A Genetic History of Continuity and Mobility in the Iron Age Central Mediterranean

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Abstract

The Iron Age saw the expansion of Phoenician and Greek colonies across the Mediterranean and the rise of Carthage as the major maritime power of the region. These events were facilitated by the ease of long-distance travel following major advances in seafaring. We know from the archaeological record that trade goods and materials were moving across great distances in unprecedented quantities, but it is unclear how these patterns correlate with human mobility. To investigate population mobility and interactions directly, we sequenced the genomes of 30 ancient individuals from Carthaginian and Etruscan port cities around the central Mediterranean, in Tunisia, Sardinia, and central Italy. At all three locations, there is a meaningful contribution of autochthonous populations (from Bronze Age North Africa, Sardinia, and Italy, respectively), as well as highly heterogeneous ancestry including many individuals with ancestry from other parts of the Mediterranean region. These results highlight both the role of autochthonous populations and the extreme interconnectedness of populations in the Iron Age Mediterranean. By studying these trans-Mediterranean neighbors together, we explore the complex interplay between local continuity and mobility that shaped the Iron Age societies of the central Mediterranean.

Introduction

The Iron Age was characterized by a marked increase in mobility in the Mediterranean. Advances in sailing and seafaring allowed for easier and more frequent travel across the open sea, facilitating new networks of interaction - for trade, colonization, and conflict. In this paper, we use data from three key locations in the central Mediterranean -- Tunisia, Sardinia, and central Italy -- to study the genetic origins and mobility of individuals within this region. We see that these technological changes were accompanied by an increase in gene flow and genetic mobility across the Mediterranean, which impacted the ancestry makeup of the populations on its shores.

In the early Iron Age, Phoenician and Greek city-states established trading ports and colonies across the Mediterranean. One such colony, Carthage, originally founded by Tyre, became the capital of the Phoenicio-Punic territories (Fig. 1, Fig. 2A). Carthage was located at the crossroads of many trans-Mediterranean trade routes and, for 500 years, it was the dominant maritime power of the region until Roman Imperial expansion in the final centuries BCE (1, 2). Across the sea, Etruscan city-states flourished in central and northern Italy during the Iron Age. Their material culture suggests continuity with the preceding Villanovan culture, but there are also strong indications of contact with other cultures, both by sea and land (3).

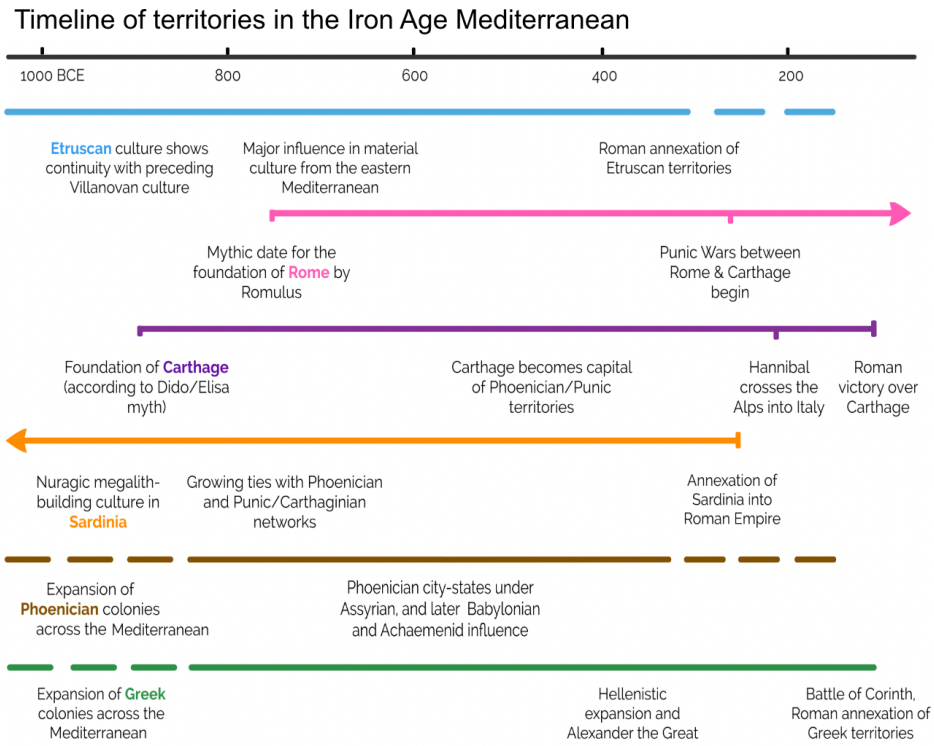

Fig. 1. Overview of historic events in the Iron Age Mediterranean. *Each line corresponds to a cultural group, indicated in the labels below the lines. Dashed lines represent gradual processes that do not have a definitive start or end date.*

While contemporary scholarship (and geopolitics) often regards the Mediterranean as a border and siloes research along country and continent boundaries, these divisions are modern in their making. Carthage and the Etruscans were trading partners and, at times, allies united against Greek and Roman colonial expansion. However, insights into the relationship between these trans-Mediterranean neighbors are limited. Few Punic or Etruscan written sources have survived to the present day, and most of these are

short inscriptions (3, 4). As a result, much of the historical record is filtered through the lens of Greek and Roman sources (5, 6). Iron Age history was, quite literally, written by the victors, and archaeogenetic research adds a new line of evidence to our understanding of the people of this dynamic time period.

Until recently, the genetic makeup of Iron Age Mediterranean populations has been relatively understudied in comparison to earlier cultures. New research has begun to shed light on the Iron Age peoples of central Italy and Sardinia (7–10). To date, however, there has been no whole-genome ancient DNA research on Northwest Africa from this, or adjacent, time periods, despite the major historical importance of Carthage and the complex history of the region. Mitochondrial DNA from an Iron Age individual from Carthage is the only existing ancient DNA data from the region (11).

Our goal here is to explore the relationships among the peoples of the central Mediterranean at a time when the developments in seafaring were facilitating new levels of connection across the “Great Sea”. Was there genetic continuity between the Iron Age populations of Tunisia, central Italy, and Sardinia and their predecessors? How were these populations shaped by the emerging trans-Mediterranean networks of trade, conflict and colonization? For instance, did the expansion of Phoenician and Greek colonies to the central Mediterranean lead to population replacement? To what extent was the increased movement of trade goods observed archaeologically paralleled by an increasing mobility of the human populations among these different communities of the Mediterranean?

To explore these questions, we generated 30 new genomes from four archaeological sites in the central Mediterranean - from Tunisia, central Italy and Sardinia - to study the effects of the increasingly interconnected nature of humans in the Great Sea, particularly in terms of human mobility and interaction (Fig. 2). Notably, our dataset includes 12 Iron Age genomes from Tunisia; these start to fill a major gap in ancient DNA sampling from North Africa during the Bronze or Iron Ages. This trans-Mediterranean approach illuminates the complicated interplay between continuity and mobility resulting in extensive genetic exchange across the Iron Age central Mediterranean and allows us to directly examine relationships between local and diasporic people of the Iron Age.

Results

Data generation

We generated double-stranded DNA libraries after partial uracil-DNA glycosylase (UDG) treatment. Libraries were sequenced on an Illumina NovaSeq 6000 sequencing platform to generate whole-genome shotgun data, with an average genome-wide coverage of 1.1x (range: 0.61 - 1.9x). We successfully obtained data from 30 individuals from 4 central Mediterranean Bronze and Iron Age archaeological sites, showing in Fig. 2A: Kerkouane in Tunisia (n=12), Sant’Imbenia in Sardinia (n=3), and Pian Sultano (n=4) and Tarquinia in central Italy (n=11). In our analyses, the new data are supplemented with 9 previously published Iron Age Sardinians dating from 818- 208 BCE (7, 8) and 11 additional Iron Age Italians dating from 963 - 200 BCE (9).

We radiocarbon-dated 19 individuals (Fig. 2b, Dataset S3). These confirm the archaeologically attested dates of use for the Kerkouane necropolis to the mid-Iron Age (650 - 250 BCE), when Carthage was the Mediterranean's dominant maritime power. In Italy, the individuals from Tarquinia spanned the Iron Age, from the city’s growth in the early Iron Age through its incorporation into the growing Roman Republic in the 3rd century BCE. The burials at Pian Sultano date to the middle Bronze Age; these are the first available Bronze Age samples from central Italy with adequate genome coverage, and thus provide important context for Italian populations before the Iron Age. Individuals at Sant’Imbenia, Sardinia date to the Bronze/Iron Age transition on the island (1115 - 775 cal BCE). We identified one close kinship

relationship: a pair of first-degree relatives (R11104 and R11105) from a shared burial at the Bronze Age site of Pian Sultano (Table S1).

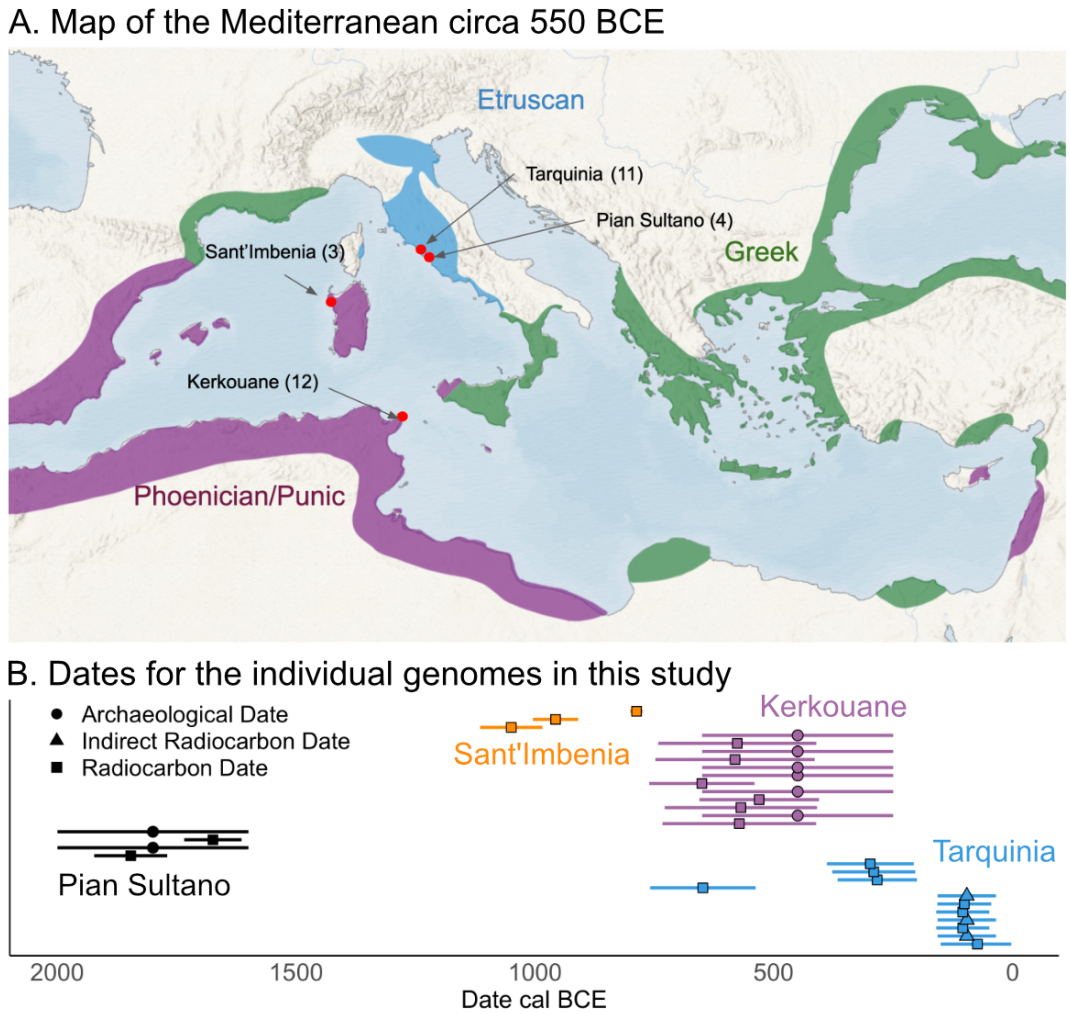

Fig. 2. Data overview and relevant geography and chronology. ***A.*** *Locations of the 4 archaeological sites examined here, as well as a map of the territorial extent of major Mediterranean political players in the mid-Iron Age (around 550 BCE).* ***B.*** *Timeline showing the dates for the newly reported individual genomes, with the methods of dating indicated. Indirect radiocarbon dating refers to using the radiocarbon date of a different individual in the same tomb.*

Overview of the genetic structure

To contextualize these individuals within the genetic landscape of contemporaneous populations, we curated a set of published ancient genomes spanning the Iron Age Mediterranean (7–9, 12–16). We organized these data into 5 regional groupings: the Italian Peninsula, Sardinia, Northwest Africa/Maghreb, the Levant, and Iberia. To compare with the preceding period, we also curated the available Bronze Age samples for each of these regions (17–19). For the Maghreb, we included Late Neolithic individuals as there are no published Bronze Age data (20). This resulted in a set of 332 reference genomes to contextualize the new data (more information about these and our curation of the metadata can be found in Dataset S2).

We generated a Principal Component Analysis (PCA) reference space using modern populations from around the Mediterranean, Europe, the Middle East, and North Africa (21), and projected the Bronze and Iron Age Mediterranean individuals onto the modern reference space (Fig. 3, Fig. 4A).

This regional time series approach allows us to make some general observations about changes occurring across these periods. In particular, we observe a marked increase in heterogeneity in the Iron Age, relative to the preceding Bronze Age Mediterranean populations; and consistent with this, in the Iron Age there is much more overlap between individuals from different regions. We also observe a number of individuals who project into the center of the PCA space, a region unoccupied by modern populations. We interpret this as representing autochthonous north African ancestry, based on analyses discussed below.

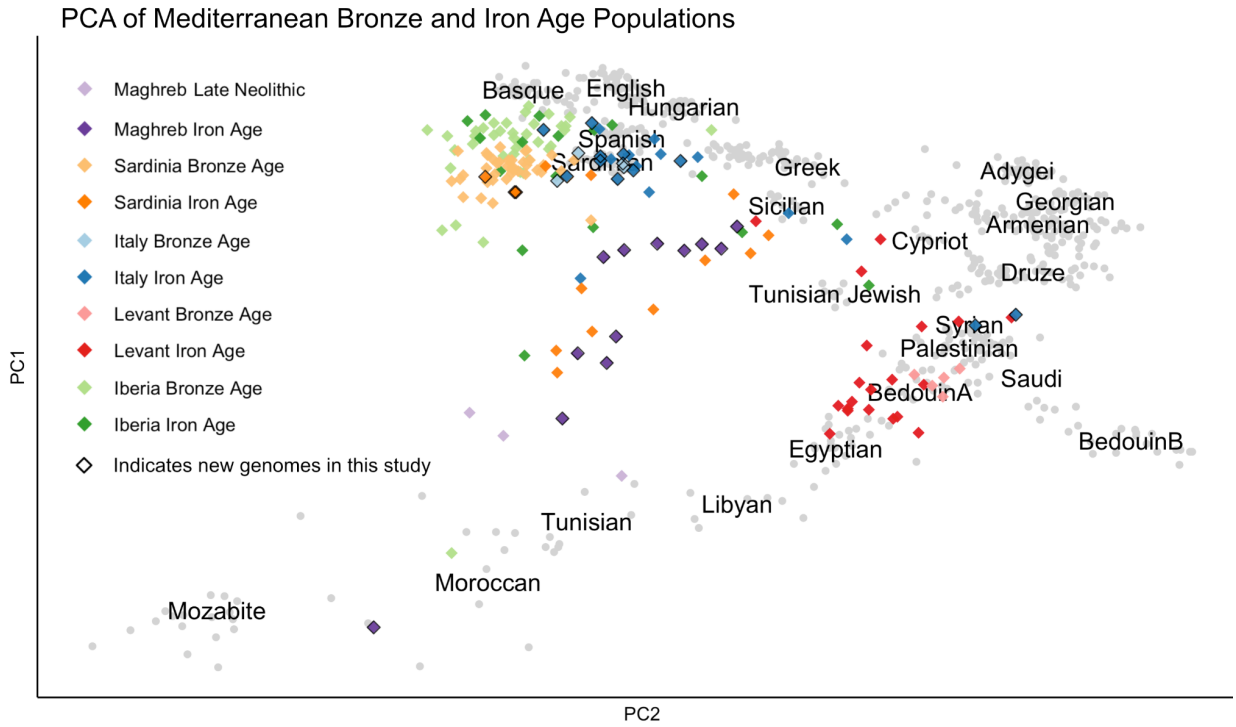

Fig. 3. Principal Component Analysis of relevant samples. *Newly reported individual genomes (diamond-shaped points) are projected onto a PCA space of present-day individuals (gray points) using smartPCA. Ancient genomes are shown in paired sets of colors by region, with the earlier time period (Bronze Age or Neolithic) represented by the lighter colors and the later time period (Iron Age) represented by the darker colors.*

To further explore these patterns, we modeled the ancestry of the new and published individual genomes from the central Mediterranean with qpAdm admixture modeling, shown in Fig. 4 (22). We chose a set of distal source populations previously shown to be informative for understanding the diversity of the Mediterranean during this period: Western Hunter-Gatherer (WHG), Yamnaya Samara, Anatolian Neolithic, Iranian Neolithic, and Neolithic farmers from Morocco (7, 9, 14).

We next used this same set of source populations to perform pairwise qpWave on all individuals to test whether pairs of individuals form a clade with each other, relative to a set of reference populations (Supplementary Methods). We used 1 - log(p-value) to calculate distances between each pair of individuals and performed clustering (Fig. 5). Given the genetic heterogeneity that characterizes the Iron Age Mediterranean, these groupings identified in qpWave help identify genetically similar individuals across regions.

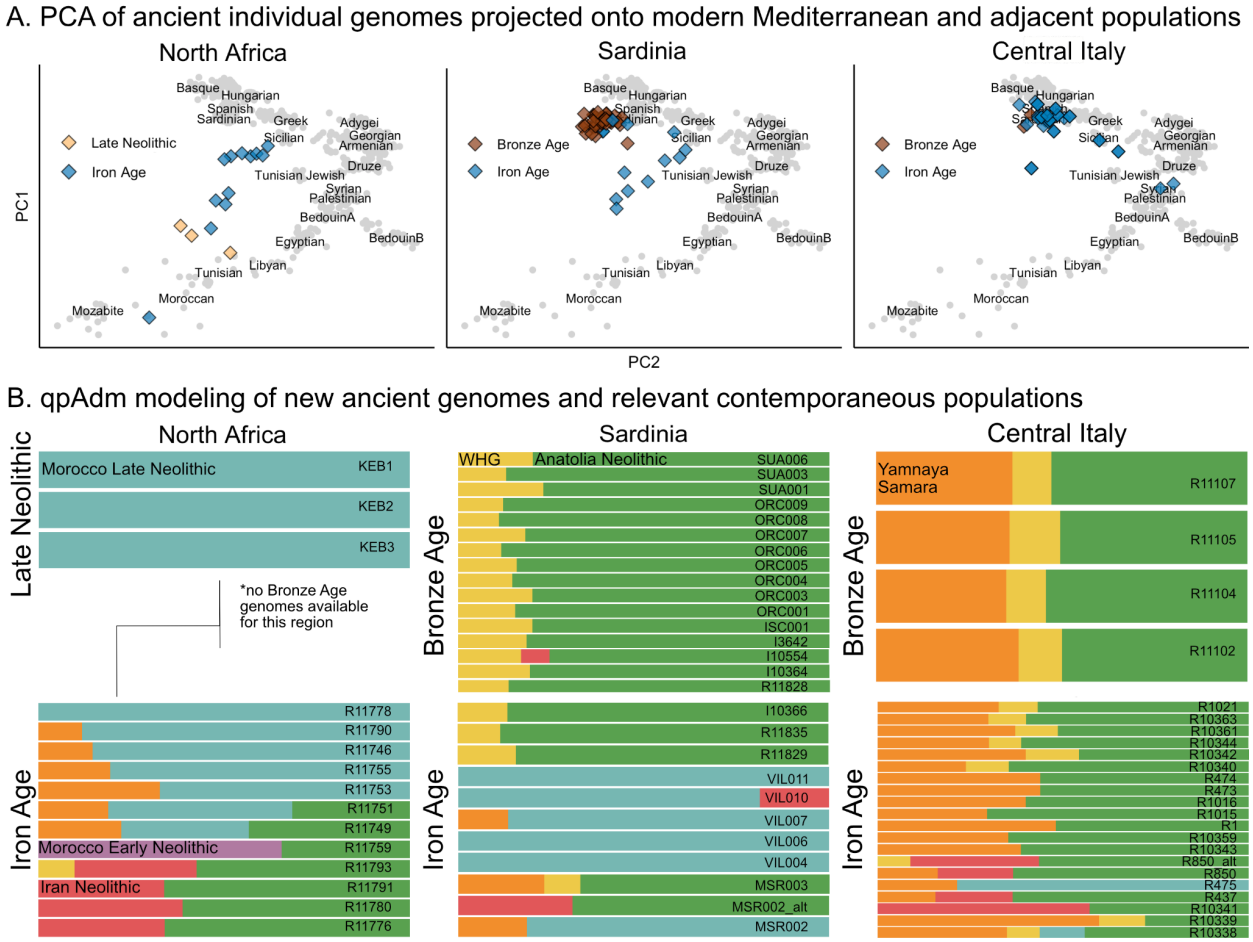

Fig. 4. Genetic overview of central Mediterranean populations during the Bronze and Iron Ages. *Both PCA and qpAdm illustrate the shift from relative genetic homogeneity in the Bronze Age to heterogeneity in the Iron Age.* ***A:*** *PCA of ancient Neolithic, Bronze and Iron Age populations from the Central Mediterranean, projected onto a modern reference space.* ***B:*** *qpAdm analyses using a set of distal source populations (Western Hunter-Gatherer (WHG), Yamnaya Samara, Anatolia Neolithic, Iranian Neolithic, Morocco Late Neolithic, and Morocco Early Neolithic).*

Characterizing the Genetic Heterogeneity at Kerkouane

At Kerkouane, a Carthaginian town on the Cap Bon peninsula in Tunisia (see extended description in Materials), we observe a highly heterogeneous population, spanning across the PCA space in Fig. 3 from modern Mozabite populations to modern Sicilian populations, consisting of three primary genetic clusters. One of the genetic groups we identified includes four individuals who have genetic continuity with preceding Maghrebi neolithic farmers, suggesting that these individuals represent an autochthonous North African population (Fig. 4). One individual, R11778, can be modeled in qpAdm with 100% Morocco Late Neolithic farmer ancestry, while three individuals, R11746, R11755, R11790, can be modeled predominantly with this component, along with the addition of 15 - 20% Steppe-related ancestry. A second cluster, visible in PCA (Fig. 3 and Fig. S5) and identified in qpWave (Fig. 5), contains seven individuals who are genetically similar to Bronze Sicilian and central Italian populations, as well as some individuals from the Hellenistic Iberian Greek colony of Empúries (14, 23).

For R11759, who projects near modern Mozabite and Moroccan populations in PCA space, there were no working distal qpAdm models with the original set of 5 distal source populations (Fig. 5). We replaced

Morocco Late Neolithic with Morocco Early Neolithic farmers and a hunter-gatherer individual from Ethiopia from ~4500 BP (24), both of which produced working models. Using competition modeling (where possible sources are rotated to the outgroup), the best model uses ~70% Morocco Early Neolithic ancestry and ~30% Anatolia Neolithic (Fig. 4, Fig. S6). When compared to other ancient individuals using qpWave analysis (Fig. 5), this individual forms a clade with ancient Canary Island inhabitants thought to be representative of the original founding population (25). The Canary Islands were originally settled in the 1st millennium BCE by a population genetically ancestral to today's Amazigh populations of Saharan Africa (26).

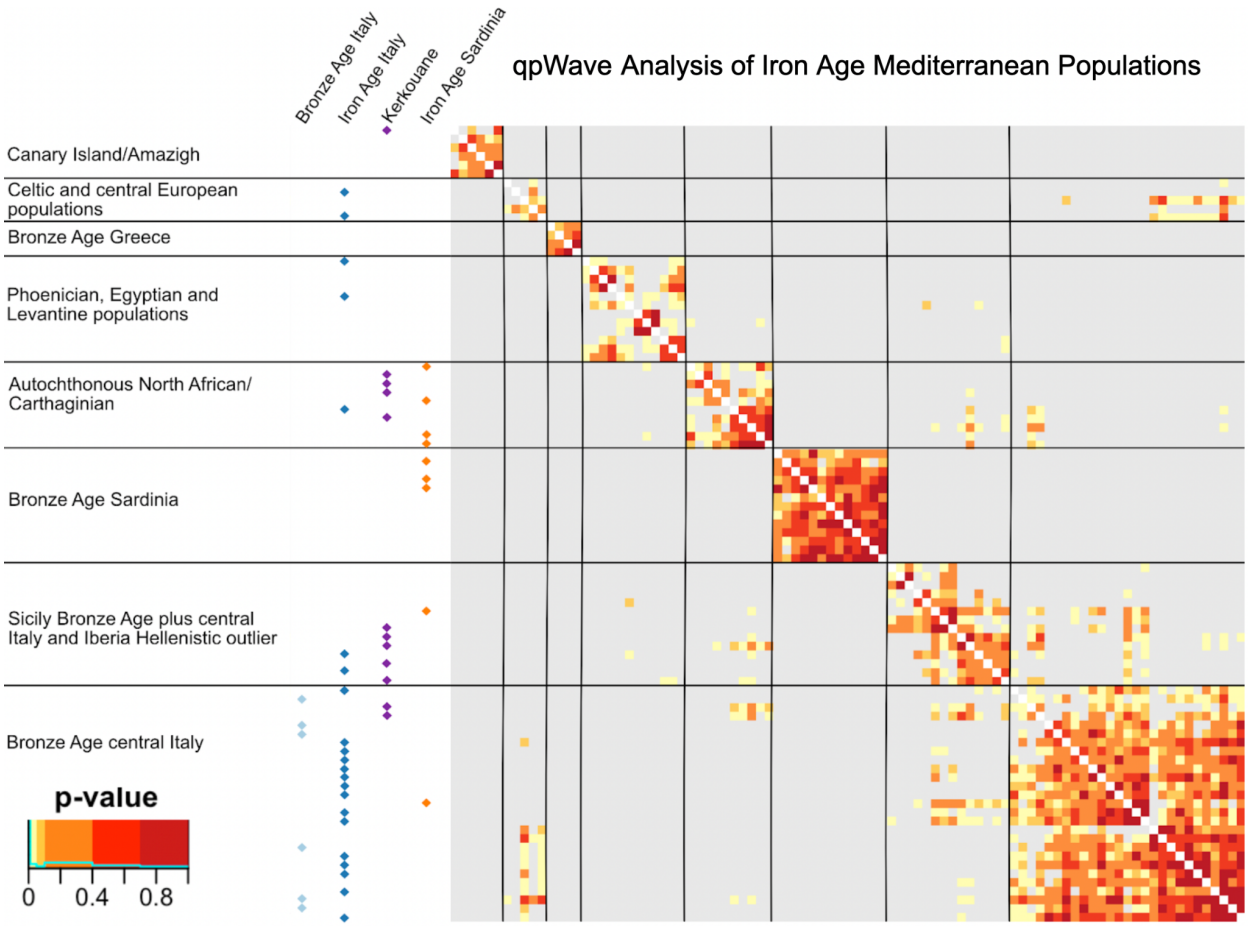

Fig. 5. qpWave Analysis of Mediterranean Populations. *The plot shows clustering of Bronze Age and Iron Age genomes from the central Mediterranean with other relevant populations. Heatmap values indicate whether each pair of individuals can be modeled with the same ancestry components in qpAdm in comparison to a set of reference populations. Individual labels are shown in Fig S7.*

Insights into the Genetic Impact of Carthaginian Expansion (Sardinia, Ibiza)

The Iron Age was a dynamic period in Sardinia - initially independent, and later incorporated first as a Phoenicio-Punic/Carthaginian colony and then as a Roman one. A short distance across the sea from Carthage, Sardinia was a relatively genetically homogenous population through the Bronze Age, with increasing heterogeneity in the Iron Age (7, 8). By incorporating Sardinia into this study, we are able to investigate the genetic impact of Carthaginian expansion in territories beyond Tunisia.

In Sardinia, we observe a shifting genetic profile reflecting the shifting political affiliations of the island through the Iron Age (Fig. S3). The three Late Bronze Age/Early Iron Age genomes from Sant'Imbenia

(R11828.SG, R11829.SG, R11835.SG; 1115 - 775 cal BCE) show continuity with the preceding Bronze Age Nuragic population. Later individuals (818 - 392 cal BCE) from the Punic site of Villamar (VIL004, VIL006, VIL007, VIL010, VIL011) can be modeled with primarily Morocco Late Neolithic ancestry, similar to the North African cluster from Kerkouane, as well as a Punic individual from Ibiza, MS10614 (15). This timing is consistent with a previous estimate from modern genomes that African gene flow into Sardinia occurred 96 generations (~2750 years ago) (27). Contemporaneous individuals (800 - 300 BCE) from the site of Monte Sirai (MSR002, MSR003), cluster with contemporaneous central Italian and Sicilian populations (Fig 5). Following the Roman annexation of this territory from Carthage in the First Punic War (241 BCE), Roman period individuals from Sardinia project in PCA near contemporaneous individuals from mainland Italy (7, 8).

Genetic Evidence for Etruscan and Punic Interactions

The Etruscan city-states of Iron Age central Italy are thought to have developed from the preceding Villanovan culture, but there are also strong indications of contact with other cultures, both by sea and land. In Iron Age Italy we observe these same two patterns: a combination of autochthonous continuity from the Bronze Age population, along with a drastic increase in genetic heterogeneity compared to the Bronze Age. Of the 22 Iron Age central Italian genomes, 12 individuals form a clade in the qpWave and qpAdm analyses (Fig.s 4, 5) with Bronze Age central Italian individuals, meaning they can be modeled as deriving 100% of their ancestry from Bronze Age central Italians.

However, nearly half of the individuals cannot be modeled this way, and are better modeled in qpWave as having ancestry from other parts of the Iron Age Mediterranean world (Fig. 4). Among these, one individual, R475, projects near the clade of autochthonous North African individuals at Kerkouane and can be modeled with the sample distal source populations in qpAdm (~85% Late Neolithic Moroccan farmer and~20% steppe-related ancestry). Additionally, four recently published individuals from Tarquinia have northern African ancestry (10). Two individuals, R10337 and R10341, appear genetically similar to contemporaneous individuals from the Levant (Fig. 4). In ADMIXTURE modeling, these two individuals lack Steppe-like ancestry, contrasting with other contemporaneous individuals from central Italy. They both have high amounts of Iranian Neolithic ancestry (Fig. S4).

Iron Age Mobility Shaped the Present-Day Distribution of Ancestry Components of the Region

Across all three locations, we observe continuity in the Iron Age with the preceding population of the region accompanied by the presence of individuals with ancestry from other parts of the Mediterranean world and beyond. This mobility seems to have contributed to the present-day genetic structure of these populations. In qpAdm and ADMIXTURE modeling (Fig. 4, Fig. S4), three ancestral components were generally sufficient for modeling preceding populations in Tunisia, Sardinia, and Italy, but in the Iron Age, a minimum of five components was needed to model the ancestry profiles of individuals. The Morocco Late Neolithic component, which was predominantly found in North Africa previously, in the Iron Age now appears in central Italy, as well as in individuals from Carthaginian sites across the central and western Mediterranean, such as Ibiza. This component may be part of the genetic signature of Carthaginian expansion.

In central Italy, we observe the appearance of Iranian Neolithic ancestry. Two individuals, R10337 and R10341, have very high amounts of Iranian Neolithic ancestry and may be early examples of the shift towards eastern Mediterranean ancestry of the following Roman imperial period (9). This component appears in high levels in the two outlier individuals mentioned above and then in smaller amounts later in the Imperial period population of Rome (Fig. 3, Fig. S4). This suggests this ancestry may have instead been introduced through individuals traveling long distances within their own lifetimes, as we know was happening with the establishment of Phoenician and Greek colonies throughout the Mediterranean at this

time. In contrast, the appearance of steppe-related ancestry during the Bronze Age in central Italy originally occurred in small amounts, ubiquitously in the population (Saupe et al. 2021), suggesting that this ancestry may have spread gradually through small, local interactions over many generations.

Discussion

To investigate interactions among Iron Age populations, we report here the genomes of 30 ancient individuals from Carthaginian and Etruscan sites around the central Mediterranean. In combination with available published data from this and adjacent regions, we examined the patterns of mobility emerging in the central Mediterranean and how these shaped the populations on its shores (Fig. 6, Table S1).

Kerkouane was highly cosmopolitan. We observe individuals who show genetic continuity with the preceding populations of the Maghreb. Many individuals with non-local ancestry cluster with contemporaneous Greek and Sicilian communities rather than with genomes from the eastern Mediterranean. One individual has ancestry most likely from nomadic populations of the Sahara. In contrast, in Italy, the majority of sampled individuals cluster genetically with the Bronze Age populations of central Italy, indicating a continuity of populations -- consistent with the recent findings of Posth et al. 2021 (10). This continuity is accompanied by a significant increase in heterogeneity with about one-third of individuals better modeled with ancestry from other populations of the Iron Age Mediterranean. In Sardinia we observe population shifts coinciding with its geopolitical affiliation - from independence, to a Phoenicio-Punic colony to a Roman one.

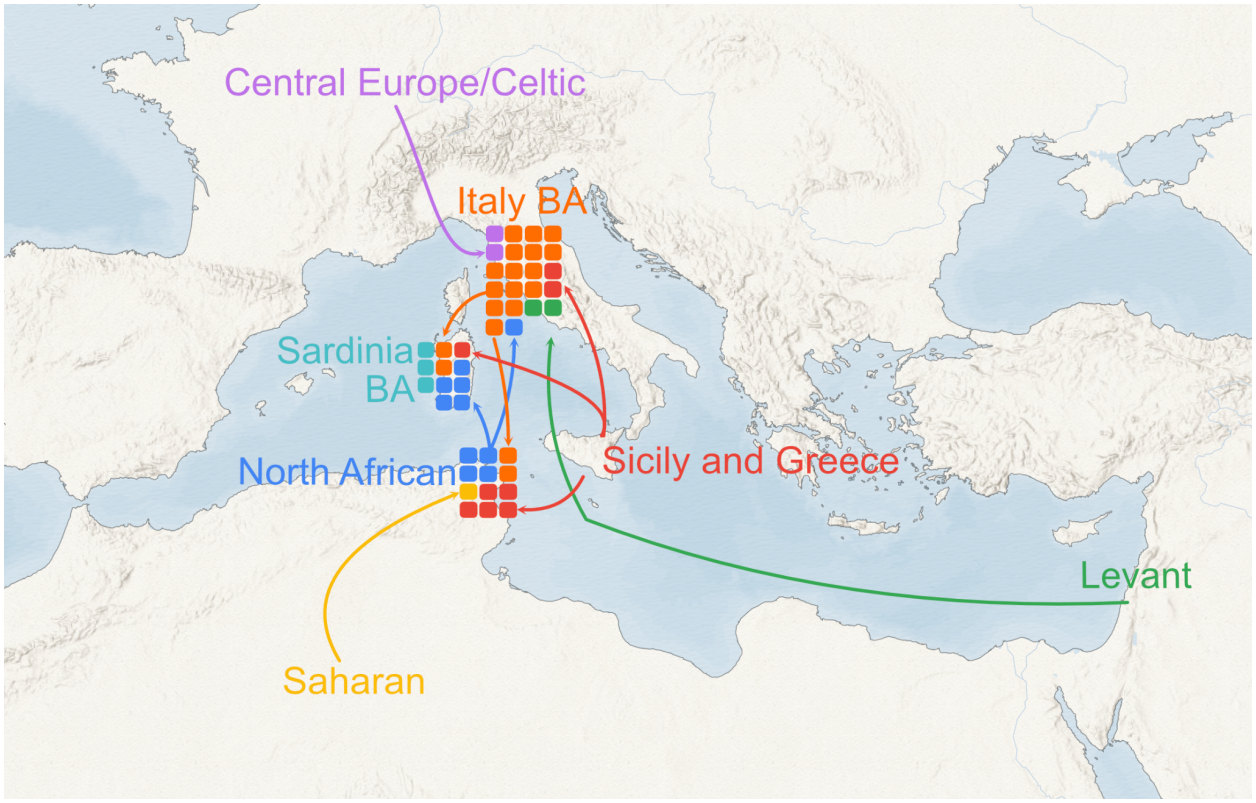

Fig. 6. Overview of results. *Map showing the memberships of central Mediterranean Iron Age individuals in different qpWave clusters, organized by site. Regional labels and arrows show likely sources of ancestries (the arrows are not intended to indicate specific routes). Colors indicate ancestry clusters as identified by qpWave.*

These results indicate that autochthonous North African populations contributed substantially to the genetic makeup of Kerkouane. The contribution of autochthonous North African populations in Carthaginian history is obscured by the use of terms like “Western Phoenicians”, and even to an extent, “Punic”, in the literature to refer to Carthaginians, as it implies a primarily colonial population and diminishes indigenous involvement in the Carthaginian Empire. As a result, the role of autochthonous populations has been largely overlooked in studies of Carthage and its empire. Genetic approaches are well suited to examine such assumptions, and here we show that North African populations contributed substantially to the genetic makeup of Carthaginian cities. The high number of individuals with Italian and Greek-like ancestry may be due to the proximity of Kerkouane to Magna Graecia, as well as key trans-Mediterranean sailing routes passing by Cap Bon (1, 28). Yet, surprisingly, we did not detect individuals with large amounts of Levantine ancestry at Kerkouane. Given the roots of Carthage and its territories as Phoenician colonies, we had anticipated we would see individuals with ancestry similar to Phoenician individuals, such as those published in (12). One possible explanation is that the colonial expansion of Phoenician city-states at the start of the Iron Age did not involve large amounts of population mobility, and may have been based on trade relationships rather than occupation. Alternatively, this could potentially be due to differential burial practices (although Phoenician burial practices were thought to have shifted from cremations to interments in the central and western Mediterranean around 650 BCE (29), predating the individuals in the study), or to a disruption in connections between Carthaginian territories and the Eastern Mediterranean, after the fall of the Phoenician city-states to Babylon.

The Iron Age genomes from Sardinia give us a glimpse into the genetic impact of Carthaginian expansion. Sardinia was comparatively more homogeneous genetically through the Bronze Age than nearby continental regions, such as Iberia and Italy, perhaps due to its insular nature. During the Iron Age, the genomic data from Sardinia shows a rapid increase in heterogeneity in ways that mirror the affiliation of Sardinia as an important territory of Carthage, and later of Rome. In the early- and mid-Iron Age, many individual genomes from the island are similar to the North African population at Kerkouane, while in the late Iron Age, individuals are similar to individuals from mainland Italy and Sicily, reflecting Sardinia’s incorporation into the Roman Empire.

Origin questions are also particularly salient for the Etruscans, who inhabited central Italy. There has been speculation about the origins of the Etruscans for over 2,500 years, with theories put forward by both ancient and modern scholars (3). Around 500 BCE Herodotus wrote that the Etruscans were people from Lydia (in modern-day Turkey) who had fled a drought. In the 1st century BCE, Livy wrote that instead they were from north of the Alps: i.e., central Europe. Also in the 1st century BCE, Dionysius of Halicarnassus wrote that the Etruscans were autochthonous and descended from previous inhabitants of the region. Recent work has started to shed light on these questions (7–10). Our analyses suggest that the long-standing theories about the origins of the Etruscans are not mutually exclusive. Etruscan material culture suggests they developed from the preceding Villanovan culture, but there are also strong indications of contact with other cultures, both by sea and land. Non-local materials, such as ostrich eggshell and ivory, as well as design motifs such as scarabs, sphinxes, palmettes, and representations of Greek, Phoenician, and Punic deities alongside the local pantheon were incorporated into Etruscan art and culture (3, 5, 6). At Chiusi, canopic jars, characteristic of Egyptian burial practice, became common. Our genetic data reveal high levels of mobility between these trans-Mediterranean neighbors.

Consistent with recent findings of Posth et al. 2021, we observe that many Etruscan individuals show continuity with the Bronze age, indicating that there was no major replacement of the Bronze Age population in Italy (10). Yet, we also see an increase in heterogeneity, with nearly 40% of the population best modeled with non-local ancestry. Thus, continuity is paired with gene flow patterned by the historical,

geopolitical, and even environmental conditions of the time, which our trans-Mediterranean approach allowed us to better characterize.

Gene Flow was Patterned by Historical Factors

By integrating our research questions across the populations of the central Mediterranean, we are able to explore how these increasing rates of mobility across the Mediterranean contributed to the genetic history of the region. The high number of individuals with North African ancestry from central Italy may reflect the close connections between Carthage and the Etruscans, both through trade and also as allies facing common adversaries, especially Greek and Roman Imperial expansion. Moorjani et al. 2011 estimated an earlier date (i.e. Bronze or Iron Age) for African gene flow into Sardinia, Spain, Portugal, and Northern Italy, when compared to other parts of Europe (27). This is consistent with archaeological evidence of Punic expansion and connections between the Etruscans with Punic territories. Interestingly, while southern Italy is geographically closer to North Africa, it may not have been as integrated into the network of Carthaginian allies and key trading partners. In particular, our findings support and add temporal resolution to previous suggestions based on modern data (27, 30) that the Iron Age was a key time for trans-Mediterranean mobility and connectivity between the regions we today call northern Africa and southern Europe. Supporting this, the presence of a number of individuals similar to contemporaneous Italian and Greek populations at Kerkouane suggests a bidirectional movement of people, especially within the central Mediterranean.

The Iron Age appears to be a key period for the formation of the current genetic structure of North Africa. Previous research suggests present-day central and western North African populations can be modeled as having four primary ancestry components: a local/autochthonous Maghrebi component derived from paleolithic hunter-gatherer populations in the region (20, 31, 32); a Near Eastern component thought to have been introduced with Arab rule of the region in the Medieval period (26); a sub-Saharan African component that was introduced in the last 5,000 years (16, 20); and a European component originally thought to have arrived due to historical population movements. While many papers have suggested the Near Eastern and European components resulted from recent historical movements, such as Arab rule in Medieval North Africa and trans-Mediterranean trade in the last 500 years, we see evidence for these components being present in North Africa in the Iron Age, around 2,500 years ago. Fregel et al. 2018 show the European component is, at least partially, linked to the farming expansion and is similar to Anatolian and early European farmers (20). Additionally, we show that both Near Eastern and sub-Saharan African components were present in North Africa earlier than previously thought, reflecting the ongoing interconnectedness of North Africa to these regions for millennia (20, 33–35).

The sub-Saharan ancestry we observe at Kerkouane may result either from direct contact or indirect contact through the nomadic populations of the Sahara. These nomadic groups, known to the Greeks as Numidians, are thought to be ancestral to Amazigh populations living in North Africa today. Trans-Saharan trade routes, made easier by a greener, less arid Sahara than today, had connected the communities of North Africa with their sub-Saharan counterparts since the Bronze Age (36, 37). Herodotus noted the coexistence of sedentary peoples and nomadic peoples in the land of the "libou" in the 5th century BCE (38). In addition to overland networks, these connections to sub-Saharan Africa also occurred by sea. Herodotus described Phoenician trade routes as extended far beyond the Mediterranean to the British Isles and West Africa via the Atlantic coast and even that a Phoenician and Egyptian expedition had circumnavigated Africa the previous century (1, 39). The Iron Age may have been a key period for gene flow across the Sahara as well.

Conclusion

The Iron Age in the Mediterranean was characterized by leaps in the ease of seafaring and, as a result, mobility. We see that these technological changes were accompanied by an increase in gene flow and genetic mobility across the Mediterranean, which impacted the ancestry makeup of the populations on its shores. By examining ancient DNA from four archaeological sites in the central Mediterranean, we observe that this technology shift was accompanied by a parallel increase in genetic heterogeneity, especially in comparison to the homogeneity of the preceding populations of the region. We observe large amounts of local continuity, with mobility and accompanying gene flow patterned by historical and environmental factors. Furthermore, we suggest that there is a connection between the trend of increasing local heterogeneity and shifts towards modern Mediterranean population structure.

In *The Making of the Middle Sea,* Cyprian Broodbank notes, “[w]ithout denying the likelihood of various constellations of social, cultural and other identities, early Mediterranean history instead comprises an ever-shifting kaleidoscope of webs of people and practices changing within and between places.” Using ancient DNA we can begin to examine these webs in the Central Mediterranean during the Iron Age. People buried at the same port towns and sharing the material culture of burial, often sharing the same tombs, have diverse and geographically distant ancestries (Table S1). Non-local ancestry doesn’t seem to have made individuals any less Carthaginian or any less Etruscan in their funerary celebrations. Perhaps, instead, this points us to a defining feature of these societies, a cultural one, and that is their embrace of those with roots at home as well as elsewhere.

Materials

Short-form site descriptions (see SupplementaryI Materials for Extended Descriptions)

Kerkouane

Kerkouane is an exceptionally well-preserved Punic town located on Tunisia's Cap Bon Peninsula and provides one of the best-surviving windows into Carthaginian daily life (29, 40–42). Originally inhabited from 650 - 250 BCE, the population of Kerkouane is thought to have been around 1,200 with an economy primarily based on the production and export of marine resources from the region, including the production and exportation of garum, salt, and the eponymous purple dye derived from locally harvested *Murex sp*. shells (43). Kerkouane was abandoned after the 1st Punic War between Carthage and Rome and was never re-inhabited or rebuilt by the Romans resulting in the excellent preservation of the original Punic architecture of the town.

Sant'Imbenia

Sant'Imbenia, a port town in northwest Sardinia (present-day Alghero) is thought to have been populated by autochthonous Nuragic and Phoenician and Punic people, and has extensive trade contacts with the Eutrsucan city-states of central Italy. This differs from the Phoenician colonies in the south and west of the island, which were thought to be a primarily colonial population. The discovery of a metal workshop and copper ingots at the site suggest it may have been a major center of ore processing.

Tarquinia

This Etruscan site was one of the largest Iron Age cities in central Italy. It was inhabited throughout the Iron Age and served as one of the primary trading ports between Etruria and the civilizations of the Mediterranean.

Pian Sultano

The Bronze Age settlement of Pian Sultano is located in central Italy, near modern-day Cerveteri (the Etruscan town of Caere). The earliest record for settlement at the site dates to 2000 BCE. Archaeological investigations of the site have uncovered artifacts indicating Pian Sultano was a farming community that also drew heavily on marine resources. Many ceramics feature design motifs characteristic of the central Italy Apennine culture. Long-distance trade is also indicated in the material culture of the site by obsidian blades, the material for which would have been procured from one of the 4 central Mediterranean island sources - Lipari, Pantelleria, Sardinia, or Sicily (44).

Methods

Extraction of DNA in Dedicated Cleanrooms

We cleaned, isolated, and powdered the cochlear portion of the petrous bone in dedicated clean rooms following the protocols described in (45, 46). Following a 30-minute uracil–DNA–glycosylase (UDG) treatment, double-stranded library preparation followed a modified version of the (47) protocol (SI, Methods). Libraries passing screening based on DNA concentration were sequenced on an initial next-Seq screening run. Computational authentication of the presence of endogenous ancient DNA was based on 1) the presence of reads mapping to the human genome (hg19 assembly), 2) on the damage patterns at the terminal ends of reads, and 3) contamination analyses using Schmutzi (48).

Newly generated data merged with published data

In total, 30 individual genomes passed endogenous preservation and quality control thresholds (Fig. 2; Dataset S1). For the analyses in the paper, we merged the newly generated data reported here with the Allen Ancient DNA Resource v44 (49) using PLINK v1.9059. We also added recently published data from

Bronze Age Italy to the reference dataset (17). We performed all subsequent analyses on autosomal data.

Population genetic analyses

Individual biological sex determination was inferred based on the ratio of reads from sex chromosomes and autosome coverages, and kinship analysis was performed using READ (Relationship Estimation from Ancient DNA) (50). We carried out *qpAdm* and *qpWave* analyses using ADMIXTOOLS2 (51). For modeling the distal ancestries (Fig. 4), we used Mbuti.DG, Russia_Ust_Ishim.DG, CHG, Russia_EHG, Iberia_ElMiron, Czech_Vestonice1, Russia_MA1_HG.SG, Israel_Natufian, Jordan_PPNB as outgroup populations. All individuals in these analyses and curation of the group labels for these runs can be found in Dataset S2.

Data availability

All tools and data needed to reproduce and evaluate the conclusions in this paper are presented in the main text and the Supplementary Materials. Alignment files for the DNA sequences for all newly reported individual genomes will be available at the European Nucleotide Archive (ENA) database under the accession number Project PRJEB49419. (www.ebi.ac.uk/ena/browser/view/PRJEB49419).

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Author Contributions: RP, JKP, AC and MF **designed research**; RP, SS, VO, EP, OC, KO, LD, HMM and DF **performed and supervised laboratory work**; AC and MF **designed collection strategy for archaeological material**; MF, AC, ML, FLP, FG, FC, DDA, GG, HMM, MC and SA **assembled skeletal material and provided archaeological background**; HMM, MA, SS, JPS, VO, CW, EP, BZ and ZG **curated and analyzed data with input from** JKP, RP, AC, DF and MC; HMM, JPK RP, JPS, MA, SS, CW, MC and ZG **wrote the paper** with input from all collaborators.

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