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Journal

Annals of Human Biology, 45(1)

ISSN

0301-4460

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Publication Date

2018-01-02

DOI

10.1080/03014460.2017.1416172

Peer reviewed




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To cite this article: Flavio De Angelis, Gabriele Scorrano, Cristina Martínez-Labarga, Giuseppina Scano, Fabio Macciardi & Olga Rickards (2018) Mitochondrial variability in the Mediterranean area: a complex stage for human migrations, *Annals of Human Biology*, 45:1, 5-19, DOI: [10.1080/03014460.2017.1416172](https://doi.org/10.1080/03014460.2017.1416172)

To link to this article: <https://doi.org/10.1080/03014460.2017.1416172>

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REVIEW



Mitochondrial variability in the Mediterranean area: a complex stage for human migrations

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ABSTRACT

Context: The Mediterranean area has always played a significant role in human dispersal due to the large number of migratory events contributing to shape the cultural features and the genetic pool of its populations.

Objective: This paper aims to review and diachronically describe the mitogenome variability in the Mediterranean population and the main demic diffusions that occurred in this area over time.

Methods: Frequency distributions of the leading mitochondrial haplogroups have been geographically and chronologically evaluated. The variability of U5b and K lineages has been focussed to broaden the knowledge of their genetic histories.

Results: The mitochondrial genetic makeup of Palaeolithic hunter-gatherers is poorly defined within the extant Mediterranean populations, since only a few traces of their genetic contribution are still detectable. The Neolithic lineages are more represented, suggesting that the Neolithic revolution had a marked effect on the peopling of the Mediterranean area. The largest effect, however, was provided by historical migrations.

Conclusion: Although the mitogenome variability has been widely used to try and clarify the evolution of the Mediterranean genetic makeup throughout almost 50 000 years, it is necessary to collect whole genome data on both extinct and extant populations from this area to fully reconstruct and interpret the impact of multiple migratory waves and their cultural and genetic consequences on the structure of the Mediterranean populations.

ARTICLE HISTORY

Received 22 November 2017

Revised 30 November 2017

Accepted 5 December 2017

KEYWORDS

Mediterranean Basin; complete mitochondrial genomes; Mediterranean mitochondrial lineages; mtDNA human populations

Introduction

The Mediterranean area has always played a significant role in the expansion of human populations, because it represents a bridge for humans among three continents: Europe, Africa and Asia. Indeed, during human pre-history and history, a profusion of demic movements and cultural interactions occurred in this area, contributing to shape the biological features and genetic pool of modern European populations. Since the Pleistocene, when the first out of Africa dispersal of *Homo sapiens* took place (Hublin et al., 2017), the Mediterranean territories represented a significant stage for human expansion (Sazzini et al., 2014). Even today, the Mediterranean Sea is often used as a migratory route from Africa and the Near East to Europe: globalisation, therefore, continues to increase the genetic variability of Mediterranean populations.

In this frame, the particular spatial distribution patterns of genetic lineages in contemporary human populations could provide meaningful insights into migrations and multiple gene flows contributing to demographic changes and their

related biological consequences (Cerezo et al., 2012). Accordingly, the exploration of the genetic structure of extant Mediterranean populations could be pivotal in inferring their demographic history through research comparing ancient and extant genetic data to determine possible signatures of ancient routes (Fu et al., 2016; Gamba et al., 2014; Günther et al., 2015; Hofmanová et al., 2016).

The selected genetic markers suitable for phylogenetic and phylogeographic analyses have, for a long time, been the mitochondrial DNA (mtDNA) and the non-recombining region of the Y-chromosome. The mtDNA genome and the Y-chromosome are uniparental genetic systems which provide a female and a male perspective, respectively, and since they do not recombine, they change over generations only by an accumulation of mutations, ultimately creating patrilineal and matrilineal lines called haplogroups. Different haplogroups can be geographically and chronologically observed among different human populations. Their ultimate distribution and frequency are also constrained by the effect of other factors they interact with, like cultural traits and geographical obstacles.

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📄 Supplemental data for this article can be accessed [here](#).

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Despite this surely useful approach, today such an evolutionary analysis based only on the mt- or the Y-DNA markers has been unable to adequately dissect the overall genetic makeup of worldwide human populations: the recent developments of Next Generation Sequencing (NGS) techniques fully accomplish this task, providing several whole genomes from extant and ancient samples.

Indeed, a great deal of data on whole genomes has been generated by international consortia for both modern populations, such as the '1000 Genome Project' (The 1000 Genomes Project Consortium, 2015), and ancient people (Allentoft et al., 2015; Fu et al., 2016; Haak et al., 2015; Hofmanová et al., 2016). These advances make possible a merged data analysis that could contribute to a holistic understanding of genetic variability in present and ancient human groups (Rasmussen et al., 2010; Reich et al., 2010).

Nonetheless, certain important questions about human movements in the Mediterranean area are still far from being fully addressed, mainly because available data about whole genomes related to past Mediterranean populations are scarce. As the ancient DNA data from the Mediterranean people have been to date almost exclusively derived from the analysis of well, but heterogeneously covered mitochondrial genomes, the purpose of this review is to broadly depict the variability of mtDNA in the Mediterranean region and to better describe the genetic landscape of the leading human migration routes. The analytical strategy to accomplish this goal is the evaluation of the frequency distributions of the leading mitochondrial haplogroups, because comparisons among heterogeneously covered mtDNA data could lead to misinterpretations if they are matched at the haplotype level (Schueneman et al., 2017). Moreover, the understanding of the variability of U5b and K lineages has been deepened by placing each sequence in the accepted mtDNA phylogeny

(van Oven & Kayser, 2009) to produce unambiguous trees able to support inferences on where the direct (or indirect) descendants of the ancient sequences have appeared.

Pre-history of the Mediterranean

Homo sapiens originated in Africa ~200 thousand years ago (kya) (Hublin et al., 2017; Mellars, 2006) and began to colonise the remaining continents ~60–80 kya. The first modern humans moved to Europe ~43 kya, carrying M and N mitochondrial haplogroups (Benazzi et al., 2011; Higham et al., 2014; Ingman et al., 2000; Stoneking, 2008) (Figure 1) and succeeded in the replacement of the existing species, *Homo neanderthalensis* (Hublin, 2011), whose mitochondrial sequences fall outside the mtDNA variability of *Homo sapiens* (Green et al., 2008; Prüfer et al., 2017), even though at the nuclear DNA level a certain degree of interbreeding has been detected (Sankararaman et al., 2012). A drastic climatic event, the Last Glacial Maximum (LGM), occurred 27–19 kya during the Upper Palaeolithic period and markedly influenced the demographic dispersal and genetic variability of early European hunter gatherers (Tallavaara et al., 2015). If the pre-LGM European populations were characterised by human mitochondrial variability, mainly constituted by R, U and M lineages (Posth et al., 2016; Seguin-Orlando et al., 2014), the LGM represented a bottleneck for genetic diversity: U sub-haplogroups were the sole lineages identified in early post-LGM palaeolithic people. Only in the late post-LGM phase there was a demic diffusion and a consequent flourishing of U sub-haplogroups, particularly those derived from U5. Thus, the overall genetic landscape of the first Europeans seems to suggest that *Homo sapiens* experienced an early and significant expansion before the LGM, as supported by the M

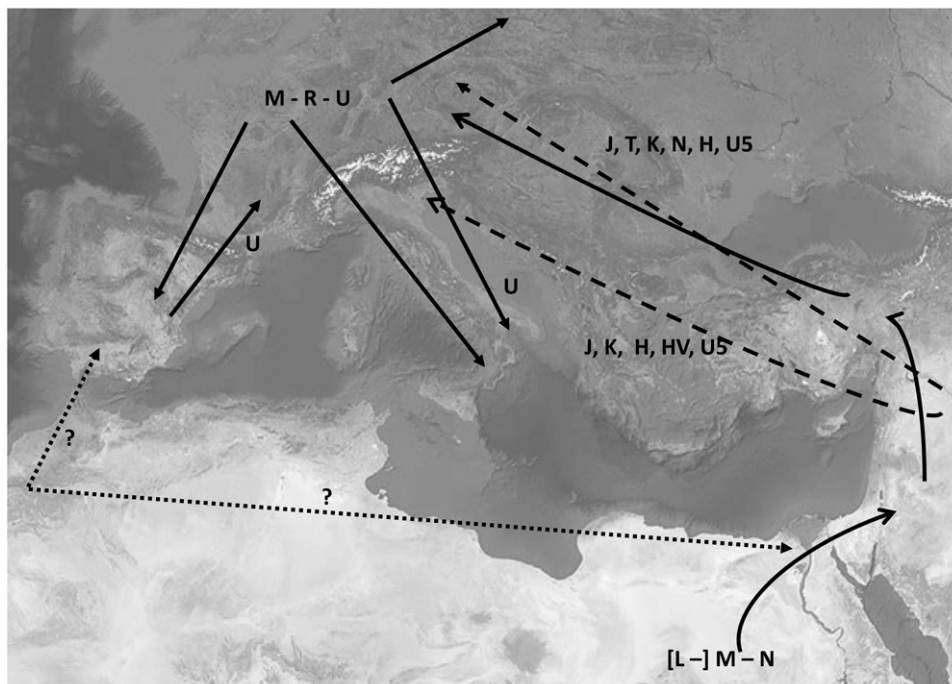


Figure 1. Major migratory routes with the associated mtDNA haplogroups for the Upper Palaeolithic (solid lines) and the Neolithic (dashed lines) chronologies. Other hypothetical migratory routes are presented with dotted lines (see text for more details).

haplogroup distribution (Macaulay et al., 2005), followed by a climate-driven recession that forced people into the few ice-free areas (Southwestern Europe, the Mediterranean coastline, the Balkans, the Levant and Eastern Europe). This contraction led to a reduction in population size and a consequent loss of differentiation in mtDNA lineages, which then experienced re-expansion into the rest of Europe after the LGM (Posth et al., 2016).

The analysis of extant mitochondrial data in Europe indicates that most of the mtDNA lineages originated after the LGM, probably from the small populations that survived in these refuges (Hewitt, 2000). Despite the belief that most of the mtDNA lineages that expanded into Western and Central Europe, such as H and V, came from the Franco-Cantabrian refuge, these haplogroups are lacking in post-LGM hunter-gatherers and there are not enough genetic data to support this scenario. A possible exception is U5b3, which probably expanded from the Italian refuge (Pala et al., 2009). Palaeolithic mitochondrial lineages represent to date an interesting genetic fingerprint in modern Europeans, but they only marginally contributed to the variability of the extant European mtDNA gene pool (Günther & Jakobsson, 2016).

Around 10 kya, the Neolithic revolution took place, which had major impacts on Mediterranean groups. The first Neolithic societies rose 12 to 10 kya in the area of the Near East traditionally known as the 'Fertile Crescent'. From this region, Neolithic farming technology rapidly spread to Anatolia, reaching the rest of Europe in less than 3000 years via two main routes (Fernandez et al., 2014). The Danubian route associated with the Linearbandkeramic (LBK) archaeological complex spanned the central plains up to Scandinavia, even though the genetic changes associated with the Neolithic transition in the Baltic region also seem to be associated with populations that most likely migrated from northern Eurasia and the Pontic (Jones et al., 2017). The Mediterranean route was associated with the Cardial-Imprinted cultural complex, and spread Neolithic technology along the Mediterranean coast up to the Iberian Peninsula. It is worth noting that the term *Neolithic* does not so much denote a specific chronological period as it does a suite of cultural characteristics, such as the rise of farming and breeding strategies. The term also indicates a tipping point in the pre-history of Mediterranean populations, representing one of the greatest economic revolutions ever to occur in Western and Central Europe. The introduction of a farming lifestyle promoted population growth (Skoglund et al., 2012), albeit at the cost of increased health problems (Cohen & Armelagos, 1984). To date, there is still no comprehensive understanding of how the Neolithic transition occurred, representing an unresolved argument from the 1960s, when two models were proposed, mainly based on cultural or demic diffusion (Childe, 1964; Zvelebil, 2001).

Recent analyses of ancient genomic data support the hypothesis of a predominantly demic rather than purely cultural process in the Neolithic transition, characterised by the assimilation of local hunter-gatherers (Günther et al., 2015) and the migration of early farmers from the Fertile Crescent (Bramanti et al., 2009; Gamba et al., 2014; Haak et al., 2015; Hofmanová et al., 2016; Lazaridis et al., 2014; Mathieson

et al., 2015; Skoglund et al., 2014), although no firm conclusion has yet been reached (Pinhasi et al., 2005).

Several mtDNA lineages arose in Neolithic societies, such as the J, J1c, T, K, N1, N1a1a1, H, U5 and U5a1 lineages (Gamba et al., 2014; Haak et al., 2015; Hofmanová et al., 2016; Omrak et al., 2016) (Figure 1).

Other genetic components were introduced into Europe ~4.5 kya during the early Bronze Age by a Pontic-Caspian steppe population related to the westward-moving Yamnaya culture (Allentoft et al., 2015; Haak et al., 2015). This migration has been tentatively linked to the development of Indo-European languages and horseback riding in Western and Northern Europe (Allentoft et al., 2015; Haak et al., 2015), but the genetic components of the Mediterranean Basin do not seem to have been deeply modified by this large migration of herders from the steppes. The rate of admixture between the inhabitants of Southern Europe and the Yamnaya population seems to have been very low (Allentoft et al., 2015; Haak et al., 2015), although the mtDNA haplogroups most influenced by this demic introgression were some U sub-lineages, H, and W, with an increase in the T2 haplogroup (Allentoft et al., 2015; Haak et al., 2015; Sokolov et al., 2016): with the exception of H, these lineages are infrequently found in extant Mediterranean populations.

North Africa has seen extensive bidirectional migrations, receiving migrants from neighbouring Mediterranean Europe and Southwestern Asia. Its pre-history is quite complex and is linked to Aterian, Iberomaurusian and Capsian cultural facies, which created inter-mixed periods of population continuity and discontinuity. The Gibraltar area played a significant role in this history in determining the permeability of the border between Europe and Africa, despite the common argument against pre-historic human flow based on the adverse environmental conditions in this area (Hernández et al., 2014).

Thus, in pre-historic times, three significant waves seem to have synergistically shaped the genetic makeup of the Mediterranean: the first hunter-gatherers' movements from Africa and the last ice-free refuges, the Neolithic farming societies who potentially moved from the east, and the herders who came from the Caucasian steppes during the Bronze Age. These three components are currently reflected to various extents in the mtDNAs of extant populations (Günther et al., 2015; Lazaridis et al., 2014; Skoglund et al., 2012; 2014).

History of the Mediterranean

Around 3 kya, the collapse of Bronze Age societies allowed the rise of two populations that began to spread across Europe: the Phoenicians from the coastal Levant (Cunliffe, 2001) and the Greeks from the Aegean Sea (Murray, 1993).

The Phoenician population dominated east-to-west trade routes along the Mediterranean coast of North Africa and the Iberian Peninsula until ~700 BCE. During this period, new cities were built, including Tyre, Sidon, Byblos, and Arwad, which still exist today in Lebanon and southern Syria. In an effort to discern the complete mitochondrial genome of a Phoenician from Carthage, Matisoo-Smith et al. (2016)

identified the U5b2c1 haplogroup; this finding was particularly interesting because no other U5b2c haplotypes have been recorded in North Africa and currently this haplogroup is found in only a few modern populations (Behar et al., 2012; Herrstadt et al., 2002; Marques et al., 2015). Remarkably, this lineage seems to be linked to Iberian populations, where western hunter-gatherer mtDNA lineages, including U5b2c1, resisted the Eurasian Steppe-derived replacement (Haak et al., 2015; Hervella et al., 2012) and might have moved to North Africa (Matisoo-Smith et al., 2016). Indeed, multiple population movements seem to have occurred in the Africa/Iberian Peninsula region, connected at the narrow Strait of Gibraltar (a 14 km-wide marine barrier). This geographic proximity has often facilitated coast-to-coast human movements, promoting a rich bidirectional migration history to North Africa that resulted in a consistent contribution to human, cultural and commercial exchange (Hernández et al., 2014, 2015). Although a southward route was mentioned, the bidirectional movements between Africa and Western Europe cannot be denied. The findings presented by previous studies identified H1, H3 and V lineages as resulting from southward migrations and U6 and L lineages in the opposite direction. Taken together, these lineages seem to represent useful proxies to explain the different ancestries of each side of the Mediterranean Basin (Hernández et al., 2017; Ottoni et al., 2010).

Greek expansion reached its climax with the establishment of colonies in Southern Italy, collectively known as Magna Grecia, but also involved the central and western Mediterranean Basin (King et al., 2011) until 700 BCE. This migration should have had a great impact from both a cultural and genetic point of view, but little about the genetic pool of the populations associated with this migration is definitively known. In spite of this issue, there is no doubt that the Greeks' influence in Southern Italy represented a significant part of its genetic makeup. Indeed, Italian genomes seem to reflect a series of pivotal demographic changes occurring in the Mediterranean Basin, because the Italian Peninsula has experienced a long and varied history of colonisation and migration (Fiorito et al., 2016). The Italian population displays a wider genomic variability than other European countries as a result of the synergistic effects of its geographic isolation due to its mountainous topography and the historical events that triggered its demography. Certainly, the Roman Empire (27 BCE–476 CE) could be put forth as the leading influence on Italian demography; its decline was followed by a considerable reduction in population size that initiated a series of migratory waves across Italy and the central Mediterranean area.

A well-defined assumption is that the increased frequency and differentiation of haplogroup H and related sub-groups date to this period (Di Bernardo et al., 2009; Gamba et al., 2008; Martiniano et al., 2015; Vernesi et al., 2001). The complex genomic mosaic of Italy bears witness to other population movements. Although it is difficult to genetically discriminate between Northern, Central and Southern Italians with precision, due to the internal migration that occurred during the last two generations, some differences can be still identified. Recent ancestry and identical-by-descent analyses provide evidence related to the admixture pattern of Italians

(Northern, Central and Southern) with three major ancestries, mostly represented by Northern Europeans, Southern Europeans and Middle Easterners, respectively (including a small North African component for Southern Italy), with no restricted boundaries between these regions, allowing continuous gene flow in multiple directions (migratory waves to and from Italy) (Fiorito et al., 2016). The Middle Eastern ancestry in Southern Italians most likely originated at the time of the Greek colonisation and, to a smaller extent, during subsequent Arab rule. Central-Northern Italy seems to have been influenced by the admixture of indigenous residents with populations from Central Europe.

This hypothetical admixture event should have occurred ~3 kya, involving populations coming from the Caucasus and the Middle East and populations that lived in Central Italy (Tuscany and Latium) and this was interpreted as possible evidence of the Middle Eastern (or Anatolian) origin of the Etruscans (accordingly to Herodotus' hypothesis) (Brisighelli et al., 2009; Pardo-Seco et al., 2014). Moreover, the mingling of Northern-Central European ancestry with Italian genomes is estimated to have occurred after the Roman Empire collapsed (476 CE): the Barbarian Invasions kicked off as migratory waves from Northern-Central Europe to Northern-Central Italy iteratively occurred, even though a certain amount of Northern-Central European ancestry in contemporary Italians could be attributed to subsequent Italian population growth. This led to the establishment of settlements of Central Europeans in Northern Italy that might have contributed to the foundation of some communities in Northern Italy (Boattini et al., 2015).

Eventually, admixture events involving Southern Italian communities were hypothesised to have mainly occurred ~1 kya, coinciding with the Norman conquest of Southern Italy (Di Gaetano et al., 2009). This conquest is strictly related to the Arab expansion, which increased the cultural heterogeneity along the Mediterranean Basin and is generally considered one of the most important migratory events in that region. This migration occurred at the end of the Roman Empire, when the Arabs quickly spread along the southern coasts of the Mediterranean Sea, extending their rule through the whole of North Africa (Hitti, 1990). They arrived in Europe around 700 CE, ruling the Iberian Peninsula as Moors and Southern Italy as Saracens (Norman, 1975): their control of Mediterranean commercial routes continued until the 15th century CE, when they were driven out by the Catholics (Norman, 1975).

The short description above highlights only a fraction of the demic complexity of the Mediterranean area. Despite the fact that pre-historic and historic events occurring in the Mediterranean area should reveal how the genetic variability of modern Mediterraneans was generated, a full understanding of the mode by which these various migrations contributed to the extant gene pool is far from complete.

Materials and methods

In order to compare the genetic data of ancient populations, whole mitochondrial genomes of people who lived in the

Mediterranean area were accessed through publicly available databases. They mainly referred to the Upper Palaeolithic and Neolithic periods, as there is little mitochondrial genomic data currently available for the Bronze Age and the Roman period. The ancient genomes are, thus, clustered culturally and topographically into the following groups: pre-LGM Hunter-gatherers, post-LGM Hunter-gatherers, Neolithic Central Europe, Neolithic Eastern Europe, Linear Pottery culture, Yamnaya culture and Romans.

The haplogroup frequencies have been matched with a matrix of available ones scored in extant Mediterranean populations, thus homogeneously covering the Mediterranean area ([Supplementary Table 1](#)) in order to assess putative diachronic similarities.

Moreover, these frequencies have been used to compile a similarity matrix by Euclidean distance that allows us to draw up a bidimensional representation (nmMDS, Kruskal, 1964) of the genetic affinities across human groups, while Correspondance Analysis (CA) was used to account for the influence of the frequency of each lineage in past populations (Past software v.2.08 b; Hammer et al., 2001). Population clustering has been evaluated by AMOVA package in Arlequin v.3.5 (Excoffier et al., 1992, 2005).

U5b and K haplogroup complete mtDNA sequences have been compared with the accepted mtDNA phylogeny available at the last release of Phylotree (van Oven & Kayser, 2009) to produce unambiguous trees that can support inferences on putative descendants of the ancient sequences.

Whole mitochondrial genomes of extant Mediterranean populations available in the MitoTool database (Fan & Yao, 2013) and ancient people ([Table 1](#)) have been downloaded.

When the ancient mt genomes were not already available from previous publications or databases, we extracted the mtDNA sequences from whole genome data using SAMtools (Li et al., 2009) and generated a consensus mtFASTA sequence relative to the reference NC_012920, also known as the revised Cambridge Reference Sequence (rCRS: Andrews et al., 1999). We identified variants and then assigned haplogroups with the online tool Haplogrep2 (Weissensteiner et al., 2016), via the last release of PhyloTree (mtDNA tree Build 17; van Oven & Kayser, 2009).

Results and discussion

Although the DNA in the Mediterranean samples was generally poorly preserved because of the warm climate, the recovery of the complete ancient mitogenomes gives some basis to speculate about the genetic history of Mediterranean communities (Olalde et al., 2015). The list of the haplogroup frequencies among synchronic and diachronic populations indicates a wide heterogeneity that does not exclusively relate to different time periods ([Supplementary Table 1](#)). Pre-LGM hunter-gatherers were characterised by three predominant haplogroups: M (13%), R (8.7%) and several branches of the U lineage (ranging from 13% for U8 to 26.2% for U2). Whereas haplogroup M completely disappeared thereafter, excluding the Roman age, R lineage was retained by eastern Neolithics. The frequency of the U branches varied drastically,

Table 1. Ancient specimens information for U5b and K haplotypes evaluation. Mediterranean ancient specimens are noted by *.

Tree code	Specimen description
U5b HAPLOGROUP	
1	mtDNA (U5b) El Miron, Spain (De la Rua et al., 2015; Fu et al., 2016)*
2	mtDNA (U5b2b) Paglicci71, Italy (Posth et al., 2016)*
3	mtDNA (U5b2b) Villabruna, Italy (Fu et al., 2016)*
4	mtDNA (U5b2b) Rochedane, France (Posth et al., 2016)*
5	mtDNA (U5b2b) Iboussieres39, France (Posth et al., 2016)*
6	mtDNA (U5b1) Iboussieres31-2, France (Posth et al., 2016)*
7	mtDNA (U5b2a) Iboussieres25-1, France (Posth et al., 2016)*
8	mtDNA (U5b1) Ranchot88, France (Posth et al., 2016)*
9	mtDNA (U5b1a) BerryAuBac1, France (Posth et al., 2016)*
10	mtDNA (U5b1b) CuiryLesChaudardes1, France (Posth et al., 2016)*
11	mtDNA (pre-U5b1i) I0408, Spain (Haak et al., 2015)*
12	mtDNA (U5b1d1) Bockstein, Germany (Posth et al., 2016)
13	mtDNA (U5b1d1) Ofnet, Germany (Posth et al., 2016)
14	mtDNA (U5b2c1) HohlensteinStadel, Germany (Posth et al., 2016)
15	mtDNA (U5b2a) Falkenstein, Germany (Posth et al., 2016)
16	mtDNA (U5b2b(2)) Blätterhöhle11, Germany (Bollongino et al., 2013)
17	mtDNA (U5b2b2) Blätterhöhle14, Germany (Bollongino et al., 2013)
18	mtDNA (U5b1a) Loschbour, Belgium (Fu et al., 2013)
19	mtDNA (U5b1a) Loschbour, Belgium (Lazaridis et al., 2014)
20	mtDNA (U5b1h) Bichon, Swiss (Jones et al., 2015)
21	mtDNA (U5b2c) NE1, Hungary (Gamba et al., 2014)
K HAPLOGROUP	
1	mtDNA (K1a18) I1414, Jordan (Lazaridis et al., 2016)*
2	mtDNA (K1a4b) I0867, Israel (Lazaridis et al., 2016)*
3	mtDNA (K1a2a) CB13, Spain (Olalde et al., 2015)*
4	mtDNA (K1a2) I1583, Turkey (Mathieson et al., 2015)*
5	mtDNA (K1a) I1579, Turkey (Mathieson et al., 2015)*
6	mtDNA (K1a3a) I1102, Turkey (Mathieson et al., 2015)*
7	mtDNA (K1a) I1100, Turkey (Mathieson et al., 2015)*
8	mtDNA (K1a or K1a1) I0746, Turkey (Mathieson et al., 2015)*
9	mtDNA (K1a2) I0727, Turkey (Mathieson et al., 2015)*
10	mtDNA (K1a4) I0724, Turkey (Mathieson et al., 2015)*
11	mtDNA (K1a4) I0707, Turkey (Mathieson et al., 2015)*
12	mtDNA (K1a2) Klei10, Greece (Hofmanová et al., 2016)*
13	mtDNA (K1a2) Bar8, Turkey (Hofmanová et al., 2016)*
14	mtDNA (K1c) Theo1, Greece (Hofmanová et al., 2016)*
15	mtDNA (K1c) Theo5, Greece (Hofmanová et al., 2016)*
16	mtDNA (K1b1a1) I0407, Spain (Haak et al., 2015)*
17	mtDNA (K1a1b1) I0405, Spain (Haak et al., 2015)*
18	mtDNA (K1a2a) I0411, Spain (Haak et al., 2015)*
19	mtDNA (K1a18) I1701, Jordan (Lazaridis et al., 2016)*
20	mtDNA (K1a12a) I1671, Iran (Lazaridis et al., 2016)
21	mtDNA (K1a) KO2, Hungary (Gamba et al., 2014)
22	mtDNA (K1a3a3) NE6, Hungary (Gamba et al., 2014)
23	mtDNA (K1a2) I1550, Germany (Mathieson et al., 2015)
24	mtDNA (K2b2) I1544, Germany (Mathieson et al., 2015)
25	mtDNA (K1a1b2a) I1534, Germany (Mathieson et al., 2015)
26	mtDNA (K1b1b1) I1103, Turkey (Mathieson et al., 2015)
27	mtDNA (K1a2c) I0060, Germany (Haak et al., 2015)
28	mtDNA (K1a) I0048, Germany (Haak et al., 2015)
29	mtDNA (K3) Satsurblia, Georgia (Jones et al., 2015)

but remained detectable. The post-LGM period has been characterised by a high frequency of haplogroups U5b (38.2%) and U8 (28.7%), although both are virtually absent in modern samples. Haplogroups K, J, H and T, often along with some sub-clades, rose throughout the Neolithic until reaching frequencies of 30.3%, 14.3%, 18.2% and 21.4%, respectively, in different clustered communities.

The H lineage was specifically lacking in previous hunter-gatherers, but its frequency reached 9.4% in eastern Neolithics, rose to 14.3% and 18.2% in Linear Pottery culture people and Central European Neolithics, respectively, and was highest at 21.4% in Yamnaya people. The migration of the nomadic herders led to the rise of haplogroup W, previously absent or rare in Neolithic communities. Haplogroup T

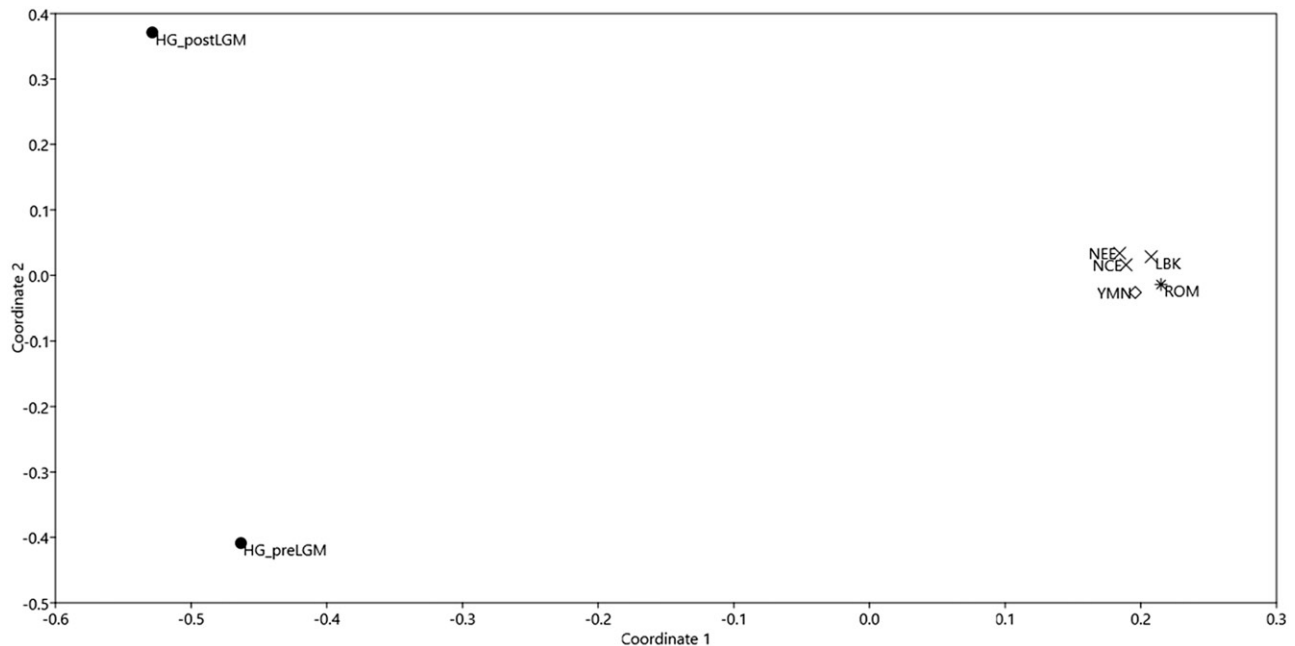


Figure 2. Non-metric Multidimensional Scaling (stress value: 0.14) by Euclidean distance matrix from frequency distributions of ancient populations. HG_preLGM, Hunter-gatherers pre-LGM; HG_postLGM, Hunter-gatherers post-LGM; NCE, Neolithic Central Europe; NEE, Neolithic Eastern Europe; LBK, Linear Pottery culture; YMN, Yamnaya; ROM, Romans.

is wholly represented by the T1, T2, T2b sub-branches: T1 is present in eastern Neolithics (9.4%), but totally absent in other farming people; T2 reached 21.4% in Linear Pottery people, while T2b reached 14.3% in Yamnaya people. Lineage J is found constantly in Neolithics, ranging from 9.4% in eastern people to 14.3% in Linear Pottery ones. The latter is characterised by a low percentage of the K line, which, in contrast, is appreciable in Central and Eastern European Neolithics (16.4% and 30.3%).

If haplogroup H appeared in the Neolithic, it tended to be the most common mitochondrial lineage in the Roman age, at frequencies of up to 40%.

The raw haplogroup frequencies have been used to compile a Euclidean distance matrix that can be adequately represented by a non-metric Multidimensional Scaling (nmMDS, Kruskal, 1964).

Figure 2 shows the totally different mitochondrial genetic component among pre-historic and historic populations: this scenario is consistent with the extreme LGM filtering that drastically changed the haplogroup frequencies. Afterwards populations were totally differentiated with respect to the hunter-gatherers and seem to be more similar to what concerns their mtDNA makeup (stress value = 0.14).

This peculiar scenario is consistent with the CA plot based on the haplogroup frequencies (Figure 3): the entire hunter-gatherer sample is located at one edge of the distribution, while the other ancient populations appear quite separated.

Pre-LGM and post-LGM groups are similar to what concerns the first axis encompassing 34.2% of the total variance, but they are split into different quadrants roughly characterised by two different ancient haplogroups like R and M, although the post-LGM location is mainly ascribed to U5b and U8 lineages and pre-LGM location is partially influenced by some U sub-lineages (U, U5 and U2). All the Neolithics are

located in the same area of the plot, suggesting a rough similarity that could be ascribed to the flourishing of several lineages such as N1, J, K and T.

The Yamnaya and Roman populations are located in the same area of the plot due to the strong influence of the H lineage, even though Yamnaya is surrounded by W, U4 and U5a, which are quite rare in the other chronologies. It is worth noting that it appears the Romans' location is not so far away from Neolithic communities. The wide range of related haplogroups seems to be consistent with the nuanced genetic landscape of the Roman Empire, whose genetic characteristics should be partially derived by extensive gene flow from people all over Europe who became Roman citizens.

The haplogroup distribution of past populations should be compared with that of extant human groups. The intense population movements occurring in historical times served to merge different human groups with consequently extensive gene flow, thus homogenising their genetic components. Despite this confounding effect, present-day Mediterranean populations could be divided into two large groups, based on their current geographical distributions, namely the North and South Mediterranean regions (AMOVA $F_{ct} = 0.019$, $p < 0.001$).

At first glance the Northern Mediterranean populations exhibit typical European haplogroups, where H reaches frequencies of more than 30%. Southern Mediterranean populations are characterised by a higher frequency of L and L-derived lineages, typical of African populations (Soares et al., 2010), although there are several confounding factors that could potentially complicate this picture.

The matrix plots (Figure 4) depicting frequency distributions of each lineage show that H is the leading macro-haplogroup in both Northern and Southern/Eastern

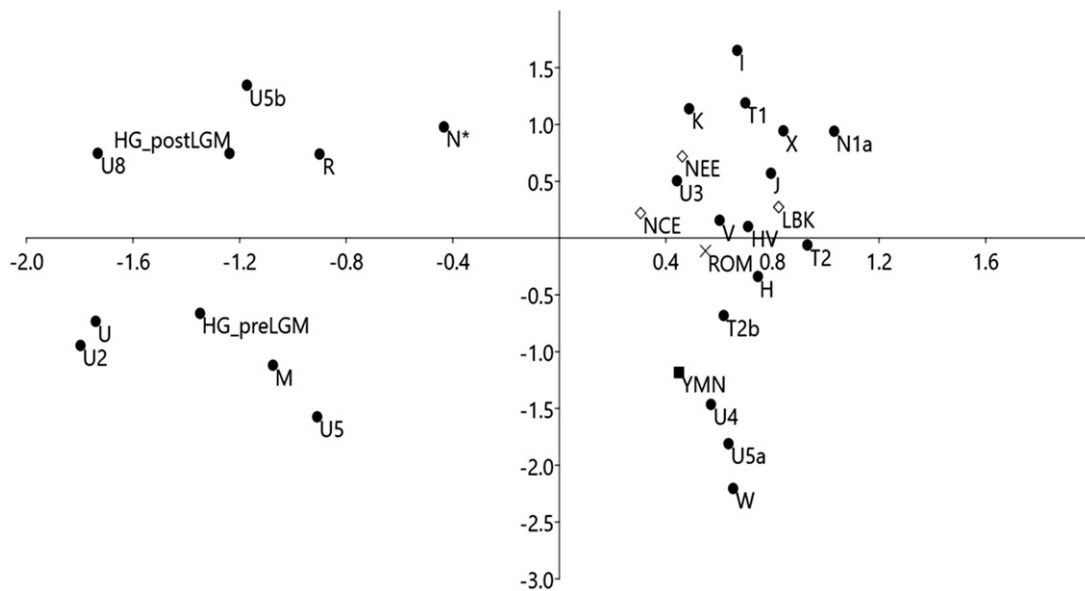


Figure 3. Correspondence analysis (CA) (Component 1: 34.2%; Component 2: 21.6% of variance) based on the mitochondrial DNA haplogroup frequencies of the ancient populations.

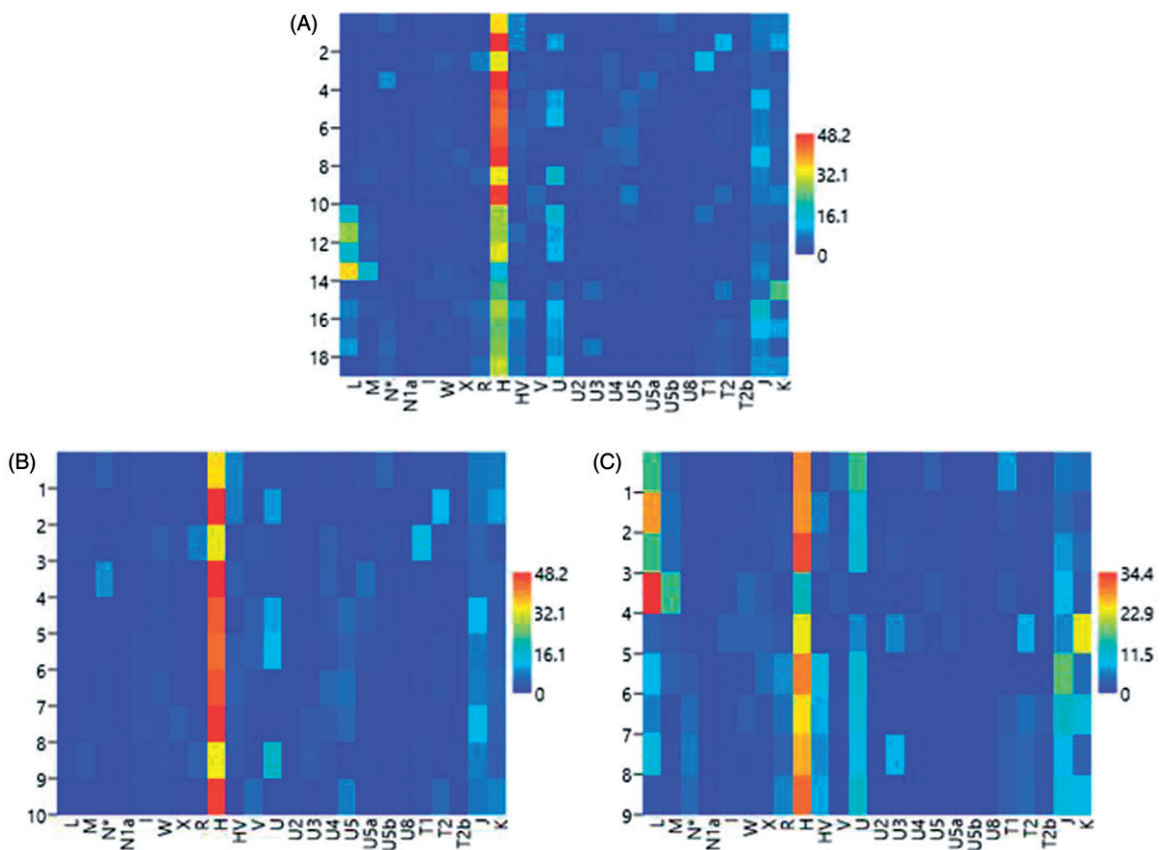


Figure 4. Matrix plots built up with the frequency distribution for each lineage as reported on the horizontal axis. (A) All the Mediterranean populations. Vertical Axis: 1: Spain; 2: Italy; 3: Albania; 4: Bosnia Herzegovina; 5: Slovenia; 6: Croatia; 7: Serbia Montenegro; 8: Greece; 9: Turkey; 10: France; 11: Morocco; 12: Algeria; 13: Tunisia; 14: Egypt; 15: Cyprus; 16: Palestine; 17: Syria; 18: Jordan; 19: Lebanon. (B) All the Northern Mediterranean populations. Vertical Axis: 1: Spain; 2: Italy; 3: Albania; 4: Bosnia Herzegovina; 5: Slovenia; 6: Croatia; 7: Serbia Montenegro; 8: Greece; 9: Turkey; 10: France. (C) All the Southern and Eastern Mediterranean populations. Vertical Axis: 1: Morocco; 2: Algeria; 3: Tunisia; 4: Egypt; 5: Cyprus; 6: Palestine; 7: Syria; 8: Jordan; 9: Lebanon.

Mediterraneans. Northern groups seem to differentiate themselves mainly by the uneven presence of the U and sub-U lineages, whereas the L lines are characteristic of Southern groups that are, thus, differentiated from Eastern groups

(Northern Mediterraneans vs Southern Mediterraneans vs Eastern Mediterraneans; AMOVA $F_{ct} = 0.025$; $p < 0.01$).

The nmMDS plot built by similarity index among extant populations (Figure 5) seems to be consistent with the rough

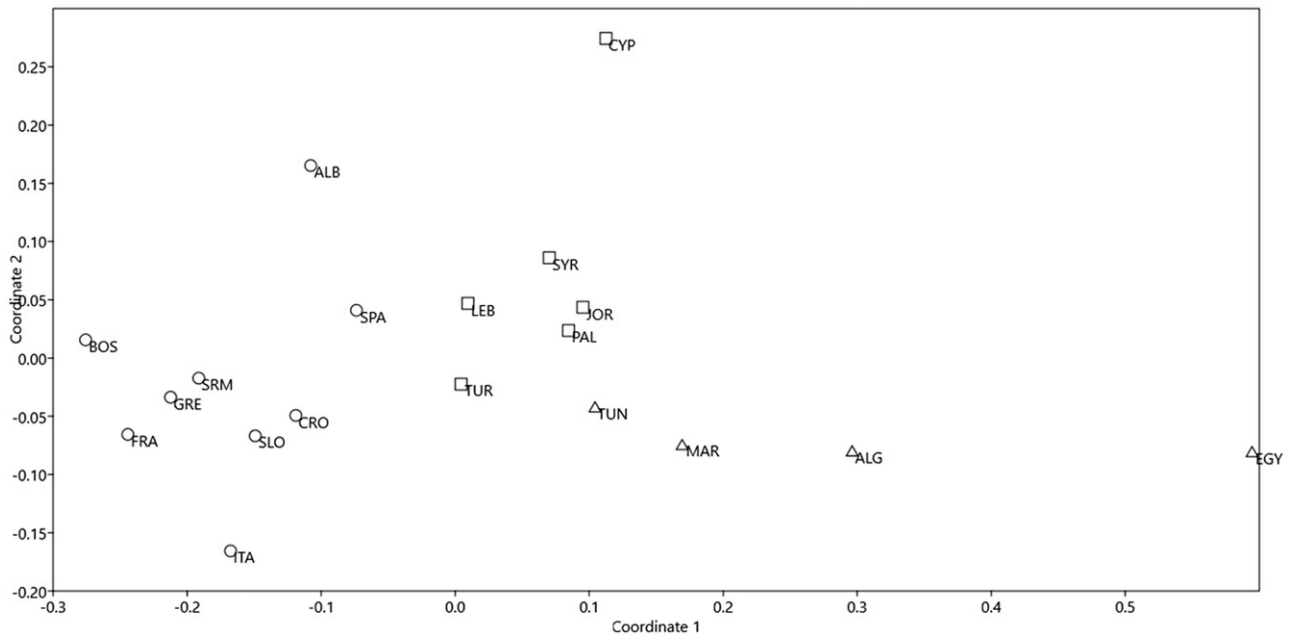


Figure 5. Non-metric Multidimensional Scaling (stress value =0.12) by Euclidean distance matrix from frequency distributions of modern populations. For full length names and details of samples and populations, see [Supplementary Table 1](#).

geographical clustering elaborated above, allowing us to significantly dissect Mediterraneans by geographical location: Northern Mediterraneans lie on one side and the Southern Mediterraneans on the other, while the Eastern ones are in the middle of the distribution (stress value =0.12).

The Egyptians' location on the plot edge is consistent with the appreciable frequency of haplogroup M, which is broadly unrepresented in other Northern African samples. The presence of M along with L lineages is probably the outcome of frequent southward commercial trades and consequent demic intermingling, which make Egyptians matchless with respect to their neighbours (Badro et al., 2013; Schueneman et al., 2017; Scorrano et al., 2016). Moroccans, Tunisians and Algerians are quite close to each other: North African mitochondrial genetics is characterised by the predominant influence of lines such as U6 and M, which seems to wire North Africa and Southwestern Asia: this makes sense, given the proximity of Eastern human groups such as Palestinians and Jordans. The latter populations are both close to Syrians, and could be grouped together by the appreciable presence of K and J haplogroups that may be linked to the original Neolithic expansion over the extant Near Eastern and European genetic landscape, suggesting a possible Neolithic pioneer seafaring colonisation of Europe (Fernandez et al., 2014). Cypriots are quite dissimilar from the other Eastern Mediterraneans, but this discontinuity could be ascribed to the geographic isolation of its people along with the confounding effect of the minimum sample size that could lead to inaccurate speculations.

The European populations located in the central Mediterranean area are tightly clustered and this reflects a rough genetic homogeneity that was probably increased by intensive gene flow affecting those countries (Fiorito et al., 2016; Lao et al., 2008). A middle position is occupied by a group including Turkey, Spain, Albania and Lebanon (This cluster vs Northern vs Eastern vs Southern; AMOVA

$F_{ct} = 0.022$; $p < 0.01$). The westward routes developed by Phoenicians could be partially diachronically mirrored in the similarities between Lebanon, Turkey and Spain (Cunliffe, 2001). Even the Albanians are included in this group, suggesting that Balkan populations show different mitochondrial haplogroup frequencies through complex isolations and genetic drift events in the past (Bosch et al., 2006; Jankova-Ajanovska et al., 2014; Scorrano et al., 2017).

In order to identify the possible contribution of ancient human groups to the present-day Mediterranean mitochondrial genetic pool, this similarity evaluation has been complemented by their haplogroup frequencies (nmMDS stress value =0.09; Figure 6). The resulting plot shows how the hunter-gatherers' genetic composition, both for the pre-LGM and post-LGM periods, are very dissimilar to what concerns their haplogroup frequencies. None of the Neolithics are so close to extant populations, suggesting how this revolution marked the ancient Mediterranean peopling, but its genetic fingerprint has not been extensively retained in present day populations. A comparable contribution seems to come from the genetic mitochondrial makeup of steppe people, while a larger influence was provided by the Romans, whose incredibly influential rule surely played a significant role in modifying the demic Mediterranean melting pot.

Among the mtDNA haplogroups, two lineages could be considered as suggestive for people migrations. The U5b lineage was previously proposed as a post-glacial expansion marker from the Iberian Peninsula (Posth et al., 2016; Rootsi et al., 2004; Torroni et al., 2001), whereas haplogroup K is widely known as a relevant Neolithic pointer with decreasing frequencies westward of the Levant (Isern et al., 2017).

The availability of a thorough mtDNA database for U5b and K lineages allowed us to properly identify the exact phylogeographic location of every sequence according to the latest accepted mtDNA phylogeny ([Supplementary Figures 1 and 2](#)).

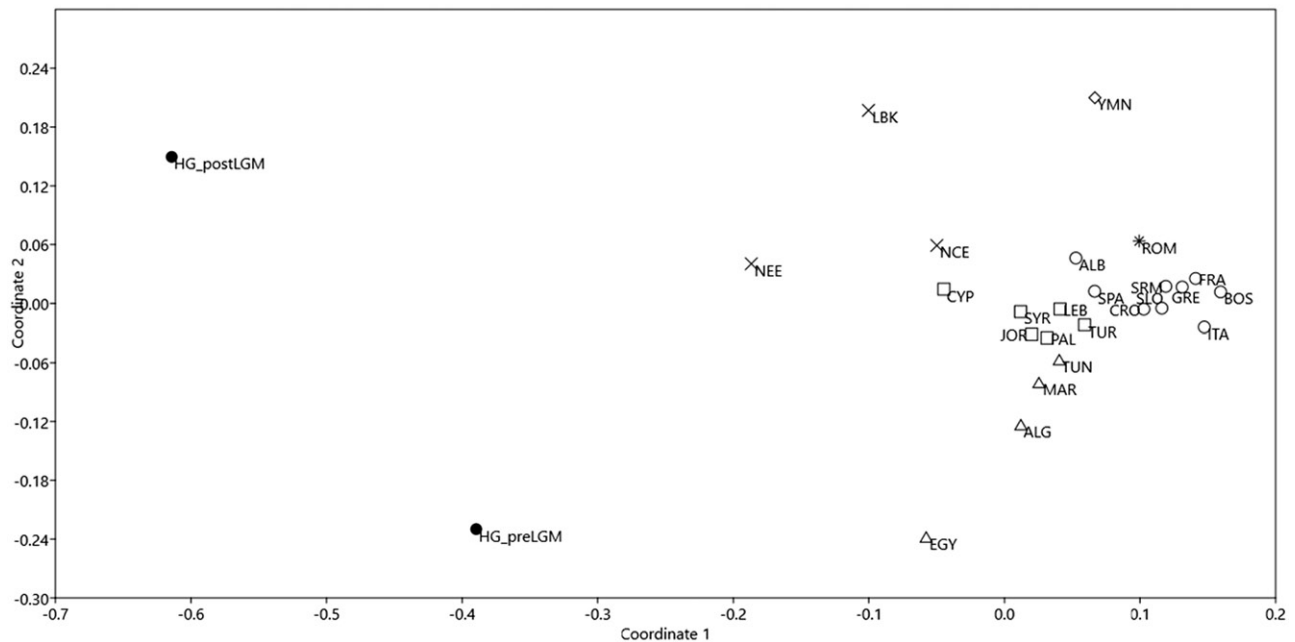


Figure 6. Non-metric Multidimensional Scaling (stress value =0.09) by Euclidean distance matrix from frequency distributions of ancient and modern populations. For full length names and details of samples and populations, see [Supplementary Table 1](#). Nomenclature for ancient populations: HG_preLGM, Hunter-gatherers pre-LGM; HG_postLGM, Hunter-gatherers post-LGM; NCE, Neolithic Central Europe; NEE, Neolithic Eastern Europe; LBK, Linear Pottery culture; YMN, Yamnaya; ROM, Romans.

Despite this comprehensive approach, these unambiguous trees failed to highlight specific geographic patterns, suggesting putative genetic continuity among ancient and extant people. The selection of the sole shared branches among ancient Mediterranean genomes and extant worldwide ones (Figures 7 and 8) permitted us to visualise the rough location of each ancient haplotype related to those identified in Northern, Southern or Eastern people.

U5b ancient sequences are totally grouped with Northern Mediterranean haplotypes (Figure 7), as expected due to the demic waves that occurred from European LGM ice-free refuges. These refuge areas represented the starting point of the human post-glacial dispersion, indicated by a significant diffusion of this sub-haplogroup (Maca-Meyer et al., 2001) that did not spread in the southern Mediterranean regions.

K ancient sequences are placed in a slightly more geographically restricted scenario (Figure 8). Two ancient Spanish haplotypes cluster in K1a2a branches along with Northern Mediterraneans, as well as K1a18 cluster which encompasses two ancient Jordan sequences and three Eastern Mediterranean haplotypes. Moreover, a Spanish Neolithic mtDNA exclusively shares the K1b1a1 node with sequences related to people from northern Mediterranean areas. The nuanced scenario highlighted for the other K ancient sequences seems to be consistent with the largest effect provided by historical and modern demic movements in significantly shaping the mitochondrial DNA make-up of present day populations.

The heterogeneous mean coverage of ancient mitogenomes could also be partially responsible for this picture because some specific mtDNA segments could be inaccurately covered, although the extensive presence of global private variants makes every haplotype, both ancient and

extant, quite totally diachronically and even synchronically unshared.

The overall descriptive evaluation is, thus, consistent with the known evidence that the first hunter-gatherer populations did not substantially contribute to the extant Mediterranean mitochondrial genomes. It is widely known that human mitochondrial DNA lineages in all present-day non-Africans belong to two basal branches (M and N) and their derived lineages (van Oven & Kayser, 2009). These two ancestral clades seem to indicate an early southern spread of modern humans, followed by a later non-African diffusion of the N clade perhaps via a northern route (Maca-Meyer et al., 2001), through a fast dispersal across Eurasia where the Western façade would have been reached only when M was lost (Macaulay et al., 2005). The drastic climate constraints of the LGM acted as a narrow filter of human mitochondrial variability, mainly affecting specific lineages such as M and some U sub-branches. These climatic changes forced humans to contract their geographic dispersion to specific refuges, with a decline in population size from ~330 000 people before the LGM to 130 000 afterward (Tallavaara et al., 2015). These refuge areas represented the starting point of the human post-glacial dispersal, indicated by a significant diffusion of major sub-haplogroups (U5a and U5b) (Maca-Meyer et al., 2001) that are still present in current people, although represented by different haplotypes (Figure 7). Human dispersal in Europe has been widely studied, suggesting a complex replacement scenario (Achilli et al., 2004; Dolukhanov, 1993; Gamble et al., 2004; Soares et al., 2010; Torroni et al., 2001). The importance of the H lineage in the European mitochondrial landscape is undoubtable; its Mediterranean dispersal seems to be linked to the neolithisation process as well as to the rising frequencies of several specific clades

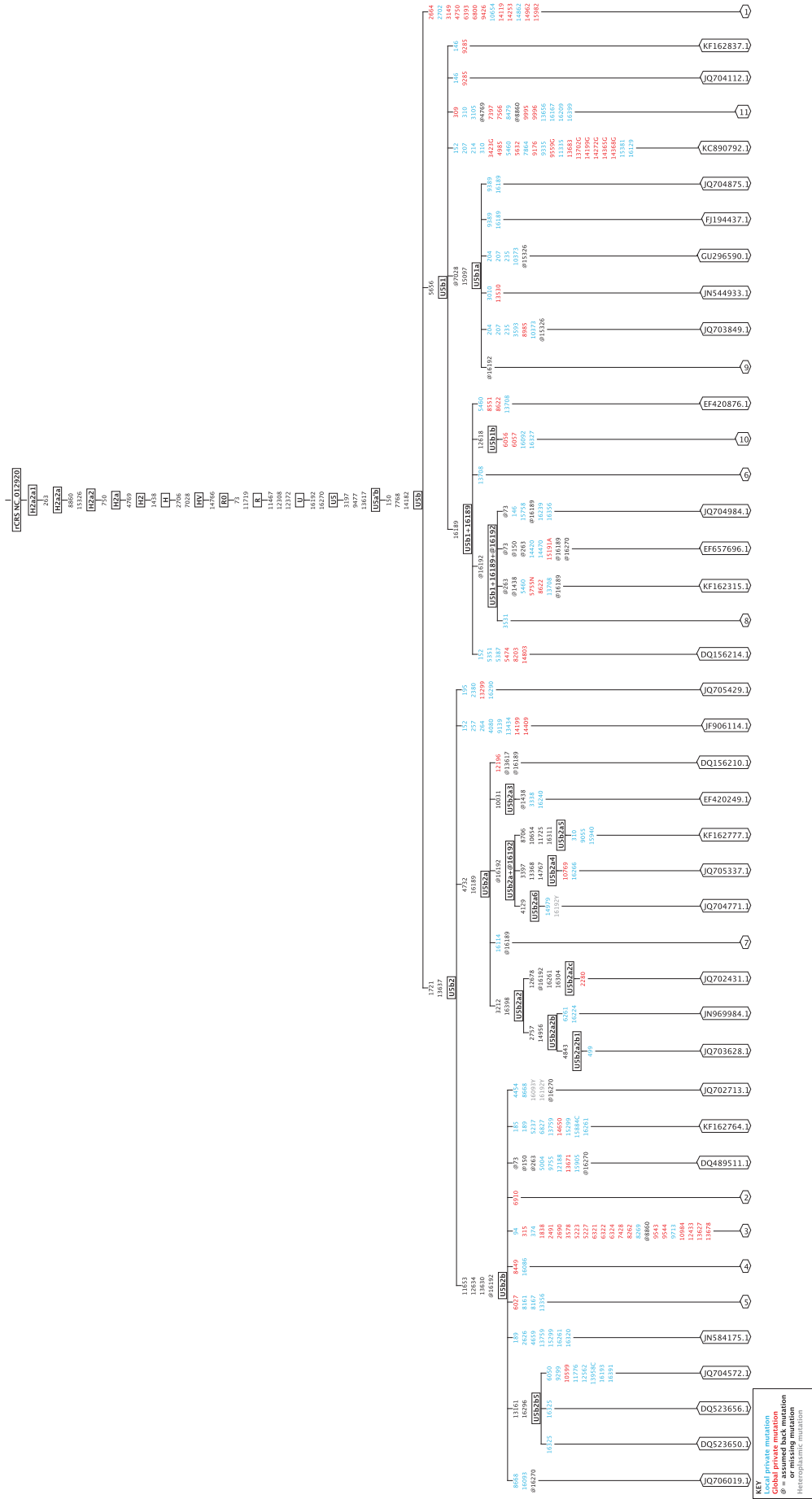


Figure 7. Phylogeny tree built with U5b haplogroup complete mtDNA sequences available in MitoTool database (Fan & Yao, 2013) and Mediterranean ancient people (Table 1) by Haplogrep2. Codes for ancient haplotypes are in Table 1.

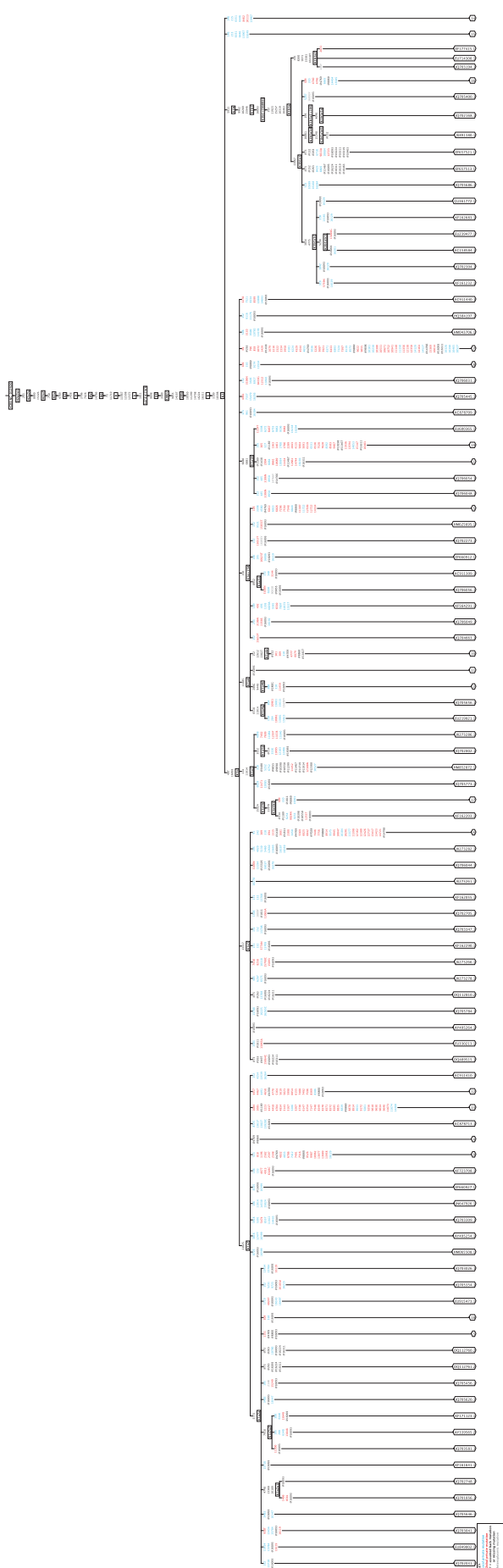


Figure 8. Phylogeny tree built with K haplogroup complete mtDNA sequences available in MitoTool database (Fan & Yao, 2013) and Mediterranean ancient people (Table 1) by Haplogrep2. Codes for ancient haplotypes are in Table 1.

that characterise both the northern and southern shores of the Mediterranean sea (Gamba et al., 2014; Haak et al., 2015; Hofmanová et al., 2016; Omrak et al., 2016), spreading westward from Anatolia and the Levant.

The Western European section is a very complex area characterised by a plethora of demic interactions that could be mirrored by a significant geographic sub-structure on the Iberian Peninsula. While haplogroup H has a strong presence, its frequency gradually decreases, moving from the Atlantic area towards the Mediterranean one. Haplogroups J and T also show some distinctive geographic patterns: they are more prevalent in the Mediterranean area and this specific genetic fingerprint was probably set in Mesolithic and Neolithic times (Barral-Arca et al., 2016).

Trans-Iberian flows seem to be the main factor responsible for the sub-structure of Spain: for instance, the Arab conquest of the Iberian Peninsula contributed to a rise in frequency of eastern lineages (M1) as well as L haplotypes. These are more frequent in the Mediterranean regions of the Iberian Peninsula, confirming genome-wide data supporting the Muslim expansion as one of the leading factors in shaping the genetics of southern Spain due to multiple coast-to-coast human movements. Remarkably, U6, which is a typical Western Northern African lineage, has been widely linked to early dispersal movements from southwestern Asia towards North Africa (Schueneman et al., 2017), even though recent analysis (Hernández et al., 2014) does not allow us to rule out the Northwest Iberomaurusian African culture influence spreading in the North Sahelic area in more recent times (20 kya–9 kya).

The Neolithic changes represented a dramatically complex genetic landscape and we are far from a holistic interpretation and understanding of that period in the Mediterranean Basin. However, a definite, but not deeply influential, replacement of people occurred in the Bronze Age.

The Yamnaya migrations have been linked to major cultural changes such as the expansion of Indo-European languages (Anthony, 2007; Kristiansen, 2007; Mallory, 1987; Renfrew, 1987). Archaeological and whole genome analysis suggest early European farmers were largely replaced by the Yamnaya culture, especially in Central Europe (Allentoft et al., 2015; Haak et al., 2015), although mitogenome data do not show such clear and significant results in Mediterranean groups. Indeed, today it is clear that the Bronze Age was an extremely dynamic timeframe involving large-scale population migrations and replacements throughout Europe and Asia, responsible for significantly shaping the present-day demographic structure of Central Europe and Asia. However, these impacts on the matrilineal marker composition in Southern European populations appear to be of a lower magnitude.

The impact and genetic influence of multiple historical phenomena certainly represent significant modifier effects on Mediterranean mitogenomics and their specific role in shaping the mitochondrial DNA variability in the present day population.

The elucidation of these historical movements is hard to discern for the complex multidimensional demic migrations, but some speculations could be made. For example,

Phoenician commercial trade could have contributed to the genetic affinity between Middle-Eastern regions and the Western fringe of Europe and North Africa (Carpenter, 1958; Matisoo-Smith et al., 2016). The partial similarity in North Africans gives another hint: Carthage was located in Tunisia and dominated for a long time (Matisoo-Smith et al., 2016), even though the Berbers genetically and culturally influenced Algerian and Moroccan populations (Brett & Fentress, 1996), supporting their mitochondrial genetic affinity (Figures 5 and 6).

The lack of classical Greek genetic data does not allow a proper evaluation of the influence of the ancient Greek world (Di Gaetano et al., 2009; Tofanelli et al., 2016), although Mycenaeans (former Greeks) and Minoans (the ancient culture on the island of Crete) could be a valuable proxy for supporting evidence about ancient Greek expansion. Recent evaluations show a widespread homogeneity between these two populations, corroborating the hypothesis that modern Greeks are the results of admixture between those ancient populations and Neolithics (Lazaridis et al., 2017), even though mitochondrial data restrict the farmers' influence in the maternal background of Greeks that lie close to Balkan populations (Figures 5 and 6; Di Gaetano et al., 2009; Hughey et al., 2013; Tofanelli et al., 2016).

The ancient Romans seem to be close to Northern Mediterranean people, sharing the typical European mitochondrial pattern rich in H lineages in accordance with the cosmopolitan nature of the Roman Empire. Unfortunately, consistent genomic data about Mediterranean Romans are still scant and the data reported could refer to people living outside the Italian Peninsula and of course at the edge of the *Urbs* central power. It is conceivable that, in the Roman Empire, the expansion and the massive increase in effective population size resulted in an overall homogeneity of mitochondrial lineages among some Mediterranean colonies, leading to extant populations. The need for Ancient Roman genomes from Rome will soon be fulfilled by the ongoing research of a multidisciplinary consortium (De Angelis et al., 2018), aiming to improve the knowledge about the genomic landscape of this outstanding population that surely dominated the demographic and cultural stage of the Mediterranean Basin for a long time, even to cross the Empire boundaries and reach far Eastern territories (Rezakhan, 2010).

Thus, to summarise the evidence drawn together by this review, ancient mitogenomic contributions offer insights into the genetic makeup of Mediterranean populations reaching back almost 50 kya, although they are not completely sufficient for interpreting multiple migratory waves and their genetic and cultural consequences. The multiple historical and contemporary demic movements probably led to an overall genetic affinity among several geographic areas, confounding our understanding of the extent of ancient mitochondrial genetic contributions with different present-day human groups.

Nonetheless the buildup of data on ancient mitogenomes as well as the availability of whole genomes would surely be a significant aid in the interpretation of the past genetic variability of Mediterraneans. A notable direction for future work

will be the generation of similar ancient DNA data from human groups in Mediterranean Europe (Macchiardi et al., 2016; Modi et al., 2016), North Africa (Schueneman et al., 2017) and the Near East to gain a more comprehensive picture of the ancient population history of the most important and crowded natural hub for human genetic and cultural expansion (Sazzini et al., 2014).

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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