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Genetic Diversity of a Late Prehispanic Group of the Quebrada de Humahuaca, Northwestern Argentina

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Summary

This palaeogenetic study focused on the analysis of a late prehispanic Argentinean group from the Humahuaca valley, with the main aim of reconstructing its (micro)evolutionary history. The Humahuaca valley, a natural passageway from the eastern plains to the highlands, was the living environment of Andean societies whose cultural but especially biological diversity is still poorly understood. We analyzed the DNA extracted from 39 individuals who populated this upper valley during the Regional Development period (RDP) (between the 11th and 15th centuries CE), to determine their maternal and paternal genetic ancestry. Some mitochondrial and Y-chromosomal haplotypes specific to the Andean region are consistent with an origin in the highlands of Central Andes. On the other hand, a significant genetic affinity with contemporary admixed communities of the Chaco area was detected. Expectedly, recent demographic events, such as the expansion of the Inca Empire or the European colonization, have changed the original mitochondrial gene pool of the ancient Humahuaca Valley community. Finally, we identified a particular geographical organization of the prehispanic populations of Northwestern Argentina. Our results suggest that the communities of the region were divided between two different spheres of interaction, which is consistent with assumptions made by means of craniometric traits.

Keywords: Palaeogenetics, ancient DNA, Andes, microevolution, migration, mitochondrial DNA, Y-chromosome

Introduction

During the past two decades, significant progress has been made regarding the understanding of the peopling of America (O'Rourke & Raff, 2010; Perego et al., 2010; Bodner et al., 2012). Recent archaeological discoveries (Waters & Stafford, 2007; Lahaye et al., 2013), as well as the increase in studies focusing on the genetic diversity of Amerindians (Reich et al., 2012) have bettered our understanding of Native American prehistory. Despite these advances, many controversies persist, in particular concerning the micro-evolutionary processes experienced by pre-Columbian South Amerindian populations after they settled in the various environments of the continent (Tarazona-Santos et al., 2001; Fuselli et al., 2003).

Direct access to DNA molecules preserved in ancient human provides invaluable information about the biological history and population dynamics of ancient communities (Pääbo et al., 2004; Bramanti et al., 2009; Shapiro & Hofreiter, 2014). Ancient DNA (aDNA) combined with archaeological and anthropological evidence provides a unique perspective on the interactions between communities, their organization, or subsistence. The peculiarities of aDNA can make its study difficult (Pääbo, 1989; Hofreiter et al., 2001; Willerslev & Cooper, 2005), especially for human DNA. These facts can explain, among other factors, why there are

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relatively few palaeogenetic studies on pre-Colombian Andean populations (Shimada et al., 2004; Moraga et al., 2005; Shinoda et al., 2006; Kemp et al., 2009; Carnese et al., 2010; Fehren-Schmitz et al., 2010a, 2010b; Casas-Vargas et al., 2011).

To enlarge the reconstruction of the history of pre-Columbian Andean populations, we studied the genetic diversity of ancient communities of Northwestern Argentina, and in particular the Quebrada de Humahuaca. The Quebrada de Humahuaca is a narrow and fertile valley situated in the Andean zone of the province of Jujuy (Northwestern Argentina; Fig. 1). Its strategic position made this valley a natural crossroad for cultural, economic, and social communication from the time of hunters and gatherers to the Inca and Hispanic periods. Archaeological evidence shows that the Quebrada de Humahuaca was used as a major passage for the transport of goods and ideas, connecting the high Andean region (Bolivian Altiplano and Argentinean Puna) to the eastern forests (Andean foothills) and plains (Chaco) (Cocilovo et al., 2001). The first traces of human occupation, found in caves or rock shelters, date back more than 10,000 years ago when nomadic groups of hunter-gatherers settled the valley (Nielsen, 2001). These nomadic groups of hunter-gatherers evolved during the following millennia, leading to the emergence of sophisticated production and subsistence systems, based on agriculture, pastoralism, and long-distance exchange, making the valley one of the most densely populated regions of the Southern Andes (Nielsen, 2001).

The Regional Development Period (RDP) (CE 1000–1450) is characterized by significant social changes, leading to the development of distinctive local cultures. The relatively egalitarian social formations of the Formative Period (FP) (500 BCE–CE 650) and Regional Integration Period (RIP) (CE 650–1000), gave way to complex multi-community corporate polities (Nielsen, 2006; Seldes, 2012). During the 13th and 14th centuries, many groups of the Southern Andes, including the Humahuacas, were involved in chronic warfare, probably in response to a cycle of severe droughts that triggered conflicts over the control of more stable water sources for irrigation (Nielsen, 2001). This time of turmoil was brought to an end with the annexation of the Central and Southern Andes by the Incas, who displaced entire groups in order to control local communities (D'Altroy, 2003). Soon after the arrival of the Incas, the first European settlers arrived in the region (CE 1536), leading to massive demographic shifts (Catelli et al., 2011; Hunley & Healy, 2011; Avena et al., 2012).

The main purpose of this study was to determine the genetic diversity of a late prehispanic group of the Quebrada de Humahuaca, studying both maternal and paternal lineages to reconstruct the demographic processes undergone by this ancient community. We aimed to assess the genetic relationships of this group with extinct and extant Amerindian communities from South America. On a regional scale, some anthropological studies suggest the presence of two sets of biologically differentiated populations in Northwestern Argentina: one including the communities of Humahuaca and of the Puna and another including the communities of the Calchaqui valley and of Pampa Grande (Fig. 1) (Cocilovo et al., 2001, 2009; Varela et al., 2008). We explored the biological interactions between Northwestern Argentinean communities through the study of additional DNA samples from neighboring areas (from the site of Doncellas in the Puna and from three sites of the Calchaqui valley), and by comparison with the data obtained in a previous study on the ancient group of Pampa Grande (Carnese et al., 2010).

Material and Methods

Authentication and Prevention of Contamination

Sample processing and analyses were performed using standard precautions (Cooper & Poinar, 2000; Gilbert et al., 2005) to minimize the risk of exogenous DNA contamination and ensure the reliability of the results. All analyses were performed in a laboratory dedicated to analyses of ancient DNA, following strict precautions including a separation of pre- and post-PCR laboratories and the use of disposable protective clothing, face masks, and disposable laboratory gloves. Workbenches and all other laboratory equipment were cleaned with bleach, rinsed with ultrapure water and irradiated with UV light before each manipulation.

Each sample was extracted at least two times, and then amplified at least twice, at different times, to test the reproducibility of results. The results were replicated from two different samples from the same individual. Blank extraction and amplification controls were used throughout the analysis.

Archaeological Sites and Samples

This study included 39 ancient samples excavated from nine archaeological sites in the Quebrada de Humahuaca, a valley situated in the province of Jujuy connecting the eastern plains to the Puna and the southern mountain hills area (Fig. 1). Nine other samples came from two archeological sites in the Calchaqui Valley, which is situated in the neighboring province of Salta, whereas three other samples were obtained from the site of Doncellas situated in the Puna region (Fig. 1). On the basis of absolute radiocarbon dating and archaeological contexts, it was established that all of these individuals dated to the RDP. Table 1 summarizes the information about the analyzed samples. For each individual, two teeth still included in mandibles or jaws were collected and analyzed separately, as described above.

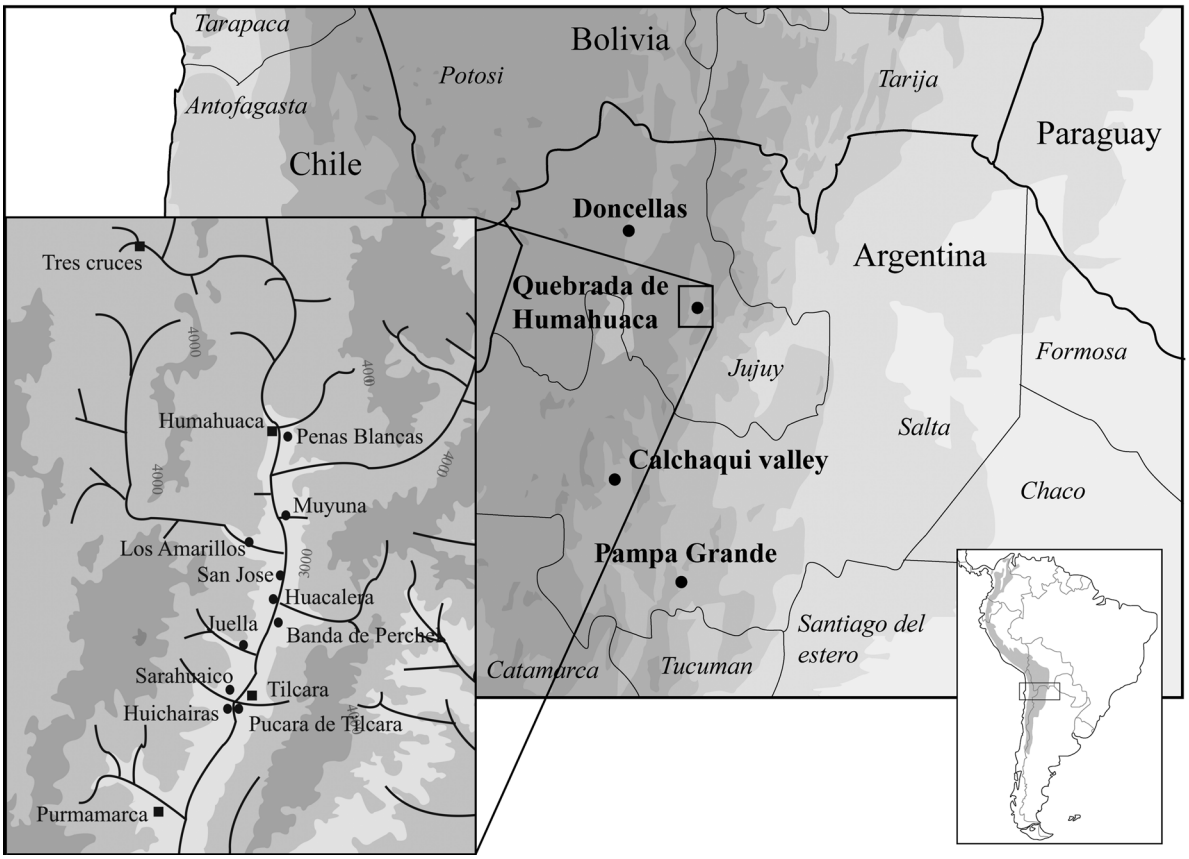

Figure 1 Location of the archaeological sites studied.

Sample Preparation and Ancient DNA Extraction

Tooth samples were first cleaned with bleach and rinsed with ultrapure water, before being exposed to UV light for 15 min on each side to remove contaminant DNA molecules from the outer surface. Each tooth was then entirely powdered in a grinder mill under liquid nitrogen (6870 SamplePrep Freezer Mill®, Fisher Bioblock, Illkirch, France). The DNA was carefully extracted from approximately 200 mg of powdered teeth samples, according to a protocol previously described (Mendisco et al., 2011). Two or more independent DNA extractions were carried out for each sample. Thus, for each individual, a minimum of four extractions were obtained from two different teeth.

Mitochondrial DNA Polymorphisms

Mitochondrial DNA (mtDNA) haplotypes were determined through the sequencing of a 359 bp fragment of the mitochondrial hypervariable region 1 (HVR1) (nucleotide positions 16024–16383), using two overlapping primer pairs. Primer sequences for the amplifications and PCR conditions have been described in a previous study (Mendisco et al., 2011). All segments were sequenced with the ABI Prism BigDye Terminator Cycle Sequencing kit 3.1 (Applied Biosystems, Forster City, CA, USA), and read on an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems), according to the manufacturer's instructions.

To confirm and refine their assignment to haplogroups we additionally analyzed specific single nucleotide polymorphisms (SNPs) characterizing founding Native American haplogroups A2, B2, C1b, C1c, C1d, and D1 (Tamm et al., 2007; Achilli et al., 2008). The screening of these mtDNA-SNPs was performed using the iPLEX Gold technology (Sequenom Inc., San Diego, CA, USA) as described in a previous publication (Mendisco et al., 2011). All primers used for this analysis are presented in Table S1.

Y-Chromosome Polymorphisms

Y-chromosome haplotypes were determined from the analysis of 17 Y-chromosome short tandem repeats (STR) loci using the AmpF*l*STR®Yfiler™ PCR Amplification Kit

Table 1 Main information of the sampled archaeological sites.

Archaeological site	Location	Site type	N[1]	Period[2]	Radiocarbon dates (cal. AD)[3]
Banda de Perchel (BPe)	Quebrada de Humahuaca, Jujuy	Domestic terrace	2	RDP	1036–1281[a]
Huacalera (Hua)	Quebrada de Humahuaca, Jujuy	Residential area	1	RDP	n.d.[4]
Juella (Jue)	Quebrada de Humahuaca, Jujuy	Urban area	5	RDP	1066–1613[b]
Los Amarillos (LAm)	Quebrada de Humahuaca, Jujuy	Urban area	20	RDP	980–1467[b]
Penas Blancas (PBl)	Quebrada de Humahuaca, Jujuy	Domestic terrace	1	RDP	n.d.
San Jose (SJo)	Quebrada de Humahuaca, Jujuy	Residential area	1	RDP	1020–1271[b]
Sarahuaico (Sar)	Quebrada de Humahuaca, Jujuy	Domestic terrace	2	RDP	1164–1413[b]
Tilcara (Til)	Quebrada de Humahuaca, Jujuy	Urban area	6	RDP	989–1222[b]
Huichairas (Hui)	Quebrada de Humahuaca, Jujuy	Urban area	1	RDP	n.d.
Total Humahuaca			**39**		
Fuerte Alto (FAl)	Calchaqui valley, Salta	Residential area	2	RDP	n.d.
Tero (SSal Cac 14) (Ter)	Calchaqui valley, Salta	Urban area	7	RDP	n.d.
Total Calchaqui			**9**		
Doncellas (Don)	Puna, Jujuy	Urban area	3	RDP	750–1500[c]

[1]Number of sampled individuals; [2]RDP: Regional Development period; [3]Maximum time interval obtained from different dates; [4]Not determined.
[a]Rivolta, 2007; [b]Nielsen,2001; [c]de Micou, 2001.

(Applied Biosystems). These included: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and Y GATA H4. Assays were run on an ABI Prism 3500 system (Applied Biosystems) and data analysis performed with the GeneMapper 4.1 software (Applied Biosystems). Consensus haplotypes were determined from several amplifications (amplifications were performed on three or four different DNA extracts for each sample) by preserving the alleles having been typed at least in more than half of the genetic profiles.

To identify the paternal lineages, we analyzed eight Y-chromosome SNPs using the iPLEX Gold technology (Sequenom). The selected Y-SNPs are characteristic of the Y-haplogroups C-M216, Q-M242, Q-M346 (Q1a2), Q-M3 (Q1a2a1a1), Q-M19 (Q1a2a1a1a), Q-M194 (Q1a2a1a1b), Q-M199 (Q1a2a1a1c), Q-SA01 (Q1a2a1a1e) (Underhill et al., 1996; Tarazona-Santos et al., 2001; Seielstad et al., 2003; Jota et al., 2011). All primers used for this analysis are presented in Table S1.

Data Analysis

A database compiling mitochondrial DNA data from seven ancient and 33 contemporary Amerindian communities was established on the basis of sequences available in the literature for the HVR-1 region between positions 16024 and 16365 (Table S2). We compared the Y-chromosomal data with 24 South American populations selected from the literature (Table S3). These databases were used to identify haplotypes shared between the ancient samples analyzed by us, and ancient and contemporary groups from previous studies. Regarding the Y chromosome, given the lack of harmonization of Y-STR analyses in the literature (Table S3), we considered only the minimal Y-haplotype (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385ab) for the comparisons.

The ARLEQUIN software v3.5.1.3 (Excoffier et al., 2005) was used to calculate two within-group diversity indices [nucleotide (π) and haplotype diversity(H)] and biological distances between the ancient Humahuaca group and the comparative populations. Genetic distances were calculated from mitochondrial haplotypes employing the Tamura and Nei distance model (Tamura & Nei, 1993) with the suggested correction gamma value of 0.26 for mitochondrial HVR1 data (Meyer et al., 1999). All analyses excluded np 16182 and np 16183 because these positions are often dependent on the presence of a C mutation at np 16189 (Pfeiffer et al., 1999). To visualize biological relationships, pairwise F_{ST} genetic distances were plotted in a multidimensional scaling plot (MDS) using XLSTAT-PRO 7.5 software. Furthermore, to visualize more easily the phylogenetic relationships between mitochondrial haplotypes, networks were generated for mitochondrial DNA haplogroups A2, B2, C1, and D1 from the ancient Huamahuaca and native South Amerindian sequence data using the median-joining algorithm of Network, v.4.6 (Bandelt et al., 1999). Following the weighting scheme suggested by Bandelt et al.

(2002), higher weights were assigned to the least variable polymorphisms and lower weights to the more hypervariable sites in our dataset. The datasets were preprocessed using the star contraction option (Forster et al., 2001), and networks were created using MP processing (Polzin & Daneschmand, 2003).

Considering the Y-chromosomal data, genetic distances were calculated with the Arlequin software from the complete minimal Y-haplotypes, which is an informative Y-STR core set (Kayser et al., 1997). To visualize the distances between each group, the genetic distances were used to create a Neighbor Joining tree (Saitou & Nei, 1987), using MEGA v.6 (Tamura et al., 2013), and according to the algorithm described by Nei and Kumar (2000).

Results

Mitochondrial DNA Sequence Variation

From a total of 51 individuals, the mitochondrial haplogroup status of 41 samples was reproducibly retrieved (Table 2). Each of these 41 individuals belongs to one of the Amerindian major founding mitochondrial haplogroups (Tamm et al., 2007; Achilli et al., 2008; Perego et al., 2010). Eighteen individuals were associated with maternal lineage A2, 12 with lineage B2, six individuals belonged to lineage C1b, and finally, five belonged to lineage D1. Reproducible complete HVR-1 sequences were obtained for 33 of the 51 individuals tested. These 33 sequences were classified into 16 different haplotypes, of which 10 were carried by a single individual whereas six were shared by at least two individuals (Table 2).

All of the 16 mitochondrial haplotypes were compared with our reference database, revealing exact matches for nine haplotypes. Five of these shared haplotypes were not informative, concerning the origin or the affinities of the individuals. Indeed, these haplotypes (H1, H8, H9, H14, and H16) are identical or close to the founding haplotypes, distributed throughout all the regions of South America, as we can see in the networks (Fig. S1). Haplotype H4 was detected in contemporary populations inhabiting the Andean foothills (Bert et al., 2004) and the plains located east of the study area (Chaco area and plains of Argentina and Brazil) (Cabana et al., 2006; Marrero et al., 2007). Haplotypes H10 and H11, belonging to haplogroup B2, were shared exclusively with ancient and contemporary Andean populations (Cabana et al., 2006; Alvarez-Iglesias et al., 2007; Afonso Costa et al., 2010; Barbieri et al., 2011; Gaya-Vidal et al., 2011). Finally, haplotype H12 was shared with two contemporary Andean individuals (Afonso Costa et al., 2010; Barbieri et al., 2011) and three individuals of the extant Guahibo population (Vona et al., 2005). Haplotype H5 was not found in the reference database, although it contains a particular combination of mutations (16145A-16156A-16157C) that we previously described for various ancient samples of Pampa Grande located in the province of Salta, in the Andean foothills (Carnese et al., 2010), and that has also been detected for one contemporary individual of the "Criollos" population of the Chaco province of Argentina (Sevini et al., 2013).

The mitochondrial haplogroup distribution of the late prehispanic group of Quebrada de Humahuaca, composed of 32 individuals, was as follows: 46.9% A2, 25% B2, 12.5% C1, and 15.6% D1. This pre-Columbian population showed a genetic diversity, calculated from the HVR1 sequence data, slightly lower ($h = 0.837$) than seen in most of the populations from the Central Andean region (whose diversity varies between 0.867 and 0.981) (Table S2). This reduced diversity may be due to the over-representation of haplotype H1 (10 individuals out of the 32). It can be also explained by more significant genetic exchanges within the Valley than with groups from other regions, as will be discussed later. An MDS plot representing the inter-population genetic distances (HVR1 data) is shown in Figure 2. The ancient Humahuaca group was not included in the cluster formed by all of the ancient and contemporary groups from Central Andes. Instead, its position was intermediate between some Andean groups and others from the Chaco or Andean foothills. The lowest genetic distances were found with the communities of Guarani, Salta, Criollos, and Chaco from Argentina, the Tayacaja from Peru, and the Cayapa from Ecuador, with F_{ST} values between 0.02 and 0.06 (all F_{ST} values are presented in Table S4).

Y-Chromosome Data

From a total of 51 individuals, the Y-haplogroup status of 22 individuals was reproducibly determined (Table 3). Eighteen out of the 22 male individuals belonged to the most frequent haplogroup within Amerindian populations, Q-M3 (Battaglia et al., 2013). The remaining three individuals were associated with the haplogroup Q-M346 (Q1a2).

Twenty more or less complete haplotypes were reproducibly obtained for the 22 male individuals. The 10 complete minimal Y-haplotypes (haplotypes 1 to 10) were compared to the reference database and exact matches were found for three haplotypes. Haplotype 3 has been observed in an individual from the Bolivian Andean foothills, in the Beni province (Tirado et al., 2009). Haplotype 4 has been observed in one Toba from the Argentinean Chaco (Toscanini et al., 2008), one Andean Kichwa from Ecuador (Gonzalez-Andrade et al., 2009), and four Andean Uros from Peru (Sandoval et al., 2013). Finally, haplotype 9 was identical to

Table 2 MtDNA haplotypes and haplogroups inferred for each sample.

													Quebrada de Humahuaca								Calchaqui		
Ht[1]	HVR-1 (16024-16383 bp)									Mt-SNPs	Hg[1]	n	BPe	LAm	Hui	Til	Jue	Sar	SJo	Hua	Ter	FAl	Don
H1	16111T	16223T	16290T	16319A	16362C					12007A – 64T	A2	**11**	1	8	.	1	.	.	.	.	1	.	.
H2	16111T	16125A	16223T	16290T	16319A	16362C				12007A	A2	**1**	.	1	.	.	.	.	.	.	.	.	.
H3	16111T	16188T	16193T	16223T	16290T	16319A	16362C			12007A – 64T	A2	**1**	.	.	.	.	.	.	.	.	.	1	.
H4	16111T	16223T	16266T	16290T	16319A	16362C				12007A – 64T	A2	**1**	.	.	1	.	.	.	.	.	.	.	.
	n.d.[2]									12007A – 64T	A2	4	.	3	.	.	.	.	.	.	.	1	.
H5	16092C	16145A	16156A	16157C	16182C	16183C	16189C	16217C	16295T	3547G	B2	**2**	.	.	.	.	.	.	.	.	2	.	.
H6	16111T	16126C	16183C	16189C	16217C					3547G	B2	**1**	.	.	.	1	.	.	.	.	.	.	.
H7	16179T	16183C	16189C	16217C						3547G	B2	**1**	.	.	.		1	.	.	.	.	.	.
H8	16182C	16183C	16189C	16217C						3547G	B2	**2**	.	1	.	1	.	.	.	.	.	.	.
H9	16183C	16189C	16217C							3547G	B2	**2**	.	.	.		.	2	.	.	.	.	.
H10	16183C	16188T	16189C	16217C						3547G	B2	**1**	.	.	.	1	.	.	.	.	.	.	.
H11	16183C	16189C	16217C	16289G						3547G	B2	**1**	.	.	.		.	.	.	.	.	.	1
	n.d.									3547G	B2	2	.	.	.	1	.	.	.	.	.	.	1
H12	16129A	16223T	16298C	16325C	16327T					493G	C1b	**1**	.	.	.	.	.	.	.	.	1	.	.
H13	16183C	16189C	16223T	16298C	16311C	16325C	16327T			493G	C1b	**2**	.	1	.	.	.	.	.	.	.	.	1
H14	16223T	16298C	16325C	16327T						493G	C1b	**1**	.	.	.	.	1	.	.	.	.	.	.
H15	16223T	16292T	16298C	16325C	16327T					493G	C1b	**1**	.	.	.	.	.	.	1	.	.	.	.
	n.d.									493G	C1b	1	1	.	.	.	.	.	.	.	.	.	.
H16	16223T	16325C	16362C							2092T	D1	**4**	.	2	.	.	1	.	.	1	.	.	.
	n.d.									2092T	D1	1	.	.	.	1	.	.	.	.	.	.	.
Total												**41**	**2**	**16**	**1**	**6**	**3**	**2**	**1**	**1**	**4**	**2**	**3**

[1]Ht = Haplotype; Hg = Haplogroup.
[2]Haplotype not determined reproducibly.

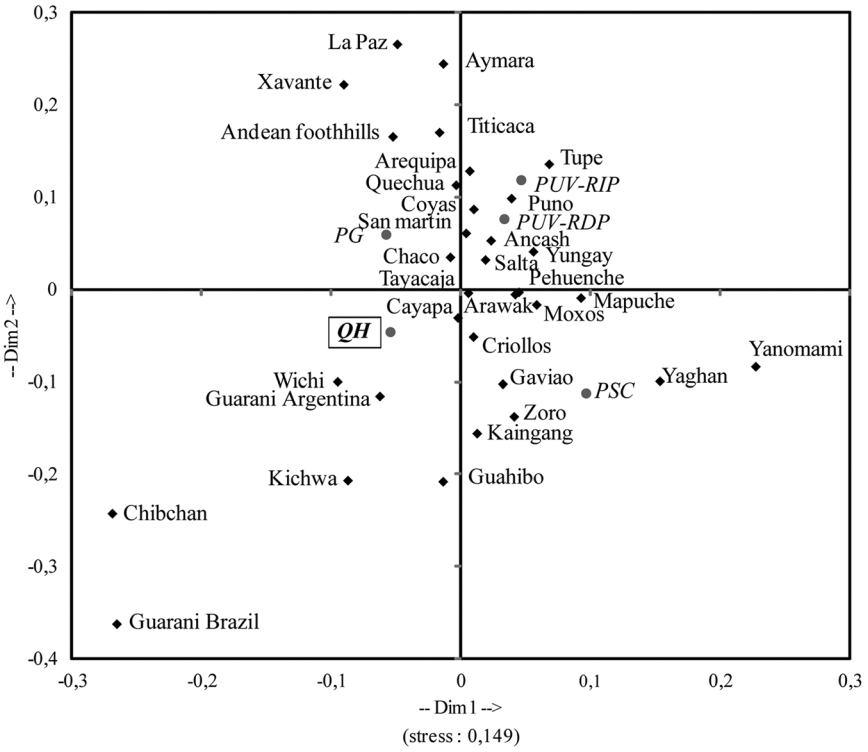

Figure 2 MDS plot based on pairwise F_{ST} values derived from mtDNA HVR1 sequences. Ancient populations, represented by grey dots, are abbreviated as follows: QH, Quebrada de Humahuaca; PG, Pampa Grande; PSC, Peruvian South Coast; PUV-RIP, Peruvian Upper Valleys Regional Integrative Period; PUV-RDP, Peruvian Upper Valleys Regional Development Period.

a haplotype found in a Peruvian sample (Iannacone et al., 2005).

The ancient Huamahuaca group was composed of 18 male individuals belonging to Q-M3 (15 individuals) and Q-M346 (2 individuals). The complete minimal Y-haplotypes available for the male individuals from Humahuaca were used to calculate pairwise distances with current Amerindian groups. This analysis, illustrated in Figure 3, shows a clustering of the ancient group from Humahuaca with contemporary Andean populations such as the Uros, Aymara, and Quechua. While the Humahuaca group also has affinities with the Argentinean Guarani and also, to a lesser extent, with Chacoan groups (Toba and Wichi), it diverges from current groups from Northwestern Argentina, such as the Diaguitas or the Collas.

Discussion

Thanks to good preservation of the samples and to the implementation of an effective strategy of analysis, we can present original aDNA data from the Humahuaca Valley samples. The results presented in this study can be reasonably considered authentic given the various precautions followed during the analysis. In addition, (i) all consensus haplotypes and haplogroups were determined from many replications (from different extracts and amplifications performed at different times and from different samples), (ii) no contaminating sequence was detected in negative controls, and (iii) European-specific sequences originating from the researchers who directly participated in this study were never observed during the analyses.

The mitochondrial DNA diversity of the late prehispanic group of Quebrada de Humahuaca is dominated by a high frequency of haplogroup A2 (46.9%). This lineage is generally not as widespread among ancient highland groups (Fehren-Schmitz et al., 2010b) and contemporary Andean populations (Fuselli et al., 2003; Lewis et al., 2005, 2007; Alvarez-Iglesias et al., 2007; Corella et al., 2007; Afonso Costa et al., 2010; Barbieri et al., 2011; Gaya-Vidal et al., 2011). The majority of the contemporary Andean populations are characterized by a high frequency of haplogroup B2 (Table S2), which is detected at a frequency of 25% in this RDP group from Humahuaca. Surprisingly, the pattern of distribution of

Table 3 Y-haplogroups and Y- STR haplotypes inferred for the 22 male samples.

Ht[1]	Sample	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS385a	DYS385b	DYS437	DYS438	DYS439	GA-H4TA	DYS448	DYS456	DYS458	DYS635	Q-M242	Q-M346	Q-M3	Q-M19	Q-M194	Q-M199	Q-SA01	Hg[1]	Y-SNP
1	Ter-2	13	13	29	24	10	14	13	14	14	14	11	11	12	20	14	19	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
2	Til-5	13	13	30	23	9	14	14	13	16	14	11	11	11	20	15	16	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
3	LAm-24	13	13	30	23	10	14	13	14	16	14	11	11	12	20	17	16	.	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
4	Jue-4	13	13	30	24	10	14	13	14	18	14	11	11	11	20	15	17	23	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
5	Jue-5	13	13	30	25	10	14	12	14	17	14	12	12	12	.	15	15	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
6	FAl-1 ; LAm-4	13	13	30	25	10	14	13	14	14	14	11	12	13	20	15	20	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
7	LAm-21	13	14	31	25	10	14	12	15	17	14	11	11	11	20	15	17	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
8	LAm-10	13	14	32	23	9	14	13	13	18	14	11	11	12	20	15	15	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
9	LAm-9 ; LAm-20	13	14	32	24	11	14	13	15	16	14	11	11	12	20	15	17	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
10	Til-1	13	14	33	25	10	14	13	14	19	14	11	11	11	20	16	17	23	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
11	LAm-7	.	14	33	25	10	14	13	14	18	14	.	11	11	.	16	18	.	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
12	Hua	13	13	.	25	10	14	12	14	15	14	12	13	12	20	16	15	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
13	Til-4	13	13	30	23	10	.	13	14	17	14	11	.	10	.	15	15	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
14	LAm-17	14	14	30	24	10	.	13	16	17	14	11	11	11	19	16	17	22	**T**	**G**	C	T	T	DEL	C	Q1a2*
15	SJo	12	13	31	.	10	.	13	14	14	14	.	11	11	.	16	16	.	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
16	Ter-5	13	12	.	24	10	.	13	15	15	14	.	10	12	.	14	15	.	**T**	**G**	C	T	T	DEL	C	Q1a2*
17	Sa-1	13	13	29	.	10	.	13	16	17	14	11	11	11	.	17	15	22	**T**	**G**	C	T	T	DEL	C	Q1a2*
18	Don-1	.	13	.	.	.	.	9	14	15	.	.	.	.	.	15	16	-	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
19	LA-12	.	13	.	25	10	.	13	14	14	14	11	.	13	.	15	20	22	**T**	**G**	**T**	T	T	DEL	C	Q1a2a1a1*
20	LA-22	.	13	.	.	10	.	13	13	18	13	.	.	12	.	16	.	.	**T**	**G**		T		DEL	C	Q1a2*

[1]Ht = Haplotype; Hg = Haplogroup.

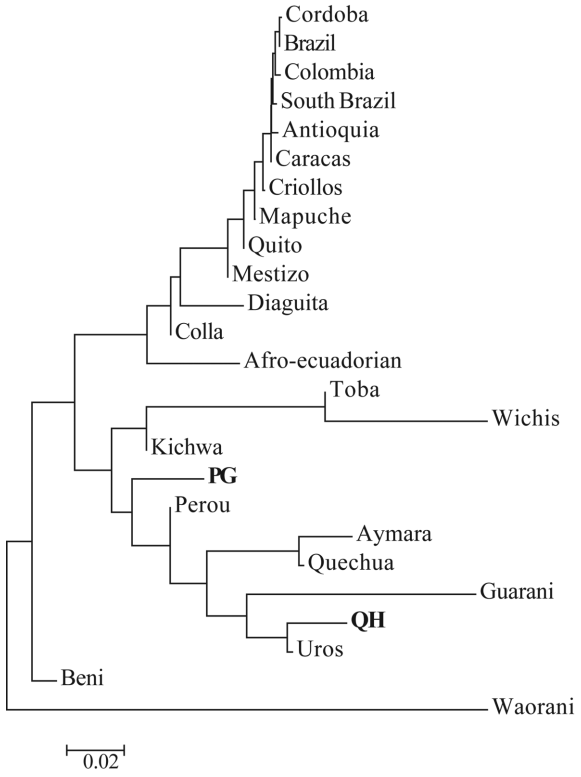

Figure 3 Unrooted NJ tree depicting relationships among 25 populations based on genetic distances derived from the Y-chromosome "minimal" haplotypes.
Ancient populations are abbreviated as follows: QH, Quebrada de Humahuaca; PG, Pampa Grande.

mitochondrial haplogroups observed for this ancient group is not typical of Andean populations but is similar to that reported for some non-Andean contemporary populations such as the Guahibo (Vona et al., 2005) or the Guarani (Sala et al., 2010). The high frequency of mitochondrial lineage A2 is also not found in current populations that inhabit the region [Salta (22.2% of haplogroup A2) or Collas (13.1% of haplogroup A2)]. The paternal lineages detected in this ancient group are the most prevalent autochthonous Native American haplogroups (Q-M3 and Q-M346), currently widely distributed in America (Bisso-Machado et al., 2012). It is interesting to note that the Q-SA01 paternal lineage, specifically distributed in the Andes (Jota et al., 2011), was not detected in our sample.

The detected mtDNA and Y-chromosome haplogroups suggest that the biological diversity of the studied group was shaped by different populations, not only from the Andes, but also from other neighboring regions, such as the Chaco. However, the resolution of haplogroup analyses, especially in America, is not sufficient to discuss precisely the (micro)evolutionary processes that shaped the gene pool of ancient groups. The distribution and the sharing of mitochondrial and Y-chromosome haplotypes between ancient and contemporary Amerindian communities can provide more reliable evidence to understand the demographic history of the late prehispanic Humahuaca community.

Recent studies, based on different lines of evidence, proposed that the initial settlement of the Andean region was done by a single migration wave, along the Cordillera (e.g., see the review by Rothhammer & Dillehay, 2009). This assumption implies that the groups from the Humahuaca Valley have common ancestors with populations from Central Andes. The genetic data that we obtained do not refute this hypothesis, given the Andean affinities detected both at the mitochondrial and Y-chromosome level. The phylogenetic analyses carried out show that several haplotypes retrieved in the ancient Humahuaca samples are present exclusively in the Andean region, or, at least, are very common in this region (Fig. S1). It is interesting to note that an ancient individual of Humahuaca, a sample from Tilcara (H10), presents a particular variant of the mitochondrial lineage B2 which is characterized by a specific mutation in position C16188T. This mutation is widely distributed within Andean populations like the Aymara and the communities of the Atacama region (Fuselli et al., 2003; Bert et al., 2004; Alvarez-Iglesias et al., 2007; Afonso Costa et al., 2010; Barbieri et al., 2011; de Saint Pierre et al., 2012). Moreover, considering the Y-chromosomal data, the ancient group of Humahuaca and the extant Andean Uros (Sandoval et al., 2013) are genetically highly related. While affinities are detected with current Andean groups, the mitochondrial DNA results suggest that the ancient group studied is genetically differentiated from all ancient Andean groups (Shimada et al., 2004; Moraga et al., 2005; Shinoda et al., 2006; Kemp et al., 2009; Carnese et al., 2010; Fehren-Schmitz et al., 2010a, 2010b; Casas-Vargas et al., 2011). Given the heterogeneity of the ancient Andean groups studied (culturally, geographically, and chronologically), this result is not surprising. Only one individual from the archaeological site of Doncellas, situated in the Argentinean highlands (Puna), shares a mitochondrial haplotype (H11) with three samples from the Laramate site located in the highlands of Peru (Fehren-Schmitz et al., 2010b). Considering the geographical distance between the two archaeological sites, this genetic link is more likely indicative of a common origin than of gene flow between the two groups. The paleogenetic data obtained to date show that the Andean territory was occupied by genetically heterogeneous groups during prehispanic periods. In particular, the groups which occupied the southern parts of the Central Andes evolved locally without experiencing significant gene flow from successive civilizations of the Central Andes. This study shows that contemporary Andean populations, which are genetically

very homogeneous (Fuselli et al., 2003; Afonso Costa et al., 2010; Barbieri et al., 2011; Gaya-Vidal et al., 2011), do not necessarily reflect the diversity of ancient Andean populations.

The mitochondrial data suggest that, despite their closeness to some Andean communities, the Humahuaca group diverges from Central Andean populations (Fig. 2). This observation can be explained by the fact that, following its settlement in Northwestern Argentina, this group has been isolated genetically from the Central Andean groups and/or has established specific exchange networks with other regions. It is noteworthy that some A2 haplotypes are shared between ancient individuals of the Humahuaca group and contemporary groups located in eastern areas, including individuals of the Chaco area (Cabana et al., 2006; Sevini et al., 2013) and Guarani communities of Argentina (Sala et al., 2010). In addition, mitochondrial haplotype data show that the ancient Humahuaca group is not very differentiated from the Chaco group ($F_{ST} = 0{,}067$), or the Criollos of the Chaco region ($F_{ST} = 0{,}050$) and cannot be significantly differentiated from the Guarani ($F_{ST} = 0{,}023$) (Table S4).

Several hypotheses can be proposed to explain the genetic affinity observed between these communities. The idea that some groups from the Southern Andes settled the Gran Chaco through Northwestern Argentina has been mentioned in the literature (Rothhammer et al., 2001; Cabana et al., 2006; Bodner et al., 2012). However, rather than resulting from a common origin, the affinity between these two regions is more likely related to more recent events. Indeed, one of the peculiarities of the prehispanic groups of Humahuaca was their active involvement in long distance exchange of goods from the Pacific coast to the eastern Chaco (Nielsen, 2013). These long distance trades may have been accompanied by gene exchange, resulting in the genetic affinity observed between the ancient Humahuaca community and the contemporary populations of the Chaco area. Moreover, several studies, based on craniometric traits, showed that the diversity of the Humahuaca groups is the result of the contribution of various groups native of different regions (Bordach & Cocilovo, 1991; Cocilovo et al., 2001; Varela et al., 2004, 2008). Alternatively, the Humahuaca and Chaco groups may have been particularly related by matrimonial exchanges, a practice that could allow the introduction of uncommon lineages in the Humahuaca Valley, such as some A2 haplotypes. The affinity with the populations of the Chaco region (Chaco, Guarani, Criollos) seems a little more pronounced for the maternal lineages than for the paternal lineages. This would emphasize that foreign women arrived more frequently than men to the communities of Humahuaca, a pattern consistent with the patrilocal residential norm that is most common in the Andes (Baca et al., 2012). However, this analysis should be interpreted with caution because of the lack of data for the Y-chromosome. The results obtained may be slightly biased by the small size of our population (10 male samples) but also by the low number of males in the reference data.

Finally, it is necessary to keep in mind that recent demographic events have influenced the gene pool of prehispanic groups. Population movements driven by the Incas in the region could be responsible, at least partially, for the dilution, or the loss, of some genetic lineages (Corella et al., 2007; Barbieri et al., 2011). This phenomenon was likely accentuated by the arrival of the European settlers (Hunley & Healy, 2011). We can indeed note that some mitochondrial (H2, H3, H5, H6, H7, H13, and H15) and Y-haplotypes (1–2, 5–8, and 10) were not detected in the reference database. The absence of these haplotypes in the current groups of the region may be indicative of the loss of diversity caused by conflicts and relocation of communities related to the colonization of the territory. However, we cannot exclude the possibility that this discrepancy is the result of a sampling bias in the ancient and contemporary groups. European colonization had a significant impact on the demography of Native South Americans, causing the displacement of entire groups (Corach et al., 2010; Catelli et al., 2011; Avena et al., 2012). In particular, the Criollos and Guarani, who share a strong affinity with the ancient group of Humahuaca, did not live in the Chaco or eastern plains of Argentina during prehispanic periods. These groups migrated to new areas after the European colonization. In particular, the Criollos, considered as the descendants of the first "mestizo" inhabitants of the northeast region of the Andes, colonized the region of the Chaco in the early 20th century (Sevini et al., 2013). The affinity observed at the mtDNA level with the Criollos, or with the Guarani, on the Y chromosome, may have resulted from gene flow, before or during the RDP period, while these groups occupied neighboring territories.

To summarize, according to the paleogenetic data obtained, we can hypothesize that the communities from the Humahuaca Valley are descendants of central Andean groups, but have subsequently evolved locally such that they no longer have strong affinities with the Central Andean populations. The later establishment of long-distance trade networks allowed the exchange of genes and the introduction of new lineages from neighboring regions, such as the Chaco. Finally, recent events, which took place after the arrival of Europeans in the Argentinean territory, could have increased the affinities observed with some groups of the eastern Argentinean plains.

One of the objectives of this study was to understand the organization of the ancient groups living in the Southern Andean region. In spite of the small sample size, our genetic data can be compared with the available anthropological data. Several authors have suggested a significant divergence between groups from different ecological environments of Northwestern Argentina (Varela et al., 2008; Cocilovo

et al., 2009). Their studies reveal two differentiated groups, one including the ancient communities of the Puna and Humahuaca and another including the populations of the Calchaqui valley and Pampa Grande. Mitochondrial DNA data from the Humahuaca ancient group appear quite divergent from that of the Pampa Grande group (F_{ST} = 0.12), which we previously studied (Carnese et al., 2010). No mitochondrial haplotypes are shared between Humahuaca, the Calchaqui sites, or the neighboring site of Pampa Grande. One Humahuaca individual shares a mitochondrial haplotype (H13) with an individual from Doncellas (Puna) of the same period. To date, this haplotype has not been described in any other extant South American population. On the other hand, it is interesting to note a particular sequence motif of haplogroup B2 (characterized by the mutations: 16145A 16156A 16157C) that is shared by two samples of Calchaqui (H5), seven samples of Pampa Grande (Carnese et al., 2010), and one Criollo individual from the Chaco region. These genetic results are consistent with the anthropological data available (based on craniometric traits). However, we must remain cautious in making this interpretation, given our small sample size. In particular, the Y chromosome data obtained lead us to qualify the proposed hypotheses. Keeping in mind the small samples sizes (10 and five samples for Humahuaca and Pampa Grande, respectively), the analysis of Y-STR haplotypes underlines the fact that the Humahuaca and Pampa Grande groups are not closely related. However, two individuals, one from Humahuaca (LA-4) and the other from Calchaqui (FA-1), share the same Y-STR haplotype (6), which reflects a close kinship. Long distance llama caravan trade, traditionally a male activity, was particularly intense during the RDP (Dillehay & Núñez, 1988; Yacobaccio, 2012), a phenomenon which could favor some gene flow between the two valleys studied. The study of additional samples will be necessary to really understand the organization of these groups in the territory.

Conclusion

In this aDNA study, we provide original data from a late prehispanic group of the Argentinean Humahuaca Valley. This study demonstrates that aDNA is a powerful tool to reconstruct the population history of prehistoric groups. The analysis of maternal and paternal genetic lineages reveals how microevolutionary processes played an important role in the shaping of the gene pool of the ancient communities from the Humahuaca Valley. These ancient groups, with a typical Andean basic component, share an affinity with contemporary populations of the Chaco region, which can be explained by recent population movements. The genetic comparison with neighboring populations of the Northwestern Argentinean region indicates a particular organization of populations in the territory. Two different spheres of interaction may have led to strong biological affinities between the groups of the Humahuaca and Puna areas and divergence from groups of the Calchaqui Valley and the Pampa Grande site. Finally, we show that there is a discontinuity with current local populations, of a part, at least, of the mitochondrial DNA gene pool, which can be explained by the significant demographic shifts caused by European colonization. Therefore, it is essential to continue palaeogenetic studies in the region and throughout South America to obtain the most accurate view of the genetic diversity of the prehispanic Amerindian populations.

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Supporting Information

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

Table S1. PCR and SBE primers used for the 15-plex iPLEX assay (Sequenom).

Table S2. References of the ancient and contemporary Amerindian populations included in the mtDNA analyses.

Table S3. References of the modern day South American populations used for comparison and analyses of Y chromosome data.

Table S4. F_{ST} values, from mtDNA and Y-chromosome data, calculated between Humahuaca and ancient and modern South American populations.

Figure S1. Median Joining Networks analyses on mitochondrial haplogroups A2, B2, C1, and D1.

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