

Human Mitochondrial DNA Diversity in an Archaeological Site in *al-Andalus*: Genetic Impact of Migrations from North Africa in Medieval Spain

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KEY WORDS aDNA; haplotypes; Andalusia; Iberia

ABSTRACT Mitochondrial DNA sequences and restriction fragment polymorphisms were retrieved from three Islamic 12th–13th century samples of 71 bones and teeth (with >85% efficiency) from *Madinat Baguh* (today called Priego de Cordoba, Spain). Compared with 108 saliva samples from the present population of the same area, the medieval samples show a higher proportion of sub-Saharan African lineages that can only partially be attributed to the

historic Muslim occupation. In fact, the unique sharing of transition 16175, in L1b lineages, with Europeans, instead of Africans, suggests a more ancient arrival to Europe from Africa. The present day Priego sample is more similar to the current south Iberian population than to the medieval sample from the same area. The increased gene flow in modern times could be the main cause of this difference. *Am J Phys Anthropol* 131:539–551, 2006. © 2006 Wiley-Liss, Inc.

The origin of the genetic diversity of human populations in Europe is still very controversial, despite the multidisciplinary approach of the research being used to address this question. At this point, ancient DNA studies can contribute by providing at least a piece of the genetic landscape at a precise time in the past, and so they can help shed light on the origin of the genetic composition of present populations.

Most of the research regarding the origin and evolution of the Iberian population is based on modern DNA analysis of autosomic markers, Y-chromosome, and mitochondrial DNA (Côrte-Real et al., 1996; Comas et al., 2000; Flores et al., 2000; Bosch et al., 2001; González et al., 2003). For mitochondrial DNA, it shows a high similarity with other European populations (Richards et al., 1996), although perhaps the most important difference is due to the presence, though at low frequencies, of haplogroups U6 and M1 (Macaulay et al., 1999; González et al., 2003), which were probably introduced from North Africa, where U6 and M1 are predominant (Rando et al., 1998). This distribution indicates moderate levels of gene flow from North Africa to the Iberian Peninsula (Côrte-Real et al., 1996; Rando et al., 1998) that would have occurred during the Muslim occupation of the Peninsula (Bosch et al., 2001; Larruga et al., 2001), but also in prehistoric times (González et al., 2003) in proportions that remain unknown.

The frequency of African lineages in Iberia is highest in Andalusia (7.7%), and the frequency of the European haplogroup H is also more similar to North Africa than to the North of the Iberian Peninsula.

Iberia was conquered by Muslims of northwest (NW) African and Arab origin at the beginning of the eighth century A.D., and their political domination over part of the Peninsula extended until the 15th century. During these eight centuries the limits of the area under their political control changed considerably, with the South, and in particular Andalusia, being the region where the

occupation lasted the longest. The historical records describe how Arabs and Berbers settled the conquered territory. Arabs were a minority who arrived mainly during the conquest, while Berbers immigrated throughout during the whole period of Muslim domination in significantly higher numbers. Berbers, together with the mainly Christian, but also Jewish, local population, adopted the Islamic religion and culture, although at different rates and degrees depending on the areas. It has been proposed that by the 10th century, Islam would have been the religion of 50% of the population (Carrasco et al., 2002). The last important military efforts for the Islamic control of the area occurred during the 11th and the 13th centuries when *al-Andalus* (the Arabic term for the territory under Muslim domination) became a part of the *Almoravide* and *Almohade* empires. Both political movements originated in Berber regions of North Africa and were probably accompanied by new waves of Berber immigrants.

The archaeological excavations held between 1995 and 2000 in Priego de Cordoba (Andalusia, Spain) (Carmona and Luna, 1996; Carmona et al., 1998; Carmona, 1999) enabled us to obtain human remains from three different

Grant sponsor: The Spanish Ministerio de Educación; Grant number: 2002 EX 9/30/02; Grant sponsor: Ministerio de Ciencia y Tecnología; Grant number: BMC2001-3511; Grant sponsor: Gobierno de Canarias; Grant number: COF2002-015.

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Received 11 October 2005; accepted 21 March 2006.

DOI 10.1002/ajpa.20463

Published online 9 May 2006 in Wiley InterScience (www.interscience.wiley.com).

Muslim burial areas dating from *Almohade* times. Their state of preservation is very good and the archaeological documentation is excellent. From the archaeological records there is no doubt about the culture and religion of these individuals, but they are not informative about the biological background of the population. The main objectives of this study are a) to determine the genetic composition of the medieval population, b) to evaluate the impact of North African migrations, and c) to assess the evolutionary changes of the population until present. To this end, osseous and dental remains from 71 medieval individuals and 108 saliva samples from the present population of the same geographic area were analyzed using mtDNA sequences from the first hypervariable region (HVRI) and diagnostic restriction-fragment polymorphisms.

MATERIALS AND METHODS

Medieval samples

Bone and teeth samples belonging to 71 individuals were collected from the burial sites of El Palenque (45), La Cava (8), and El Castillo (18), of the Islamic medieval town *Madinat Baguh* (today called Priego de Cordoba). The chronology of the human remains was determined by the archaeological records as well as by calibrated ^{14}C dating of human bones (cal. A.D. 1218, 2σ : 1025–(1218)–1291; and cal. A.D. 1214, 2σ : 1022–(1214)–1289). Dates were consistent with the *Almohade* epoch, and therefore the samples were considered a sole population.

In two of the sites, La Cava and El Palenque, the skeletons were perfectly individualized and in anatomical connection. At the third site, El Castillo, samples from individualized burials were collected (4), but also teeth from isolated jaws from no individualized complete skeletons (14).

Femur and tibia in good macroscopic preservation conditions, i.e., without fractures and with, at most, moderately porous cortices, were the preferential bones selected for sampling. Pieces of $\sim 4\text{ cm}^2$ were cut from the front of the shaft in minimum informative areas from an anthropological perspective, i.e., without pathological signs and not corresponding to muscular or ligament insertion areas. The sampled teeth were taken directly from their alveoli, and for those from incomplete skeletons, only inferior teeth were collected from complete jaws, to be sure they belonged to different individuals. The selected teeth showed no cracks, fractures, or caries lesions.

For some individuals, two bones or teeth of the same skeleton were collected, so that independent extractions could be prepared.

Modern samples

Saliva samples of 108 unrelated individuals who gave their informed consent were collected in FTA cards (FTA[®], Whatman). All the donors and their maternal ancestors at least for two previous generations came from Priego de Córdoba or nearby villages within 30 km of the town.

Extraction

Prior to extraction, around 1 mm of the surface of the bone sample was removed with a dentist drill to prevent contamination.

At the ancient DNA laboratory of the University of Oslo (Norway), following the recommendations of Hagelberg (1994), bone samples were broken into small fragments ($< 1\text{ cm}$) with a hammer and grounded up in a cryogenic impact grinder (Spex Industries, Edison, NJ) in grinding vials with a capacity for 2–4 g of bone. Grinding vials and impactors of the mill were cleaned with Deconex[®] (Borer Chemie AG) after use, to prevent sample carryover. After grinding, bone powder was stored in sterile tubes at -20°C . Bone powder (0.8 g) was washed twice by centrifugation with 10 ml of 0.5 M EDTA (pH, 8.5), then set for lysis in 10 ml volume and finally used for a DNA phenol-chloroform extraction (Hagelberg and Clegg, 1991). The aqueous phase was desalted and concentrated using Centriplus[®] Centrifugal Filter Devices (Amicon, Millipore) according to the manufacturer's instructions. Several rounds of dilution of the retentate with autoclaved ddH₂O and centrifugation were necessary to get a final volume of 100–200 μl with a salt concentration $\leq 1\text{ mM}$. One blank control was included.

At the ancient DNA laboratory of the University of La Laguna (Tenerife, Spain) the whole surface of each bone sample was exposed to UV light for 5 min, while teeth were thoroughly washed with 15% HCl, rinsed with UV-treated ddH₂O, and then exposed to UV light for 5 min. Then, the sample (bone or tooth) was placed between two sterilized metal plates and crushed with a hammer (Maca-Meyer et al., 2005). The pieces were kept in 15-ml sterile tubes (Costar). Alternatively, after exposure to UV, and instead of crushing, bone samples were cut with an electric saw, and from inside the section, bone was powdered with a dentist drill. Also alternatively, dental material from inside the crown was powdered, by practicing just a small entrance from the inferior limit of the crown. Powder of bone or teeth was collected in 1.5-ml sterile eppendorf tubes. Then DNA was extracted according to a modified silica-based protocol (Höss and Pääbo, 1993; Maca-Meyer et al., 2004). Briefly, 1–2 ml of a commercial guanidine thiocyanate solution (DNAzol[®]; Chomczynski et al, 1997) was added into each tube and incubated at room temperature for 2–3 days. After this incubation, the supernatant was passed through commercial silica columns QIAquick[®] (Qiagen) (Yang et al., 1998), according to the manufacturer's instructions. For some samples, part of the bone powder was processed as described, and another part was previously washed with 0.5 M EDTA for 10 min at room temperature, followed by centrifugation at 10,000 rpm for 1 min, before adding DNAzol. The use of alternative extraction protocols allowed us to compare the efficiency of crushing versus powdering as well as EDTA decalcification versus no decalcification. To quantify these alternative procedures, twofold serial dilutions were tested in parallel for positive PCR amplification.

Amplification

At the Oslo laboratory, two overlapping fragments of HVRI mtDNA with sizes of 278 and 281 bp, respectively, were amplified from the ancient extracts with primers A1(L15997)/B2(H16237) and A2(L16159)/B1(H16402), kindly provided by Eva Staalstrøm (Forensics Department, Rikshospitalet, Oslo), and used routinely in Forensics. A1 and A2 were as described by Wilson et al. (1995), while the sequence of B1 is TGATTTACGGAGGATGATG and that of B2 is CTTTGGAGTTGCAGTTGAT,

TABLE 1. Primer pairs used for RFLP PCR amplifications of modern and historic samples, with their annealing temperature, product size, and analyzed position

Primer sequence (5' → 3')	T (°C)	Product size (pb)	Analyzed position
L6909 ^a AAGCAATATGAAATGATCTG	50	167 + 75 (137 + 30 + 75)	7025 <i>AluI</i> (7028) + noH
H7115 ^a CGTAGGTTGGTCTAGG			
L6977 ^b GGCCTGACTGGCATTGTATTA	45	115 (100 + 15/70 + 30 + 15)	
H7052 ^b CGTAGGTTGGTCTAGG			
L12238 CAAGAAGCTGCTAACTCATGCC	52	119 (91 + 28)	12308 <i>HinfI</i> (12308) + U/K
T9 ^{*a} TACTTTTATTGGAGTTGCACCAAGATT			
L12253 ^b ATGCCCCCATGTCTAACAAC	48	103 (75 + 28)	
T9 ^{*a} TACTTTTATTGGAGTTGCACCAAGATT			
L11486 ^c AAAACTAGGCGGCTATGGTA	48	275 (252 + 23)	11718 <i>HaeIII</i> (11719) + preHV, HV, H, V
H11720 [*] AGAATAGTAATGAGGATGTAGG			
L11675 AGCCATTCTCATCCAAACCC	48	86 (64 + 22)	
H11720 [*] AGAATAGTAATGAGGATGTAGG			
L10403 ^c AAAGATTAGACTGAACCGAA	48	460 +53 (438 + 53 + 22)	10873 <i>TaqI</i> (10873)+ N, no L, no M
H10874 [*] TGATTTGGTTAAAAAATAGTCC			
L10814 CTACCACTGACATGACTTTCCA	48	103 (81 + 22)	
H10874 [*] TGATTTGGTTAAAAAATAGTCC			

L12238, L10814, L11675, H11720^{*}, and H10874^{*} have been designed at La Laguna, the last two to create new restriction sites.

^a Torroni et al., 1996.

^b Maca-Meyer et al., 2004.

^c Maca-Meyer et al., 2001.

this one being only slightly different from that used by Wilson et al. (1995). Amplifications were done in 25 μ l reactions, using 1 μ l of DNA extract in 1 \times GeneAmp PCR Gold buffer (15 mM Tris-HCl (pH 8.0), 50 mM KCl), 2.5 mM Cl₂Mg, 0.2 mM dNTPs, 0.8 mg/ml BSA, 6–10 pmol of each primer and 0.85 U of Taq Gold Polymerase (Applied Biosystems). Forty amplification cycles were performed, each consisting of 1-min denaturation (94°C), annealing (56°C), and extension (72°C) steps; the denaturation step of the first cycle was increased to 5 min and the final extension at 72°C to 10 min. One negative PCR control as well as the corresponding negative extract control were included in each amplification in order to detect possible contamination of the PCR reagents and extractions. PCR products were separated by electrophoresis in 1.5% agarose gels and visualized (Maniatis et al., 1982) with ethidium bromide staining. Positive amplifications were purified with commercial silica columns (Wizard[®] SV Gel and PCR Clean-Up System, Promega), following the manufacturer's protocol.

HVRI fragments from mtDNA of the ancient samples were amplified by PCR at the laboratory at La Laguna, following the protocol described in the work of Maca-Meyer et al. (2005). We used the same primers and PCR conditions detailed by the authors, and we included two negative controls and the corresponding extraction blank control at each amplification. Products were separated by electrophoresis in 6% polyacrylamide gels and UV visualized with ethidium bromide staining. Positive amplifications were purified with ammonium acetate (Maniatis et al., 1982) and pellets were resuspended in different volumes (15–30 μ l) of TE (10 mM Tris – 1 mM EDTA, pH 8.0), depending on the PCR yield.

HVRI (632 bp) from modern samples was amplified with a pair of primers HVI(L15840) and HVII (H16436) (González et al., in press) in 50 μ l reactions, using 5 μ l of DNA extract in 1 \times PCR buffer (16.6 mM (NH₄)₂SO₄ and 67 mM Tris-HCl, pH 8.8), 3 mM Cl₂Mg, 0.2 mM dNTPs, 0.8 mg/ml BSA, 5 pmol of each primer, and 1 U of Taq Polymerase (Ecogen/Bioline). Thirty-five amplification cycles were performed, each consisting of 15-s denaturation, annealing, and extension steps at 94, 54, and 72°C, respectively. One negative control was included at each amplification. Prod-

ucts were loaded in 1% agarose gels, stained with ethidium bromide, and UV visualized. Positive amplifications were purified with ammonium acetate (Maniatis et al., 1982).

To solve ambiguities when ascribing sequences to haplogroups, restriction-fragment polymorphism (RFLP) analysis was designed on specific sites of the haplogroups most common in Europe and NW Africa. The analyzed positions are 7025 *AluI* (7028), 12308 *HinfI* (12308), 11718 *HaeIII* (11719), and 10873 *TaqI* (10873). PCR amplifications were done in 10–20 μ l reactions and with 2 μ l of template using the primers mentioned in Table 1. PCR conditions were 35 cycles, each one consisting of 15-s steps, with denaturation at 94°C, annealing at the corresponding temperature (Table 1), and extension at 72°C. Amplifications were directly digested in reactions of 15–20 μ l with the corresponding restriction enzyme and following the manufacturer's recommendations. Products were loaded in 6 and 10% polyacrylamide gels, separated by electrophoresis, and detected by ethidium bromide staining.

Sequencing

Both strands of the PCR fragments were sequenced, with the same pair of primers used in the corresponding amplification. Ambiguous positions were resequenced. At La Laguna, the sequencing reactions were prepared using the BigDye v3.1 Terminator Cycle Sequencing kit, (Applied Biosystems) and the products were ethanol precipitated and run on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). At Oslo, the sequencing reactions were prepared with 11 μ l template, 1.1 μ l primer (5 μ M), and 8 μ l DYEnamic ET; the products were cleaned and desalted using spin columns supplied by Amersham, Autoseq96, and then run on a MegaBACE 1000 DNA sequencing system (Amersham Pharmacia Biotech). The minimum region sequenced extended from position 15978 to 16400 and from 15841 to 16435 of the HVRI region, for the ancient and modern samples, respectively.

Contamination prevention and monitoring

To prevent contamination from modern sources, ancient samples were prepared in a dedicated room. Surfa-

TABLE 2. HVRI sequences and RFLP status of the medieval and modern samples of Priego de Cordoba^a

Haplogroup (motif) ^b /Haplotype	MP	PP	SIP	NWA	IPI ^d	EU ^d	NE ^d	AFR ^d
Sample sizes	61	108	310	784	2281	4915	1231	2622
CRS (H/HV/preHV*/R*) [between 16065 and 16365]								
CRS	11	17	✓	✓				
37		1	✓	✓				
42		1	✓	✓				
184d	1		✓	✓				
366		1	✓	✓				
399		1	✓	✓				
Total CRS	12	21						
H/HV*/U*/R* except CRS -7025 AluI								
104		1*						
114	1			✓				
126	2		✓					
129	2	3	✓	✓				
162		1			✓	✓	✓	
172		1		✓				
176		1	✓					
189		1	✓	✓				
183C 189		2	✓	✓				
192	1	2	✓					
193	1			✓				
220		2			✓			
234		1			✓	✓	✓	
256	1			✓				
259		1			✓	✓		
261	1		✓					
304	2		✓	✓				
311	1	2	✓	✓				
362		2	✓	✓				
067 129	1							✓
093 126	1	1	✓					
051 183C 189 294		1			✓	✓		
183C 189 294		1			✓	✓		
189 311		1	✓					
189 362	1	1				✓		
235 291		1			✓	✓		
260 286		1*						
278 311	1		✓					
058T 304 362		2				✓	✓	
093 192 311		1*						
172 343 362		2*						
294 304 320		3		✓				
174 189 190 192 294	1*							
Total H/HV*/U*/R* except CRS	17	35						
pre-HV (126 362) +14766 MseI + 11718 HaeIII +12705 NdeII				✓				
126 362		1						
Total pre-*HV	0	1						
V (298) +7025AluI/72								
298	1		✓	✓				
(15904) 298		1	✓	✓				
192 298	1		✓					
(15904) 234 298		3		✓				
(15904) 240 298		3			✓	✓		
Total V	2	7						
U2 (051 129C) (129C 189) +12308 HinFI								
(15907) 051 092 129C 182C 183C 189		1			✓	✓		
(15907) 051 129C 183C 189 362		2	✓	✓				
Total U	0	3						
U4 (356) +12308 HinFI								
356		1	✓	✓				
179 356		3	✓					
Total U4	0	4						
U5* (270) (+9477TspI) +12308 HinFI								
270	1		✓	✓				
093 270		1	✓					
224 270 311	1					✓		
(15905) 224 270 362		2				✓		
Total U5*	2	3						

(continued)

TABLE 2. (Continued)

Haplogroup (motif) ^b /Haplotype	MP	PP	SIP	NWA	IPI ^d	EU ^d	NE ^d	AFR ^d
U5a1 (192 256 270)								
192 256 270 320	1					✓		
Total U5a1	1	0						
U5a1a (256 270 399)								
227 256 270 316 320 399		1*						
Total U5a1a	0	1						
U5b (189 270)								
189 192 270 311	1				✓			
172 183C 189 270 274 311 325 357		1			✓			
Total U5b	1	1						
U6c (169 172 189) +12308 <i>Hinf</i> I								
U6c1								
129 169 172 183C 189		1		✓				
Total U6c1	0	1						
K (224 311) -9052 <i>Hae</i> II								
224 311	1	2	✓	✓				
(15924) 093 224 311		2	✓	✓				
189 224 311		1		✓				
182C 183C 189 224 311		2		✓				
182C 183C 189 224 260 311		1*						
Total K	1	8						
T* (126 294) +4216 <i>Nla</i> III								
126 294	3		✓	✓				
126 294 325	1*							
126 189 294 296	1					✓	✓	✓
(15928) 126 189 294 296		1				✓	✓	✓
(15928) 126 288 294 296		1				✓		
Total T*	5	2						
T1 (126 163 186 189 294)								
126 163 186 189 294	2		✓	✓				
(15928) 126 163 186 189 294		1	✓	✓				
(15928) 126 163 186 189 294 304		2			✓			
Total T1	2	3						
T2 (126 294 304)								
(15928) 126 294 304		1			✓	✓	✓	
Total T2	0	1						
J* (069 126) +4216 <i>Nla</i> III								
069 126	4	1	✓	✓				
069 126 366	1		✓	✓				
069 126 189	1		✓	✓				
069 126 227 278	1*							
Total J*	7	1						
J1a (069 126 145 231 261)								
069 126 145 231 261	1	1	✓	✓				
Total J1a	1	1						
J2 (069 126 193)								
069 126 193 278		2	✓	✓				
Total J2	0	2						
N1b (145 176G 223)								
111 126 145 176G 223	1*							
Total N1b	1	0						
W (223 292) -8994 <i>Hae</i> III +8249 <i>Ava</i> II								
(15884C) 223 234 292		1				✓	✓	
(15884C) 192 223 292 325		2	✓					
Total W	0	3						
X (189 223 278) +14465 <i>Acc</i> I -1715 <i>Dde</i> I								
108 182C 183C 189 223 255 278		1	✓					
Total X	0	1						
N*/M*/L3* (223)								
223	2		✓					
223 311	1				✓	✓	✓	✓
Total N*/M*/L3*	3	0						
L3d (124 223) -8616 <i>Mbo</i> I, -10084 <i>Taq</i> I								
124 223	1			✓				
Total L3d	1	0						
L3f (209 223 311/15940d)								
(15940dT) 209 223 311		1		✓				
Total L3f	0	1						

(continued)

TABLE 2. (Continued)

Haplogroup (motif) ^b /Haplotype	MP	PP	SIP	NWA	IPI ^d	EU ^d	NE ^d	AFR ^d
L3h (223 256A)								
179 223 243 256A 284 311 320		1		✓				
Total L3h	0	1						
L3e5 (041 223) +2349 MboI								
041 223		1	✓					
041 172 223		1		✓				
Total L3e5	0	2						
L3x2								
(15928) 169 193 195 223 243 261		4			✓			
Total L3x2	0	4						
L1b (126 187 189 223 264 270 278 311) -7055AluI								
126 187 189 223 264 278 311	1			✓				
126 175 187 189 223 264 270 278 311	1*							
Total L1b	2	0						
L1b1 (126 187 189 223 264 270 278 293 311)								
126 175 187 189 223 264 270 278 293	1*							
126 162 187 189 223 264 270 278 293 311 362		1*						
Total L1b1	1	1						
L2 (223 278 390)								
223 278 311 355 368 390	1*							
Total L2	1	0						
L2a1β3 (189 192 223 278 294 309 390)								
189 192 223 278 294 309 390	1			✓				
Total L2a1β3	1	0						

CRS indicates Cambridge reference sequence (Anderson et al., 1981); MP, medieval Priego de Cordoba; PP, Present population Priego de Cordoba; SIP, South Iberian Peninsula: South Portugal (Pereira et al., 2000; González, et al. 2003) + Andalusia (Côrte-Real et al., 1996; Larruga et al., 2001; Plaza et al., 2003); NWA, Northwest Africa: Algeria (Côrte-Real et al., 1996; Plaza et al., 2003) + Morocco (Plaza et al., 2003; Pinto et al., 1996; Rando et al., 1998; Brakez et al., 2001; Thomas et al., 2002) + Tunisia (Fadhlaoui-Zid et al., 2004; Plaza et al., 2003) + West Sahara (Plaza et al., 2003; Rando et al., 1998) + Mauritania (Rando et al., 1998); IPI, Iberian Peninsula + Madeira + Azores + Canary Islands; EU, Europe; NE, Near East; AFR, Africa, except Northwest. In brackets, positions that could be read in some of the ancient sequences.

✓Matches between the medieval and/or present population of Priego de Cordoba and current populations from other geographical regions.

^a Haplotypes shared with South Iberian and/or Northwest African populations are also indicated. When no matches were found in these areas, shared haplotypes with wide geographical areas are indicated.

^b HVRI motif and RFLP status of each haplogroup.

^c Mutations are indicated by the position according to Anderson et al. (1981) minus 16000.

^d References are listed in Appendix.

* Exclusive haplotypes.

ces were washed with bleach before preparing each sample, and all the instruments that came into direct contact with the osseous and dental tissues were systematically washed with bleach before use. The samples were collected in sterile tubes to be sent to the laboratories.

Extractions and PCR reactions were set in a physically separated area in specific laboratories only for ancient DNA work, using dedicated pipettes. Before work, all the surfaces were cleaned with bleach, and at La Laguna, the laboratory was also constantly UV irradiated. PCR reactions were set in a laminar flow cabinet previously irradiated with UV. Solutions were commercially acquired and tubes were purchased sterile and DNA-free (Oslo) and sterilized by autoclaving (La Laguna). Metallic and glass material were sterilized at 200°C in an oven for at least 2 h. At all times laboratory coats, face shields, hats, and sterile gloves were used.

To monitor contamination, one extraction negative control and one (Oslo) or two (La Laguna) PCR negative controls were processed for each group of extractions and PCR reactions respectively. To test the authenticity of the sequences, duplicates of 10 individual samples processed in Oslo were extracted and amplified at the Genetics Department of La Laguna University. Also to test reproducibility, bone samples of four individuals were double extracted, amplified, and sequenced in La Laguna.

The HVRI mtDNA of the operators working with the medieval samples (MJC in Oslo and RF in La Laguna) were sequenced in order to be compared with the ancient sequences obtained.

Sequence analysis

Sequences were ascribed to haplogroups as described in the work of Richards et al. (2000), and their frequencies were calculated. To estimate the relationship between populations, the haplotype frequency-based linearized F_{ST} (Slatkin, 1995) was calculated, with the Arlequin 2000 program. All the estimates of distance were calculated taking into account the positions between 16069 and 16365. For the comparisons, published HVRI mtDNA data from present populations were pooled in two groups (Table 2), here referred as NW Africa, including data from Algeria, Morocco, Tunisia, Western Sahara, and Mauritania (Côrte-Real et al., 1996; Pinto et al., 1996; Rando et al., 1998; Brakez et al., 2001; Thomas et al., 2002; Plaza et al., 2003; Fadhlaoui-Zid et al., 2004), and Southern Iberia, including data from South Portugal and Andalusia, Spain (Côrte-Real et al., 1996; Pereira et al., 2000; Larruga et al., 2001; González et al., 2003; Plaza et al., 2003).

Those sequences from Medieval and present population of Priego not found in NW Africa or Southern Iberia were searched in other HVRI mtDNA data bases from large geographical areas (Table 2, Appendix), considering the positions between 16065 and 16365.

Divergence time estimations, based on control region mtDNA, were calculated as the mean divergence ρ (Morral et al., 1994) from inferred ancestral sequence types and converted into time by assuming that one transition within the nucleotide positions 16090–16365 corresponds to 20,180 years (Foster et al., 1996).

RESULTS

From the 71 medieval samples available, 46 were analyzed in Oslo and 35 in La Laguna. Ten of them were replicated in both the laboratories. Four samples failed in the extraction process. Five more were also discarded for being too incomplete as to be ascribed to haplogroups and, in Oslo, two samples were discarded for including the haplotype of the person handling the samples. The four duplicates in La Laguna gave identical HVRI sequences. Nine of the ten sequences replicated in both the laboratories were consistent. As the tenth corresponded to one of the two samples that, in Oslo, gave the sequence of the operator, the sequence from this duplicate processed in La Laguna was included in the study. Therefore the final sample size of medieval Priego was 61, giving an extraction efficiency of 93%, and a final efficiency of 85.9%.

To test powdering versus crushing and previous EDTA decalcification versus no EDTA decalcification, four sample replications were carried out in La Laguna. Although we could not directly quantify the DNA extraction yield, powdering and no previous decalcification of the samples resulted in at least a twofold higher PCR amplification efficiency when compared with crushing and washing with EDTA solution before DNA extraction.

A mean of two PCR amplifications, for each fragment, were carried out in Oslo. Usually, only one PCR amplification was enough in La Laguna except for fragments 4 and 6 (Maca-Meyer et al., 2005) for which, in some individuals, the amplification procedure had to be repeated up to three times.

PCR contamination was sporadic and could be overcome by cleaning the extraction laboratory and leaving it inoperative for at least 48 h and using new aliquots of PCR reagents and buffers in the repeated reactions. Contamination of the extraction blank was detected only with contamination of the PCR negative controls, and was also always successfully overcome upon repeating the amplifications with new aliquots.

Haplotype classification and distribution

The number of different haplotypes found in medieval (MP) and current (PP) population of Priego de Cordoba (Table 2) was 41 and 65 respectively. Gene diversities are practically identical in both the historical (0.95 ± 0.02) and present day (0.96 ± 0.01) samples, and not significantly different from South Iberia (0.96 ± 0.01) or NW Africa (0.98 ± 0.00). These results confirm that the sampling procedure followed to obtain the medieval remains was adequate to avoid sampling repetitions.

TABLE 3. Linearized F_{ST} between the medieval and present populations of Priego de Cordoba, South Iberian Peninsula, and Northwest Africa

	MP	PP	SIP	NWA
MP		0.00258	0.00150	0.00361*
PP			0.00121	0.00476**
SIP				0.00287***

MP indicates medieval Priego de Cordoba; PP, present population Priego de Cordoba; SIP, South Iberian Peninsula; NWA, Northwest Africa.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Seven haplotypes were found in both medieval and present day Priego samples but are absent in the reference European and North African database. They account for 17% and 11% of the lineages respectively. Twelve haplotypes are present in both medieval and current Priego samples, which represent 29% for the medieval sample and 19% for the present population. However, none of these haplotypes was exclusively shared between both samples. Haplotypes shared with South Iberia and/or NW Africa and those absent in these areas but found in other regions are indicated in Table 2. Although the majority of the lineages of medieval (33; 80%) and present day (58; 89%) Priego samples are of Eurasian origin, the African contribution is higher in the historic sample (8; 20%) than in the current population (7; 11%).

F_{ST} distances based on haplotype frequencies (Table 3) show NW Africa as the only area significantly different from the others. The closest sample to NW Africa is medieval Priego. Congruently, in Iberia the largest distance are those where MP participate.

Haplogroup frequency comparisons

Haplogroup frequencies in the historic and current Priego samples and in geographically related areas are listed in Table 4 and graphically represented in Figure 1.

The characteristic North African U6 haplogroup (Rando et al., 1998; Maca-Meyer et al., 2003) was not detected in MP, and only once in the PP sample. However, the percentage of African haplogroups in MP (15%) is significantly ($P < 0.05$) higher than in the present day south Iberia (6%) and half of that found for NW Africa (32%). Furthermore there is a significant difference (Fisher test, $P = 0.02$) in the proportion of sub-Saharan Africa lineages between the historical and present day Priego samples. This could be due to the Castilian repopulation after the Christian reconquest of this region.

In relation to the Eurasian haplogroup distribution, it is worth mentioning that frequencies for T and J haplogroups are higher in MP than in PP, whereas the K haplogroup is more frequent in PP than in MP, with the former being similar to the South Iberian (SIP) sample (Table 4). Although there is a rare haplotype match (189 362) between Priego samples, in general, haplogroup frequencies of the PP sample are more in accordance with the current SIP distribution than with the MP sample (Table 4). The increased gene flow among areas in modern times could be the main cause of this difference. However, differences in the Eurasian haplogroup frequency distribution between both Priego samples are not statistically significant ($P = 0.08$).

TABLE 4. Haplogroup frequencies of medieval (MP) and present population (PP) of Priego de Cordoba, compared with those of present populations of South Iberian Peninsula (SIP) and Northwest Africa (NWA)

Haplogroup	MP	PP	SIP	NWA	Haplogroup	MP	PP	SIP	NWA
CRS	19.7	19.4	19.0	13.4	W	—	2.8	1.6	0.1
H/HV*/U*/R* (except CRS)	27.9	32.4	25.5	17.5	X	—	0.9	3.2	1.5
pre-HV	—	0.9	0.6	1.0	M1	—	—	2.3	3.2
V	3.3	6.5	4.8	6.4	N*/M*/L3*/L3e5	4.9	1.9	1.0	3.1
U1a	—	—	0.3	0.5	L3b	—	—	0.3	1.4
U1b	—	—	—	0.1	L3b1	—	—	0.3	1.9
U2	—	2.8	1.0	0.1	L3d/d2	1.6	—	—	1.1
U3	—	—	1.0	2.4	L3d1	—	—	—	0.1
U4	—	3.7	1.6	0.8	L3e1	—	—	—	0.3
U5*	3.3	2.8	0.6	0.9	L3e1a	—	—	—	0.1
U5a	—	—	1.3	0.1	L3e2	—	—	—	0.3
U5a1	1.6	—	1.6	0.4	L3e2b	—	—	1.3	0.4
U5a1a	—	0.9	0.6	0.3	L3f	—	0.9	—	0.4
U5b	1.6	0.9	1.6	1.7	L3f1	—	—	0.3	0.4
U6a	—	—	0.3	3.1	L3h	—	0.9	—	0.1
U6a1	—	—	—	5.1	L3x2	—	3.7	—	—
U6b	—	—	0.3	0.1	L0a1a	—	—	—	0.3
U6c	—	—	—	0.4	L0a1	—	—	—	0.1
U6c1	—	0.9	—	0.1	L1b	3.3	—	0.3	0.5
U7	—	—	0.3	0.3	L1b1	1.6	0.9	1.0	2.4
K	1.6	7.4	6.8	6.5	L1c3	—	—	—	0.3
T*	8.2	1.9	0.6	0.8	L1c2	—	—	0.3	—
T1	3.3	2.8	2.3	3.8	L1c3b1	—	—	—	0.4
T2	—	0.9	2.6	0.6	L2	1.6	—	—	0.9
T3	—	—	1.3	1.1	L2a	—	—	1.0	0.4
T4	—	—	0.3	—	L2a1	—	—	1.3	2.3
T5	—	—	0.3	0.1	L2a1a	—	—	—	0.5
J*	11.5	0.9	4.5	3.1	L2a1b	—	—	—	0.1
J1	—	—	—	0.6	L2a1beta3	1.6	—	—	1.0
J1a	1.6	0.9	0.6	0.3	L2a1d	—	—	—	0.1
J1b	—	—	1.6	0.1	L2b	—	—	—	0.3
J1b1	—	—	0.3	0.1	L2b1	—	—	—	0.1
J2	—	1.9	1.9	0.9	L2c1	—	—	—	0.1
N1a	—	—	0.3	0.1	L2d1	—	—	0.3	0.5
N1b	1.6	—	—	1.0	L2d2	—	—	—	0.3
I	—	—	1.3	1.1	Sample size	61	108	310	784

DISCUSSION

Although among present European populations Iberia is genetically the closest to North Africa and genetic flow has been detected between them, they show a clear genetic differentiation when analyzing different DNA markers (Flores et al., 2000; Bosch et al., 2001). We have also detected this genetic differentiation, as shown by F_{ST} data. However, the medieval Priego differentiation from NW Africa is lower than that of the present day population. Assuming that NW Africa has not suffered important demographic changes since the 12th–13th centuries, this difference could be explained by the migratory movements from NW Africa during the Muslim occupation of the Iberian Peninsula, and the posterior movements toward Africa. In fact, it is well known from the historical records (Carmona, 1997) that important population movements from and toward Priego de Cordoba took place between 13th and 17th centuries due to political causes: the revolt of the Islamic population (*mudéjares*) and their movement to the South while Castilians were encouraged to repopulate the area (13th century), the definite Christian conquest (14th century), the instability of the borders with the remaining Moslem territories (14th–15th centuries), the forced conversion or expulsion of the *mudéjares* in 1502, and the definitive expulsion of around 3,000 Christianized Moors (*mor-*

iscos) in 1611. As a result, the current population is more divergent from NW Africa than in medieval times.

Considering haplotypes and their frequencies, MP is the most divergent Iberian sample. Medieval North African immigrants in Priego should not have been numerous, and therefore their haplotype frequencies would not have been representative of the original population. They were probably integrated into quite an isolated local population, whose haplotype frequencies could also be divergent when compared with global Andalusia. So, this differentiation of MP seems to be the result of several genetic drift effects.

For the above reasons, genetic drift could also explain the absence of U6 haplogroup in the medieval sample of Priego, as the frequency of this haplogroup in all NW Africa is also low. On the other hand, the only U6 sequence of current Priego has been found in three Canarian individuals (Rando et al., 1999; Maca-Meyer et al., 2003) and only one in all continental NW Africa, which seems to indicate its Canarian origin and that it most probably arrived in the Iberian Peninsula because of the aborigine slave trade of Canary natives after the conquest of the Canary Islands in the 15th century.

The significant higher number of sub-Saharan African lineages (L1 and L2, Table 2) in MP, two of which are also found in NW Africa, points to the important role of Moslem migrations in the present gene distribution in

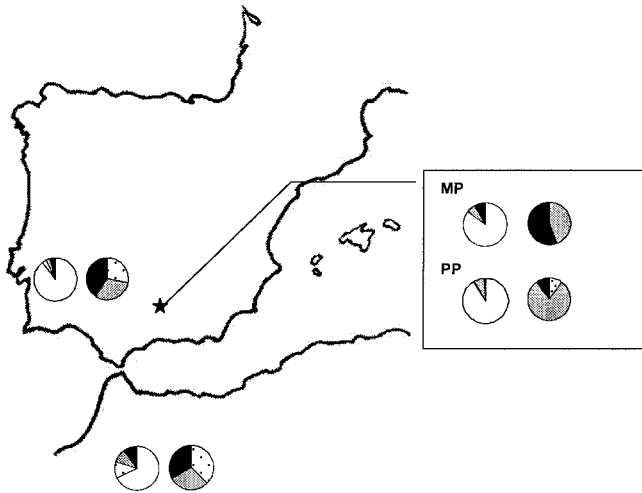


Fig. 1. Frequency distribution of mtDNA haplogroups in South Iberian Peninsula, Northwest Africa as well as Medieval (MP) and present (PP) Priego de Córdoba. The star indicates the geographical location of Priego de Córdoba in Spain. Left pie graphs represent the relative frequencies of Eurasian (white), Nor-African (dotted), sub-Saharan (black), and African L3 (grey) haplogroups. Right pie graphs represent the relative frequencies of African haplogroups in each sample, with colors as in left charts.

Iberia. The unique sharing of L1b (126 127 189 223 264 278 311) with the Sahara points to this area as the most probable origin. Nevertheless, the high number of non-shared lineages impedes the determination of the precise African origin. Moreover, the fact that the closest sequences to the two L1b haplotypes with the 16175 transition are not in Africa but in Germany (Richards et al., 2000) and Russia (Malyarchuk et al., 2004) clouds the origin of these haplotypes. It could be simply that they are also present in Africa but not yet detected. However, when we revised published and our unpublished HVRI data from Europe (11,511) and Africa (4,566), we collected 31 L1b European haplotypes, four of which are 16175 carriers, giving a frequency of 0.129 for this type in Europe. On the contrary, from 310 L1b Africans none of them has the 16175 transition. Supposing that Africa has the same frequency as in Europe, the binomial probability of not finding this motif in the African sample would be 2.5×10^{-19} . Therefore, the most probable situation is that the ancestor of this motif (Fig. 2) arrived from Africa to Europe where the 16175 mutation occurred. The divergence time estimation for this clade in Europe is around $20,180 \pm 16,144$ years, pointing to a prehistoric arrival, in Europe, of the basic African motif. It could also be that L1b with the 16175 transition was carried to the Iberian Peninsula with the Muslim invaders and that this motif was lost in Africa. However, there are not important NW African demographic movements registered in recent times that could explain this loss. Furthermore, this clade has a relatively high diversity in Europe (three different haplotypes from four individuals); therefore, if these types had arrived from Africa in recent times, we would have to suppose that this great diversity should already be in Africa. Moreover, the presence of a North African M1 representative, recently detected, in historic Basque remains that pre-date the Muslim invasion (Alzualde et al., in press) is

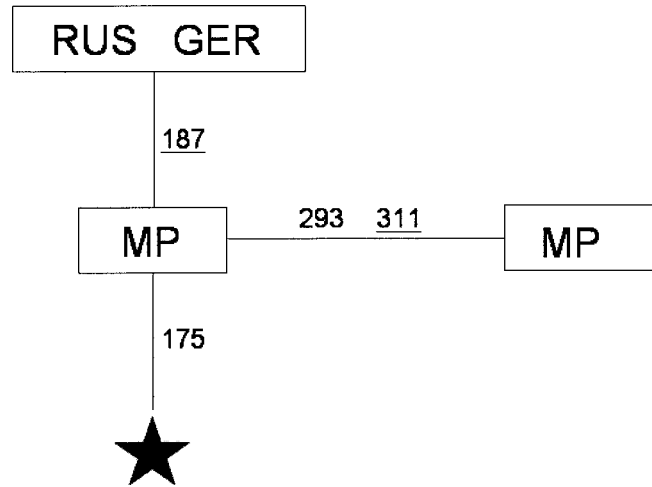


Fig. 2. L1b network constructed with the European haplotypes carrying the 16175 transition. Star represents the basic HVRI L1b motif (126 187 189 223 264 270 278 311). Numbers along links refer to nucleotide positions (mentioned in Anderson et al. 1981) minus 16,000. GER, Germany; RUS, Russian; MP, Medieval Priego.

congruent with this proposed African pre-Muslim arrival to Europe.

Regarding those L3 African lineages only found in current Priego and NW Africa (belonging to L3h and L3e5 haplogroups), their limited distribution favors a more recent arrival to Iberia, in this case from Tunisia, as both lineages have only been found in Berber populations from this country.

The results of this research have shown how population studies on ancient DNA can contribute to a better understanding of the origin of present genetic composition of human populations. Studies combining genetic data from different periods should be a new objective in human evolutionary studies, as they may help to solve some of the controversies surrounding the arrival of specific markers, and the impact of different human migrations on the present genetic structure of the populations.

CONCLUSIONS

The medieval Priego sample showed greater affinities to North-Africa than other Iberian Peninsula samples including that of present day Priego. Haplotype analysis revealed that some African haplotypes detected in medieval Priego have matches with samples of precise north-African origin as Tunisia, west-Sahara or the Canary Islands pointing to well documented historic connections with this area. However, medieval Priego L1b lineages carrying the 16175 transition have their most related counterparts in Europe instead of Africa. The coalescence age for these L1b lineages is compatible with a minor prehistoric African influence on Priego that also reached other European areas.

ACKNOWLEDGMENTS

We thank the valuable scientific assistance and support of Vicente M. Cabrera in carrying out this research. We are grateful to Emmanuel Cleuvenot and Rafael Carmona for the scientific contribution to the anthropologi-

cal and archaeological documentation, respectively, of the medieval remains. Ancient samples were available thanks to the Museum, the Association *Amigos del Museo de Priego de Córdoba*, and local authorities of Priego de Córdoba. Antonio Alcalá and the donors made the collection of modern DNA samples possible. We also acknowledge the unconditional support of Nils C. Stensteth to the project. Ian Frame gave all his support to this research, and Eva Staalstrøm provided with A1B2 A2B1 primers.

APPENDIX

List of populations, and related literature, included in the broad geographical areas for HVRI mtDNA comparisons. Numbers at the beginning of lines indicate the size of the samples included in the analysis.

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