



Research article

Y-chromosome analysis recapitulates key events of Mediterranean populations

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ABSTRACT

The remarkable geographical situation of the Mediterranean region, located between Europe, Africa, and Asia, with numerous migratory routes, has made this area a crucible of cultures. Studying the Y-chromosome variability is a very performant tool to explore the genetic ancestry and evaluate scenarios that may explain the current Mediterranean gene pool. Here, six Mediterranean populations, including three Balearic Islands (Ibiza, Majorca, and Minorca) and three Southern Italian regions (Catanzaro, Cosenza, and Reggio di Calabria) were typed using 23 Y-STR loci and up to 39 Y-SNPs and compared to geographically targeted key reference populations to explore their genetic relationship and provide an overview of Y-chromosome variation across the Mediterranean basin. Pairwise R_{ST} genetic distances calculated with STRs markers and Y-haplogroups mirror the West to East geographic distribution of European and Asian Mediterranean populations, highlighting the North-South division of Italy, with a higher Eastern Mediterranean component in Southern Italian populations. In contrast, the African populations from the Southern coast of the Mediterranean clustered separately. Overall, these results support the notion that migrations from Magna Graecia or the Byzantine Empire, which followed similar Neolithic and post-Neolithic routes into Southern Italy, may have contributed to maintaining and/or reinforcing the Eastern Mediterranean genetic component in Southern Italian populations.

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1. Introduction

In recent years, the Y-chromosome has been extensively studied to explore human migrations [1]. Because it is inherited from the father, Y-chromosome polymorphisms largely avoid recombination. This maintains a straightforward record of male ancestry over time, making them useful for studying the geographical distribution of populations globally [2]. Combined with the widespread practice of patrilocality in many societies, the Y-chromosome holds significant biogeographical importance [2,3].

Single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) are the primary markers under study [3]. Y-SNPs, which mutate at a rate of around $2 \cdot 10^{-8}$ per locus per generation, are relatively stable and their combination determines haplogroups, which are useful for studying human migrations and inferring their geographic origin [1,4,5]. Conversely, Y-STRs, which mutate at a rate of $1 \cdot 10^{-3}$ per base per generation, offer greater discriminatory power among unrelated paternal lineages, making them valuable in forensic genetics. Moreover, Y-STRs haplotypes are valuable tools in population structure studies and allow the study of the divergence within a haplogroup [1,2,5].

Population movements can imprint themselves on the human genome and affect genetic diversity [6]. Consequently, examining the gene pool of contemporary populations can reveal information about past migratory events [7]. The selection of suitable genetic markers on the Y-chromosome depends on the time frame of the events being studied [7,8]. Y-SNPs, with their low mutation rates, are suitable for analyzing ancient population movements. However, for more recent events where greater detail is necessary, Y-STRs, with their higher mutation rates and greater variability, are more useful [7,9]. Hence, the study of both types of markers provides an overview in which both ancient and recent events that have taken place are examined simultaneously.

Studying the genetics of human populations in the Mediterranean region is particularly intriguing because of its unique geographic position, serving as a crossroads between Europe, Africa, and Asia. This location has benefited from maritime expansions, making it a migratory route of great relevance [10]. The rich historical background of the Mediterranean basin, characterized by continuous migrations of diverse people and cultures, has transformed it into a cultural and genetic melting pot. Although the genetic variation of the current Mediterranean populations probably is a consequence of the Neolithic demographic diffusion in this region approximately 9000 years before present (YBP), posterior events, including colonization, expansions, isolations, bottleneck processes, admixtures, etc., may also have contributed to this gene pool [10–13].

For this reason, Y-chromosome variation is well-defined in many Mediterranean populations, which have been the subject of numerous studies aiming to reconstruct their genetic history [12–20]. However, there are still some populations within this area, such as the Balearic Islands in Eastern Spain and the Calabria Region in Southern Italy, which are of great value because of their location and their variable cultural isolation, but for which not much information is available in the Y-chromosome haplotype reference database (YHRD). The study of these populations may shed light on questions such as the genetic impact of the Crown of Aragon in Southern Italy, the Hellenic contribution in the Mediterranean, or the numerous movements from the Middle East towards Southern Europe since the Neolithic expansion, providing an overview of Y-chromosome variation across the Mediterranean.

Here, an extensive analysis of the Y-chromosome pool in six Mediterranean populations (Fig. 1), including three Balearic Islands (Ibiza, Majorca, and Minorca) and three Southern Italian regions (Catanzaro, Cosenza, and Reggio di Calabria) was carried out. We



Fig. 1. Populations under study, including the Balearic Islands of Ibiza, Majorca, and Minorca, and the Southern Italian regions of Catanzaro, Cosenza, and Reggio di Calabria.

analyzed 23 Y-STR loci and 39 Y-SNPs to increase the Y-STR haplotype database of these populations, which means an improvement in forensic genetics. Additionally, we aim to evaluate the genetic makeup of these communities, their relationship with their close neighbors and the effect of their migration histories.

2. Material and methods

2.1. Sample collection and DNA isolation

The samples were collected from 461 unrelated males belonging to six populations in the Mediterranean area (Fig. 1), including three Spanish populations from the Balearic Islands: Ibiza (N = 102), Majorca (N = 99), and Minorca (N = 62), and three Southern Italian populations: Catanzaro (N = 60), Cosenza (N = 37), and Reggio di Calabria (N = 101). The study received approval from the General Directorate of Research + Development + Innovation of the Government of the Balearic Islands, Spain (Ref. AAEE procedure 12099/2003) and complied with Italian Law D.lgs. 196/2003. All participants provided informed consent prior to inclusion in the study, adhering to the ethical guidelines of the Declaration of Helsinki for the protection of human subjects. Additionally, participants gave informed consent to the use of their samples in subsequent population-based studies. DNA extraction was performed using QIAamp spin columns (Qiagen, Hilden, Germany) according to the manufacturer's instructions. NanoDrop™ One (ThermoFisher Scientific, MA, USA) was used for DNA quantitation. Once quantified, DNAs were diluted in Milli-Q water to 1 ng/μL and stored at -20 °C.

2.2. Reference populations

For comparison with the Y-STRs analyzed in this study, data from 32 geographically targeted key reference populations were used (Supplementary Fig. 1). Supplementary Table S1 provides details on the geographical locations, population abbreviations, number of individuals, and references.

2.3. Y-STR haplotyping

A total of 23 Y-STR loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS635, Y-GATA H4, DYS576, DYS481, DYS549, DYS533, DYS570, and DYS643) were analyzed. For this purpose, the 17 to 23 mini Y-STR panel was applied, as previously described [21], when samples had 17 Y-STR loci available (previously analyzed with AmpFlSTR Yfiler system, AB/LT/TFS, MA, USA). When samples had only 12 Y-STR loci available (previously analyzed with PowerPlex® Y System, PPY, Promega Corporation, WI, USA), the 12 to 23 multiplex panel described in Navarro-López et al. [22] was applied.

In both cases, PCR was carried out using a GeneAmp9800 (AB/LT/TFS) under the same cycle conditions as the 17 to 23 mini Y-STR panel [21]. The resulting amplicons were then separated on an ABI Genetic Analyzer 3130 (AB/LT/TFS) with the GeneScan 500 LIZ (AB/LT/TFS) serving as the internal size standard. Fragment lengths were determined using GeneMapper v4.0 (AB/LT/TFS). Allelic nomenclature adhered to the guidelines of the International Society for Forensic Genetics (ISFG) (www.isfg.org).

2.4. Accession numbers

Haplotypes for all the individuals of the six Mediterranean populations are included in the Y-STR Haplotype Reference Database (YHRD) at <https://yhrd.org/> under the following accession numbers: Ibiza YA003484-2; Majorca YA005613 Y23; Minorca YA006026 Y23; Catanzaro YA003493 Y23; Cosenza YA003492 Y23; Reggio di Calabria YA003490 Y23.

2.5. Y-SNP analysis

Y-STR haplotypes were used to predict their most likely haplogroup using the Nevgen Y-DNA Haplogroup Predictor (<https://www.nevgen.org/>). Due to the concerns that have been raised about the accuracy of Y-haplogroup predictors, tentative haplogroups obtained with Nevgen were then confirmed through the analysis of 39 bi-allelic markers (CT-M168, C-M130, DE-M145, E-P170, E1b1b-M215, E1b1b-V13, G-M201, G2a2-L1259, H1a-M69, IJ-P126, I-M258, J1a-Z2214, J2-M172, J2a-M410, J2b-CTS488, K-M9, L-M11, N-M231, O-M175, P1-M45, Q-M242, R-M207, R1b-M269, R1b-U106, R1b-S116, R1b-DF27, R1b-U152, R1b-M529, R1b-L238, R1b-DF19, R1b-Z196, R1b-L881, R1b-L617, R1b-Z220, R1b-M153, R1b-M167, R1b-S68, R1b-DF17 and T-M272). The analysis was performed using High Resolution Melt (HRM) and Sanger sequencing (Supplementary Table S2) or SNaPShot mini sequencing as previously described [23,24]. The Y-SNP markers were systematically scored to establish the Y-haplogroup of each sample. The assignment and naming of Y-SNP haplogroups followed the guidelines of the International Society of Genetic Genealogy (ISOGG v15.73), accessible at <https://isogg.org/> and can be found in Supplementary Table S8.

2.6. Statistical analysis

Using the software Arlequin v3.5.2.2 [25], allele frequencies, single-marker genetic diversity (GD), haplotype diversity (HD), and different, unique, and population-specific haplotypes were assessed. The DYS385a/b alleles were treated as haplotypes in these

calculations. To analyze Mediterranean Y-chromosomal distribution patterns, the global discrimination capacity (DC) was determined by dividing the number of different haplotypes by the total number of individuals in each population (Supplementary Table S1). Since not all populations collected in the literature had the same number of loci typed, two groups were made for phylogenetic analysis, one including populations with 17 markers and the other with 23 Y-STRs. Pairwise genetic distances (R_{ST} values) for Y-STRs and corresponding P -values were computed using the AMOVA & MDS tool provided by the YHRD (<https://yhrd.org/>). The Y-STR frequency distributions of the Ibiza, Majorca, Minorca, Catanzaro, Cosenza, and Reggio di Calabria populations were evaluated within 32 geographically targeted reference populations.

Pairwise comparisons between populations were assessed for significance at a level of 0.01 using 10,000 permutations. To mitigate the risk of false positives and type I statistical errors, the Bonferroni correction was applied ($\alpha = 0.05/\{n \times (n-1)/2\}$; n = number of populations). Haplotype diversity calculations excluded DYS385 due to the inability to distinguish alleles at the DYS385a and DYS385b loci with the commonly used Y-STR PCR primers. The number of repeats at DYS389II was determined by subtracting the number of repeats at DYS389I. Moreover, any samples containing non-consensus alleles were excluded from the R_{ST} calculations.

Next, multidimensional scaling (MDS) was performed to explore population relationships (Supplementary Table S1). The MDS plot was generated with PAST v4.04 [26] based on the R_{ST} pairwise values.

Regarding the Y-SNP haplogroups, the frequency within each population under study was determined by dividing the count of each haplogroup by the total number of individuals in that population. Each of the six populations was considered individually, and their clustering into Spanish populations (Ibiza, Majorca, and Minorca) and Italian populations (Catanzaro, Cosenza, and Reggio di Calabria).

3. Results

3.1. Allelic frequencies and haplotypes

Supplementary Table S3 summarizes the allele frequencies and gene diversity (GD) of the 23 Y-STRs loci for the six Mediterranean populations. Supplementary Table S4 presents the 23-loci haplotypes of the individuals from Ibiza ($N = 102$), Majorca ($N = 99$), Minorca ($N = 62$), Catanzaro ($N = 60$), Cosenza ($N = 37$), and Reggio di Calabria ($N = 101$).

3.2. Population genetic parameters

Population genetic parameters of the studied populations using the PPY 12-loci, Y-filer 17-loci, and PPY23 23-loci haplotypes are compiled in Table 1. As anticipated, the values for the total haplotypes, unique haplotypes, population-specific haplotypes, haplotype diversity, and discrimination capacity increased with the inclusion of additional loci. When PPY23 haplotypes were considered, the six Mediterranean populations exhibited high levels of haplotype diversity, with values close to 1.00, and the discrimination values were in the range of 0.8710–0.9730.

The enhancement to the 23-loci level of the PPY23 system compared to the 12-loci of the PPY and 17-loci of the Y-filer systems

Table 1

Diversity parameters obtained for the populations analyzed. N = sample size, HD = haplotype diversity, DC = discriminatory capacity.

Population	Ibiza ($N = 102$)	Majorca ($N = 99$)	Minorca ($N = 62$)	Catanzaro ($N = 60$)	Cosenza ($N = 37$)	Reggio di Calabria ($N = 101$)
PowerPlex Y 12-loci						
Different haplotypes	55	90	47	55	33	87
Unique haplotypes	33	84	37	48	30	74
Population specific haplotypes	48	77	36	49	29	82
HD	0.9802 ± 0.0046	0.9973 ± 0.0021	0.9889 ± 0.0054	0.9972 ± 0.0036	0.9925 ± 0.0088	0.9970 ± 0.0019
DC	0.5392	0.9091	0.7581	0.9167	0.8919	0.8614
Yfiler 17-loci						
Different haplotypes	78	93	49	56	35	89
Unique haplotypes	61	87	38	52	33	78
Population specific haplotypes	77	91	49	55	34	89
HD	0.9936 ± 0.0025	0.9988 ± 0.0017	0.9921 ± 0.0044	0.9977 ± 0.0035	0.9970 ± 0.0071	0.9974 ± 0.0018
DC	0.7647	0.9394	0.7903	0.9333	0.9459	0.8812
PowerPlex Y23 23-loci						
Different haplotypes	91	96	54	56	36	93
Unique haplotypes	81	93	46	52	35	86
Population specific haplotypes	90	95	54	56	36	93
HD	0.9977 ± 0.0018	0.9994 ± 0.0016	0.9958 ± 0.0037	0.9977 ± 0.0035	0.9985 ± 0.0067	0.9982 ± 0.0018
DC	0.8922	0.9697	0.871	0.9333	0.973	0.9208

improves discrimination resolution across all six populations, with particularly notable benefits observed in the Ibiza and Minorca groups. In Catanzaro, the haplotype diversity and the discrimination capacity increase over PPY12, but no improvement is found when comparing these values obtained with Y-filer with those obtained with PPY23.

3.3. Y-STR genetic distances in Mediterranean populations

Supplementary Table S5 includes pairwise R_{ST} genetic distances and the corresponding P -values among Ibiza, Majorca, Minorca, Catanzaro, Cosenza, Reggio di Calabria, and 32 other Mediterranean populations. Various analyses were conducted across different population sets to explore potential scenarios that may contribute to the current genetic diversity within the Mediterranean region.

On the one hand, the six Mediterranean populations under study were analyzed with Aragon and Barcelona to determine the potential genetic impact of the relationships between the historical expansion of the Crown of Aragon (12th to 18th centuries). After applying the Bonferroni adjustment for potential type I errors ($\alpha = 0.05/28 = 1.7857 \times 10^{-3}$), R_{ST} distances (Supplementary Table S5a) indicated non-significant differences between the Spanish populations, except Ibiza with Aragon. Previous studies have shown a considerable distance between Ibiza and other Spanish populations, which has been associated with the demographic and historical differences of this island [8,20,27,28]. When considering only the Italian populations, non-significant differences were detected either. Nevertheless, genetic distances were statistically significant between the Spanish and the Italian communities, suggesting that the gene flow derived from the Crown of Aragon's expansion did not significantly impact the current Y-chromosome pool of the Southern Italian populations here analyzed. This absence of genetic affinity is clearly observed in the MDS plot (Fig. 2), in which the Spanish populations are to the right of the X-axis and Italian groups to the left of the X-axis.

On the other hand, the six populations studied here were compared within a Mediterranean context to assess Y-chromosomal distribution patterns using the populations listed in Supplementary Table S1. As previously mentioned, two analyses were performed, using 17 and 23 loci, respectively. Not all populations were included in all analyses. Populations with only 17 Y-STRs were considered in the first analysis, whereas those with data from 23 markers were included in both analyses.

When pairwise comparisons of the Mediterranean populations based on 17 loci were carried out, and after applying the Bonferroni adjustment for potential type I errors ($\alpha = 0.05/703 = 7.1124 \times 10^{-5}$), R_{ST} distances (Supplementary Table S5b) indicated non-significant genetic differences of the three Balearic Islands with the other Spanish populations as well as with the French and Northern Italian ones. Non-significant genetic differences are also found between the Balearic Islands and two Southern Italy populations (Catania and Trapani), maybe due to the small size of these populations (Catania $N = 17$ and Trapani $N = 39$). In contrast, there are statistically significant genetic differences between Balearic populations (Majorca, Minorca, and Ibiza) with certain populations from Central-Southern Italy (Belvedere, Catanzaro, Cosenza, Latium, Marche, Offida, Puglia, Reggio di Calabria, and Sicily), suggesting reduced gene flow between these sets of populations. Significant differences are also found between the populations of the Southern Mediterranean (Libya, Northern Egypt, Algeria, and Tunisia) and Eastern Mediterranean (Greece, Cyprus, Lebanon, and Turkey).

Regarding the Southern Italian populations under study, this analysis showed non-significant differences between Catanzaro and Reggio di Calabria with several Central-Southern Italian (Belvedere, Catania, Cosenza, Latium, Marche, Offida, Puglia, Trapani, and Sicily) and Eastern Mediterranean populations (Greece, Cyprus, Lebanon, and Turkey). Non-significant genetic differences are also found between Catanzaro and Reggio di Calabria, with some Northern Italian (La Spezia and Liguria) and Northern Egyptian populations. In contrast, Catanzaro and Reggio di Calabria exhibit statistically significant genetic differences with other Northern Italian

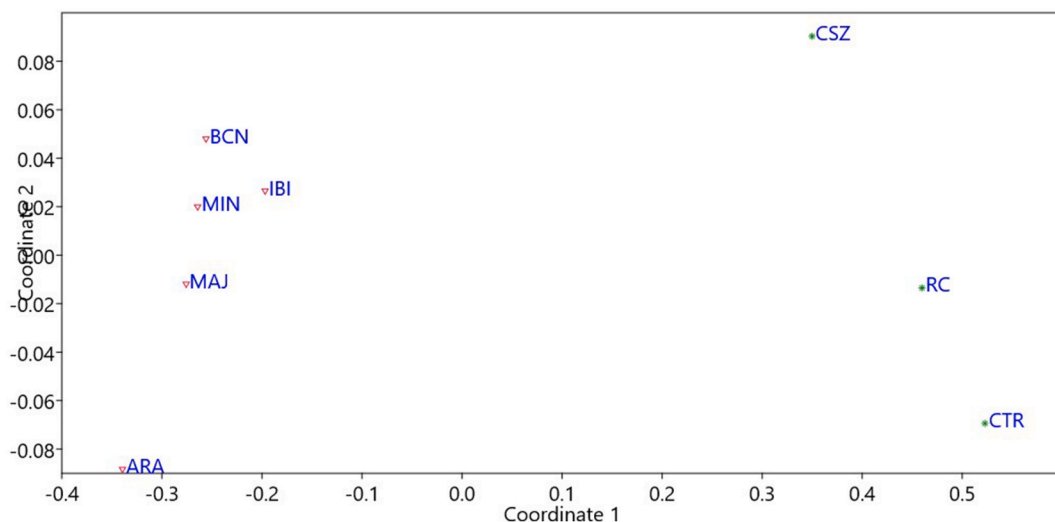


Fig. 2. MDS plot based on R_{ST} distances considering the Crown of Aragon. Spanish populations are represented by an inverted triangle, while an asterisk represents Italian populations.

(Emilia-Romagna, Tuscany, Ravenna, Udine, and Veneto), the Western Mediterranean (Spain and France) and the Southern Mediterranean populations (Libya, Algeria, and Tunisia).

The R_{ST} pairwise distances among these populations were represented using MDS analysis based on the R_{ST} distances (Fig. 3a). In this graph, the populations are organized in an arc. Following the X-axis from left to right, the Spanish populations are positioned first, followed by the French ones. Then the Italians appear, showing a North-South division. While Northern Italian populations are more to the left, Southern ones are to the right. Among the populations of Southern Italy are also the Greek, Lebanese, Cypriot, and Turkish ones. Finally, in the extreme right part of the arc are the Southern Mediterranean populations, including Northern Egypt, Libya, Algeria, and Tunisia.

Similar results were obtained when analyzing the 23 loci. After the application of the Bonferroni adjustment for potential type I errors ($\alpha = 0.05/300 = 1.6667 \times 10^{-4}$), R_{ST} distances (Supplementary Table S5c) indicated significant differences of the three Balearic

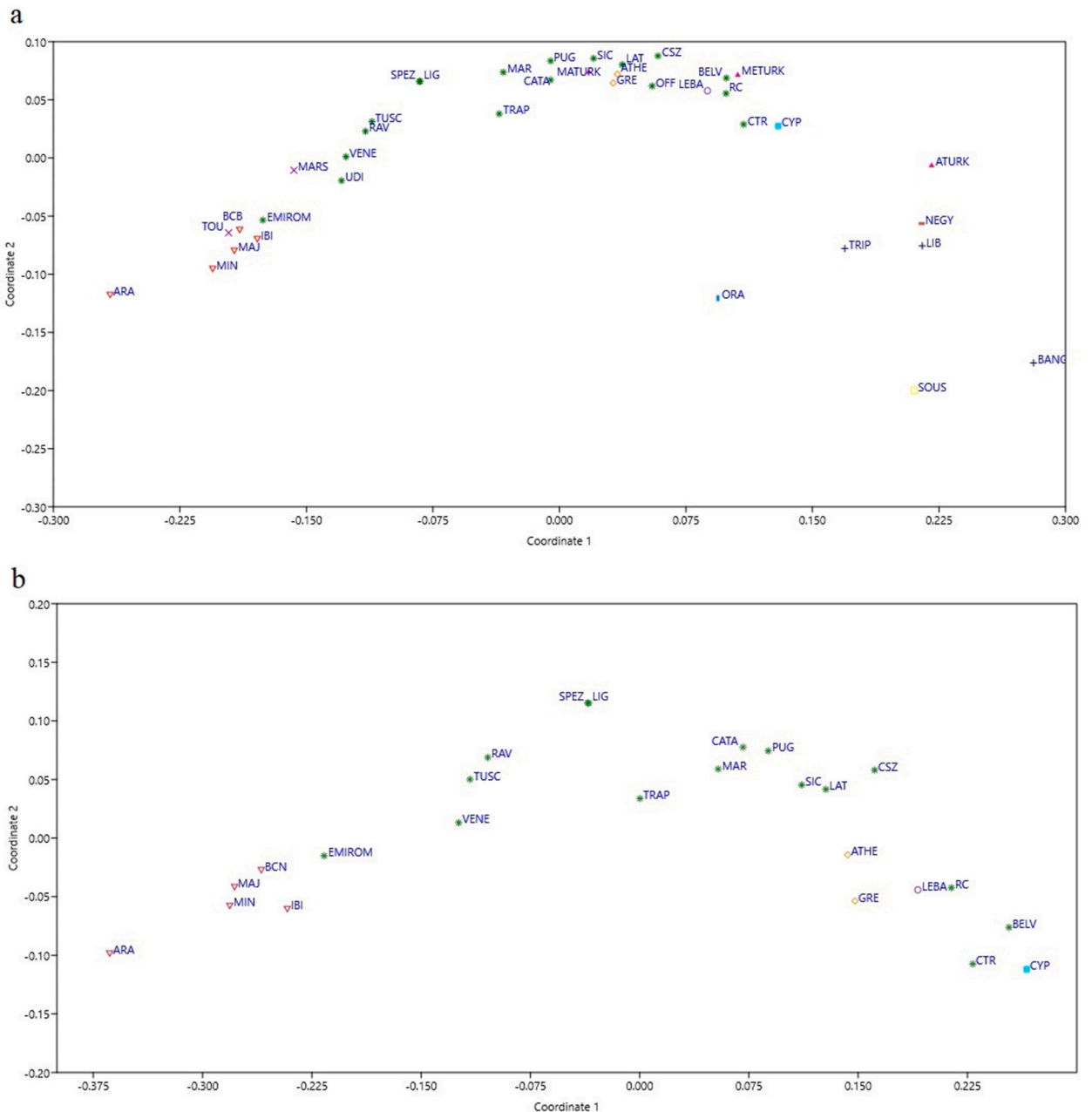


Fig. 3. MDS plot based on R_{ST} distances considering Mediterranean populations and 17 loci (a) or 23 loci (b). Spanish populations are represented by an inverted triangle, French populations by an X, Italian populations by a star, Greek populations by a diamond, Lebanese populations by a circle, Cyprus by a filled square, Turkish by a filled triangle, Egyptian by a dash, Libyan by a plus, Algerian by a bar and Tunisian by a square.

Islands with specific populations of Central-Southern Italy (Belvedere, Catanzaro, Latium, Puglia, Reggio di Calabria, and Sicily) and the Greek, Cypriot and Lebanese ones. On the other hand, these analyses showed significant differences between Catanzaro and Reggio di Calabria and certain Northern Italian (Emilia-Romagna, Ravenna and Veneto) and the Spanish populations.

Using the R_{ST} values, a MDS plot was obtained (Fig. 3b). The resulting graph is quite similar to the one shown above. The same arc shape is observed, with the Spanish populations on the far left, followed by those of Northern Italy and the Southern Italy populations, along with the Greeks and Lebanese ones. Finally, in the extreme right part of the arc are the Cypriots.

In summary, both graphs (Fig. 3) show a distribution of populations mirroring their geographical locations from West to East (Spanish, French, Italian, Greek, Cypriot, Lebanese, and Turkish populations). In contrast, African Mediterranean populations are clustered separately (Supplementary Fig. S1).

3.4. Y-SNP haplogroups

The Y-STR haplotypes were used to infer its most likely Y-SNP haplogroups using the Nevgen Y-DNA Haplogroup Predictor (<https://www.nevgen.org/>). Next, up to 39 bi-allelic markers were analyzed to validate the tentative haplogroups obtained with the Nevgen haplogroup predictor and to determine the exact haplogroup of each individual. This haplogroup predictor tool applied to the studied populations showed an accuracy rate of 99.13 % (Supplementary Table S8).

Supplementary Table S6 displays the Y-SNP haplogroups identified, while Supplementary Table S7 provides their respective frequencies. Across the six populations under study (Ibiza, Majorca, Minorca, Catanzaro, Cosenza, and Reggio di Calabria), eight major haplogroups (C, E, G, H, I, J, R, and T) were detected. Haplogroup R-M207 was the most prevalent, ranging from 67.74 % in Minorca to 18.33 % in Catanzaro. However, if populations are grouped into Spanish (Ibiza, Majorca, and Minorca) and Italian (Catanzaro, Cosenza, and Reggio di Calabria), differences in the most frequent haplogroups between these two groups are observed.

The R-M207 haplogroup continues to be the most abundant in the Spanish populations, ranging from 67.74 % in Minorca to 65.69 % in Ibiza, and its subhaplogroup R1b-DF27 appears with a high frequency, ranging from 50.00 % to 47.48 %. The observed frequencies of this lineage are consistent with those reported in other regions of the Iberian Peninsula, mainly Northern and Eastern areas, with particular emphasis on the native population of the Basque Country, where R1b-DF27 reaches its highest frequencies (70 %) [16, 24, 29]. The only other haplogroups that exhibit frequencies higher than 10.00 % in one of the three Spanish islands examined are E1b1b-M215 (xV13) (Minorca, 14.52 %) and G2a2-L1259 (Ibiza, 18.63 %). All the other haplogroups are below 10.00 %.

In the Italian populations, the J2a-M410 haplogroup presents the highest frequency, ranging from 31.67 % in Catanzaro to 21.62 % in Cosenza. R-M207 is the second most abundant haplogroup, ranging from 27.03 % in Cosenza to 18.33 % in Catanzaro. These data are in agreement with haplogroup R being one of the most frequent throughout Italy [30]. E1b1b-M215 (xV13) is found at frequencies ranging from 17.82 % to 8.33 %. Its subhaplogroup E1b1b-V13 appears with frequencies ranging from 13.33 % to 4.95 %. Several Southern communities show the presence of E1b1b-V13, which is characteristic of the Balkan Peninsula. The frequency distributions observed of haplogroups J2-M172, R, and E are in accordance with these haplogroups being the three main haplogroups found in the whole country [30]. Other haplogroups that exhibit frequencies higher than 10 % in Reggio di Calabria and Catanzaro are G2a2-L1259 (13.86 % and 13.33 %, respectively) and J1a-Z2214 in Cosenza (10.81 %). Both haplogroups could have dispersed into Italy across the Mediterranean from the East, too [12, 31, 32]. All other haplogroups are observed in low frequencies (<10.00 %) throughout the Italian populations. The presence of past migrations can be traced in light of the distributions of Y-SNP haplogroups in the studied Balearic and Southern Italian populations. The presence of Middle Eastern haplogroups (G and J), associated with East-West migratory movements, stands out in all populations. A higher incidence of these haplogroups in the Southern Italian communities is observed, possibly due to other later events following similar routes.

4. Discussion

A review of Y-chromosome data shows a need to increase the number of Y-STRs in population for forensic purposes, as it has been observed that a limited number of Y-STRs may not adequately distinguish between male lineages [21].

With this purpose in mind, we have increased the number of Y-STRs in six Mediterranean populations. When analyzing the number of haplotypes and unique haplotypes in these populations, it was noted that these diversity parameters increased as the number of loci augmented, with the highest values being obtained when considering 23 Y-STRs. As a result, a greater genetic resolution was achieved by providing higher haplotype diversity and discriminatory power.

Moreover, the study of Y-chromosome markers in these Mediterranean populations not only enhances forensic genetics but may also shed light on how the different distributions of these haplotypes may have been shaped by the genetic contributions that came from their migratory histories (Supplementary Fig. S2).

The Mediterranean basin, located between Europe, Africa, and Asia, offers the chance to explore the population genetic dynamics of a migratory crossroad of great relevance [10]. The exceptional historical context of the Mediterranean has made it the subject of many studies to reconstruct its genetic history. Nevertheless, there are still some populations that have not been extensively studied that can be quite useful to provide an overview of Y-chromosome variation across the Mediterranean [12–20].

In this sense, different scenarios that may explain the current Mediterranean gene pool have been evaluated through the determination of genetic distances of the six Mediterranean populations under study (the Spanish populations of Ibiza, Majorca, and Minorca and the Italian populations of Catanzaro, Cosenza, and Reggio di Calabria) and other previously studied Mediterranean populations, based on Y-STR data. On the one hand, the possible influence of the Crown of Aragon in the current genetic pool of the populations under study was investigated. This Aragonese empire in the Mediterranean expanded from present-day Eastern Spain

(Aragon and Catalonia) to current Southern France, Balearic Islands, Sicily, Corsica, Sardinia, Malta, Southern Italy, and parts of Greece during the 12th–18th centuries. Therefore, this past expansion could be reflected in the genetic legacy from the current Mediterranean populations for the Y-chromosome. With this purpose, the six Mediterranean populations under study were compared to the Aragon and Barcelona (Catalonia) populations. Examination of the R_{ST} values indicates that Eastern Spain and Southern Italy populations are not closely related. Indeed, combinations involving all these populations exhibit significant genetic differences between the Spanish and the Italian groups, suggesting that the potential genetic impact of the Crown of Aragon in Southern Italy is currently not detected. A possible explanation for this absence of impact can lay in the specificities of the political system within the Crown of Aragon, which was established in the late Middle Ages. The autonomy of every kingdom and territory incorporated into the Crown, either by conquest or inheritance, was respected. Therefore, no major transfers of population between these territories was promoted, but small groups of bureaucrats or merchants [33].

On the other hand, in a wider context, including populations from the Western, Eastern and Southern Mediterranean previously studied by other authors, examining the R_{ST} values based on Y-STR data exhibits a West-to-East genetic differentiation of this area. It should be pointed out that this differentiation mirrors the geographical distribution of these populations in the Mediterranean region (Fig. 3 and Supplementary Fig. S1).

It is also relevant to note the clear North-South division in Italy found in previous studies. Northern Italian populations were found to be more closely related to Western Europe, while Southern ones showed more genetic affinity with the Eastern Mediterranean [13, 17, 18, 34]. This is consistent with the results obtained in this study, where Northern Italian populations are grouped with Spanish and French ones (Western Mediterranean), and Southern Italy is clustered with Greek, Cypriot, Lebanese, and Turkish populations (Eastern Mediterranean). These North-South differences can be traced back to population movements during the Neolithic and Post-Neolithic periods [13, 17, 19, 34]. Nevertheless, other later events, such as Magna Graecia, from the 8th century BC, or the Byzantine Empire from the 5th century AD, followed similar Neolithic and Post-Neolithic routes into Southern Italy and also involved gene flows [14, 17, 19, 35]. It seems that these migratory events, instead of contributing to a new ancestry, help to maintain and/or reinforce the differential distribution already present in Italy [17, 19]. The fact that no significant genetic differences are found between certain Northern and Southern Italy populations (e.g., La Spezia and Liguria with Catanzaro and Reggio di Calabria), could be explained by internal migrations. It is known that there were population movements Southwards to the North, looking for better economic opportunities [13].

As previously mentioned, the Y-chromosome is highly suitable for studying human migrations and inferring paternal biogeographic ancestry. Because Y-SNPs have lower mutation rates, geographical ancestry traces are much longer maintained in these markers before being diluted by mutations than in Y-STRs. As a result, Y-SNPs are often more appropriate tools for understanding population origins and migration history [36, 37].

Analysis of the Y-haplogroups present in the Mediterranean populations under study (Ibiza, Majorca, Minorca, Catanzaro, Cosenza, and Reggio di Calabria) indicates differences in the most frequent haplogroups observed among the Spanish and the Italian populations.

Spanish populations have a high frequency of haplogroup R1b-DF27, ranging from 50.00 % to 47.48 %. However, in the Italian populations, although R-M207 is the second most abundant haplogroup, subhaplogroup R1b-DF27 appears with a low frequency, ranging the R1b-DF27 from 0.99 % to 5.41 % in the Italian populations here studied. These data are in accordance with those found in previous studies. On the one hand, R1b-DF27 reaches its highest frequencies in the Iberian Peninsula. In fact, it has been suggested that it originated in this region [16, 24, 29, 38]. On the other hand, R1b-U152 is relatively abundant in Northern Italy [39–41], so it is not surprising that it is also found in Southern populations.

E1b1b-M215 haplogroup exhibits frequencies higher than 10 % in Spanish (Minorca) and Italian populations. The presence of this haplogroup could be due to certain subhaplogroups, such as E1b1b-M81 or E1b1b-M34. The subhaplogroup E1b1b-M81, characteristic of North African regions, may be associated with the genetic flow between Maghreb and Iberia favored by Carthage expansion [8, 42–45]. The centuries of Muslim domination of the Iberian Peninsula and South Italy in the Middle Ages must also be considered. This conquest was made from the Maghreb, and there was a population transfer from that area, with fluid contacts between both sides of the Strait of Gibraltar during these centuries [46]. On the other hand, the subhaplogroup E1b1b-M34, which is widespread among Jews, is also found in Spain. This is consistent with the Jewish presence, deep-rooted in the Iberian Peninsula since the Roman period [8, 42, 44].

In Italy, subhaplogroup E1b1b-V13 appears with higher frequency than in Spain. This subhaplogroup is distinctive in the Balkan Peninsula, particularly in Greece, and serves as a marker for assessing the Hellenic influence in the Mediterranean [14, 15]. Several evidences suggest an initial migration of E1b1b-V13 from Anatolia to the Balkans, where it expanded demographically and then spread to Southern Italy [30, 47, 48]. However, the occupation by the Hellenes of Magna Graecia from the 8th century BC [14, 15, 30] and the fluid contact between Southern Italy and the Greek areas even during the time this region was under the dominion of the Crown of Aragon (12th to 18th centuries) [49, 50], may have helped to maintain and/or reinforce the presence of this subhaplogroup in Italy. In fact, before the Roman Empire, one in every 10–13 inhabitants in Southern Italy was Greek [35]. Hence, it makes sense that E1b1b-V13 is mainly found in Catanzaro, Cosenza and Reggio di Calabria populations.

G2a2-L1259 is the last haplogroup observed in Spanish (Ibiza) and Italian (Catanzaro and Reggio di Calabria) populations with a frequency above 10 %. These results are in accordance with its distribution, which has been related to the spread of agriculture during the Neolithic from the Near East across the Mediterranean coast [12, 42, 51]. It represents the primary male lineage among early European farmers, preceding the Y-chromosome turnover during the Bronze Age [52]. Ibiza's large difference in frequency compared to Majorca and Minorca in this haplogroup is consistent with the findings in Adams et al. (2008) [8].

Finally, J2a-M410 and J1a-Z2214 exhibit a significant presence but only in the Southern Italian populations. Subhaplogroups of J2, including J2a-M410, have been associated with successive waves of migrations from the Neolithic to the Bronze Age, ended up in

Western Mediterranean [31,53]. This mainly affected Greece, Crete and Southern Italy, so it is reasonable that Spain does not show such a great incidence [15,30,54]. The presence of subhaplogroup J1a-Z2214 characteristic of the Arabic-speaking populations of Near East and North Africa, could be explained in Southern Italy by a gene flow between these populations [30].

5. Conclusions

The present study has analyzed Y-chromosome markers of six Mediterranean populations, including three Balearic Islands (Ibiza, Majorca, and Minorca) and three Southern Italian regions (Catanzaro, Cosenza, and Reggio di Calabria). The analyses performed in this investigation have demonstrated that the 23 Y-STR loci improve the resolution of discrimination in paternal lineages compared to multiplex systems with 12 and 17 loci in all six study populations. On the other hand, here we have assessed the male gene pool of these populations and inferred the relationship to their close neighbors and the effect of their migratory histories. Recognizing the inherent limitations of this investigation is crucial. Regarding data availability, certain Mediterranean populations were omitted from the study due to insufficient or absent marker analysis. However, we included 32 geographically targeted reference populations, thereby ensuring a comprehensive Mediterranean perspective. A subset of our sampled populations exhibited modest sizes, suggesting the need for further investigations including a larger number of samples. Nevertheless, efforts were made to compensate this concern during statistical analyses, resulting in sufficient significance power attainment. Overall, the studied Y-STRs and Y-SNPs mirror the West to East geographical distribution of European and Asian Mediterranean populations, emphasizing the North-South Italian division, with a larger Eastern Mediterranean genetic component in Southern Italian populations. In contrast, the African Mediterranean populations are clustered separately. Finally, our results support the notion that the Neolithic and Post-Neolithic expansion from the Middle East towards Southern Europe has been maintained and/or reinforced in Southern Italy populations, possibly due to other later events, such as Magna Graecia or the Byzantine Empire.

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Code availability

Not applicable.

Ethics declarations

- This study was reviewed and approved by the General Directorate of Research + Development + Innovation of the Government of the Balearic Islands, Spain (Ref. AAEE procedure 12099/2003) and conducted in accordance with Italian Law D.lgs. 196/2003.
- All participants provided informed consent prior to participate in the study and for the publication of their anonymized case details.
- Additionally, all participants also gave their informed consent for the samples to be used in the population-based follow-up studies.
- Under current Spanish legislation, samples collected before 2007 can be utilized for research purposes if anonymized. Blood samples used in this study were collected before 2007 and anonymized in accordance with the Law 14/2007 on Biomedical Research.

Data availability statement

The datasets generated during this study are available within the published article and its supplementary files. They are also deposited in the YHRD repository under the following accession numbers: Ibiza YA003484-2; Majorca YA005613 Y23; Minorca YA006026 Y23; Catanzaro YA003493 Y23; Cosenza YA003492 Y23; Reggio di Calabria YA003490 Y23. <https://yhrd.org/>

CRediT authorship contribution statement

B. Navarro-López: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **M. Baeta:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **O. Moreno-López:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **T. Kleinbielen:** Writing – review & editing, Methodology, Investigation. **C. Raffone:** Writing – review & editing, Methodology, Investigation. **E. Granizo-Rodríguez:** Writing – review & editing, Methodology. **J.F. Ferragut:** Writing – review & editing, Resources, Methodology. **O. Alvarez-Gila:** Writing – review & editing, Writing – original draft. **A. Barbaro:** Writing – review & editing, Resources, Methodology. **A. Picornell:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization. **M.M. de Pancorbo E:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing

interests: Marian M de Pancorbo reports financial support was provided by Basque Government. Marian M de Pancorbo reports a relationship with Basque Government that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e35329>.

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