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Echoes from Sepharad: signatures on the maternal gene pool of crypto-Jewish descendants

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The majority of genetic studies on Jewish populations have been focused on Ashkenazim, and genetic data from the Sephardic original source, the Iberian Peninsula, are particularly scarce. Regarding the mitochondrial genome, the available information is limited to a single Portuguese village, Belmonte, where just two different lineages (a single one corresponding to 93.3%) were found in 30 individuals. Aiming at disclosing the ancestral maternal background of the Portuguese Jewry, we enlarged the sampling to other crypto-Jewish descendants in the Bragança district (NE Portugal). Fifty-seven complete mtDNA genomes were newly sequenced and — in contrast with Belmonte — a high level of diversity was found, with five haplogroups (HV0b, N1, T2b11, T2e and U2e) being putatively identified as Sephardic founding lineages. Therefore — in sharp contrast with Belmonte — these communities have managed to escape the expected inbreeding effects caused by centuries of religious repression and have kept a significant proportion of the Sephardic founder gene pool. This deeper analysis of the surviving Sephardic maternal lineages allowed a much more comprehensive and detailed perspective on the origins and survival of the Sephardic genetic heritage. In line with previously published results on Sephardic paternal lineages, our findings also show a surprising resistance to the erosion of genetic diversity in the maternal lineages.

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INTRODUCTION

Sepharad, the traditional Jewish word for the Iberian Peninsula, became the name of the branch of the Jewish people who can trace their origin from there.¹ Their settlement in Iberia certainly occurred a long time ago, and the oldest archeological evidence found so far has a chronology of 390 CE (http://www.uni-jena.de/en/News/PM120525_Schrifttafel.html). Thus, the Jewish presence in Portugal precedes the nation's foundation in 1139 CE,² and several documents for the period 1279–1325 testify the presence of Jewish communes in the Bragança district, NE Portugal.³ The degree of tolerance toward these communities was variable during the subsequent years and, in the sixteenth century, Iberian Jews were a demographically non-negligible minority with very heterogeneous social status. However, during that period (first in the United Kingdoms of Castile and Aragon under the rule of Catholic Kings and, shortly after, also in Portugal), Jews were forced into either conversion or expulsion. The Portuguese edict of expulsion was, however, far different from the one issued in Spain. As Jewish permanence in the country was intended, it was followed by several contradictory measures, as the forced baptism of 20 000 Jews from all over the country, who were prepared for exile and the forbiddance of inquiries on religion, during 20 years, for the *New-Christians*; hence, a consented crypto-Judaism (the secret adherence to Judaism while publicly professing another faith) was installed.^{2,4,5} Although a decree to end the distinction between *Old* and *New-Christians* was issued in 1507, the inquisition was established in 1536 and the effective abolishment of the distinction would only happen three centuries later with the Pombaline law.²

As the inquisition mainly targeted crypto-Judaism, it was especially rampant in the most remote areas of Portugal, near the Spanish

border, such as Bragança and Belmonte. The persecutions reached Bragança region, Trás-os-Montes, in 1582–1583 causing numerous accusations and arrests from the very beginning.^{6–8} In the seventeenth and eighteenth centuries, the inquisitorial processes intensified and, as a result, there was a significant exodus to other countries, particularly of manufacturers and the merchant elite,⁹ many of them preserving connections with their original communities for a long period.⁷ The Jewish community of Bragança reappeared in the early twentieth century, gathering several families from the region, who had maintained their culture and religious secret practices for centuries. The estimated number of crypto-Jews at that time was around 700 to 800 people.⁷ Although the community was dissolved shortly after, a strong sense of belonging is still well alive today among the Jewish descendants.

Contemporary Jewish communities have been genetically analyzed both from population genetics and medical perspectives by means of uniparental and recombining markers,^{10–17} and more recently also through genome-wide approaches.^{18–23} However, only a few reports have been published on Sephardic and crypto-Jewish descendants.^{24–30}

Recent analysis of paternal lineages in Iberia points to a high proportion of Jewish ancestry.¹⁰ Nonetheless, this work assumes an oversimplified parental population scenario and recognizes 'alternative possible sources for lineages ascribed a Sephardic Jewish origin'.

Concerning Portugal, little information exists. For the Y chromosome, samples from Belmonte were included in the study from Adams *et al*¹⁰ but were pooled with non-Iberian Sephardic Jews; Nogueiro *et al*²⁷ found an unexpected high haplotype diversity for an isolated, small-size population, scattered over the Bragança district

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(NE Portugal). Concerning mtDNA, previous studies identified some lineages with signatures of Sephardic origins found in Jewish communities from Portugal (Belmonte), Bulgaria, Italy, Turkey, Yugoslavia, Greece, Netherlands, Surinam and Spain¹² and, more recently, South Texas, USA, and Mexico.^{31,32} Nevertheless, data from the Iberian Peninsula, which constitutes the original geographic source of these populations, can be considered scarce: in Portugal, the information available until now was restricted to a single village, where just two different mtDNA lineages were found.¹² Here, we are deepening preliminary results on control region (CR)³³ to obtain a better picture of the Portuguese Jewish maternal lineages (Figure 1), in order to scrutinize whether the low diversity found in Belmonte is indeed a general hallmark of their mtDNA pool.

MATERIALS AND METHODS

Population sampling and DNA analyses

We sequenced complete mtDNA genomes from 57 unrelated self-designated Jews (and recognized as such by the host community) from the Bragança district, the same samples already characterized for Y chromosome²⁷ and the mtDNA CR.³³ Sampling criteria and collection method, as well as DNA extraction, are described in Nogueiro *et al.*²⁷ The study was approved by the Ethics Committee of the University of Porto (N°02/CEUP/2012) and appropriate informed consent was required from all subjects. Full mtDNA sequences were obtained using the protocol described in Ramos *et al.*^{34,35} Sequences were aligned against the revised Cambridge Reference Sequence GenBank accession number NC_012920.1³⁶ using Genious software version 5.5.8 (<http://www.geneious.com>), variants were annotated following the HGVS rules (<http://www.hgvs.org/mutnomen/>). Haplogroups were classified following the updated mtDNA phylogeny, PhyloTree, mtDNA tree Build 16³⁷ (<http://www.phylotree.org/>) and assigned haplotypes were submitted to the EMPOP database³⁸ (<http://empop.org/>). The accession number for the sequences reported in this paper is EMP00619.

Data analysis

Based on the mtDNA haplotypes found, standard and molecular diversity indices were estimated using ARLEQUIN software v3.5.1.3.³⁹ For comparative

purposes, original and publicly available data for mtDNA HVRI and HVRII (16 024–16 365 bp and 72–300 bp, respectively) were also compiled for 766 Jewish samples¹² and 884 Western Iberian non-Jews.^{40–42}

Differences in haplogroup composition, defined according to CR polymorphisms (16 024–16 400 bp for HVRI, and 073–340 bp for HVRII), among the analyzed Jews from Bragança and a large sample of the Portuguese host population,⁴⁰ were assessed through Fisher's exact tests implemented in IBM SPSS software.⁴³

Median-joining networks⁴⁴ (<http://www.fluxus-engineering.com/sharenet.htm>) of the (16 024–16 400 bp for HVRI, and 073–340 bp for HVRII) were constructed, for comparative purposes, using a compilation of original data on the Portuguese Jewish communities with publicly available data sets on the Portuguese host population,⁴⁰ Mirandese⁴¹ and NW Spain.^{41,42}

Complementarily Median-joining networks using complete sequences were initially constructed and drawn, and then — for better visualization — redrawn for the defined putative Jewish founding lineages together with available complete sequences from public databases NCBI-GeneBank (<http://www.ncbi.nlm.nih.gov/genbank/>), empop.org (<http://empop.org/>), mtDNA community (<http://www.mtDNAcommunity.org/>) and from Zhang *et al.*⁴⁵ work.

RESULTS AND DISCUSSION

Haplotype diversity

Using complete mtDNA sequences, we were able to identify 45 haplotypes and 32 different haplogroups; detailed information is shown in Supplementary Table 1. We estimated global haplotype and nucleotide diversity to be just slightly lower than in the Portuguese host population, and much higher than in the Portuguese Jewish community from Belmonte (Table 1). Theta k values (θ_k) were also calculated for both the complete mtDNA as well as for the CR (Table 1). As expected, θ_k obtained for the CR is lower than when calculated for the complete mtDNA sequences, emphasizing the importance of studying complete mtDNA sequences in order to obtain more precise information on the maternal lineage diversity, as well as a more accurate estimate of the female effective population size.

Estimated θ_k value for the total sample is similar to the one usually found in Sephardic Jews from other countries, but exceptionally high if compared with Belmonte (Table 1), and also similar to the non-Jews from the neighboring region of Miranda.⁴¹ It is worth mentioning that the estimated number of putative female founders in each village of the Bragança district is higher than the current number of Jewish families. In fact, the samples included in the present work represent, if not the whole, the vast majority of the extant lineages, as increasing the sample size to reach predicted levels of saturation^{46,47} would lead to the inclusion of closely related individuals.

The CR haplogroup frequencies of the Jews from Bragança together with the Portuguese host population and other geographical neighbors (Miranda⁴¹ and NW Spain^{41,42}) are presented in Supplementary Table 2. Differences in haplogroup composition were assessed through Fisher's exact test among the Jews from Bragança and the Portuguese populations⁴⁰ and Mairal *et al.*⁴¹ We focused on Jewish haplogroups that showed higher frequencies when compared with the Portuguese population, as they are likely to represent Sephardic origins (in accordance with Behar *et al.*)¹² Statistically significant differences were found for haplogroups HV0b (12.3% vs 0.0%; $P = 0.000$), N1 (7.0% vs 1.3%; $P = 0.014$), T2b11 (7.0% vs 0.0%; $P = 0.000$), T2e (5.3% vs 0.7%; $P = 0.019$) and U2e (7.0% vs 1.3%; $P = 0.014$). These putative Jewish founding lineages account for 38.6% of the total sampled gene pool.

Sephardic lineages

The complex history of Jewish populations along the Mediterranean area is reflected on the haplogroup diversity observed in extant

Figure 1 Geographic location of the sampling area in the Iberian Peninsula context. The zoomed area in the Iberian Peninsula represents the Portuguese district of Bragança and the Spanish province of Zamora (bold letter). Grey dots indicate the location of the sampling municipalities (uppercase) and villages (lowercase).

Table 1 Diversity indices and neutrality test results calculated for the complete mtDNA haplotypes found in the samples from Bragança district Jews (considering the total sample as well as each village separately)

Population	N	K (% K)	S	HD ± SD	$\pi \pm SD$	θ_K (95% CI)
<i>Complete mtDNA haplotypes</i>						
Portuguese Jews						
Total sample	57	45 (78.95)	286	0.987 ± 0.007	0.002 ± 0.001	96.747 (53.910;179.708)
Bragança	11	11 (100.00)	83	1.000 ± 0.039	0.001 ± 0.001	*
Argoselo	24	18 (75.00)	164	0.967 ± 0.024	0.002 ± 0.001	31.047 (13.714;73.620)
Carção	8	6 (75.00)	94	0.893 ± 0.111	0.002 ± 0.001	9.231 (2.613;34.341)
Vilarinho dos Galegos	13	11 (84.62)	108	0.974 ± 0.039	0.001 ± 0.001	30.896 (9.645;108.127)
<i>Control region—HVRI (16 024–16 365 bp) and HVRII (72–300 bp)</i>						
Portuguese Jews						
Total sample	57	35 (61.40)	61	0.967 ± 0.012	0.014 ± 0.008	37.616 (22.328;63.866)
Bragança	11	9 (81.82)	20	0.946 ± 0.066	0.011 ± 0.006	20.730 (6.297;73.767)
Argoselo	24	14 (58.33)	36	0.931 ± 0.033	0.015 ± 0.008	13.188 (6.121;28.635)
Carção	8	5 (62.50)	22	0.857 ± 0.108	0.016 ± 0.010	4.694 (1.394;16.076)
Vilarinho dos Galegos	13	11 (84.62)	27	0.974 ± 0.039	0.012 ± 0.007	30.896 (9.645;108.128)
Belmonte ¹²	30	2 (6.67)	6	0.129 ± 0.115	0.001 ± 0.001	0.279 (0.065;1.097)
Other Sephardic and non-Ashkenazi Jews ¹²						
Bulgaria	71	46 (64.79)	70	0.982 ± 0.007	0.012 ± 0.007	55.477 (34.470;90.159)
Turkey	123	85 (69.11)	109	0.985 ± 0.005	0.013 ± 0.007	120.394 (82.638;176.996)
Mediterranean	19	18 (94.74)	46	0.994 ± 0.019	0.016 ± 0.009	158.779 (40.578;673.927)
Algeria	20	15 (75.00)	45	0.968 ± 0.029	0.013 ± 0.007	25.594 (10.596;65.022)
Libya	83	36 (43.37)	63	0.922 ± 0.019	0.013 ± 0.007	23.631 (15.104;36.717)
Morocco	148	80 (54.05)	92	0.979 ± 0.005	0.012 ± 0.006	70.307 (50.484;97.941)
Tunisia	36	25 (69.44)	43	0.971 ± 0.015	0.012 ± 0.007	34.939 (18.044;69.325)
Kurdistan	12	9 (75.00)	28	0.939 ± 0.063	0.013 ± 0.007	14.686 (4.971;45.966)
Syria	4	3 (75.00)	13	0.833 ± 0.210	0.012 ± 0.009	3.766 (0.774;18.233)
Iran	82	43 (52.44)	76	0.971 ± 0.008	0.016 ± 0.008	35.821 (23.098;55.513)
Iraq	134	48 (35.82)	79	0.950 ± 0.009	0.016 ± 0.008	26.344 (18.151;37.920)
Western Iberia non-Jews ^{40–42}						
Portugal	549	353 (64.30)	171	0.986 ± 0.001	0.013 ± 0.007	425.966 (356.006;510.438)
Miranda	121	57 (47.11)	81	0.973 ± 0.007	0.011 ± 0.006	41.666 (28.672;59.784)
NW Spain	214	129 (60.28)	117	0.984 ± 0.004	0.013 ± 0.007	136.409 (103.182;180.727)

K: Number of different haplotypes; S: number of polymorphic sites; HD: haplotype diversity; π : nucleotide diversity averaged over loci; θ_K : theta estimator based on the number of different lineages; * cannot be computed when all haplotypes are different.

populations. An overview of their genetic composition is shown in Figure 2.

Until recently, haplogroup HV0, the ancestor of HV0b, was thought to have originated in Eastern Europe soon after the Last Glacial Maximum, having afterwards spread from there, following an east-west axis throughout Europe.⁴⁸ However, the low gene and nucleotide diversities found in this region and in Northern Africa, compared with the ones found in North-Central Europe, seem to exclude Eastern Europe as a possible focus of expansion.^{49,50}

In the Iberian Peninsula, HV0 is a rare haplogroup, found at low frequencies in NW Spain, Zamora (4.7%),⁵¹ a Spanish province geographically close to Bragança. Because of a lack of resolution, it is not possible to differentiate haplogroup V inside HV0 in the Portuguese population.⁴⁰ The same happens with the study of Mairal *et al*⁴¹ focused on a linguistically isolated population, Miranda do Douro, from the same geographical region (NE Portugal). Nevertheless, and considering the whole HV0 branch (haplogroup V included), we observe a frequency of 14% for this haplogroup, higher than the frequencies found in the Portuguese and in the Miranda populations (5.2% and 8.3%, respectively). Moreover,

although HV0 has very low frequencies in eastern European Jewish Ashkenazim,⁵² we found a high prevalence of this lineage within the Jewish community of Bragança. Our results are in accordance with a previous report¹² describing HV0 as a Jewish founding lineage in Portugal, as in Belmonte 93.3% of the analyzed mtDNA genomes could be traced back to a single female, carrying an mtDNA within haplogroup HV0b. Considering solely the CR, in a comparison between samples from Bragança Jews (present work), Portugal,⁴⁰ Miranda⁴¹ and NW Spain,^{41,42} our sample does not share any haplotypes with the Portuguese population (Supplementary Figure 1).

The most parsimonious tree of HV0b sub-clade, including all available mitochondrial complete sequences (Supplementary Figure S2), shows that Bragança HV0b lineage shares a common private variant m.8520A>G with Belmonte.¹² Moreover, the Bragança samples cluster together, sharing a more recent variant not previously described m.10644G>A, which seems to have arisen locally. As no ethnic information is available for the remaining HV0b samples, and no introgression seems to have occurred with the Portuguese host-population, our results support the hypothesis that at least the HV0b-8520G haplotype is a Sephardic Jewish founding lineage.

Figure 2 mtDNA haplogroup composition of the Sephardic Portuguese Jews (Bragança and Belmonte) and other Sephardic and non-Ashkenazi Jews. Haplogroups were defined for mtDNA HVRI and HVRII, 16 024–16 365 bp and 72–300 bp, respectively, according to Phylotree mtDNA tree Build 16 (other Sephardic and non-Ashkenazi Jews represent the same population as Table 1).

The high frequency of haplogroup N1 found within the Jewish descendants of Bragança (7%) differentiates this community from the general Portuguese population, which displays an average frequency of just 0.8%. Haplogroup N1, along with macrohaplogroups M and R, is one of the founder lineages of the Eurasian settlement around 50–63 kya⁵³ and comprises two major clades, N1a and N1b. N1a dates to approximately 20 kya and is a relatively rare haplogroup in Europe, reaching higher frequencies in Eastern Africa and in the Arabian Peninsula.⁵⁴ Although this haplogroup was found in Neolithic and Mesolithic skeletal remains from Central and Northwest Europe,^{55–57} it was absent in previous studies on Iberian Neolithic populations, suggesting a rather uneven genetic and geographical spread of this lineage across Europe.^{58,59} However, more recently, Gamba *et al*⁶⁰ detected this lineage in Neolithic northeast Iberia, evidencing a possible scenario of several Neolithic colonization events along the Mediterranean, from the Near East. The samples from Bragança cluster into sub-haplogroups N1a and N1b and similarly to what was observed for HV0, NJ networks of N1b revealed no CR haplotype sharing with the general Portuguese population, except for four individuals from Miranda, an isolated population from the same geographical area as well as with two samples from Zamora^{41,42} (Supplementary Figure 3).

Within N1b, the N1b2 haplogroup is considered as a founding lineage in Ashkenazi Jews with a ‘Hebrew/Levantine’ origin.⁶¹ However, an alternative European origin encompassing an assimilation of this lineage into the founding Ashkenazi population along the north Mediterranean coast has been recently proposed.⁵² Unexpectedly, we found no samples belonging to the N1b2 haplogroup among the Bragança Jews — all N1b samples fall inside the N1b1 sister-clade (N1b1a2 and N1b1a5) and the N1a1a1a2 sub-haplogroup. According to the most parsimonious tree for complete sequences (Supplementary Figure 4), within sub-haplogroup N1b1a2, a transition at position m.204T>C defines a cluster with three samples, one from this study along with one from Zamora and another with an unknown origin. To further investigate this cluster, a search for similar CR haplotypes was performed in the EMPOP database and only three sequences were found. Two of them were from the same geographical area, namely Zamora (the same sample from Supplementary Figures 3 and 4) and Miranda do Douro,^{41,42}

both places with a well-documented history of Jewish presence. The remaining one was interestingly also from a Jew, an Ashkenazi from Hungary,⁶² thus this transition could be identified as a Jewish, albeit not exclusively Sephardic founding lineage.

In the N1b1a5 sub-clade, the only matches found at EMPOP database, including the transition at position m.16311T>C, were with five samples from Miranda do Douro⁴¹ and one from Uzbekistan,⁶³ about which no further information was available. As neither the Bragança Jews nor the Mirandese population share haplotypes with the Portuguese population, and given their geographic proximity in a rather remote and isolated area, as well as the fact that there was an organized Jewish community in Miranda, at least from the twelfth century on,⁴ these individuals could easily also be Jewish descendants who lost memory of their origins or have not been detected as such, as in Mairal *et al*⁴¹ sampling did not include ethnical criteria.

Regarding the N1a1a1a2 branch, the transitions at positions m.150C>T, m.4501C>T and m.11977C>T defines a cluster that includes the Bragança Jews (with no shared haplotypes with the Portuguese population). Lineages carrying the m.150C>T transition were not found in available databases and thus represent what could be a specific feature of the Sephardic Jews from NE Portugal.

Considering that haplogroup N1 is very rare in contemporary European populations, the low number of complete available sequences from the Near East, as well as the poor definition obtained so far for this haplogroup in the Iberian Neolithic samples⁶⁰ (N*), it seems at the moment hazardous to conclude whether the lineages found here are relics brought from the Near East by the first Jewish diasporas or were assimilated into the ancestral Iberian Jewish population in Europe.

The T haplogroup in the Jewish population of Bragança is much more frequent than in Portugal⁴⁰ and pertains to the T2 branch, with two different sub-clades: T2b11 and T2e1. It has been proposed that haplogroup T, which diverged from the macro haplogroup JT around 40 kya, underwent a dramatic expansion from its geographical origin in the Near East into Europe before the Neolithic period.⁶⁴ An European indigenous dispersion has been proposed for T2 sub-clades, namely the T2b and T2e paraphyletic lineages.⁶⁴ Recently, several mitochondrial lineages, defined as the ‘Neolithic

package' including T2, were also proposed to explain a rapid change from hunter-gathering to farming, in the Early/Middle Neolithic, indicating a genetic flow from the Near East, Anatolia, and the Caucasus into Europe.⁶⁵ T2b is the most common lineage among T2, reaching higher frequencies in Western Europe than in the Near East.⁶⁴ Considering T2b branch, no haplotypes were shared between Jews and Portuguese individuals for the CR, demonstrating the absence of reciprocal introgression of T2b lineages between these two populations (Supplementary Figure 5).

Regarding the complete mtDNA genome, the Bragança samples nest in the branch defined by the transition at position m.9181A>G, along with a sample from Spain (Supplementary Figure 6). Interestingly, the Spanish sample is from the Zamora province (the same sample from Supplementary Figure 5) which, as stated above, is a geographically neighboring region to Bragança, at the Portuguese-Spanish border, and has also a well-documented Jewish past, with a well-established community dating back to 1259 CE.⁶⁶ Hence, the m.9181A>G variant within the T2b11 branch seems to be a regionally specific variant within the T2b11 branch, reflecting a Sephardic signature, given its absence from public databases. The additional motif m.4902A>G-m.8557G>A-m.16167C>T-m.16261C>T is only present among the Portuguese Jews.

Haplogroup T2e is spread throughout southern Europe and the Mediterranean, but is also found in Scandinavia, Egypt, the Near East and Arabia⁶⁴ and has been described as a founding lineage in the Bulgarian Sephardic community.¹² More recently, it was also found among Sephardic descendants in Turkey, in Northern Mexico and south Texas, USA, being interpreted as a 'Sephardic signature' inside haplogroup T.^{31,32} According to the more recent nomenclature in PhyloTree built 16,³⁷ T2e's sub-branches, T2e1 and T2e1a, are defined by the variants m.41C>T and m.2308A>G, respectively. Considering the growing number of complete mtDNA sequences available at this time, it was possible to define a new sub-haplogroup T2e1a1, based on m.15499C>T variant. It is important to notice that variants at position m.41C>T are exceptionally infrequent along the mitochondrial phylogeny. Position m.41C>T seems quite unstable inside the new defined T2e1a1 sub-branch (Supplementary Figure 6). Considering a back-mutation at position m.41C>T and taking all mtDNA genomes available to date, the m.2308A>G-m.15499C>T-m.16114C>T-m.16192C>T motif defines one of the branches (T2e1a1a1) of Portuguese Sephardic signature within T2e, previously reported for a Sephardic sample³² from Turkey, one of the Mediterranean countries that received exiled Iberian Jews. Two other samples shared this same motif, one from Mexico and another one from Texas. Without m.16114C>T, there was also a sample from Mexico. All these samples are from the study of Bedford *et al*³¹ and although the ethnicity of these three samples is not known, their Iberian ancestry seems consistent with a Sephardic origin. The Bragança Jews present two further distinct variants in the Sephardic signature, m.13135G>A and m.7133C>T, the latter not described until now. In conclusion, the back-mutation at position 41 inside this new sub-haplogroup T2e1a1 is entirely associated, so far, with Sephardic or probable Sephardic ancestry.

Inside the T2e1 branch, a new sub-haplogroup T2e1b (Supplementary Figure 6), defined by the presence of variant m.9181A>G, has been proposed in PhyloTree built 16.³⁷ T2e1b is supported by 11 complete mtDNA sequences, including the Bragança Jews. Except for two samples from mtDNA Community database,⁶⁷ without information concerning their ethnicity, all the remaining nine individuals are Jews, Sephardim or Ashkenazim.

The last putative Jewish Sephardic founding lineage belongs to haplogroup U2, particularly its European U2e sub-haplogroup. This lineage is extremely rare in modern European populations and was recently found in Late Neolithic⁶⁸ and Iron Age populations, especially in the north of Europe.⁶⁹ It also appears with rather high frequencies in Croatia,⁷⁰ western Eurasia⁷¹ and the Basque country.^{50,72} All U2e individuals in this study belong to the U2e1a1 sub-clade. A high frequency of haplogroup U2 was found in the Bragança Jews (7.0%), whereas in Portugal it only reaches a frequency of 1.3%, being found absent in the Bragança district.⁴⁰ U2e1 lineages found in the Bragança Jewish population are shared with two individuals from Miranda; accordingly, this is the only putative Jewish founding lineage shared with the Portuguese population, when only the CR is looked upon (Supplementary Figure 7).

However, when considering complete mtDNA sequences, the Bragança clade is isolated from all other U2e1a1 haplotypes, except for another Jewish Ashkenazi sample from Moldova (Supplementary Figure 8), sharing the motif m.8014A>G—m.13708G>A, seemingly Jewish specific.

The strong founder effect previously reported in the maternal lineages of Portuguese Jews from Belmonte¹² cannot be considered a general trait of the Sephardic groups in Portugal. In fact, NE communities, despite preserving a distinctive lineage profile, displayed diversity levels similar to the host population. Remarkably, for two of the founder lineages (T2e1b and U2e1a), defined by the complete mitochondrial genome, the shared sequences belong to both Sephardic as well as Ashkenazi Jews. Two possible scenarios could accommodate this finding: either the defining variants for each branch could have arisen before the separation between the two Jewish groups; or there may have been recent introgression of Sephardic lineages into Ashkenazim communities in the north of Europe. More complete sampling and complete sequences will contribute in the clarification of which one is more likely. In any case, it must be said that although not frequent, marriages between the two communities occurred (especially) in the sixteenth and seventeenth centuries, namely among the elite sugar traders, with the descendants assimilated into the Ashkenazi community.^{73,74}

The estimates of both the diversity levels and the number of female effective-population founders point at a stable size of the studied populations, in agreement with previous findings for the male counterparts.²⁷ As expected, it was possible to identify some Sephardic signatures as well as signs of introgression from the host non-Jewish population. This gene flow seems to have been mutual as the maintenance of the observed diversity levels can only be explained by a number of founders that is higher than the Jewish families, which can still be traced today.

A study of recombinant markers is now required to unveil the reproductive strategies that have sustained this ancestral signature along with a moderate degree of admixture with the host population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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