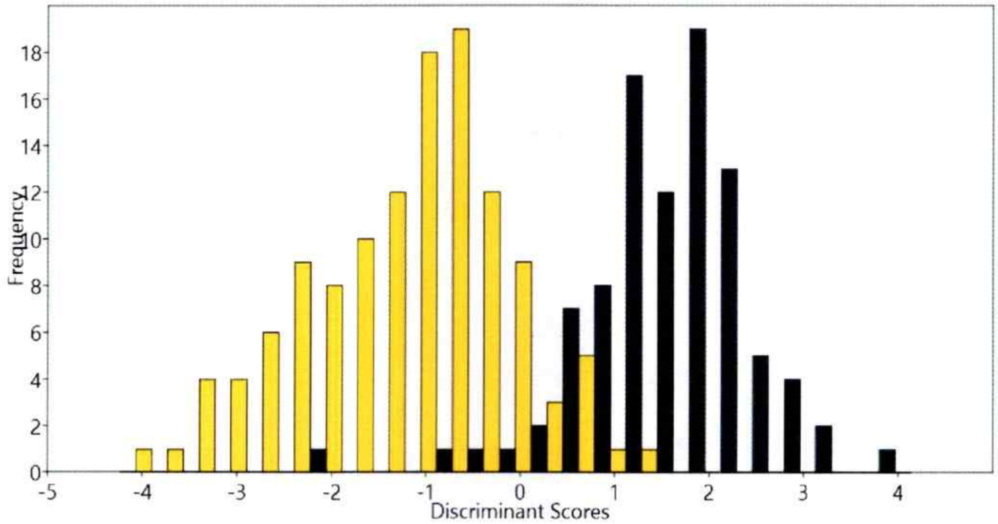


Figure 10. Histogram of discriminant scores for 10 traits in cobs. Archeological cobs are shown in yellow bars, modern cobs are shown in black bars. As shown, modern and archeological cobs are separated in two distinct groups.

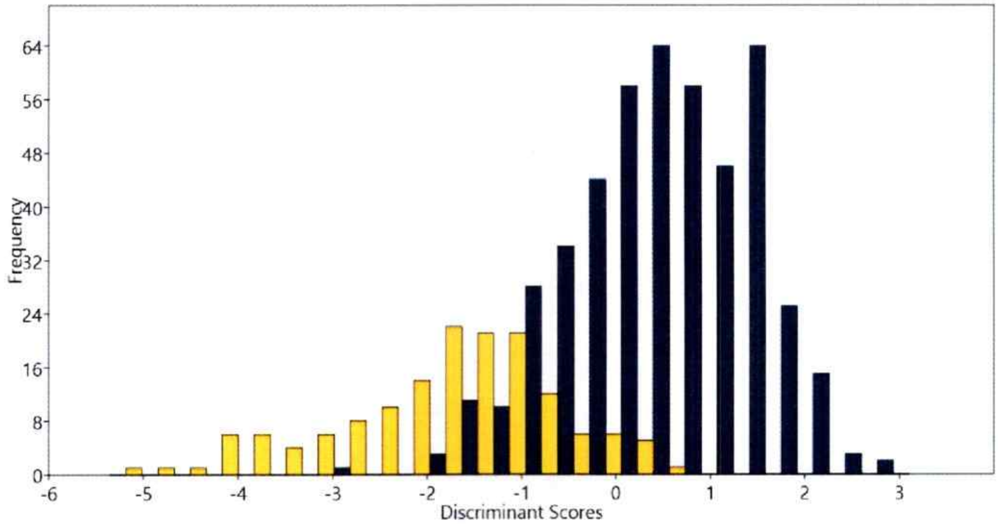


Figure 11. Histogram of discriminant scores for 5 traits in kernels. Archeological kernels are shown in yellow bars, modern kernels are shown in black bars. As shown, modern and archeological kernels are separated in two distinct groups.

3.5 Genetic diversity in modern samples

All SSR loci were polymorphic in all modern populations. The average number of different alleles for the six modern populations was 3.6. The mean number of alleles per locus was 5.1, with a minimum of 4 and a maximum of 9 (Table 4). The ratio of heterozygosity (Ho) over expected heterozygosity (He) showed an excess of homozygotes under the assumption of Hardy –Weinberg (average 0.413 [Ho] and average 0.521 [He]), except for three loci (Phi029, umc1332 and Phi034) (Table 1). The inbreeding coefficient varied between valleys; the populations of Camiña had higher values (F_{is} 0.32) than those of Tarapacá (F_{is} 0.086).

SSR	Ho	He	DNA size (bp)	N allele	aDNA size (bp)
Phi127	0.168	0.409	120-124-128-132-140	5	128
Phi063	0.117	0.579	168-172-176-180-184-188-192-196-228	9	192
Phi059	0.409	0.462	168-171-177-180	4	168-177
Phi029	0.568	0.553	156-162-166-172-174	5	*158*-166-172-
Umc1332	0.578	0.564	141-162-165-168	4	141-*153*-162-165
Phi075	0.271	0.391	248-250-260-262-270	5	*258*-262-*288*
Phi034	0.606	0.532	144-147-150-153-159	4	159
Phi056	0.586	0.681	258-261-264-267-270	5	258-261-267
Average	0.413	0.521		5.1	

Table 4. Genetic diversity in modern samples. SSR, Simple Sequence Repeats or microsatellites used in this study; Ho, Observed Heterozygosity for six modern populations; He, Expected Heterozygosity; DNA size (bp), Size range amplification of 94 specimens in modern samples; N alleles, Number of alleles per locus in modern samples; aDNA size (bp), Size range amplification in archeological samples from 12 plants.

Molecular variance and F_{ST} provided no evidence for population structure in modern samples, since 97% of the molecular variance was within populations, 3% among population and 0.18% among regions. We observed low F_{ST} values among all six populations; the highest F_{ST} value was 0.048 between population 1 and population 5. Accordingly, the Nm (the effective proportion of immigrants) is 7.4.

3.6 Genetic diversity in archeological samples

Archeological kernels showed the same allele amplification as modern samples, with the exception of allele 158 (Phi 029), allele 153 (umc1332), allele 288 and 258 (phi075) present as private alleles and not found in our modern samples. The two kernels dated for to the Late Formative Period showed the same range of amplification as the later ones (Late Intermediate Period and Late Period).

The number of different alleles in archeological samples was 2.8 and the number of effective alleles per locus was 2.3. Ho/He showed an excess of homozygotes under the assumption of Hardy –Weinberg equilibrium (average 0.164 [Ho] and 0.479 [He]).

3.7 Structure Analysis

STRUCTURE analysis produced the highest Delta K (6.777) score in $K=2$ but no spatial genetic structure was observed (Fig. 12). All populations in Structure analysis show admixture, being population 5 (modern) and population 7 (archeological) more homogenous than the rest.

This results is consistent with clustering based on genetic distance by Fst values (Fig. 13). Thus F_{ST} values segregates the archeological sample from the rest of the modern populations (Table 5). As is shown by hierarchical cluster analysis of the F_{ST} values three major clusters are displayed. The first cluster includes modern populations 2 and 5 and the second cluster includes modern populations 1, 3, 4 and 6, while the archeological sample (population 7) is distant from the rest of the clusters.

When the archeological sample is included as another population (belonging to Tarapacá valley) with the other six modern populations, the AMOVA analysis shows that 91% of molecular variance is explained within populations, 9% among populations and less than 1% among regions, indicating no significance differences between archaeological and modern populations. But, if sample is divided as archeological population being considered as one region and the whole rest of modern samples as another region, the AMOVA analysis indicates that 23% of molecular variance is explained among region, 75% within populations and 2% among populations. The percentage among regions is consistently with the results shown by Structure and F_{ST} values.

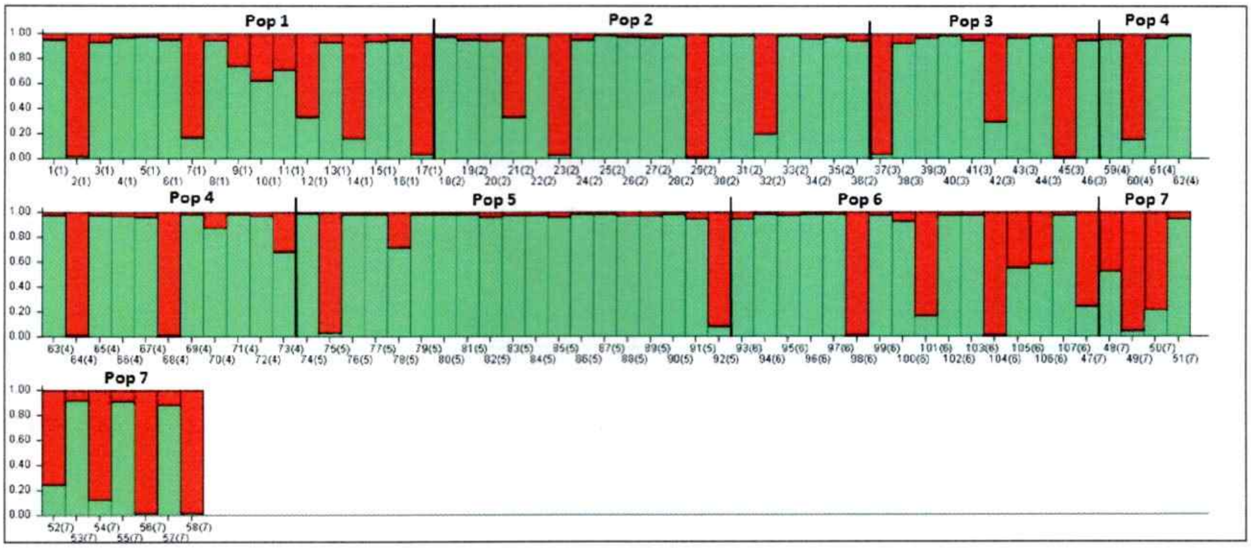


Figure 12 Estimated population structure. Each individual is represented by a bar and is individualized by number and population assigned in the lower part of the graphic. The graphic is portioned into K=2 colored segments (green and red segments). Population are separated by black bars and labeled above the figure. No spatial genetic structure is observed.

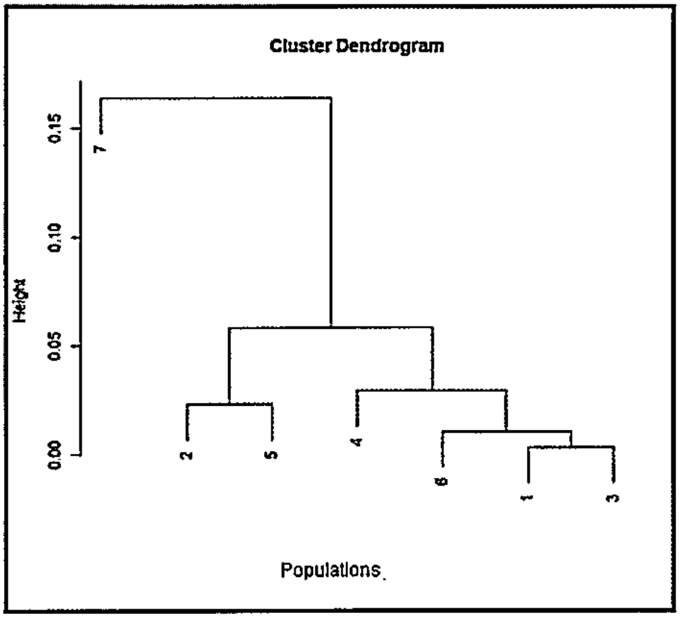


Figure 13. Cluster dendrogram for F_{ST} values for populations 1 to 7. Three major groups are observed; populations 2 and 5 grouped in one branch, populations 1, 3, 4 and 6 are grouped in a second branch and the archeological sample (population 7) is distant from the rest.

c	Pop 7	p value
Pop 1	0.132	P<0.05
Pop 2	0.191	P<0.05
Pop 3	0.135	P<0.05
Pop 4	0.166	P<0.05
Pop 5	0.214	P<0.05
Pop 6	0.144	P<0.05

Table 5. F_{ST} values for archeological sample population 7 and six modern populations.

3.7 Agronomic and Anthropological Information

Charac.	PROV	VN	SA	SH	WC	F	Hib	L. PROV	PA
Farm.									
1	L/F	*Choclo blanco*	0	Ag-Ap	SW	P	NH	Chusmiza	1
2	L	*Choclo blanco*	0	Ag-Ap	SW	P	NH	Chusmiza	1
3	L	*Choclo colorado or morado Choclo blanco*	0	Dc-My	SW	NA	NH	Limaxiña	2
4	L/F	*Choclo blanco*	1	Ag-Fe	NA	P	NH	Huaviña	3
5	L	Choclo blanco	4	Ju-Dc	NA	P	NH	Camiña	4
6	F	*Chulpi, Capio, Choclo blanco, Choclo colorado*	2	Nv-Ma	NA	P	NH	Camiña	5
7	L	*Choclo blanco*	0	Nv-Ma	S	P	NH	Camiña	6
8	L	*Choclo de Camiña*		Ju-Jn	S	P	NH	Camiña	6

Table 6. Summary of agronomical and anthropological information concerning characteristics of maize used for this study. PROV, provenance of seeds L=local, F=foreign; VN, vernacular name of the variety; SA, sample age in months. 0=collected fresh from the plant; SH, sowing and harvesting times (My-J-Ju-Ag autumn-winter, Nv-Dc-Jn-Fb-Ma-Ap spring-summer); WC characteristics of water S=salty, SW=Sweet; F, use of fertilizer presence=P, Absence=A,; Hib, hybridized (H) or not hybridized (NH); L. PROV, locality associated with maize sample; PA, Maize population associated. NOA=no information available.

The qualitative information gathered and summarized in Table 6 shows six vernacular names given to the modern varieties collected (*Choclo Blanco, Choclo Colorado, Choclo Morado, Chulpi, Capio* and *Choclo de Camiña).* As shown in Tables 1 and 5, seeds of populations 1 and 3 have a local and foreign origin and seeds for population 5 are completely from a foreign locality (Chiapa valley).

The sowing and harvesting time tends to vary from five to nine months. It was mentioned to us that almost all maize varieties need sweet water; only population 6 belonging to Camiña valley was resistant to salty water. Seven farmers indicated the use of some type of fertilizer; in one case (population 2) this information was not available. The fertilizers used were urea and potassium nitrate. Other qualitative information given by Tarapacá farmers during our visit was that Camiña maize has faster maturation time, due to longer sun exposure, than Chusmiza or Limaxiña. These localities are at higher altitudes, therefore having a shorter time of sun exposure. Also farmers from Huaviña affirm that sun exposure time affects the size of the ears, thus Chuzmisa maize is smaller than Huaviña crops according to their appreciation. This observation was mentioned to us only once and was not corroborated by PCA.

DISCUSSION

4.1 Morphological and genetic diversity in modern samples: Are there any phenotypic and genetic differences between current maize from Camiña and Tarapacá valleys?

Although farmers refer to maize samples as different landraces or varieties, PCA makes no distinction in the 10 morphological traits considered in our modern specimens, providing no evidence for morphological differentiation between Camiña and Tarapacá valleys (Fig 5). The morphological parameters selected for ears are not critical traits to show landraces of maize. However, PCA on Camiña's kernels showed that populations 5 and 6 clearly segregated from each other (Fig. 6). As explained, population 5 is presumably the only population that came entirely from a different valley (Chiapa valley) from where it was collected. It was referred to us as composed of four different varieties not found among the other populations collected (Table 5). The different provenance and its internal variability could explain the distance observed. We conclude that kernel traits alone are better traits to distinguish variability among maize races in the samples under analysis.

Genetic diversity parameters showed low variability (Table 4); F_{ST} values and AMOVA results made no distinction between valleys (regions) and/or populations. These Low F_{ST} values in modern samples are interpreted as consequence of gene flow carried out through seed exchange between farmers, within locations and between Camiña and Tarapacá valleys in current times, indicating there is no cultural or natural barrier preventing isolation by distance. Likewise, farmers interviewed during collections indicated that some populations come from different locations from those where they were being cultivated (Tables 1 and 5). This is the case for populations 1, 3 and 5. For population 1 and 3 the collection sites are situated in the same basin of Tarapacá. Population 5 (Alto Camiña) is the most singular case, since it presumably comes entirely from a different valley (Chiapa village) situated at higher altitude. Thus farmers confirmed seed exchange between localities is being intentionally and consciously performed in Tarapacá and Camiña.

The modern samples analyzed exhibited low genetic diversity parameters compared to adjacent areas. Previous analysis of 235 plants of Andean provenience showed an average of 12.4 alleles per locus and an observed heterozygosity value of 0.706 (Vigouroux et al., 2008). Similarly, Grimaldo et al. (2011) indicated an average of 6.1 alleles per locus in 176 modern Andean specimens. Although this could due to the smaller size of our sample (n=95), maize from Tarapacá may show a founder effect as has been suggested for the Andean Group (Lia et al., 2007; Freitas et al., 2003) If this is the case, we suggest that also several years of human selection would have decreased the allele frequencies (Coyac et al., 2013). The method for choosing the specimens that fulfilled cultural requirements was told by farmers during the collection of our sample and has been used by Andean farmers for centuries (Murra 1999). Thus we hypothesize that a founder effect, followed by an intensive selection and an isolation process, caused the low genetic diversity observed.

Even in the absence of a founder effect, due to the specific environmental conditions of Tarapacá, such as altitude (from 1200 to 3300 m.a.s.l), low nutrition soils, low daytime sunlight and heavy temperature gradients, only a few seeds would have adapted to the Tarapacá region, consequently decreasing genetic diversity parameters.

The low genetic diversity for Tarapacá maize is indicative of a late introduction of maize in Tarapacá valleys, therefore leaving no time to generate genetic diversity. Archeological records are consistent with the hypothesis of a late maize introduction to the region, since the earliest date for its presence in the area is just 1500 yBP in the Guatacondo and Ramaditas archeological sites (Rivera et al., 1995-1996; García et al., 2014).

Low F_{IS} values for these modern populations indicate that each population behaves as a panmictic population, probably to the open-pollinated system of maize (Pressoir & Berthaud 2004). However, higher F_{IS} values in the population of Camiña suggest an extended inbreeding process.

Traditionally, homozygote excess is attributed to consanguineous mating, population substructure and artifacts caused by null alleles (Pressoir & Berthaud 2004). The homozygote excess observed in this study may be due to the condition of isolation of Tarapacá throughout its social and agronomic history. Thus, unlike other areas of South Central Andes, the Tarapacá region shows social isolation since the Middle Horizon (ca. 1500-1000 yBP)[1]. During these 500 years a series of objects related to the Altiplano state of Tiwanaku appear in Arica and San Pedro de Atacama (adjacent regions of Tarapacá) funerary contexts. However, Tarapacá human communities do not show evidence of contact or connection with the Altiplano state, seeming impervious to their material culture (Uribe 2009) and the cultural groups under the Tiwanaku political sphere. We postulate that the low genetic diversity of Tarapacá maize (archeological and modern)

---

[1] According to the cultural parameters traditionally used to describe the Middle Horizon (1500-1000 BP) in South Central Andes, this period is not present in the cultural sequence for Tarapacá. Therefore this moment is classified as the Late Formative Period (1700-1000 BP) for Tarapacá (Uribe 2009).

could be more evidence of this process of isolation as result of low seed exchange with other neighboring sectors.

4.2 Comparison between modern and archeological samples. Are there a phenotype and genotype variations in Tarapacá maize between pre-Hispanic and current times?

The comparison of morphological traits between archeological and modern samples is consistent with an increase of cob size and kernel size of modern maize (Fig. 8). Since the genetic continuity between archeological and modern maize is untested, it is not possible to establish if the increased size of modern landraces is due to anthropic management.

We also observed differences in kernel size within the archeological samples (Fig.9). We postulate that the progressive increase of kernel size during the Late Intermediate Period and Late Period could be the result of human selection carried out by Tarapacá pre-Hispanic farmers. Additionally, some cobs and kernels from Tarapacá Viejo (Late Period) present the same size as modern specimens. These larger specimens belong to the Inca period; this is the first time in the cultural sequence of Tarapacá that corn reached the size of modern maize. Thus the morphological differences observed between archeological and modern samples are also present in the ancient maize.

The genetic results indicate that archeological samples amplified loci for all SSR identical to those observed in modern samples, with the exception of four private alleles (phi029 $_{153}$, umc1332 $_{158}$ and phi075 $_{258/288}$) only present in the archeological sample. Similar studies of archeological specimens of maize in northwestern Argentina highlighted the lack of genetic variation, exhibiting a single allelic variant identical in size to modern populations for SSR phi 127$_{112}$, phi 029$_{154}$ and phi 059 $_{157}$ (Lia et al., 2007). This is not the case for the Tarapacá region, which could be due to our larger sample and the greater number of SSR used in our study. However, F_{ST} values segregated the archeological sample from the modern sample, indicating an isolation process and therefore the generation of genetic differentiation between modern and archeological samples.

It is likely that this isolation process was caused by the time period of 600 hundred years. During this time lapse the genetic distance observed could have been caused by a) loss of alleles and alteration of allele frequencies as a result of a selection process during pre-Hispanic times and/ or b) an introduction of a new genetic pool in Tarapacá in modern times.

In summary, significant phenotypic differences are recognized between archeological and modern maize in Tarapacá. Despite the low genetic diversity present in both samples, F_{ST} values are suggestive of some processes of temporal isolation, which would have led to the genetic distinction between ancient and modern samples.

4.3 Does the phenotypic and genetic diversity of Tarapacá maize indicate the introduction of new landraces since pre-Hispanic times?

The production of maize in Tarapacá suggests that environmental conditions for its production were established since the Formative Period. The pollen record from rodent middens indicates an increase in moisture since 2,200 yBP, which would have remained until about 700 yBP. This humid period is also corroborated by stratigraphic studies in the Pampa del Tamarugal for the period between 1070-700 yBP (Maldonado & Uribe 2015). The more humid conditions recharged aquifers and streams (Houston 2001), making possible an extensive agriculture (Meighan, 1980; Rivera & Dodd, 2013; Vidal, et al. 2015) and allowing the settlement of villages and sites at low altitudes (1100-1300 meters) such as Pintados 1307, Guatacondo, Ramaditas, Pircas and Caserones. However, the low quantity of maize macro remains (3% of total plants) recovered in Pircas, Guatacondo and Ramaditas during the Early Formative Period (2500-2000 yBP) led us to postulate that corn was not the main crop at this initial agricultural stage. The low genetic variability observed in modern and archeological samples corroborates this assumption, indicating a possible late introduction and further development of maize agriculture. Massive production of maize occurred during the Late Formative Period (1700-800 yBP), during this time constituting more than 90% of the plant resources at the site of Caserones (García et al., 2014).

During the Late Intermediate Period (800-550 yBP), environmental conditions changed to a drier regime (Maldonado & Uribe 2015). Accordingly, most settlements during this time are situated at higher altitudes (2200-2600 meters), where there is more water available. Only a few settlements, such as Caserones and Tarapacá 13 (1200 meters) remained in the lower portion of the basin of Tarapacá. The cultural consequences are that Caserones became a core center of corn production with human selection operating on size (Vidal-Elgueta et al., 2016). Our results indicate that Tarapacá 13 kernels are distant from previous kernels, progressively increasing their size, maybe as the receptor of Caserones production.

During the Late Period (550-465 yBP) humid conditions returned (Maldonado & Uribe 2015) and Tarapacá Viejo basin was occupied by the Inca State (Núnez, 1983, 1984; Uribe et al., 2012; Zori 2011). It has been suggested the Inca state introduced new resources in areas under its domain, especially in agricultural production (Murra 2002; Uribe et al., 2012; Zori 2011). Our results contradict this assumption because we observed the same types of kernels in Tarapacá 13 and Tarapacá Viejo. An earlier agricultural system was well established before the arrival of the Incas in Tarapacá, as shown by Caserones farmers in their effort to enhance maize size (García et al., 2014; Núñez 1966; Vidal-Elgueta et al., 2016). However, some changes did occur under Inca presence. Thus maize in Tarapacá Viejo is larger than previous maize and reached the same size as modern specimens (Fig. 8 and Fig 9). We postulate that Inca strategies focused on increasing size of maize (specifically kernel size) and increment the quantity of maize production and pod corn types.

Although we do not know the specific biological mechanism of this size improvement for archeological specimens, we do know farmers select the largest ears and kernels for sowing in present times. This cultural practice over 200 years would have increased the size of cobs and kernels. In fact, it has been described that the high phenotypic diversity of maize is due to transposon activity and the polyploid nature of the maize genome (White & Doebley, 1998); several genes are known to contribute to phenotypic differences in several traits under selection during domestication or manipulation of the species (Doebley et al., 2006; Liu et al., 2015; Wang et al., 2013). Furthermore, some major morphological changes were reached via changes in a single locus (Doebley et al., 2006; Gross & Olsen, 2010) and experimental conditions proved this is possible to achieve in just one generation (Gremillion & Piperno 2009; Piperno et al., 2015). For example, Mexican corn cob specimens from the Ocampo Caves dated from 5550 old to 4400 yBP show that cob size increased continuously during the first 2000 years of human selection (Jaenicke-Després et al., 2003). The mechanism apparently involved P*bf* and su1 genes, which are related to protein and starch quality. The presence of these genes in Mexican pre-Hispanic cobs suggests that kernel quality and cob size were traits selected early (Jaenicke-Després et al., 2003).

Thus we propose that the Inca did not introduce new landraces of corn but reinforced the agricultural system in Tarapacá by increasing production, expanding agricultural fields and terraces, channeling water in desert landscapes, focusing on production of floury varieties of maize suitable for the preparation for alcoholic beverages and finally increasing cob and kernel size already present in the Tarapacá valley.

One interpretation for the F_{ST} values between modern and archeological samples is an introduction of a new gene pool in Tarapacá during present times. To test this possibility further analysis for the *ADh2* loci will be carried out and a larger archeological sample should be included. Nevertheless, the excess of homozygotes is concordant with an inbreeding process (Contreras 2007; Coyac et al., 2013). Homozygous excess as a result of inbreeding has also been reported for primitive races of maize (Bracco et al., 2013), crops such as rice and alfalfa (Contreras 2007) and for domesticated animals (Paredes et al., 2011). Inbreeding has been related to cultural selection in maize (Pressoir & Berthaud

2004). Therefore, the genetic distance observed could also due to a long human selection and manipulation of maize.

4.4 Archeological Implications

The scarce presence of corn during the Early Formative Period in Tarapacá, representing no more than 1% of the plant spectrum, indicates that it was not relevant in economic terms, but probably its presence is due to ritual or other extraordinary functions (García et al. 2014, Mandakovick 2016). Accordingly, recent studies conducted on isotope stable of Tarapaca-40 human remains suggest that corn would not have been relevant in the diet of people during early times (Santana-Sagredo et al. 2015). But from the Late Formative Period its frequency increases in an evident way, being the site of Caserones the main exponent of this intensive agriculture. In parallel to the agricultural intensification, there is a marked change in the size of cobs present in Caserones, suggesting that from the Late Formative to the Late Intermediate Period, Caserones-1 experimented consciously with corn, which resulted in the described changes and their singularity as a great settlement (Vidal-Elgueta et al., 2016). We suggest that the differences in size observed in Pircas, Pintados, Tarapacá 13 and Caserones corn are due to the fact that the strategies of manipulation of plant resources respond to concrete and heterogeneous historical situations (Uribe 2006) by politically autonomous groups. Thus, Caserones may have acted as a nuclear settlement for agricultural development, while the other communities have acted as recipients of this agricultural production.This hypothesis must be tested in the future.

Finally, following Uribe (2006), evaluating the continuity of maize and its transformations, reaffirms the important historical role played by the local substratum of the Formative Period, positioning marginal groups as potent agents of change in all their cultural spheres. In fact, not only the nuclear areas of American development were protagonists of the connoted cultural transformations of the pre-Columbian continent.

CONCLUSIONS

A. Archeological and modern samples shared most of the alleles for the SSR used in this study. However, the archeological sample presents four private alleles. .Overall, archaeological sample showed lower diversity parameters than the modern sample, and F_{ST} values indicated genetic distance between the two samples. Accordingly, two cluster (K=2) were identified by Structure Analysis, but no spatial genetic structure was found

B. No genetic or morphological differences were found in modern samples, probably due to cultural exchange practices, but also this could due to a late introduction of maize in Tarapacá. The only difference observed in modern samples was in the size of kernels for just one population. Therefore kernels, rather than cobs, are better traits for morphological distinction in this case.

C. The hypothesis that ancient maize (archeological) samples would show higher genetic and phenotypic diversity compared to later and modern ones is not corroborated, since the samples are genetically segregated and present clear morphological differences.

D. PCA results indicate archeological cobs and kernels are consistently smaller than modern samples. Only a few examples from pre-Hispanic times reached the size of modern samples.

E. LDA results indicate archaeological cobs and kernels are consistently separated into two groups.

F. We postulate that the increase in cob and kernel size during pre-Hispanic times is due to anthropic selection by Tarapacá farmers, enhanced by the Inca States during the Late Period.

G. Likewise, low genetic diversity and excess of homozygote in Tarapacá maize is interpreted as the result of a founder effect, continuous human selection activities over corn and historical and cultural isolation processes.

REFERENCES CITED

Adán, L., S. Urbina & M. Uribe, (2007.) Arquitectura pública y doméstica en las quebradas de Tarapacá, asentamiento y dinamismo social en el Norte Grande de Chile. En *Taller de Procesos Sociales Prehispánicos en los Andes Meridionales,* A. Nielsen, M. C. Rivolta, P. Mercolli, M. Vázquez y V. Seldes (Eds.). Editorial Bayas, Córdova, Argentina.

Bracco, M., V. Lia, L. Poggio, J. A. Cámara Hernández, A. M. Gottlieb (2013). Caracterización genética de razas de maíz autóctonas de Misiones, Argentina. *Rev. Cienc. Tecnol.* Año 15, (20), 52–60.

Bonavia, D. (2013). *Maize. Origin, Domestication, and its role in the development of culture.* United States of America: Cambridge University Press.

Contreras, M. (2007). *Efecto de una generación de endogamia sobre caracteres vegetativos y productivos en yuca (Manihot esculenta Crantz).* Tesis (Magister en Ciencias Agrarias con énfasis en Fitomejoramiento). Universidad Nacional de Colombia, Facultad de Ciencias Agropecuarias, Escuela de Posgrados, Palmira, Valle del Cauca, CO. 80 p.

Coyac, J., J. Molina, J. García & L. Serrano. (2013). La selección masal permite aumentar el rendimiento sin agotar la variabilidad genética aditiva en el maíz zacatecas 58. *Revista Fitotécnica Mexicana* (36) 1; 53-62.

Doebley, J., Gaut, B., & B. Smith (2006). The Molecular Genetics of Crop Domestication. *Cell* (127),1309-1321.

Earl, D & B. von Holdt (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genet Resourc (4):359–361.

Freitas, F. O., Bendel, G., Allaby, R. G., & T. A. Brown (2003). DNA from primitive maize landraces and archeological remains: implications for the domestication of maize and its expansion into South America. *Journal of Archeological Science*, 30(7), 901-908.

García, M. & M. Uribe (2012) Contextos de uso de las plantas vinculadas al Complejo Pica Tarapacá, Andes Centro-Sur: Arqueobotánica y agricultura en el período Intermedio Tardío (ca. 1250-1450 DC). *Estudios Atacameños* (44), 107-122.

García, M., Vidal, A., Mandakovic, V., Maldonado, A., Peña, M., & E. Belmonte (2014). Alimentos, tecnologías vegetales y paleoambiente en las aldeas formativas de la pampa del Tamarugal (ca. 900 a.C.-800 d.C.). *Estudios Atacameños* (47), 33-58.

Goodman, M., & R. M Bird (1977). The races of maize. IV Tentative grouping of 219 Latin American races. *Eco. Bot.* (31), 204-221.

Gremillion, K. J., & D. Piperno (2009). Human Behavioral Ecology, Phenotypic (Developmental) Plasticity, and Agricultural Origins Insights from the Emerging Evolutionary Synthesis. *Current Anthropology*, 50(5), 615-619.

Grimaldo, C. (2011). *Investigating the Evolutionary History of Maize in South America.* Thesis for Doctor of Philosophy presented to the University of Manchester, UK.

Grobman, A. (1982). Maíz (Zea mays). In D. Bonavia (Ed.), *Precerámico peruano. Los Gavilanes. Mar, desierto y oasis en la historia del hombre* (pp. 157-179). Lima: Corporación Financiera de Desarrollo S.A., Instituto Arqueológico Alemán.

Grobman, A. (2013). Origin, Domestication, and Evolution of maize: New perspectives from cytogenetic, genetic, and biomolecular research complementing archeological findings. In D. Bonavia (Ed.), *Maize. Origin, Domestication, and its role in the development of culture.*Cambridge University Press.

Gross, B., & K. Olsen (2010). Genetic perspectives on crop domestication. *Trends in Plant Science,* 15(9), 529-537.

Hammer, Ø. (2016). *PAST. Paleontological statistics. Version 3.4. Reference Manual.* Natural History Museum, University of Oslo.

Houston, J. 2001. La precipitación torrencial del año 2000 en Quebrada Chacarilla y el cálculo de recarga al acuífero Pampa Tamarugal, norte de Chile. *Revista geológica de Chile, 28* (2):163-177.

Jaenicke-Després, V., Ed S. Buckler, B. D. Smith, M. T. P. Gilbert, A. Cooper, J. Doebley & S. Pääbo. (2003) Early Allelic Selection in maize as Revealed by ancient DNA. *Science* 14. Vol. 302, Issue 5648, 1206-1208.

Lia, V., Confalonieri, V., Ratto, N., Hernández, J., Alzogaray, A., Poggio, L., & Brown, T. (2007). Microsatellite typing of ancient maize: insights into the history of agriculture in southern South America. Proceedings of the Royal Society of London B: *Biological Sciences*, 274(1609), 545-554.

Liu, H., Yang, X., X., Liao X, Zuo, T., Qing C., Cao S., Dong L, Zhou H., Zhang Y., Liu S., Shen Y., Lin H., Lübberstedt T., Zhang Z., & Pan, G. (2015). Genome-wide comparative analysis of digital gene expression tag profiles during maize ear development. *Genomics*, 106(52-60).

Mandakovic, V. (2016). Historias de planta: Curso bajo de la quebrada de Tarapacá entre los períodos Formativo e Intermedio Tardío. Los poblados Pircas y Caserones (400 AC – 1000 DC). Memoria para optar al título de arqueólogo. Universidad de Chile, Santiago.

Lorenz, E., Frees, K. L., & D.A Schwartz. (2001). M13-Tailed Primers Improve the Readability and Usability of Microsatellite Analyses Performed with Two Different Allele Sizing Methods. *BioTechniques*, 31(1), 25-28.

Maldonado, A. & M. Uribe (2015) Paleoambientes y ocupaciones humanas en Tarapacá durante el período Formativo y comienzos del Intermedio Tardío. *Actas del XIX Congreso Nacional de Arqueología Chilena*, pp. 193-200.

Matsuoka, Y., Mitchell, S. E., Kresovich, S., Goodman, M., & J. Doebley (2002a). Microsatellites in Zea variability, patterns of mutations, and use for evolutionary studies. *Theoretical and Applied Genetics* (104), 436-450.

Matsuoka, Y., Vigouroux, Y., Goodman, M., Sanchez, J., Buckler, E., & J. Doebley (2002b). A single domestication for maize shown by multilocus microsatellite genotyping. *Proceedings of the National Academy of Sciences*, 99(9), 6080-6084.

Meighan, C. (1980). Archaeology of Guatacondo, Chile. In C. Meighan & D. True (Eds.), *Prehistoric trails of Atacama: Archaeology of northern Chile. Monumenta Archaeologica* (Vol. 7, pp. 99-126). Los Angeles: The Institute of Archaeology, University of California.

Mostny, G. (1971). La subárea arqueológica de Guatacondo. *Boletín del Museo Nacional de Historia Natural* XXIX (16).

Murra, J. 1999 [1978]. *La organización económica del Estado Inca*. Siglo Veintiuno Editores. Sexta edición en español. México.

Murra, J. 2002 *El mundo andino: Población, medio ambiente y economía*. Instituto de Estudios Peruanos, Universidad Católica del Perú, Lima.

Núñez,. L. (1966). Caserones-I, una aldea prehispánica del Norte de Chile. *Estudios Arqueológicos* (2), 25-29.

Núñez, L. (1981). Emergencia de sedentarismo en el desierto Chileno. Subsistencia agraria y cambio sociocultural. *Creces* (11), 33-38.

Núñez,.P. (1983)..Aldeas Tarapaqueñas. Notas y comentarios. *Chungará* (10), 29-37.

Núñez, P. (1984). La antigua Aldea de San Lorenzo de Tarapacá, Norte de Chile. Chungará (13),53-65.

Paratori, O. (1990). Catálogo de recursos genéticos de maíz de Chile: INIA-EELa Platina.

Paredes, M., J. Machaca, P.J Azor, A. Alonso-Moraga, A. Membrillo & A. Muñoz-Serrano (2011). In M.A. Pérez-Cabal (eds.), *Fibre production in South American camelids and other fibre animals*. Wageningen Academic Publishers.

Peakall, R., & P.E Smouse (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, 28, 2537-2539.

Piperno, D., Holst, I., Winter, K., & O. MacMillan (2015). Teosinte before domestication: Experimental study of growth and phenotypic variability in Late Pleistocene and early Holocene environments. *Quaternary International* (363), 65-77.

Pressoir, G., & J. Berthaud (2004). Patterns of population structure in maize landraces from the Central Valleys of Oaxaca in Mexico. *Heredity*, 92(2), 88-94.

Pritchard, J., Stephens, M. & P. Donelly (2000). Inference of Population Structure Using Multilocus Genotype Data. Genetics (155), 945–959.

Rivera, M., & J. Dodd (2013). Domesticando el desierto. Medio ambiente y ocupaciones humanas en Ramaditas, desierto de atacama. *Diálogo Andino* (41), 45-60.

Rivera, M., D. Shea A., Carevic & G. Graffam, (1995-96). En torno a los orígenes de las sociedades complejas andinas: Excavaciones en Ramaditas, una aldea formativa del desierto de Atacama, Chile. *Diálogo Andino* (14-15), 205-239.

Santana-Sagredo, F.; Uribe, M.; Herrera, M.J.; Retamal, R. y Flores, S. (2015). Brief communication: dietary practices in ancient populations from northern Chile during the transition to agriculture (Tarapacá region, 1000 BC-AD900). *American Journal of Physical Anthropology* Vol. (158), 751–758.

Staller, J. (2010). *Maize Cob and Cultures: History of Zea mays* L. New York: Springer.

Timothy, D. (1961). *Races of maize in Chile* (Vol. 847): National Academies.

True, D. L. (1980). Archeological investigations in Northern Chile: Caserones. In C. W. Meighan & D. L. True (Eds.), *Prehistoric Trails of Atacama: Arcaheology of Northern Chile* (Vol. Monumenta Archaeologica, pp. 139-178. Los Ángeles: The Institute of Archaeology, University of California.

Uribe, M. (2006). Arqueología de Pica-Tarapacá (norte de Chile): Reflexiones acerca de la complejidad y desigualdad social en los Andes Centro-Sur (1000-1450 DC). *EstudiosAtacameños* (31),91-114.

Uribe, M. (2009). El período formativo de Tarapacá y su cerámica: avances sobre complejidad social en la costa del Norte Grande de Chile (900AC-800DC). *Estudios Atacameños* (37), 5-17.

Uribe, M., & L. Adán (2012). Evolución, Neolítico, Formativo y complejidad: Pensando el cambio desde Tarapacá (900 AC-800 DC). *Actas del XVIII Congreso Nacional de Arqueología Chilena.* pp. 21-31, Valparaíso.

Uribe, M., S. Urbina & C. Zori (2012) La presencia del Inca y la incorporación de Tarapacá al Tawantinsuyu (Norte Grande de Chile). *Actas del XVIII Congreso Nacional de Arqueología chilena.* pp. 217-227, Valparaíso.

Vidal, A., García, M., & P. Méndez-Quirós (2015). Vida sedentaria y oportunismo: dos estrategias de producción agrícola durante el período formativo en Tarapacá. *Actas del XIX Congreso Nacional de Arqueología chilena.* pp. 183-192. Arica.

Vidal-Elgueta, A., E. Salazar, L. F. Hinojosa, M. Uribe & S. Flores (2016). Variabilidad fenotípica en maíz (*Zea mays*) del sitio de Caserones- 1, región de Tarapacá (cal. 20-1.020 d.C). *Revista Chilena de Antropología* 34: 31-39.

Vigouroux, Y., Glaubitz, J., Matsuoka, Y., Goodman, Sánchez, J., & Doebley, J. (2008). Population structure and genetic diversity of New World maize races assessed by DNA microsatellites. *American Journal of Botany* 95(10): 1240-1253.

Wang, C., Shi, X., Liu, L., Li, H., Ammiraju, J., Kudrna, D. & Zheng, Y. (2013). Genomic resources for gene discovery, functional genome annotation, and evolutionary studies of maize and its close relatives. *Genetics*, 195(3): 723-737.

White, S., & Doebley, J. (1998). Of genes and genomes and the origin of maize Trends in *Genetics*, 14(8): 327-332.

Whitt, S., Wilson, L., Tenaillon, M., Gaut, B., & Buckler, E. (2002). Genetic diversity and selection in the maize starch pathway. *Proceeding of the National Academy of Science,* 22(9): 12959–12962.

Zori, C. (2011). *Metals for the Inka: Craft production and Empire in the Quebrada de Tarapacá, Nothern Chile*. Unpublished Phd thesis, University of California, Los Angeles.

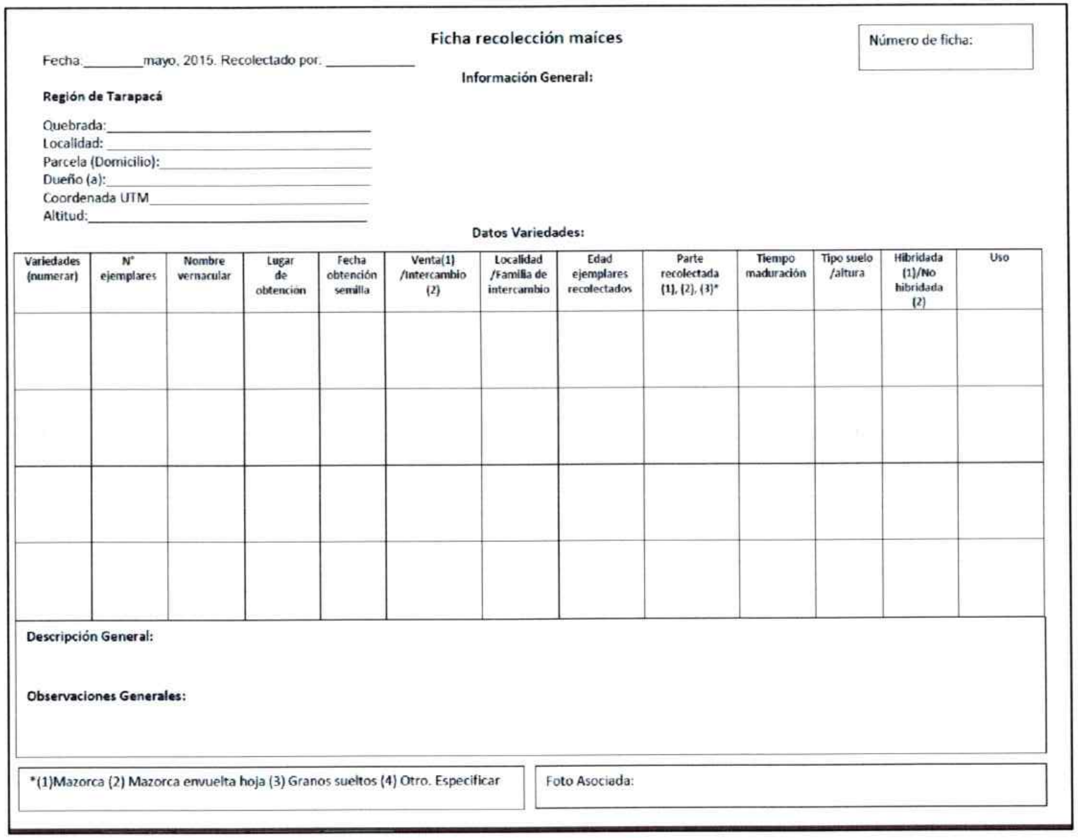
SUPLEMENTARY

Locus	Chro. Bin	5'- 3'	Type	RM	A.Tem (°C)
phi056	1	F ACTTGCTTGCCTGCCGTTAC R CGCACACCACTTCCCAGAA	Trinucleótido	CCG	55
phi029	3.04	F TTGTCTTTCTTCCTCCACAAGCAGCGAA R ATTTCCAGTTGCCACCGACGAAGAACTT	Compuesto	AG/AGAC	56
umc1332	5.04	F CCTCTTGCTTCCTCGTCATGTACT R AAGGAGCTGGAACATAAAACACCA	Trinucleótido	CTA	61
phi075	6	F GGAGGAGCTCACCGGCGCATAA R AAAGGTTACTGGACAAATATGCGTAACTCA	Dinucleótido	CT	55
phi034	7.02	F TAGCGACAGGATGGCCTCTTCT R GGGGAGCACGCCTTCGTTCT	Trinucleótido	CTT	61
phi059	10.2	F AAGCTAATTAAGGCCGGTCATCCC R TCCGTGTACTCGGCGGACTC	Trinucleótido	CCG	61
Phi127	6.07	F GGAGACGAGGTGCTACTTCTTCAA R TGTGGCTGAGGCTAGGAATCTC	Tetranucleótido	AGAC	47
Phi063	10.02	F GGCGGCGGTGCTGGTAG R CAGCTAGCCGCTAGATATACGCT	Tetranucleótido	TACT	61

Table 1. Microsatellite markers analyzed in this study. Loc, Names of loci according to MaizeGDB; Chro.bin, Chromosome bin of SSR indicated; 5'-3', Primers; Type, Number of repetitions of base pairs; RM, Repeat motif of SSR; A.Temp (°C), Annealing temperature in degrees Celsius for each SSR.

ID	280/260	260/230	ND ng/µl	QT ng/µl
ID260	1.95	1.52	1388.3	89.2
ID229B	1.94	1.87	1935.2	76.4
ID218	1.89	2.1	3675.1	67.8
ID195	1.98	1.09	1035.7	31.6
ID229A	1.76	1.61	1292.2	89
ID200	1.8	2.11	4257.9	96.6
ID223	1.88	1.63	1705.7	73
ID226	1.93	1.89	2157.2	79.4
IDPT6	1.72	1.13	820.8	96.4
ID184	1.9	1.37	508.3	60.8
IDPT5	1.52	1.32	336	*nr
IDPT4	1.5	1.57	355	*nr
IDPT1	1.82	1.43	513.1	*nr
ID216	1.43	0.77	784	*nr
IDPT3	1.77	1.69	886.5	113
ID137	1.79	1.69	53.5	*nr
ID150	1.28	0.58	53.5	*nr
ID222	1.3	0.68	54.4	*nr
IDPT2	1.31	0.72	152.8	*nr
ID236	1.53	1.54	503	*nr
IDPT6	1.72	1.13	820.8	96.4

Table 2. DNA concentrations of archeologicals sample made on NANODROP and QUBIT for 21 archeological samples. Id, Identification number of archeological sample; 260-280 Ratio of absorbance 260nm - 280nm; 230-260, Ratio of absorbance 230nm - 260nm; ND ng/ µl,DNA concentrations of NANODROP; QT ng/ µl, DNA concentrations on QUBIT.*no reading available.



Ficha recolección maíces

Número de ficha:

Fecha:________mayo, 2015. Recolectado por: ___________

Información General:

Región de Tarapacá

Quebrada:______________________
Localidad: ______________________
Parcela (Domicilio):_________________
Dueño (a):______________________
Coordenada UTM__________________
Altitud:_________________________

Datos Variedades:

Variedades (numerar)	N° ejemplares	Nombre vernacular	Lugar de obtención	Fecha obtención semilla	Venta(1) /Intercambio (2)	Localidad /Familia de intercambio	Edad ejemplares recolectados	Parte recolectada (1), (2), (3)*	Tiempo maduración	Tipo suelo /altura	Hibridada (1)/No hibridada (2)	Uso

Descripción General:

Observaciones Generales:

*(1)Mazorca (2) Mazorca envuelta hoja (3) Granos sueltos (4) Otro. Especificar

Foto Asociada:

File 1. Interview form used to gather information on agronomic and anthropological issues concerning the characteristics of maize samples collected in this study.

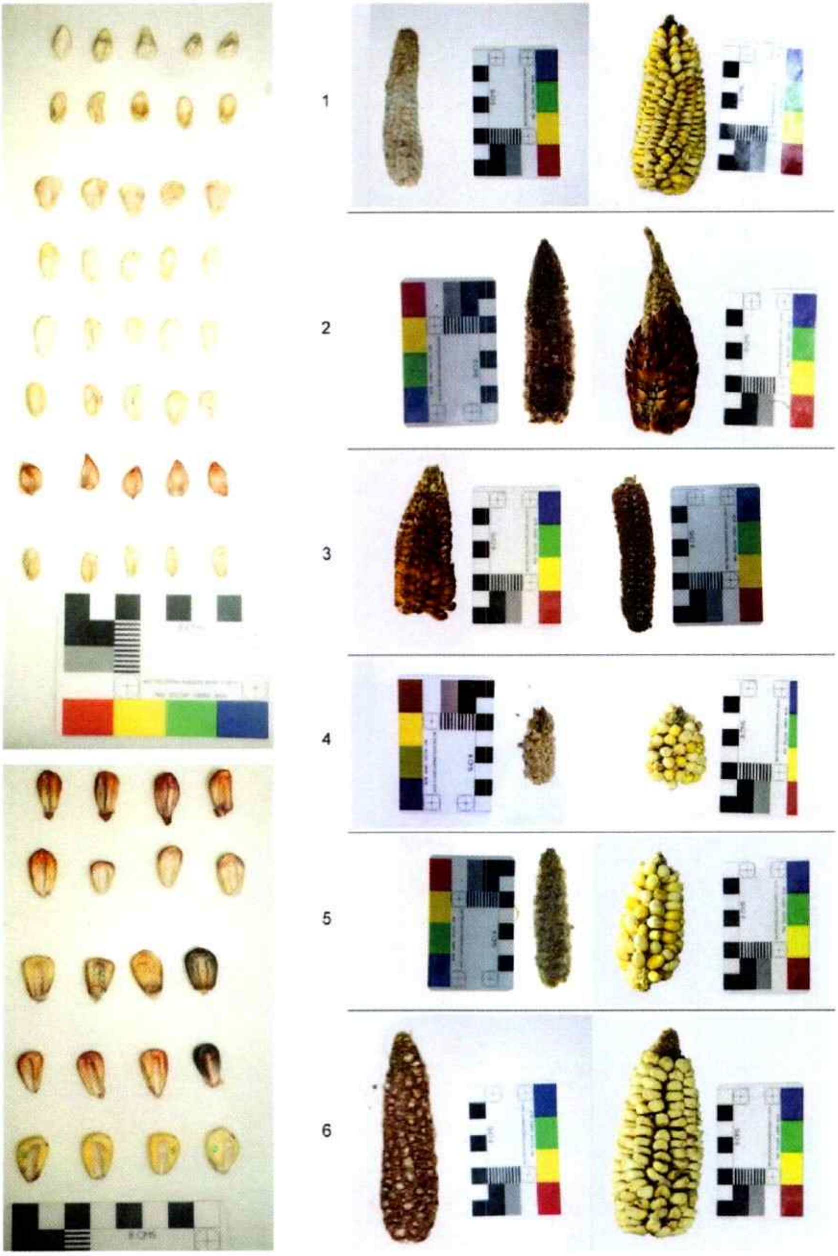


Figure 1. Some kernels, cobs and ears of population 1.

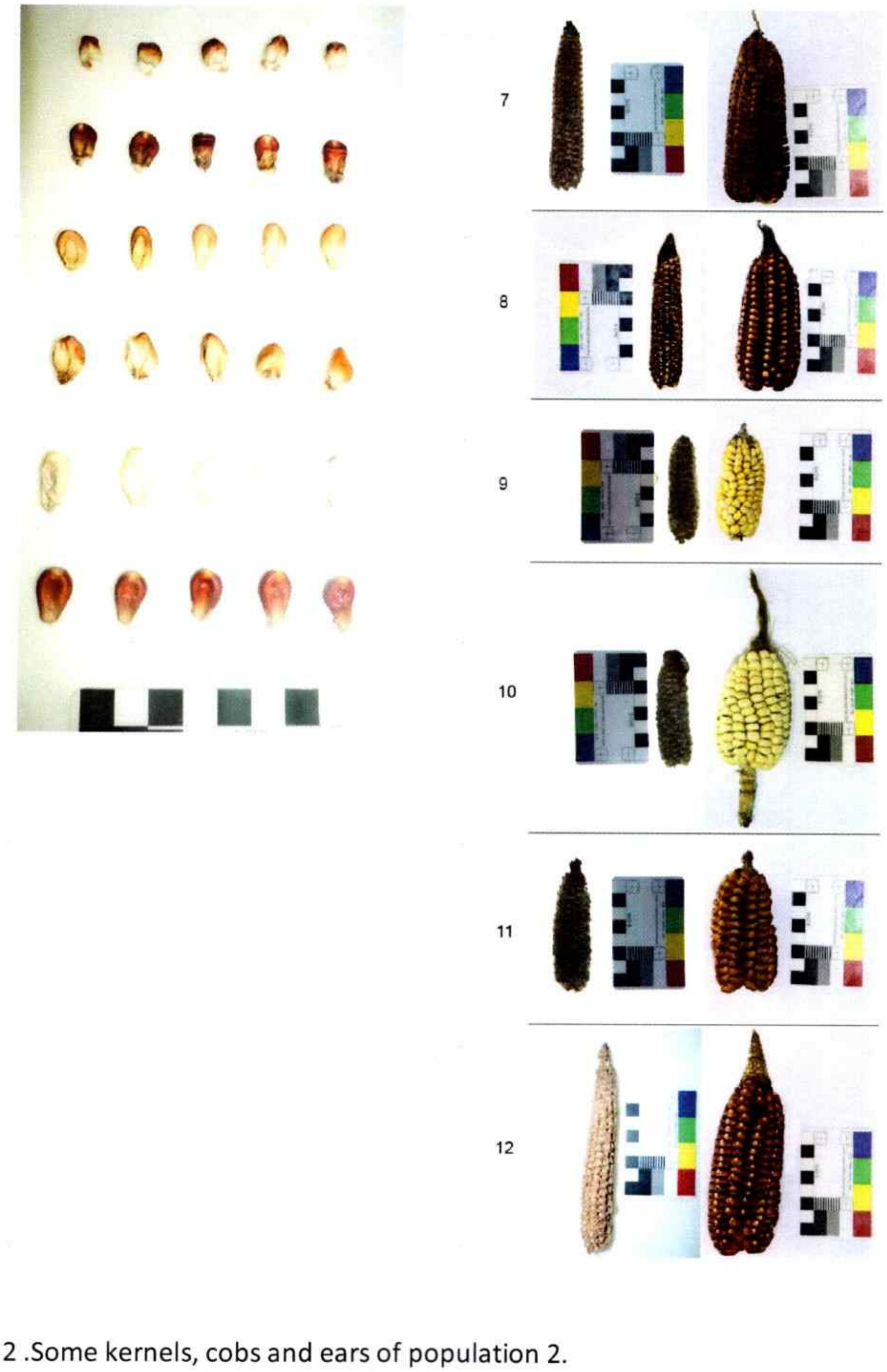


Figure 2 .Some kernels, cobs and ears of population 2.

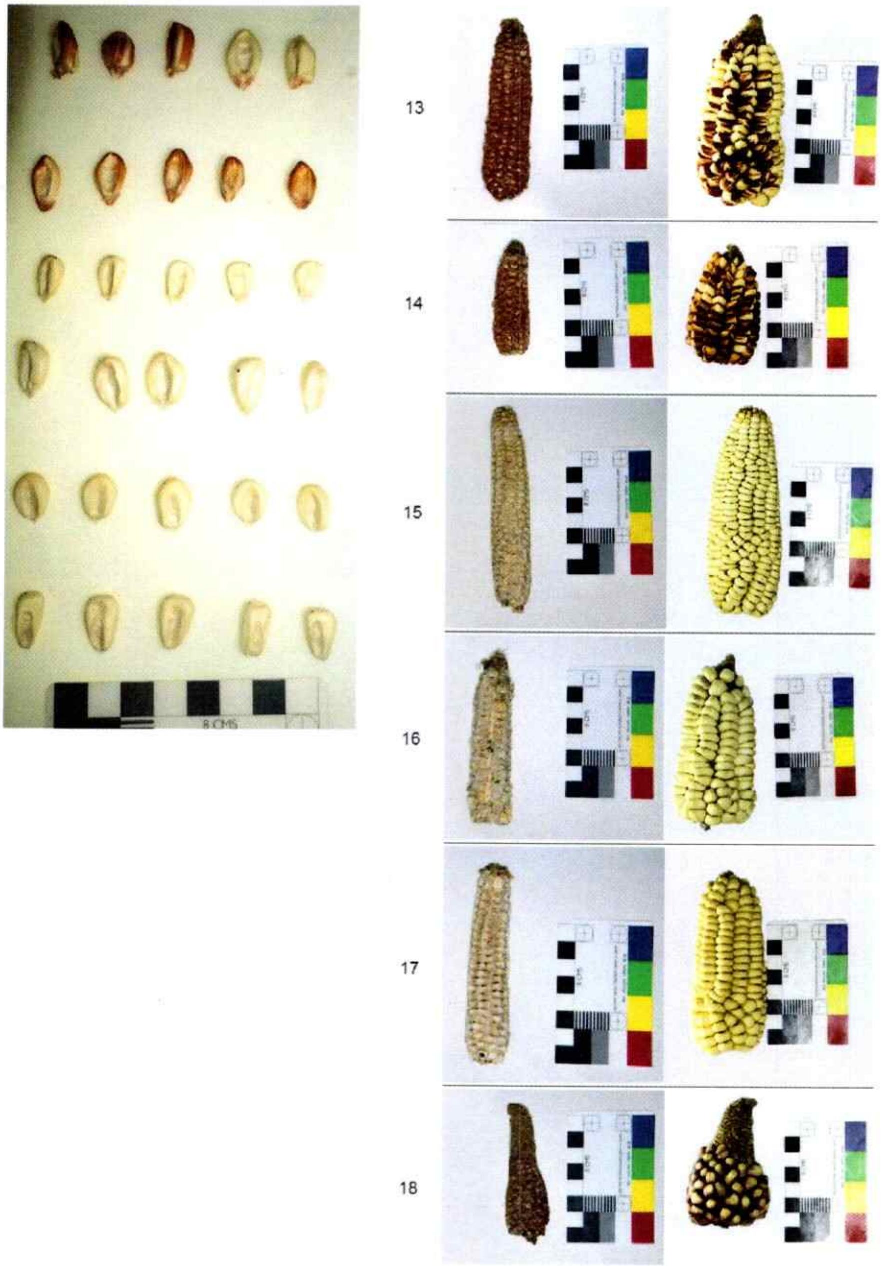
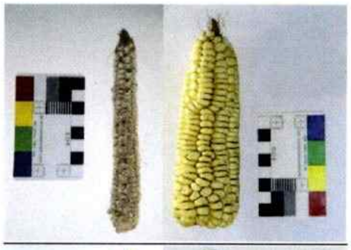
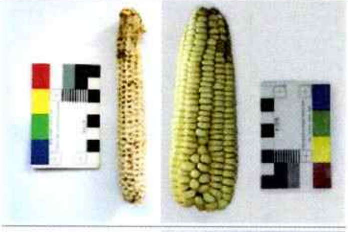
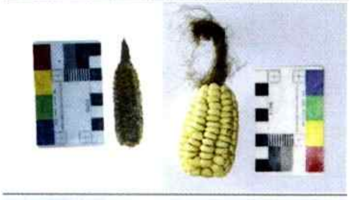
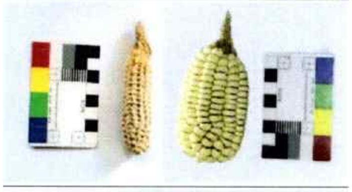


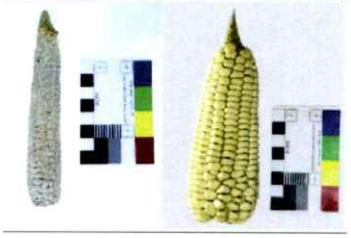
Figure 3 .Some kernels, cobs and ears of population 3.

19


20


21


22


23


24


Figure 4 .Some kernels, cobs and ears of population 4.

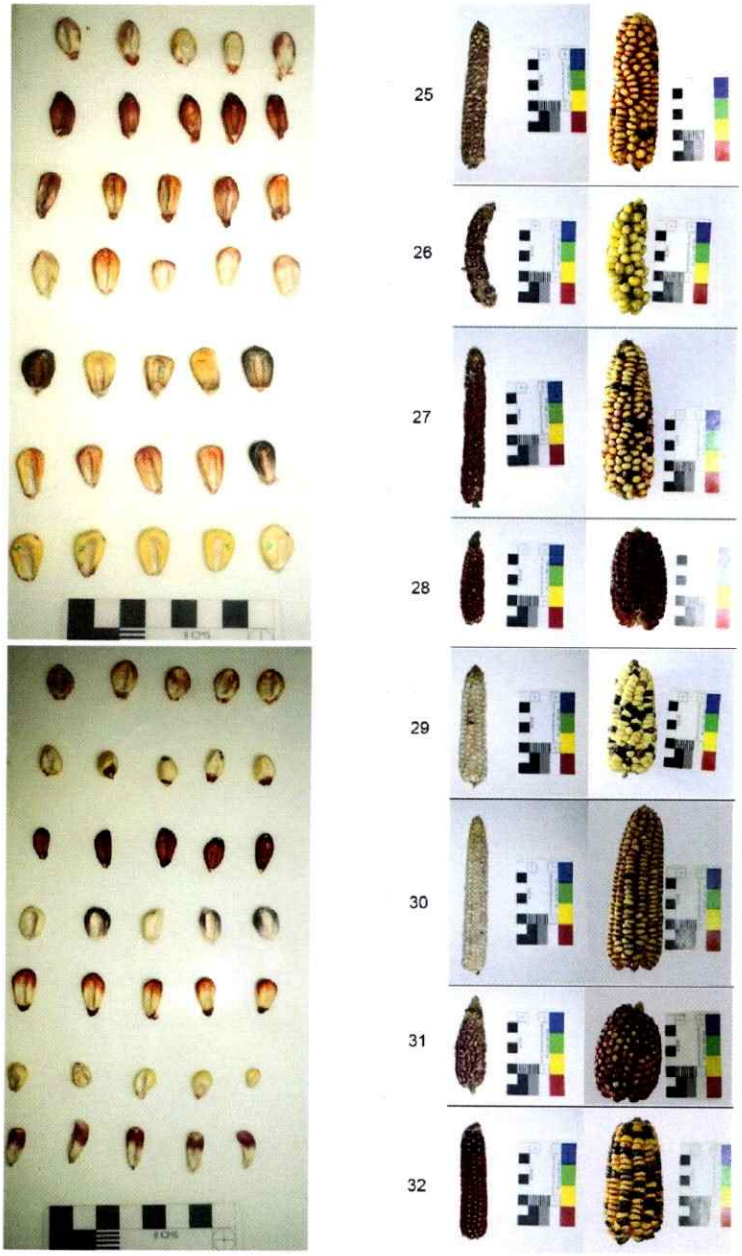


Figure 5. .Some kernels, cobs and ears of population 5.

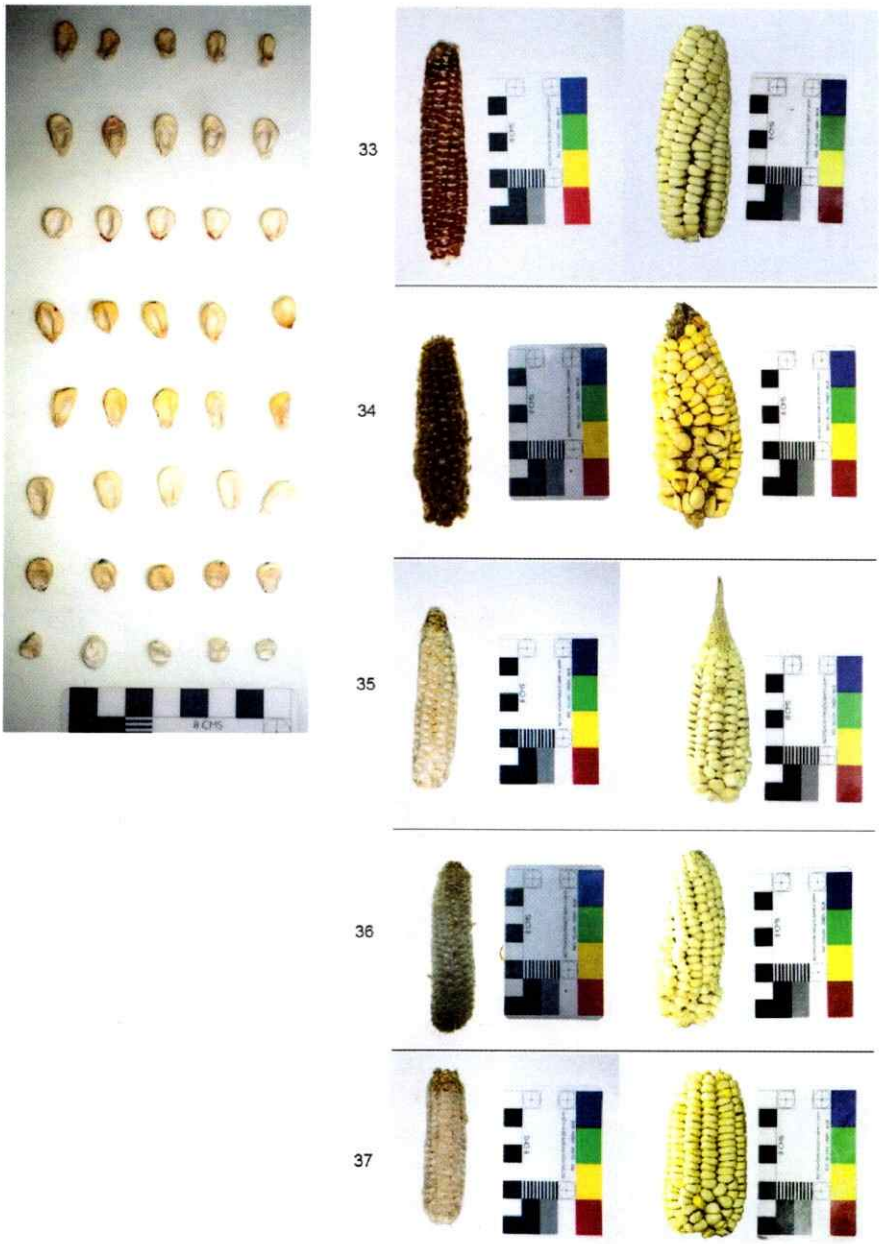


Figure 6. .Some kernels, cobs and ears of population 6.