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Y-chromosome Lineages from Portugal, Madeira and Açores Record Elements of Sephardim and Berber Ancestry

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Summary

A total of 553 Y-chromosomes were analyzed from mainland Portugal and the North Atlantic Archipelagos of Açores and Madeira, in order to characterize the genetic composition of their male gene pool. A large majority (78–83% of each population) of the male lineages could be classified as belonging to three basic Y chromosomal haplogroups, R1b, J, and E3b. While R1b, accounting for more than half of the lineages in any of the Portuguese sub-populations, is a characteristic marker of many different West European populations, haplogroups J and E3b consist of lineages that are typical of the circum-Mediterranean region or even East Africa. The highly diverse haplogroup E3b in Portuguese likely combines sub-clades of distinct origins. The present composition of the Y chromosomes in Portugal in this haplogroup likely reflects a pre-Arab component shared with North African populations or testifies, at least in part, to the influence of Sephardic Jews. In contrast to the marginally low sub-Saharan African Y chromosome component in Portuguese, such lineages have been detected at a moderately high frequency in our previous survey of mtDNA from the same samples, indicating the presence of sex-related gene flow, most likely mediated by the Atlantic slave trade.

Keywords: Portuguese, Y-chromosome, SNP, STR

Introduction

For centuries the Iberian Peninsula was a melting pot for various populations of different origins, including Celts, Germanics or Scandinavians (Swabians and Visigoths), Phoenicians, contributions from a myriad of east Mediterranean peoples, and finally Moors and Arabs (Amaral & Amaral, 1997; Oliveira Martins, 1994). Apart from these populations, there is also a well-documented input of sub-Saharan slaves into Portugal during the 15th–16th centuries. From all these peoples, three groups have been the focus for vigorous debate over their contribution to the present day Portuguese genetic gene pool: the Sephardim Jews, the Arab-Berbers and the sub-Saharans. Jews are known to have inhabited the Iberian Peninsula since at least the 3rd century AD (Tavares, 2000). Their arrival was probably the result of migrations via North Africa, where Jewish communities were already well established. During the Visigoth and Muslim periods Jews reinforced their position and flourished in the peninsular society. Expelled from Spain and Portugal in the 15th century, the Sephardim Jews (from "Sepharad" meaning Spain or "Espaniah") went back to North Africa or migrated, mainly to Amsterdam

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and Constantinople (Kayserling, 1971). However, many are supposed to have remained in Portugal under forced conversion to Catholicism, becoming known as "new-Christians" (Borges, 1998; Guerra, 2003).

Moors, or Berbers from Mauritania, started to expand into Iberia in 711 AD. However, it is generally assumed that their presence in the north of Portugal was minimal and short-lived. On the contrary, it is known that Arabs from Egypt and Yemen settled down in villages of southern Portugal (Lopes, 1928). Arab rule in Iberia ended in 1492 with the fall of Granada.

Although some sub-Saharans entered the Iberian Peninsula with the Berbers and Arabs, most of this component in Portugal dates back to the 15th century when the importation of sub-Saharan slaves accelerated, following the establishment of a commercial Atlantic network. At the height of the slave trade, 10% of the population in the south of the country, and particularly in Lisbon, was composed of sub-Saharan slaves (Godinho, 1965).

The Atlantic Archipelagos of Açores and Madeira were discovered and settled by the Portuguese in the first half of the 15th century and both archipelagos played a major role in the complex Atlantic trade network in the following centuries. The Açores also received a significant portion of male settlers from Flandria who married Portuguese women (Frutuoso, 1977). In Madeira, the non-Iberians were mainly from Italy and because of the heavy involvement with the Atlantic slave trade, especially after the discovery and settlement of Cabo Verde islands, the island also saw a considerable input of sub-Saharan slaves. In fact, it is known that the proportion of imported slaves in Madeira reached 10% of the total population by the 16th century. Inter-island movements have also been recorded, and it is known that many settlers of the Açores came from Madeira.

Mitochondrial and autosomal polymorphisms have been used to study the genetic composition of present day populations from Portugal, Madeira and the Açores (Pereira *et al*. 2000a; Spínola *et al*. 2002; Brehm *et al*. 2003; Fernandes *et al*. 2003; Gonzalez *et al*. 2003). MtDNA markers in particular showed that the Portuguese are characterized by a strong spatial heterogeneity in their mtDNA distribution. This prompted us to look for the genetic imprint left in today's populations by the male settlers, both in mainland Portugal and the Atlantic Islands of Madeira and the Açores. Using Y-chromosome biallelic markers and microsatellites, we aimed to characterize the extant paternal lineages to investigate if (1) genetic markers possibly transmitted by Sephardim Jews are detectable in today's gene pool, (2) whether there is a parallel with the strong imprint of sub-Saharan maternal markers found in Portugal and Madeira and (3) to what extent North African or Berber lineages are associated with the Muslim period. Moreover, the recently proposed phylogenetic characterization of Y-chromosome haplogroups E and J, regarding their possible origin and spread around the Mediterranean area (Cinnioğulu *et al*. 2004; Cruciani *et al*. 2004; Luis *et al*. 2004; Semino *et al*. 2004), renders possible the inference of potential sources of these lineages in the extant genetic pool of Portuguese males.

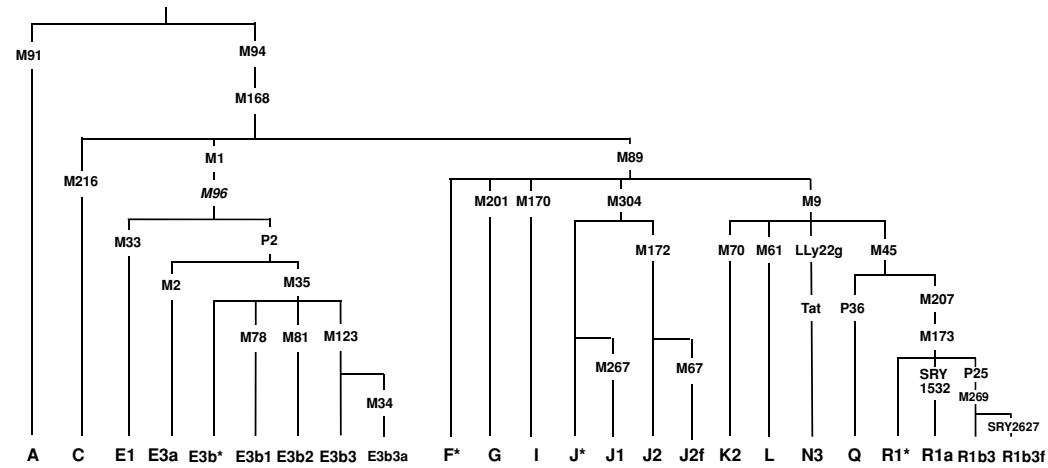
Materials and Methods

Population Samples

The populations included in this study consisted of 553 unrelated males from mainland Portugal and the North Atlantic archipelagos of Madeira and Açores. We further subdivided mainland Portugal into three regions: North, Central and South. Blood samples were collected with informed consent from volunteer males that could unambiguously certify that all relatives back to three generations were from the same island or mainland region (see Gonzalez *et al*. 2003). For comparisons of the Y-chromosome profiles prevailing in these regions we used published data on Europeans (Hammer *et al*. 2000; Underhill *et al*. 2000; Semino *et al*. 2000, 2004; Nebel *et al*. 2001; Capelli *et al*. 2003; Cruciani *et al*. 2004; DiGiacomo *et al*. 2004), North Africans (Bosch *et al*. 2001; Arredi *et al*. 2004; DiGiacomo *et al*. 2004; Luis *et al*. 2004), Middle Eastern (Nebel *et al*. 2000; Underhill *et al*. 2000; Semino *et al*. 2002; Cinnioğulu *et al*. 2004; Cruciani *et al*. 2004; DiGiacomo *et al*. 2004) and sub-Saharan African populations (Semino *et al*. 2002; Gonçalves *et al*. 2003).

DNA Extraction and Y-chromosome typing

Genomic DNA was isolated by standard protocols and the STSs containing Y-SNPs were amplified with primers described in Underhill *et al*. (2000, 2001)



	n	Gene diversity																						
MA	129	.696 (.004)	1				2	7	7	2			4	9	1		8	6	4	2	3		2	3	68	
AC	121	.647 (.004)				1		4	6		1		10	8		3	12	1	2				2		69	2
NP	101	.688 (.004)		1	3		5	8	6	2		1	5	7		1	4	3					2		53	
CP	102	.611 (.005)	1		1			6	5		4		8	5		5	3							2	62	
SP	100	.698 (.004)	1	1	1		1	1	6			1	7	7		7	6	5				1	1	2	52	
Total	553		3	2	5	1	8	26	30	4	5	2	34	36	1	16	33	15	6	2	3	1	7	7	304	2

Figure 1 Phylogenetic tree of the Y-chromosome haplotypes. Haplogroup defining mutations assayed in this study are shown along branches. Population codes are as follows: Madeira-MA, Açores-AC, North Portugal-NP, Centre Portugal-CP, and South Portugal-SP.

and Cinnioğulu *et al*. (2004). Genotyping was done using both native and engineered RFLP methods. The selected SNPs that were analyzed are shown in Figure 1 in their phylogenetic order defining the haplogroup status of each Y-chromosome. The following binary markers were assayed but derived alleles not observed: M31, M32, M75, M52, M62, M147, SRY9138, P35, M175 and M124. Six STR loci (DYS19, DYS388, DYS390, DYS391, DYS392 and DYS393) were assayed in chromosomes belonging to haplogroups E3b and J1 using standard methodology (Thomas *et al*. 1999). DYS439 and A7.1 were also tested to further define E3b1 subclusters (Cruciani *et al*. 2004), and DYS439 for J1 lineages (Table 1).

Analysis of Y chromosomal haplogroups and STR based haplotypes

In this study we follow the haplogroup nomenclature proposed by the Y Chromosome Consortium (2002) for Y chromosome typing. Frequencies of Y haplogroups for each region and gene diversity measures were obtained using Arlequin v2.000 (Schneider *et al*. 2002). These frequencies were used for comparisons with other populations and employed in an analysis of molecular variance (AMOVA) using Euclidean distances between all pairs of haplotypes (Excoffier *et al*. 1992). The total genetic variation between the populations was partitioned into hierarchical levels of grouping, and variance components were tested for significance by nonparametric randomization tests using 10000 permutations. We performed a principal component analysis (PCA) of Y haplogroup frequencies for Madeira, the Açores, and the Portuguese mainland populations, and published data sets typed with the same phylogenetic resolution: a population from North Africa (Bosch *et al*. 2001), Europeans (Capelli *et al*. 2003), Mediterranean and Middle-Eastern Sephardim Jews (Nebel *et al*. 2001) and Middle Eastern non-Jews (Hammer *et al*. 2000). To standardize the latter data sets to the level of phylogenetic resolution used in our study we deduced the proportion of J1 versus J2 (which appeared under the haplogroup designation "Med") using an estimate based on the frequencies proposed by Nebel *et al*. (2001). In this analysis we included one Jewish population of Nebel *et al*. (2001): the "Sephardic" Jews reported from the Mediterranean area and the Middle East. Superimposed on the PCA, we calculated a minimum-length spanning tree (MST) in order to detect possible distortions suggested by the

Table 1 Y chromosome haplotypes in five Portuguese populations of haplogroups E1, E3b and J* defined by 12 STR loci. Population codes are according to Figure 1

		Allele Status											Populations				
	Hg	DYS19	DYS388	DYS390	DYS391	DYS392	DYS393	GATA7.1	DYS439	DYS389I	DYS437	DYS438	AC	MA	NP	CP	SP
H1	E1	14		24	11	13	13		12	12	14	12					1
H2	E1	14		24	12	14	13		12	12	14	12			1		
H3	E1	15		21	10	11	13		12	13	14	11			1		
H4	E1	14		23	11	13	13		12	13	15	12			1		
H5	E1	15		21	11	11	14		11	14	14	11				1	
H6	E3b*	13	12	23	9	11	13							2			
H7	E3b*	13	12	24	9	11	13								3		
H8	E3b*	13	12	24	10	11	12								1		
H9	E3b*	13	12	24	10	11	13								1		1
H10	E3b1	13	12	23	10	11	13	9						1	1		
H11	E3b1	13	12	23	10	11	13	11	12				1				
H12	E3b1	13	12	23	11	11	13	9									1
H13	E3b1	13	12	23	11	11	14	11	12				1				
H14	E3b1	13	12	23	9	11	13	10	12					1			
H15	E3b1	13	12	24	9	11	13	9						1			
H16	E3b1	13	12	24	10	11	13	9					1	1	1	2	
H17	E3b1	13	12	24	10	11	13	10	12						1	1	
H18	E3b1	13	12	24	11	11	14	10	12							2	
H19	E3b1	13	12	25	10	11	13	9							1	1	
H20	E3b1	13	12	25	11	11	13	10	11				1				
H21	E3b1	13	13	23	10	11	13	10	10						1		
H22	E3b1	13	13	24	10	11	13	9						2			
H23	E3b1	13	13	25	10	11	13	9						1			
H24	E3b1	14	12	24	10	11	12	11	11						1		
H25	E3b1	14	12	25	10	11	13	11	11						1		
H26	E3b1	14	12	25	10	11	13	10	12						1		
H27	E3b2	13	12	23	9	11	13							1	1		1
H28	E3b2	13	12	24	9	11	12						1				
H29	E3b2	13	12	24	9	11	13						2	3	4	3	1
H30	E3b2	13	12	24	9	11	14						1		1		
H31	E3b2	13	12	24	10	11	13									2	
H32	E3b2	13	12	24	11	11	13						1				
H33	E3b2	13	12	24	11	11	14										1
H34	E3b2	13	12	25	11	11	13						1				
H35	E3b2	13	13	23	9	11	13										1
H36	E3b2	13	13	24	9	11	13							1			1
H37	E3b2	14	12	22	9	11	12										1

Table 1 Continued

		Allele Status											Populations				
	Hg	DYS19	DYS388	DYS390	DYS391	DYS392	DYS393	GATA7.1	DYS439	DYS389I	DYS437	DYS438	AC	MA	NP	CP	SP
H38	E3b2	14	12	23	9	11	13							1			
H39	E3b2	14	12	24	11	11	12							1			
H40	E3b3	13	12	23	10	11	12									1	
H41	E3b3	13	12	24	9	11	13									2	
H39	E3b3	13	12	24	10	11	12							1			
H40	E3b3	13	12	24	10	11	13								1		
H41	E3b3	13	12	24	10	11	14						1				
H42	E3b3	14	12	23	10	11	13									1	
H43	E3b3	15	12	24	10	11	12							1	1		
H44	J1	13	16	23	10	11	12		11								1
H45	J1	13	16	24	10	11	12		12						1		
H46	J1	14	15	23	10	11	12		9				1				
H47	J1	14	16	22	10	11	12		12								1
H48	J1	14	16	23	10	11	12		11							2	1
H49	J1	14	16	24	10	11	12		11								1
H50	J1	14	16	25	10	11	12		11							1	
H51	J*	15	15	24	10	11	12		10					1			
H52	J1	15	16	22	10	11	13		11				1				2
H53	J1	15	16	23	10	11	12		11							2	
H54	J1	15	16	24	10	13	13		12				1				
H55	J1	16	16	24	10	13	13		11								1

plot (Rohlf, 1973). PCAs for the E and J haplogroups were done with the data from Semino *et al*. (2004) and Cruciani *et al*. (2004). All the analysis was done using NTSYSpc 2.02h package.

The age of STR variation within each haplogroup was estimated as the average squared difference in the number of repeats between all current chromosomes (pooled together from all samples) and the founder (assumed to be modal) haplotype, divided by $w = 6.9 \times 10^{-4}$ per 25 years (Zhivotovsky *et al*. 2004). We computed upper and lower bounds for expansion time (the time of divergence of populations). The upper bounds were calculated using T_D (Zhivotovsky, 2001) and assuming an STR-variance in repeat scores at the beginning of the population split (*V0*) equal to zero. The lower bounds were calculated as T_D, with *V0* taken as a predicted value of the within-population STR-variance prior to the population split; the latter was computed as a linear approximation of the within-population variance in repeat scores as a function of time (L. A. Zhivotovsky, unpublished method). The minimum sample size for age calculations was 5 individuals.

Results and Discussion

Haplogroup R1b (particularly R1b3) was found to be the most dominant Y chromosomal lineage in Portugal and the North Atlantic Archipelagos of the Açores and Madeira, covering more than half of the Y chromosomal lineages in each population. The high frequency of this haplogroup is typical in all West European populations, reflecting a cline (Rosser *et al.* 2000) and likely continuity of the Paleolithic gene pool in Europe (Semino *et al.* 2000). Haplogroups I and G, also characteristic markers for many different West European populations, were found in all the populations in this study at an average frequency above 5%. Together with R1b, haplogroups J and E3b comprise 78–83% of the Y-chromosomal gene pool of each sampled population. Haplogroups J and E3b consist of lineages with differential distribution within Middle East, North Africa and Europe.

The sub-Saharan component

Typically sub-Saharan lineages are represented by haplogroups A and B, which are also the basal clades in the human Y-chromosomal tree of humans. E1-M33 has a rather limited distribution in the West sub-Saharan region, reaching its highest frequency in Mali (34%, Underhill *et al*. 2000) and north Cameroon (especially among the Fulbe and Tali groups, Cruciani *et al*. 2002). This haplogroup is virtually absent in European populations (Semino *et al*. 2004) and Northwest Africa, although it is found among Berbers (1.6–3.2%, Bosch *et al*. 2001). Haplogroup E3a is especially common in Sub-Saharan West Africa (Underhill *et al*. 2000, 2001; Semino *et al*. 2002), has been associated with the dispersal of Bantu people (Underhill *et al*. 2001, 2001) and reaches $\sim$80% in Senegal (Semino *et al*. 2002). The combined frequency of all these haplogroups in Portugal is marginally low ($\sim$0.7%), so it is highly unlikely it is a result of accidental sampling of African immigrants. Haplogroup E3a is notably absent from our sample except for a single E3a individual in the Açores, although this haplogroup constitutes the majority of Y-chromosomes in Guinea (>70%, Rosa *et al*. unpublished data) and Cabo Verde (16%, Gonçalves *et al*. 2003), the putative regions of origin for the first slaves brought to Portugal. These results sharply contrast with those obtained with mtDNA markers. mtDNA haplogroups L0–L3 and M1 that are characteristic to sub-Saharan populations are present at $\sim$12% and $\sim$14.8% in the south of Portugal and Madeira, respectively (Brehm *et al*. 2003; Gonzalez *et al*. 2003). This contrasting pattern of paternally and maternally inherited markers closely follows the situation in the Canary Islands where E3a is residual (0.9%, Flores *et al*. 2003). These Y-chromosome haplogroups have escaped detection in other populations of Iberia (Semino *et al*. 2000, 2004; Bosch *et al*. 2001), in spite of the fact that the peninsula was a recipient for sub-Saharan slaves from the 15th century onwards. These results are consistent with sex specific gene flow, probably resulting from the custom that male slaves did not mate with Iberian women while the opposite situation was common, as supported by mtDNA analysis (Brehm *et al*. 2003; Gonzalez *et al*. 2003). Three lineages belonging to haplogroup A (Y($\times$M94)) were found. The presence in Portugal of both the A and E1 haplogroups may be independent from the slave trade (otherwise E3a would be well represented since it comprises the majority of West Africa lineages). These findings either suggest a pre-Neolithic migration from North Africa or a more

Table 2 Haplogroup age estimates based on STR-variation (in ky) and expansion time of population divergence (in ky, in brackets) within haplogroups are shown. Population codes are as in Figure 1. TMRCA for all haplogroups except E1 was determined on the basis of 6 STR profiles (DYS19, 388, 390, 391, 392 and 393, see Table 1). E1 was calculated on the basis of 9 STRs (DYS19, 390, 391, 392, 393, 389I, 437, 438 and 439)

Haplogroup	N	TMRCA (±sd)
E1	5	22.9 (±7.2)
E3b*	8	4.5 (±2.9)
E3b1α	11	8.8 (±4.1)
E3b2	30	8.0 (±3.2)
E3b3	9	12.1 (±5.7)
J1	17	10.0 (±4.5)

recent origin from a founder population of small size that did not carry haplogroup E3a, which is a major component in North African populations today. TMRCA for Portuguese E1 lineages estimated as 22.9 ± 7.2 ky (Table 2) favours the first scenario, a possible parallel to mtDNA U6 cited in Gonzalez *et al*. (2003).

Haplogroup E3b

Haplogroup E3b (characterized by mutation M35) is widespread in Northwest Africa, East Africa, the Middle-East (Bosch *et al*. 2000; Semino *et al*. 2002; Underhill *et al*. 2000; Cruciani *et al*. 2004) and is also common in Europe, albeit at variable frequencies (Semino *et al*. 2000). Eastern Africa is seen as the homeland for E3b as there it has the highest number of different clades and microsatellite diversity and the almost exclusive presence of E3b*, with estimates of ∼30 ky for the age of the M35 mutation (Bosch *et al*. 2001; Cruciani *et al*. 2004). The frequency of E3b* varies from ∼3–8% in Morocco (Bosch *et al*. 2001; Cruciani *et al*. 2002; Semino *et al*. 2004) and reaches its highest frequency in Ethiopian Oromo (19.2%, Cruciani *et al*. 2002; Semino *et al*. 2002). The clustering of E3b* lineages in North Portugal (with 3.8 ± 2.2 kya) and low Y-STR variation (compared to North Africa) is a putative indication of a restricted but definite gene flow across the Strait of Gibraltar.

Haplogroup E3b1 has a significantly uneven distribution in mainland Portugal, occurring at 8–6% in Northern and Central Portugal versus only 1% in the South (x^2, $P < 0.01$). Regional sub-clustering of the STR haplotypes on the background of Portuguese E3b1-M78 suggests that these lineages could have spread from the Balkans all over Europe during the Neolithic (E-M78α in Cruciani *et al*. 2004). This is furthermore supported by the age of STR variation (8.8 ± 4.1 ky, Fig. 2) and fits into the 6.3–9.2 ky range suggested by Cruciani *et al*. (2004). The pairwise divergence times between Madeira, North and Central of Portugal differ insignificantly from each other, being on average around 6 thousand years (Table 2). The timescale for the arrival of E3b1-M78 is coincident with that pointed out by Flores *et al*. (2003). Defined on the basis of GATA A7.1 allele 9, E-M78α follows the same pattern of north-central prevalence (3% vs 1% in the south). An increased frequency of 4.6% was detected in Madeira. This may correlate with the contribution of Italians to the archipelago, as this cluster is present in ∼7% in their homeland (Cruciani *et al*. 2004). Based on the same resolution level we detected one individual in the North of Portugal having the GATA A7.1 indicated by Cruciani *et al*. (2004) as the North African E3b1ß. All the remaining E3b1 cannot be associated to any proposed geographical origin on the basis of the additional STRs.

In Northwest Africa ∼75% of the Y lineages are haplogroup E3b2-M81 (Bosch *et al*. 2001) although a much lower frequency of this clade is seen among the Arabs (32,6 % in Cruciani *et al*. 2002; 52,3 % in Bosch *et al*. 2001). E3b2-M81 in North Africa may have arisen near Egypt or Somalia and then spread westwards along the upper boundary of Africa (Sanchez *et al*. 2003; Arredi *et al*. 2004; Cruciani *et al*. 2004; Luis *et al*. 2004). This particular haplogroup has been observed at variable frequencies in Iberian populations (1–12%) and is almost absent, or occurs at very low frequencies (<6%), elsewhere in Western Europe (Underhill *et al*. 2000; Bosch *et al*. 2001; Cruciani *et al*. 2004; Semino *et al*. 2004). Surprisingly, an uniform frequency of 5–6% E3b2 was observed in Madeira, the Acores and in all Portuguese sub-populations (Figure 1), in contrast to an earlier observation of 12% in a smaller sample from Southern Portugal (Cruciani *et al*. 2004). Half of the Portuguese E3b2 haplotypes are shared with Berber and/or Arab speaking populations from Morocco (Quintana-Murci *et al*. 2004), and the age STR variation at E3b2 in the Portuguese populations is actually identical to that of North Africa (8.1 ± 3.2 ky *vs.*

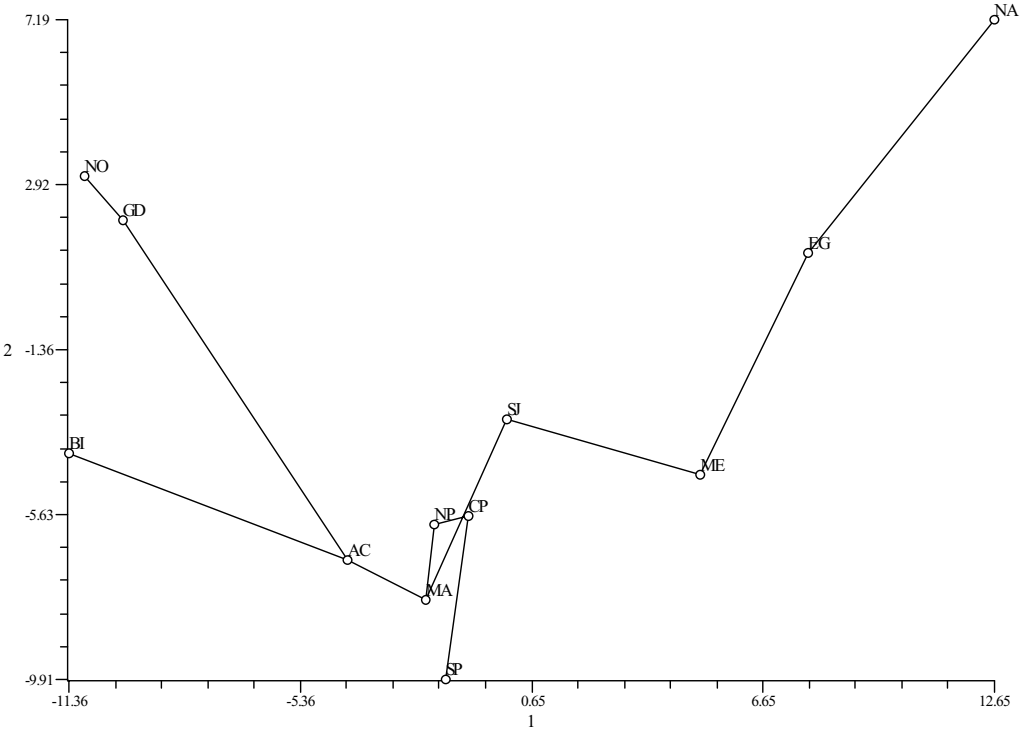


Figure 2 Principal Component Analysis based on Y-chromosome haplogroups frequencies. Axis 1 and 2 extracted 37.3% and 23% of the total variation (a third axis accounts for 17%, not shown). The MST superimposed on the ordination of populations is also shown and reveals the most likely connections between populations. Populations are as follows: MA-Madeira, AC-Açores, SP-South Portugal, CP-Centre Portugal, NP-North Portugal, ME-Middle East, BI-British Isl., GD-Germany/Denmark, NO-Norway, SJ-Sephardim Jews, EG-Egyptians and NA-North Africa.

8.6 ± 2.3 ky) (Table 2, Semino *et al*. 2004). All these findings suggest a flow of Berber-related lineages into the male Portuguese population. The lack of differentiation between Europe and Africa in relation to this haplogroup, as reported by Cruciani *et al*. (2004), makes it more plausible that extant lineages in the Portuguese most likely reflect the signature of a northwestern North African gene flow. Cruciani *et al*. (2004) explain the differences between Iberians and the remaining Europeans by an African contribution, probably resulting from Islamic influence and/or drift/founder effects. It is highly probable that the Berber dispersion in Portugal was population-structured: the average time of pairwise population divergence, more than 4 thousand years (Table 2), well predates the Arab settlement. Central and North Portugal show a lower divergence time for E3b2-STR variation (800 to 2,200 years ago); these two regions might have diverged from the same North-West African populations.

E3b3 has a clear affiliation with Mid-Eastern populations but is infrequent in Europe and North Africa (Cruciani *et al*. 2004; Semino *et al*. 2004). This haplogroup shows a considerable Y-STR variation in Turkey (Cinnioğlu *et al*. 2004) and Egypt (Luis *et al*. 2004), and again its presence in Portugal points to a Middle Eastern influence, most likely via North Africa.

Haplogroup J

More than 10% of Portuguese Y chromosomes could be classified in haplogroup J. The frequency of this haplogroup in Portugal is significantly higher than among the Spanish ($p < 0.001$) or other West European populations (Rosser *et al*. 2000), with the exception of Italy as reported by Semino *et al*. (2004) and DiGiacomo *et al*. (2004). Haplogroup J is thought to have originated in the Middle East or North East Africa

(Hammer *et al*. 2000; Malaspina *et al*. 2001; Cinnioğlu *et al*. 2004; Semino *et al*. 2004) both main J1 and J2 clusters appearing in the Levant at Neolithic times (DiGiacomo *et al*. 2004). Notably, the collection of J* lineages characterized as not bearing the mutation at M172 apportion to the J1-M267 sister clade (Cinnioğlu *et al*. 2004), and appear to have spread into North Africa from Arabia (Nebel *et al*. 2002; Semino *et al*. 2004). Haplogroup J* (= J1-M267) lineages occur in both Jewish and other Semitic speaking non-Jewish populations in the Middle East (Nebel *et al*. 2001; Semino *et al*. 2004) and notably also in the Arabs of Oman (Luis *et al*. 2004). J1 is also present in Mediterranean Europe, especially Italy (3.9%, DiGiacomo *et al*. 2004), although with a more reduced diversity than in the Middle East. The high frequency of this haplogroup among Turkish Jews and non-Jews (Hammer *et al*. 2000; Nebel *et al*. 2001; Behar *et al*. 2004) may be a signature of influence from the Middle East. As for the proposed Neolithic diffusion hypothesis, it is important to note that haplogroup J1 is weakly represented in the Balkans. A maritime dispersal, probably associated with Greeks or Phoenicians, should alternatively be postulated if one considers its higher frequency along south Mediterranean coastal areas (DiGiacomo *et al*. 2004). The history of Jewish settlement in Iberia makes it plausible that one source of the haplogroup J1 in Portugal (which reaches 7% in the South) could be related to either Jewish or Arabian ancestry. Non-Jewish North Africans are also characterized by over 10% of J1-M267 (Semino *et al*. 2004) The Portuguese J1 microsatellite combined haplotypes (Table1) include an exact match at six STR loci to the Cohen Modal Haplotype (CMH, Thomas *et al*. 1998), and do not match the Arab modal haplotypes described by Nebel *et al*. (2002). This supports the view that Portuguese J1 lineages are not due to Arab gene flow, and could have entered the country independently. DiGiacomo *et al*. (2004) indicate a putative ancestral Y-STR haplotype for J1 in Europe that is also the most representative in the Middle East, and includes the CMH. In our sample we found 3 exact matches to this Y-STR pattern (2 in the Centre, 1 in the South) and four one-step matches again in Central and South Portugal. The allelic state of 3 YSTR loci (DYS388, DYS391 & DYS439 respectively) provides a metric to distinguish the CMH (16, 10, 12) and Arab (17, 11, 11) J1 lineages (Nebel *et al*. 2002; Arredi *et al*. 2004). We observed 3 individuals displaying a similar motif to the CMH haplotype, but for the remaining ones it is not possible to assign any association with the Arab motif, further supporting our hypothesis of a loose connection to Arabs. Alternatively, an earlier but unrecorded gene flow from North Africa, or by sea from the Middle East, could be considered as likely. J1 presents a heterogeneous distribution since its frequency is higher in the South and Centre than in the North of Portugal (x^2, $P < 0.01$). Jews are supposed to have arrived in Iberia at least in the 3rd century via North Africa (Tavares, 2000). Bring longer ago than the possible arrival time of the Jewish lineages, the divergence time of J1 in Central and South Portuguese populations (between 4.8 ky and 11.1 ky, Table 2) suggests that they were imported from distinct founding populations or even through different waves from different Middle-Eastern populations that had diverged much earlier elsewhere. J2 has a higher representation than its sister clade in Madeira, the Açores and South Portugal. Turkey and the Aegean areas are supposed to have acted as a secondary source for this originally Middle-Eastern haplogroup (DiGiacomo *et al*. 2004), from where it spread to Europe. An association with Greek and/or Phoenician eras should also be considered in Portugal.

Population structure and Principal Component Analysis

An AMOVA between populations pooled by geographic regions (mainland Portugal, the two Archipelagos of Madeira and Açores, and data from North Africa (Bosch *et al*. 2001)) attributes ~33% of variance to differences among groups, but only 1% to differences among populations within each group (overall FST value of 0.335, $P < .0001$). Population pairwise differences were all statistically significant ($P < 0.0001$). Excluding North Africans, the variance attributed to differences among insular and mainland populations drops to only ~2%. This amount of variance is mainly due to the lack of R1b in North Africa. These results are also evident in the PCA (Figure 2). The Portuguese populations reveal themselves as a highly heterogeneous group, clearly separated from other European populations (GD,

NO and BI, see Figure 2 for population codes) but also from North Africa and Mid-Eastern populations obviously denoting an intermediate status. Interestingly, both North Africans and Middle-Easterners join the Portuguese populations through the "Sephardim" Jews as indicated by the MST. The most likely lineage groups responsible for differences on the axis distinguishing European-Iberian-MidEast populations are E3b (a characteristic of NA) and R1b (typical of West Europeans). The latter haplogroup is almost absent in North Africa and Middle East.

Overall, the Portuguese show an affinity to Middle Eastern populations. So far, the degree of genetic contribution from peoples of Jewish, North African and sub-Saharan ancestry to the present day Portuguese has been uncertain. Our data suggests only minor influences from sub-Saharan males in Portugal and the Atlantic Islands, despite historical records indicating that slaves constituted at least 10% of the total population in Madeira and the South of the country in the 15th century. This contrasts mtDNA and HLA data, but provides genetic support to the view that mixing was highly asymmetrical by sex. The North African component at least for mtDNA, is mainly concentrated in the North of Portugal. The mtDNA and Y data indicate that the Berber presence in that region dates prior to the Moorish expansion in 711 AD. Our Y chromosome results are also consistent with a continuous and regular assimilation of Berbers in North of Portugal. This argues against previous interpretations of Moorish mediated contributions, based on Y chromosome data (Bosch *et al*. 2001; Pereira *et al*. 2000b; Cruciani *et al*. 2004) and provides an alternative view of an earlier Berber presence in the North of Portugal. Haplogroup J* distribution in Portugal runs opposite to haplogroup E3b2, corroborating the hypothesis that J1 lineages were not introduced with North African E3b2 lineages during the Arab/Berber expansion. In addition, the J1 lineages are distinct from the common Arab haplotype, consistent with an independent source, possibly Sephardim and/or other Near East peoples. Until now, the only evidence supporting the presence of Berbers in Iberia was the high frequencies of haplogroup U6 in Northern Portugal. Our data indicate that male Berbers, unlike sub-Saharan immigrants, constituted a long-lasting and continuous community in the country.

Note Added in Proof

While under review, two papers appeared on Y-chromosome profiles from the Açores (Montiel *et al*. 2005, Pacheco et al 2005). Montiel *et al*. (2005) found a haplogroup composition similar to ours but they recognize that a lack of information or resolution of haplogroups E and J prevents a better understanding of the putative origin of islanders (North African versus Europeans), which has been resolved in our research.

The second article (Pacheco *et al*. 2005) does not achieve a reasonable level of Y-chromosome discrimination and presents serious inconsistencies with the most recently updated phylogeny and nomenclature system of the YCC. There is an obvious mis-assignment of samples under P* (most of them are likely R1b3 if M269 had been tested) or K*. The lack of resolution is most obvious for E3b, which are pooled. A more correct clarification of the differential origin of this group could have be achieved only with the use of Y-STRs, as performed in our research.

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