

Pre-Hispanic Mortuary Practices in Quebrada de Humahuaca (North-Western Argentina): Genetic Relatedness among Individuals Buried in the Same Grave

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Summary

Almost all pre-Hispanic societies from Quebrada de Humahuaca (north-western Argentina) buried their defuncts in domestic areas, demonstrating the importance of death and its daily presence among the living. Presumably, the collective graves contained related individuals, a hypothesis that can be tested through the study of ancient DNA. This study analyzes autosomal and uniparental genetic markers in individuals from two archaeological sites in Quebrada de Humahuaca occupied during the Late Formative (1450–1050 BP) and Regional Developments I (1050–700 BP) periods. Mitochondrial and Y-chromosome haplotypes were compared in order to establish possible maternal and paternal relatedness. Genotypes for 15 autosomal STRs were used to calculate pairwise relatedness coefficients and pedigree probabilities. High kinship levels among individuals buried in the same graves were found in both sites. Although only two particular cases were analyzed, these results represent an important contribution to the study of mortuary practices in the region by means of ancient DNA.

Keywords: Ancient DNA, north-western Argentina, kinship relationships, burial practices

Introduction

Mortuary practices remain one of the main subjects in archaeological studies of pre-Hispanic Andean societies. They reflect social structure and can be understood as an approximation to the knowledge of different cultural, economic, and political features of a community (Baldini & Baffi, 2006; Seldes, 2014). Burial architecture and grave goods, which are the two main approaches, have contributed to understanding complex phenomena such as ancestor cult (Cremonte & Gheggi, 2012), social hierarchy (Palma, 1997–1998), and community

resistance to domination (Acuto et al., 2004; Cremonte & Gheggi, 2012; Seldes, 2014), among others.

Early archaeological studies in north-western Argentina have focused on grave goods but in a context of evolutionary and cultural-historical approaches. These involved separation of individuals from their burial artifacts, making the reconstruction and integration of both types of evidence more difficult (Baldini & Baffi, 2003; Seldes, 2014). In the last few years, influenced by the postprocessual framework, archaeological studies began to include questions about socio-political processes, social practices, and rituals, among others. Consequently, the integration of this region to Andean studies has allowed a better understanding of the complex dynamics which characterized Andean societies.

Sepulchres in domestic areas were a common feature among many pre-Hispanic groups (Nielsen, 2001; Seldes, 2014), which could be evidence of household integration between

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the worlds of the living and the dead (Parker Pearson, 1993; Acuto et al., 2011). In addition, the reutilization of graves would demonstrate a nearly constant and close presence of the deceased in daily activities (Zaburlin et al., 2006).

Specifically in Quebrada de Humahuaca (Fig. 1), this practice was extended along all pre-Hispanic times since the first settlements (Formative Period; 2450–1050 BP) to Inca domination (520–414 BP) with only a few exceptions in the Regional Developments period (1050–520 BP) like Pukara of Tilcara and Pukara of Volcán, where burials were held in segregated areas (Seldes, 2014).

These societies were organized in extended family groups, called *ayllus*, which collectively managed resources such as crops, water, and pastures (Moseley, 2001). Minor *ayllus* contained hundreds of families inhabiting diverse communities, which were also grouped into bigger *ayllus*, and so on, into the escalating hierarchical levels of an inclusive organization (Nielsen, 2006). In line with this, we can assume some level of relatedness among individuals buried in the same enclosure. A valuable tool to test this hypothesis is the genetic analysis of individuals recovered from archaeological sites.

In the past decades, ancient DNA (aDNA) analysis of human remains has expanded to include a large number of sites in the South-Central Andes area and the rest of South America (García-Bour et al., 2004; Rothhammer et al., 2009; Figueiro, 2011; Sans et al., 2012; Baca et al., 2014; Dejean et al., 2014; Fehren-Schmitz et al., 2014; Gonçalves et al., 2014; Mendisco et al., 2014; Postillone et al., 2014 and references cited). Most of them focused on mitochondrial DNA (mtDNA), since its recovery from archaeological remains would be more feasible due to the large number of copies of mtDNA molecules in cells, allowing an increased survival considering postmortem DNA damage.

Despite the difficulties, advances in aDNA recovery and analysis favor an increase in available information and its resolution (e.g., Fehren-Schmitz, 2015; De Sarkissian et al., 2015). In this sense, recent studies have contributed to answering questions about lifestyle and kinship relationships in Inca communities from Peru, and found a patrilineal social organization and also that individuals buried in the same grave were more related than those from different sepulchres (Baca et al., 2012, 2014). Different molecular markers such as mtDNA, Y-chromosome, and autosomal markers are needed for this type of study.

The aim of this study was to contribute to the study of mortuary practices in two archaeological sites in Quebrada de Humahuaca, in north-western Argentina, belonging to the Late Formative Period (1450–1050 BP) and Regional Developments I Period (1050–700 BP), both with a pattern of domestic sepulchres and lacking cemeteries or segregated burial areas (Seldes, 2007).

In both periods societies may have been organized into agropastoral communities with absence of structural inequalities and a centralized authority. During the Formative Period, people settled in small villages close to resources and distributed along subsidiary valleys, while in the Regional Developments I Period populations started concentrating in larger settlements located in elevated areas across the central Río Grande valley, with a concomitant increase in goods exchange among different groups (Nielsen, 2001).

Materials and Methods

We analyzed samples from two archaeological sites in the central sector of Quebrada de Humahuaca (north-western Argentina): Flores 1 and Muyuna (Fig. 1), belonging to two different occupational periods. For both sites, we have the explicit permission for sample analysis and transfer granted by the Secretaría de Cultura de Jujuy (Province of Jujuy) and Secretaría de Cultura de la Nación (Argentina).

This is a brief description of methodological procedures. A more detailed description can be found in Supporting Information.

Flores 1

The site is in the urban area of the current Tilcara locality, a city built over the habitation structures occupied during the Formative Period. Human remains were rescued in an archaeological campaign back in 2003 (Zaburlin et al., 2006), stemming from house-building activities. According to the associated material culture, it has been proposed that sepulchres would have been located in domestic areas. The recovered ceramics correspond to the Late Formative Period (1450–1050 BP) (Seldes, 2007) which agrees with datings from Til 22, a very close site with similar material culture and dates of 940 ± 60 BP, 1025 ± 140 BP, and 1190 ± 90 BP (Rivolta, 1996; Tarragó & Albeck, 1997).

We analyzed teeth from the jaws of two adult individuals found in the same grave (Table 1). The remaining individuals recovered (two sub-adults and two adults) were in a poor state of preservation (Seldes, 2007).

Muyuna

The site is located on a high terrace of the Grande River's left margin (approximately 400 m. away from the current river course and 2850 m.a.s.l.). It was a residential settlement with habitation structures densely grouped in a nearly one-hectare area. Except for one individual sepulchre in Enclosure 2, all

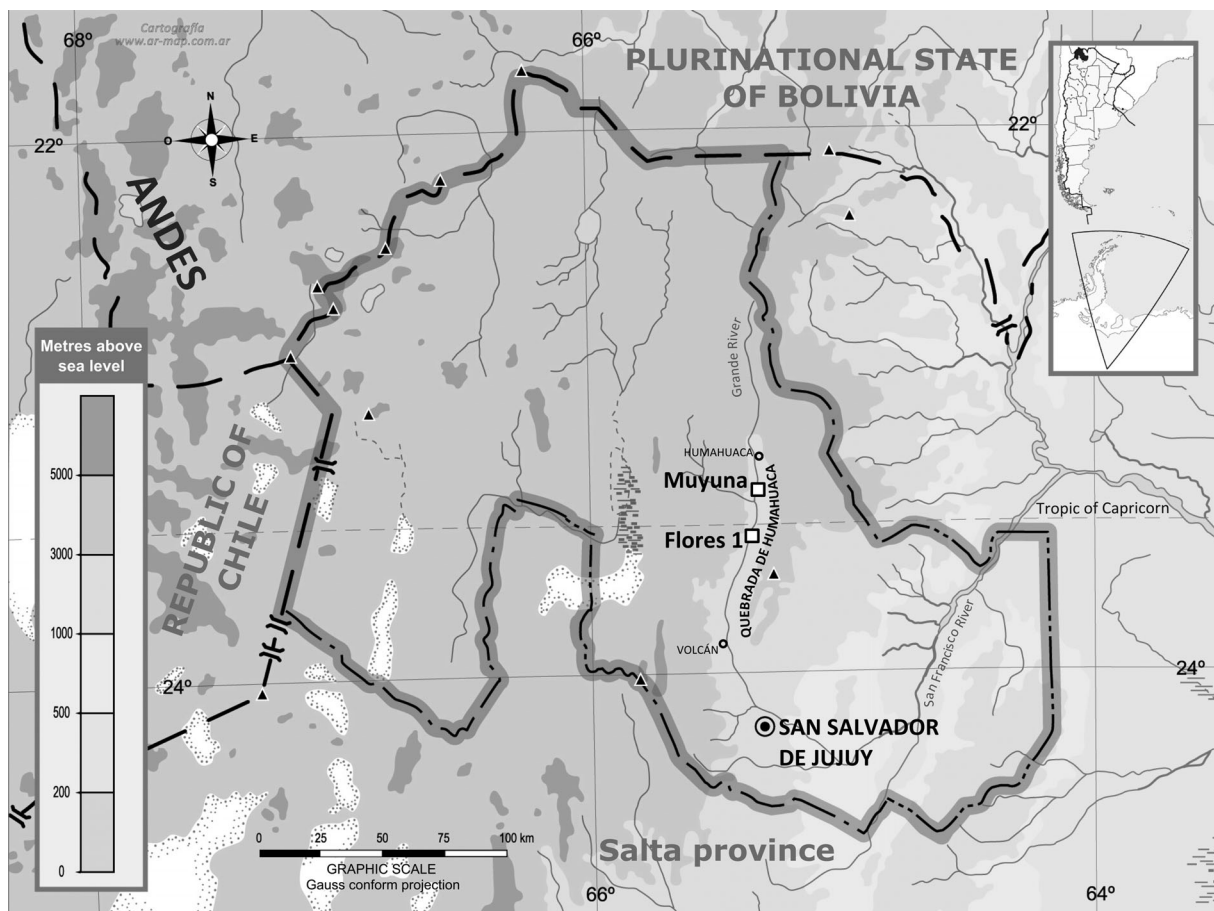


Figure 1 Location of the studied sites (squares) in Quebrada de Humahuaca, province of Jujuy, Argentina. The circles denote current localities. (Taken and modified from <http://mapoteca.educ.ar/>.)

Table 1 Samples analyzed in this study.

| Sample | Individual* | Sample type | ID | Archaeological site | Grave |
|--------|-------------|-------------|-----------------|---------------------|-------|
| FLO1 | 1 | Lower jaw | F1 15–38 | Flores 1 | 15 |
| FLO2 | 2 | Upper jaw | F1 15-14 | Flores 1 | 15 |
| MUY1 | 3 | Lower jaw | UP214 IC3 | Muyuna | R7 |
| MUY2 | 4 | Lower jaw | UP220 Ind. Sup. | Muyuna | R9 |
| MUY3 | 5 | Lower jaw | UP220 IC1 | Muyuna | R9 |
| MUY4 | 5 | Upper jaw | UP220 IC0 | Muyuna | R9 |
| MUY5 | 6 | Upper jaw | UP220 IC3 | Muyuna | R9 |
| MUY6 | 7 | Upper jaw | UP221 IC2 | Muyuna | R10 |
| MUY7 | 8 | Upper jaw | UP221 IC3A | Muyuna | R10 |
| MUY8 | 8 | Lower jaw | UP221 IC3B | Muyuna | R10 |

* Samples MUY3/4 and MUY7/8 probably belong to the same individual.

human remains were found in Enclosure 3. The analyzed samples came from the latest occupancy layer, which was sealed with green clay deposited on the last graves before the place was abandoned (Seldes, 2007).

Material culture recovered during site excavations allows assignment to the Regional Developments I Period (1050–700 BP), which is also supported by the radiocarbon dates obtained for Enclosure 3: floor 900 ± 40 14C BP, cal. 2σ

1040–1280 a.C.; roof 880 ± 60 14C BP, cal. 2σ 1040–1290 a.C. (Nielsen, 2007).

The sampled jawbones belonged to at least six adult individuals (Table 1) buried in different graves of Enclosure 3. In two cases, teeth from both lower and upper jaw were analyzed separately in order to confirm whether samples belonged to the same individual (MUY3/4 and MUY7/8 in Table 1). For this reason, they will be referred to as “samples” instead of “individuals” since we cannot determine exactly the number of individuals analyzed.

Sample Preparation and DNA Extraction

Teeth were washed with bleach solution, rinsed with deionized water, and finally UV irradiated for 15 min each side to remove surface exogenous DNA molecules. Tooth powder was obtained through total milling in liquid nitrogen (6870 SamplePrep Freezer Mill[®], Fisher Bioblock, Illkirch, France). DNA extraction was performed using 200 mg of powder following a previously published protocol (Mendisco et al., 2011). For each sampled jaw, two teeth were analyzed and for every tooth two independent extractions were done. Thus, for each sample, four extracts were available. From each extract, an analysis of the HVR-I and amplification of STRs were carried out systematically.

Mitochondrial DNA Analysis

A 359 bp fragment (between nucleotide positions 16024 and 16383) of Mitochondrial Hypervariable Region I (HVR-I) was sequenced, using two overlapping pairs of primers. Primers used, PCR amplification, and sequencing conditions are described in a previous study (Mendisco et al., 2011). Haplogroup determination was performed with Haplogrep (Kloss-Brandstätter et al., 2011) and by comparison with published data (Achilli et al., 2008, 2013; O’Rourke, 2009; Perego et al., 2009, 2010; O’Rourke & Raff, 2010). The obtained haplotypes have been deposited in GenBank under accession numbers: KX216510–KX216511.

Autosomal STR Analysis

Fifteen autosomal microsatellites or short tandem repeats (STRs) were analyzed with the AmpFISTR[®] Identifier[™] kit (Applied Biosystems), which allows the simultaneous amplification of: D2S1338, D3S1358, vWA, FGA, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, TH01, CSFPO, and TPOX. The AmpFISTR[®] MiniFiler[™] kit (Applied Biosystems), which allows the simultaneous amplification of nine mini-STRs (D3S1358,

vWA, FGA, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11), was used to complete and confirm individual profiles. Both kits were also used to amplify the Amelogenin gene region used for sex determination. PCR amplifications were performed following the manufacturer’s protocol except for the use of 34 cycles instead of 28 for the Identifier[™] kit and a final reaction volume of 12.5 μ l. A consensus genotype was obtained for each sample from four PCR products.

Y-Chromosome Analysis

For all males, the Y-chromosome haplotype was determined by amplification of 17 STRs (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and Y GATA H4) with the AmpFISTR[®] Y-filer[™] Kit (Applied Biosystems). Reaction conditions and analysis were previously described (Mendisco et al., 2011; 2014). Every sample’s consensus haplotype was determined based on those alleles that were present in at least two out of four amplifications.

Haplogroup determination was performed using Haplogroup Predictor (Athey, 2005; 2006) and Haplogroup & Haplo-I Subclade Predictor (Cullen, 2008).

Kinship Determination

As a first step, the matrilineal and patrilineal affinities within an enclosure were assessed by analyzing mitochondrial and Y-chromosome haplotypes, respectively. Haplotypes were compared with those found in other pre-Hispanic and contemporary populations (Table S1) in order to evaluate the frequency of occurrence. A total of 333 sequences from at least 13 pre-Hispanic groups and 1591 sequences from 29 contemporary populations were used for mtDNA comparisons. A total of 46 profiles obtained for pre-Hispanic individuals from at least four groups were used for Y-chromosome haplotype comparison, and also a search was conducted in the Y-Chromosome STR Haplotype Reference Database (YHRD; <http://yhrd.org>) where 2116 Native American haplotypes are stored.

Research on kinship relationships was conducted through autosomal STR analysis. First, pairwise relatedness coefficients (r) were estimated with four methods: two moment estimators (Queller & Goodnight, 1989; Lynch & Ritland, 1999), a dyadic likelihood estimator (Milligan, 2003), and a triadic likelihood estimator (Wang, 2007). Several methods were used because while the moment estimators are traditionally used and were also employed for other pre-Hispanic populations (Baca et al., 2012), likelihood estimators can be more precise (Wang, 2014). All calculations were performed

with COANCESTRY v1.0.1.5 (Wang, 2011), which supports the use of inbreeding. r being considered a continuous quantity, it is unclear which threshold value should be used to identify “related” or “lowly related” individuals, but since it can be interpreted as a correlation coefficient (Wang, 2014), values above 0.7 were defined as high, between 0.7 and 0.6 as moderate, and below 0.6 as low.

Second, the probabilities of different putative pedigrees were evaluated using Familias3 (Kling et al., 2014). The likelihood ratio (LR) and posterior probabilities of different pairwise relationships vs. the corresponding no-relation hypothesis were calculated. Following recommendations of the software developers, the “Extended stepwise” mutation model and the default priors were used, with two exceptions: the number of “Max generations” was set to 2 in order to emphasize short pedigrees, and the “Inbreeding” parameter was set to 1.5 because it increases the probability of endogamous constellations.

The population allele frequencies needed for calculations were estimated based on previously published data from other pre-Hispanic and contemporary populations (Albeza et al., 2002; Corella et al., 2008; Crossetti et al., 2008; Carnese et al., 2010; Sala et al., 2010; Callegari-Jacques et al., 2011; Mazieres et al., 2011; Baca et al., 2012, 2014; Muñoz et al., 2012; Fehren-Schmitz et al., 2015; Table S2). Contemporary groups were included since there are not enough autosomal pre-Hispanic data. On the other hand, groups self-identified as native, inhabiting rural areas and/or speaking native languages were selected in an attempt to reduce the admixture effect.

Authenticity Criteria

All analyses were conducted at laboratories exclusively dedicated to aDNA studies following strict protocols to prevent contamination (Cooper & Poinar, 2000; Gilbert et al., 2005). Pre- and post-PCR working areas are physically isolated; sample processing was done wearing protective disposable clothes, sterile gloves and face masks; all materials used during analysis were sterilized by autoclave and a long ultraviolet (UV) exposure; extraction and amplification blanks were used as negative controls in each step; and all results were replicated at different times, from multiple extracts and amplifications of the same sample.

Results

Flores 1

Mitochondrial DNA

Reproducible HVR-I sequences of the two analyzed individuals (Table 2) were obtained. Both mitochondrial lineages

belong to haplogroup B2 but with an additional mutation in nucleotide position 16111G, not present in other pre-Hispanic or contemporary populations (Table S1) nor in the PhyloTree database (van Oven & Kayser, 2009).

Nuclear DNA

As shown in Table 2, complete STR profiles were not obtained in any of the two individuals and only one was sex-determined as male, although the Y-chromosome profile was not achieved.

Kinship determination

Since only maternal lineage information was obtained for these individuals, a complete kinship determination was not achieved. Nevertheless, these two individuals share a rare haplotype not found in other populations to date, so it can be hypothesized that they shared a close maternal relationship.

Muyuna

Mitochondrial DNA

Reproducible HVR-I sequences were obtained from six out of eight analyzed samples, all sharing the same haplotype of maternal lineage D1 (Table 2). This particular haplotype was only reported in one individual from Jauranga, a Peruvian site in the south-central coast (approximately 1500 km away in a straight line) assigned to Early Horizon (2750–2150 BP) (Fehren-Schmitz et al., 2010). Due to its apparently low frequency in the area, all individuals might share a recent common maternal ancestor.

Nuclear DNA

Complete autosomal STR profiles were obtained for two samples and three others were amplified for a minimum of 11 different STRs (Table 2). Sex determination was accomplished in those five samples, detecting two females and three males.

Males share the same Y-chromosome haplotype that belongs to Q haplogroup (100% frequency with Haplogroup Predictor) and specifically to Q1a3a (Q-M3) haplogroup (10% with Haplogroup & Haplo-I Subclade Predictor). Despite the low percentage obtained with Cullen's predictor, this haplotype can be classified as Q-M3 since it has been proposed that haplotypes belonging to Q-M242 haplogroup can be predicted through STRs with high accuracy (Wang et al., 2015). Interestingly, no match was found among 2116 Native American haplotypes from YHRD or among 46 from the other pre-Hispanic populations analyzed (Table S1), probably due to the rare allele 26 at DYS390. On the basis of the

Table 3 Pairwise relatedness coefficients obtained with moment and likelihood estimators.

| Pair | LinchRd | QuellerGt | DyadML | TrioML |
|-------------|---------|-----------|--------|--------|
| MUY2 - MUY3 | 0.6000 | 0.7256 | 0.6583 | 0.6721 |
| MUY2 - MUY4 | 0.6000 | 0.7256 | 0.6583 | 0.6720 |
| MUY2 - MUY5 | 0.4906 | 0.7497 | 0.7504 | 0.7165 |
| MUY2 - MUY7 | 0.2722 | 0.4592 | 0.4310 | 0.4702 |
| MUY3 - MUY4 | 0.9871 | 0.9504 | 0.9636 | 0.9559 |
| MUY3 - MUY5 | 0.3014 | 0.5525 | 0.5758 | 0.5585 |
| MUY3 - MUY7 | 0.3326 | 0.5431 | 0.6448 | 0.6449 |
| MUY4 - MUY5 | 0.3327 | 0.6091 | 0.6169 | 0.6074 |
| MUY4 - MUY7 | 0.2541 | 0.4201 | 0.5330 | 0.5313 |
| MUY5 - MUY7 | 0.5751 | 0.6759 | 0.6295 | 0.6338 |

LinchRd, Linch & Ritland's estimator; QuellerGt, Queller & Goodnight's estimator; DyadML, dyadic likelihood estimator; TrioML, triadic likelihood estimator.

above, the males analyzed here probably share a close paternal relationship.

Kinship determination

Similar results were obtained by using different methods of relatedness coefficient estimation (Table 3) except for Linch & Ritland's (see below). All r estimates indicated that samples MUY3 and MUY4 are the closest ($r > 0.95$ in all cases) since they only differ in one allele at D19S433 (Table 2), suggesting these samples belong to the same individual. This corroborates the archaeological determination made on the lower jaw and upper jaw (Seldes, 2007). The other two samples (MUY7 and MUY8), which according to archaeological determination probably belong to the same individual, could not be compared since STR profiles were only obtained for one of them (MUY7).

Comparisons between MUY2-MUY3, MUY2-MUY4, MUY2-MUY5 and MUY4-MUY5 also yielded moderate to high r values ranging from 0.6 to 0.75 (except for Linch & Ritland's coefficient) (Table 3). This is interesting since these samples belong to one female and at least two males buried in the same grave (Table 1).

Finally, other pairwise comparisons resulted in moderate r values between samples from different graves: the females (MUY5 and MUY7), and one male (MUY3) with one female (MUY7) (Table 3).

In order to further investigate kinship relationships in Enclosure 3, different pedigrees were tested. As shown in Table 4, all pairs that yielded moderate to high r estimates also got the highest posterior probabilities for parent-offspring or sibling relationships. In addition, males buried in the same grave (MUY2 and MUY3/4) had the greatest probability of sibling relationship (Table 4).

Discussion

The analysis of mortuary practices in pre-Hispanic populations requires comparison of several types of archaeological evidence. The architectural characteristics and grave goods, for example, are two of the main features usually examined in order to understand the social patterns that may be reflected in burial practices.

In north-western Argentina domestic sepulchres were common across different periods (Seldes, 2014), and populations were organized into *ayllus*, such as throughout the Andean area (Nielsen, 2006). Generally, individuals buried in the same grave are thought to be related, although a proper inference of kinship relationships is possible with aDNA analysis. This study analyzed individuals from two archaeological sites in Quebrada de Humahuaca from two early periods (Formative and Regional Developments I), and found high levels of kinship relationships among those buried in the same grave in both cases. Despite the small sample size and limitations of this study, the findings are of great value since these types of study are scarce in the region.

The results presented in this study were determined from many replications (from different extracts and amplifications completed at different times); no contamination was detected in negative controls, and the profiles originating from the researchers who directly participated in this study were never observed in the analysis. The apparent discordance found at locus D19S433 between MUY3 and MUY4 (Table 2) may be due to intrinsic errors of the method employed (such as false alleles, allelic dropout or null alleles), which are over-represented in low quality samples such as aDNA samples. This further highlights the importance of using several samples from the same individual in order to conduct a more comprehensive analysis. Some errors, such as dropout, may be taken into consideration in the calculations in Familias software (Dørum et al., 2015). This does not seem necessary in this case, however, since samples MUY3 and MUY4 share the genotype for all other markers.

Further studies of pre-Hispanic populations in the region may also consider using shorter primers for mtDNA amplifications and probably sequencing clones, although the advantage of cloning has been questioned as an authenticity criterion (Winters et al., 2011).

In Flores 1 only mitochondrial HVR-I sequences were retrieved from both individuals, but it can be assumed that they shared a maternal ancestor since both have a rare haplotype (Table 2). This might support the idea that maternally related individuals lived together in this Late Formative population, although more individuals and nuclear DNA data are needed to confirm this assumption.

In MUYuna high levels of kinship relationships were found in both females and males, especially among those samples

Table 4 Posterior probabilities and likelihood ratios (LR) obtained for every putative pedigree evaluated.

| Individual 1 | Individual 2 | Relationship | Posterior | LR |
|--------------|--------------|------------------|-----------|------------------|
| MUY2 | MUY3 | Parent-Offspring | 0.0000000 | 0.000 |
| MUY2 | MUY3 | Siblings | >0,999999 | 2594172.787 |
| MUY2 | MUY4 | Parent-Offspring | 0.0000000 | 0.000 |
| MUY2 | MUY4 | Siblings | >0,999999 | 2594172.787 |
| MUY2 | MUY5 | Parent-Offspring | 0.9997992 | 4980.046 |
| MUY2 | MUY5 | Siblings | 0.9996859 | 3183.112 |
| MUY2 | MUY7 | Parent-Offspring | 0.0000000 | 0.000 |
| MUY2 | MUY7 | Siblings | 0.9198783 | 11.481 |
| MUY3 | MUY4 | Parent-Offspring | >0,999999 | 284813750.100 |
| MUY3 | MUY4 | Siblings | >0,999999 | 354560856000.000 |
| MUY3 | MUY5 | Parent-Offspring | 0.0000000 | 0.000 |
| MUY3 | MUY5 | Siblings | 0.9949812 | 198.251 |
| MUY3 | MUY7 | Parent-Offspring | 0.9987886 | 824.460 |
| MUY3 | MUY7 | Siblings | 0.9795445 | 47.887 |
| MUY4 | MUY5 | Parent-Offspring | 0.0000000 | 0.000 |
| MUY4 | MUY5 | Siblings | 0.9990919 | 1100.215 |
| MUY4 | MUY7 | Parent-Offspring | 0.0000000 | 0.000 |
| MUY4 | MUY7 | Siblings | 0.8954103 | 8.561 |
| MUY5 | MUY7 | Parent-Offspring | 0.0000000 | 0.000 |
| MUY5 | MUY7 | Siblings | 0.9993180 | 1465.171 |

from the same grave in Enclosure 3. Since males share the Y-chromosome haplotype and all individuals have the same mitochondrial lineage (Table 2), a patri- or matrilocality settlement pattern was not determined for this group. Further, moderate and even high relatedness coefficients were found among both males and females, and between both groups (Table 3). Nevertheless, the highest posterior probabilities (Table 4) were found for sibling relationship among males, which might indicate a patrilocality organization. This is consistent with findings in three different Inca sites in southern Peru, where males buried in the same grave shared identical Y-chromosome profiles (Baca et al., 2012) and there is evidence of a primary female dispersal (Baca et al., 2014). A patrilocality organization was previously described in ethnohistorical studies of Andean populations (e.g., Hickman & Stuart, 1977; and for a discussion see Weismantel, 2006). However, since in Muyuna probably only one family was analyzed, it is necessary to increase sample size to confirm this hypothesis.

Increasing sample size may also improve estimation of Lynch & Ritland's coefficient as it has been shown to be sensitive to the number of individuals analyzed (Csilléry et al., 2006). Although population allele frequencies were calculated based on at least 239 individuals, and up to 898 (depending on locus), increasing the number of samples of pre-Hispanic populations is crucial since they amount to less than 10% of all individuals in our database.

Also, the effect of a small reference population sample was analyzed by conducting kinship analysis on Muyuna individuals using allele frequencies of pre-Hispanic groups only (data not shown). Posterior probabilities of the most probable pedigrees were found to decrease with sample size and more notably, some improbable relationships (those with a null posterior on Table 4) became probable due to the reduction in STR loci available for analysis in ancient samples. Despite this disadvantage of using only data on pre-Hispanics as the reference population, it is important to consider that contemporary samples can also bias the results. A poor selection can add alleles absent in pre-Hispanic groups and present in contemporary groups due to subsequent European or African admixture. In addition, using a large area such as the south-central Andes and adjacent lowlands as a reference might be adding a geographical effect, on the basis of the high STR mutation rates found in these markers.

All things considered, this study represents an important contribution to increasing local genetic data of the pre-Hispanic populations from north-western Argentina and highlights the need to analyze more individuals from pre-Hispanic periods. Further, this research represents the first contribution to the study of kinship relationships and burial practices in the region through aDNA.

Results obtained here support the assumption that the domestic burial of related individuals was an extended practice both in the Formative and Regional Developments Periods.

Since we cannot rule out a matrilineal nor patrilineal organization, settlement patterns may have been more complex than thought or even variable in different periods, and across the whole Andean area. The possibility of an overestimation in the kinship calculations due to the probably high inbreeding levels in pre-Hispanic populations in Quebrada de Humahuaca should be considered. Although inbreeding in kinship calculations has been considered, the value used may be an underestimation of the true inbreeding rate of the pre-Hispanic Quebrada de Humahuaca population and, more important, the poor representation of this region in our database may produce an artificially low allelic frequency for some markers.

Finally, the importance of adding STR typing to aDNA analysis should be emphasized. Although its use to determine phylogenetic affinities among populations has been discussed (Demarchi, 2009), STR markers are highly used in forensic sciences and paternity studies (Corach, 2010) turning them, along with the traditionally used mtDNA, into an interesting tool for unraveling the history of pre-Hispanic populations from a genetic approach.

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Author's contributions: Study design: VS; Data collection: FM; Data analysis: MGR; Results discussion and manuscript preparation: MGR, VS, SAA, CBD.

Conflict of Interest

The authors have no conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Populations used for mtDNA haplotype comparisons. For some pre-Hispanic groups (*), Y-chromosome information was available and was used for comparison along with the haplotypes stored in YHRD. n: sample size (maximum number of individuals analyzed).

Table S2. Reference populations used to estimate allele frequency for autosomal STR analysis. n: sample size (maximum number of individuals analyzed).

Text S3: Sample processing

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