

A genetic history of continuity and mobility in the Iron Age central Mediterranean

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The Iron Age was a dynamic period in central Mediterranean history, with the expansion of Greek and Phoenician colonies and the growth of Carthage into the dominant maritime power of the Mediterranean. 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. Here, to investigate population mobility and interactions directly, we sequenced the genomes of 30 ancient individuals from coastal cities around the central Mediterranean, in Tunisia, Sardinia and central Italy. We observe a meaningful contribution of autochthonous populations, as well as highly heterogeneous ancestry including many individuals with non-local ancestries from other parts of the Mediterranean region. These results highlight both the role of local populations and the extreme interconnectedness of populations in the Iron Age Mediterranean. By studying these trans-Mediterranean neighbours together, we explore the complex interplay between local continuity and mobility that shaped the Iron Age societies of the central Mediterranean.

The first millennium BCE 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 Article, we use data from three key port and coastal cities in the central Mediterranean: Kerkouane (Tunisia), Sant’Imbenia (Sardinia) and Tarquinia (central Italy), to study mobility within this region.

In the late Bronze and early Iron Age (1250–800 BCE), Phoenician and Greek city-states established trading ports and colonies across the Mediterranean. Carthage was located at the crossroads of these

trans-Mediterranean trade routes and, for 500 yr (Fig. 1a), was the centre of a trading network spanning much of the central and western Mediterranean. Carthage was the dominant maritime power of the region until Roman Imperial expansion in the final centuries BCE^{1,2}. Across the sea, Etruscan-speaking city-states flourished in central and northern Italy. 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 land³. Insights into the relationship between these trans-Mediterranean neighbours are limited. Few Punic or Etruscan written sources have survived to the

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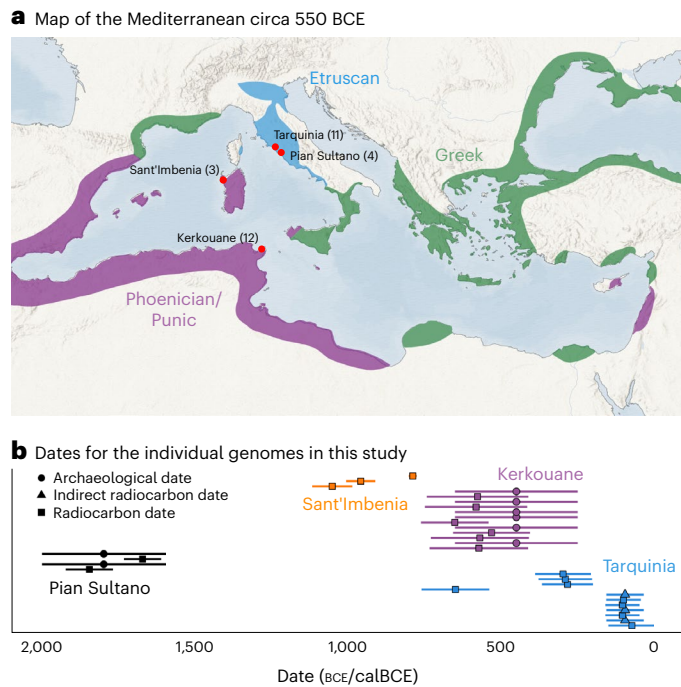


Fig. 1 | Data overview and relevant geography and chronology. **a**, Locations of the 4 archaeological sites examined here, as well as a map of the areas settled by Phoenician, Greek and Etruscan speakers by 550 BCE. **b**, Timeline showing the dates for the newly reported individual genomes, with the methods of dating indicated. Indirect radiocarbon dating refers to using the radiocarbon date of a different individual in the same tomb. A single date estimate for each individual in the study was determined by the average of lower and upper estimates of the 95% confidence interval when using accelerator mass spectrometry radiocarbon dates and the average of the lower and bound inference dates when using archaeological and historical context for dating. The full 95% confidence intervals are reported in Supplementary Dataset 3. The map was generated using the World_Basemap_v2 from the ArcGIS online platform⁶⁴.

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, Roman and Egyptian sources⁵. Archaeogenetic research adds a new line of evidence to our understanding of the people and their interactions during this dynamic period.

Until recently, the genetic makeup of Iron Age Mediterranean populations has been relatively understudied in comparison to earlier periods. New research has begun to shed light on the Iron Age peoples of central Italy and Sardinia^{6–10} but there is very limited information about the Iron Age North African communities. So far, there has been no whole-genome ancient DNA research on Northwest Africa from this time period, 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 region¹¹.

We illuminate the complicated interplay between continuity and mobility and document extensive genetic exchange across the central Mediterranean, which allows us to directly examine relationships between local and diasporic people of the Iron Age. More specifically, we observe heterogeneity at the port cities of the Iron Age central Mediterranean, in accord with historically and archaeologically attested connections. One surprising deviation from this is the absence of individuals with Levantine ancestries at Kerkouane, Sant'Imbenia and other published Iron Age sites in Sardinia. We also explore whether these mobility patterns varied by sex and examine indications of admixture between individuals of a diverse range of genetic ancestries.

Results

Data generation

We generated double-stranded DNA libraries after partial uracil–DNA–glycosylase treatment. After authentication and screening, 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 archaeological sites, shown in Fig. 1a: 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 to 208 BCE^{6,7} and 33 additional Iron Age central Italians dating from 963 to 200 BCE^{8,9,12}.

We conducted radiocarbon dating on 19 individuals (Fig. 1b and Supplementary Dataset 3). These confirm the archaeologically attested dates of use for the Kerkouane necropolis to the mid-Iron Age (650–250 BCE), a key period for the growth of Carthage and its role as a primary node in the trade networks of the Mediterranean. 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 third century BCE. The burials at Pian Sultano date to the middle Bronze Age and thus provide important context for central 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).

To contextualize these individuals within the genetic landscape of contemporaneous populations, we curated a set of published ancient genomes spanning the Iron Age Mediterranean^{6–8,13–18}. 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^{12,19,20}. For the Maghreb, we included late Neolithic individuals as there are no published Bronze Age data²¹. This resulted in a set of 330 reference genomes to contextualize the new data (more information about these and our curation of the metadata can be found in Supplementary Dataset 2).

Increased genetic heterogeneity in the Iron Age

We generated a principal component analysis (PCA) reference space using modern populations from around the Mediterranean, Europe, the Middle East and North Africa²², and projected the Bronze and Iron Age Mediterranean individuals onto this modern reference space (Figs. 2 and 3a). 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. While Bronze Age populations in this region, especially Iberia, had some outlier individuals, they were relatively few and sporadic. In the Iron Age, however, there is much more overlap in PCA space between individuals from different regions.

To further explore these patterns, we modelled the ancestry of the new and published individual genomes from the central Mediterranean with qpAdm admixture modelling, shown in Fig. 3 (refs. 23,24). 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^{6,8,15}. 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 $1 - \log(P \text{ value})$ to calculate distances between each pair of individuals and performed clustering (Fig. 4). Given the genetic heterogeneity that characterizes the Iron Age Mediterranean, these groupings identified in qpWave identify genetically similar individuals across regions.

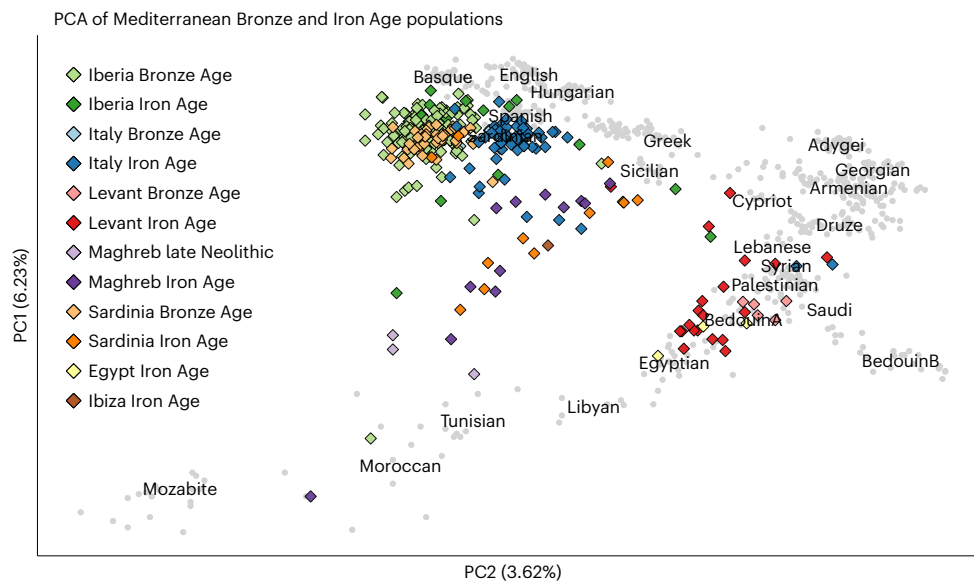


Fig. 2 | PCA of Bronze and Iron Age ancient genomes. Newly reported and previously published ancient genomes are projected onto a PCA space of present-day individuals (grey points) using smartPCA. Ancient genomes are

shown in paired sets of colours by region, with the earlier time period (Bronze Age or Neolithic) represented by the lighter colours and the later time period (Iron Age) represented by the darker colours.

Characterizing the genetic heterogeneity at Kerkouane

Kerkouane is an exceptionally well-preserved town located on Tunisia's Cap Bon Peninsula and provides one of the best-surviving windows into daily life in this period^{25–28}. Originally inhabited from 650–250 BCE, the population of Kerkouane is thought to have been ~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, lime and Tyrian purple dye derived from locally harvested *Murex* sp. shells²⁹. Kerkouane was abandoned after the First Punic War between Carthage and Rome and was never re-inhabited or rebuilt by the Romans, resulting in the excellent preservation of the original architecture of the town. Scholars have also observed the presence of Phoenician, autochthonous North African and Greek influences in the material culture recovered at the site³⁰.

Examining 12 individual genomes from 4 shared tombs, we observe a highly heterogeneous population, projected across the PCA space in Fig. 2, consisting of three primary genetic clusters. One of the genetic groups we identified (labelled 'Cluster 1') includes four individuals who project near preceding (late Neolithic) Maghrebi farmers. We used qpAdm modelling to test whether there was genetic continuity with preceding populations (Fig. 3). One individual, R11778, can be modelled in qpAdm with 100% Moroccan late Neolithic farmer ancestry, while three individuals, R11746, R11755 and R11790, can be modelled predominantly with this component, along with the addition of 15–20% steppe-related ancestry. This suggests that these individuals represent an autochthonous North African population, with some evidence for admixture with individuals of steppe-related ancestry. Of the 4 individuals in Cluster 1, two have mitochondrial haplogroups which were also identified among the preceding Maghrebi populations²¹. R11778 carries haplogroup L3e2bA, which is most common in western Africa, indicating potential trans-Saharan connections during this period³¹. The biologically male individual in Cluster 1 carries Y-haplogroup R1b, which is associated with Bell Beaker cultures in Europe and common in central Italy at this time and became prevalent in southern Iberia by the Bronze Age³⁸. This supports the possibility that the steppe component observed at Kerkouane may have been introduced through mobility occurring in the central and western Mediterranean, as also suggested in ref. 6. This is also supported by proximal qpAdm modelling, which finds Bell Beaker populations from

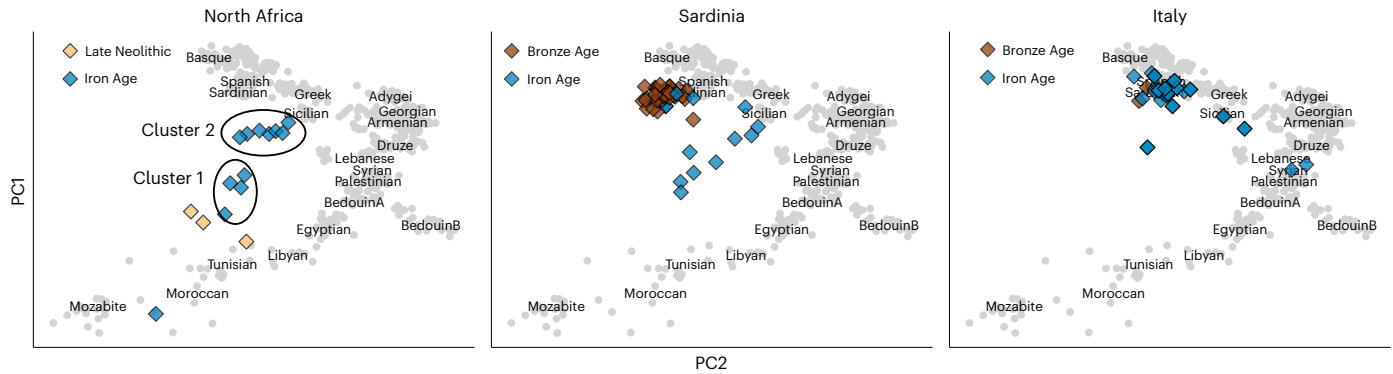
Iberia and France as suitable proximal sources for this component (Supplementary Table 4).

Due to the historically attested connection between the Levant (via the expansion of Phoenician-speaking colonies) and Iron Age North Africa, we also tested whether Bronze or Iron Age Levantine populations might serve as either distal or proximal source populations for the individuals at Kerkouane. First, we tested whether adding a Bronze or Iron Age Levantine component to the 5 distal source populations would improve qpAdm models for the individuals at Kerkouane. We did not find any working models for the individuals or ancestry clusters at Kerkouane that included these populations: Lebanon_IAII, Lebanon_IAIII, Israel_IA1_Ashkelon, Israel_IA2_Ashkelon, Megiddo_MLBA, Megiddo_IA (Supplementary Methods and Supplementary Dataset 5). To further test whether our distal set might 'mask' a Levantine component, we attempted to model the same Bronze and Iron Age Levantine populations mentioned above with the 5 distal source populations, but no working models were found.

A second group, labelled 'Cluster 2' in PCA (Fig. 3 and Supplementary Fig. 5) and identified in qpWave (Fig. 4), contains 7 individuals who cannot be modelled using the preceding north African population and are better modelled as genetically similar to Bronze Age Sicilian and central Italian populations, as well as some contemporaneous individuals from the Iberian Greek-speaking colony of Empúries^{15,32}. We cannot know whether these individuals with non-local ancestries moved in their lifetime or were part of multi-generational settlements in the region. Of the 7 individuals in the non-local ancestry cluster (Cluster 2), all individuals carry mitochondrial haplogroup HV, H and I5, all thought to have originated in the Mediterranean region³³. All biological males in this cluster carry J2b, which has been reported at high rates in Sardinian and Levantine Bronze Age populations and has been suggested to be associated with the Phoenician expansion. Interestingly, R11790 (in Cluster 1, characterized by genetic continuity with previous populations of the region) also carries HV13, which suggests admixture between the local and diasporic populations at the site.

R11753 and R11791, both from Cluster 2, show strong evidence of inbreeding, with runs of homozygosity segments over 50 Mb in length. In both cases, with over 12% of the genome being homozygous (segments >5 Mb in length), the parents were probably second-degree relatives. Consistent with the homozygosity analysis, both individuals

a PCA of ancient individual genomes projected onto modern Mediterranean and adjacent populations



b qpAdm modelling of new ancient genomes and relevant contemporaneous populations

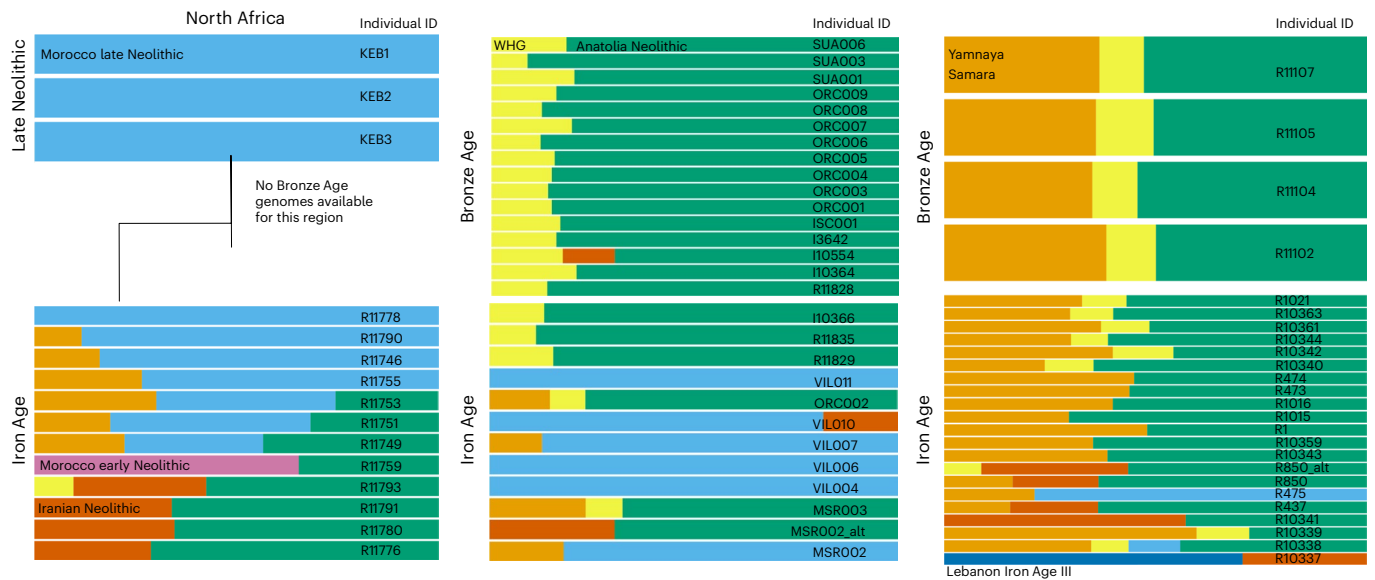


Fig. 3 | Genetic overview of central Mediterranean populations during the Bronze and Iron Ages. Both PCA and qpAdm illustrate the shift from relative genetic homogeneity in the Bronze Age to heterogeneity in the Iron Age. **a**, PCA of ancient Neolithic, Bronze and Iron Age populations from the central Mediterranean, projected onto a modern reference space. **b**, qpAdm analyses

using a set of distal source populations (the WHG, Yamnaya Samara, Anatolia Neolithic, Iranian Neolithic, Morocco late Neolithic and Morocco early Neolithic). See Supplementary Text 2 and Supplementary Dataset 5 for standard errors and statistical fits.

also have very low conditional heterozygosity (Supplementary Fig. 9). While it has been shown that endogamous marriage practices were common in the contemporaneous ancient Greek world³⁴, less is known about such practices in the Carthaginian world due to fewer surviving written records³⁵.

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. 3). We replaced Morocco late Neolithic with Morocco early Neolithic farmers and a hunter-gatherer individual from Ethiopia from ~4500 BP³⁶, both of which produced working models. Using competition modelling (where possible sources are rotated to the outgroup), the best model uses ~70% Moroccan early Neolithic ancestry and ~30% Anatolia Neolithic (Fig. 3 and Supplementary Fig. 5). When compared to other ancient individuals using qpWave analysis (Fig. 4), this individual forms a clade with ancient Canary Island inhabitants thought to be representative of the original founding population³⁷. The Canary Islands were originally settled in the first millennium BCE by a population genetically ancestral to today's populations of North Africa³⁸. This individual carries U6, a common northern African mitochondrial haplogroup. On the basis

of the study of mitochondrial haplogroups, we observe high rates of mobility, accompanied by admixture (Supplementary Figs. 11 and 12). This parallels the findings of increasingly heterogeneous mitochondrial DNA haplogroups at other Mediterranean sites during the Iron Age, particularly in Sardinia, Sicily and central and southern Italy^{39–44}.

Iron Age communities in Sardinia and Ibiza

The Iron Age was a dynamic period in Sardinia, which was initially independent and later incorporated into the Phoenicio-Punic/Carthaginian trading world (eighth century BCE) and then the Roman empire (mid-third century BCE). Sardinia was a relatively genetically homogeneous population through the Bronze Age, with increasing heterogeneity in the Iron Age^{6,7}, and this new genetic information about contemporaneous North Africa allows us to directly explore the connections between Sardinia and North Africa in this period.

The three newly reported 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 site of Villamar (VIL004, VIL006, VIL007, VIL010, VIL011) can be modelled with primarily



Fig. 4 | qpWave analysis of Mediterranean populations. The plot shows the clustering of Bronze Age and Iron Age genomes from the central Mediterranean with other relevant populations by qpWave P value. Heat map values indicate whether each pair of individuals can be modelled with the same ancestry components in qpAdm in comparison to a set of reference ('right') populations. Clustering was generated using $1 - \log(P \text{ value})$ to calculate distances between individuals, with clusters called by the cutree tool in R and the black lines representing these groupings. Unfilled models have a P value < 0.01 and were

rejected. Values over 0.01 (shown in yellow, orange and red) indicate that a pair of individuals can be modelled with the same ancestry components in qpAdm in comparison to a set of reference populations (Mbuti.DG, Russia_Ust_Ishim.DG, CHG, Russia_EHG, Iberia_ElMiron, Czech_VestoniceI, Russia_MAI_HG.SG, Israel_Natufian, Jordan_PPNB, WHG, Yamnaya Samara, Anatolian Neolithic, Iranian Neolithic and late Neolithic farmers from Morocco). Individual labels are shown in Supplementary Fig. 7.

Moroccan late Neolithic ancestry⁷, similar to the North African cluster from Kerkouane (Cluster 1), as well as an individual from Ibiza, MS10614¹⁶, which mirrors the strong political and trading connections at the time with Carthaginian North Africa. This timing is consistent with a previous estimate from modern genomes that African gene flow into Sardinia occurred 96 generations (~2,750 yr) ago⁴⁵. Contemporaneous individuals (800–300 BCE) from the site of Monte Sirai (MSR002, MSR003) cluster with contemporaneous central Italian and Sicilian populations (Fig. 4). 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^{6,7}. We observe a shifting genetic profile reflecting the shifting political affiliations of the island through the Iron Age (Supplementary Fig. 3).

Connection across the central Mediterranean

In Iron Age Italy, consistent with the findings in ref. 9, we observe a combination of genetic continuity from the Bronze Age population, along with a drastic increase in genetic heterogeneity compared with the Bronze Age. Of the 22 Iron Age central Italian genomes, 12 individuals form a clade in the qpWave and qpAdm analyses (Figs. 4 and 5) with Bronze Age central Italian individuals and thus can be modelled as deriving 100% of their ancestry from Bronze Age central Italians. However, nearly half of the individuals are best modelled in qpWave with ancestry from other parts of the Iron Age Mediterranean world (Fig. 3). Among these, one individual, R475, projects near the clade of autochthonous North African

individuals at Kerkouane and can be modelled with the sample distal source populations in qpAdm (~80% late Neolithic Moroccan farmer and ~20% steppe-related ancestry). Additionally, 4 recently published individuals from Tarquinia have northern African ancestry⁹. Two individuals, R10337 and R10341, both dating to the second or first century BCE (Supplementary Dataset 1), appear genetically similar to contemporaneous individuals from the Levant (Figs. 3 and 4). In qpAdm (Fig. 3) and ADMIXTURE modelling (Supplementary Fig. 4), these two individuals lack steppe-like ancestry, contrasting with other contemporaneous individuals from central Italy. R10337 can be modelled with Levantine (Lebanon Iron Age II) and Iranian Neolithic sources and R10341 with Iranian and Anatolian Neolithic sources (Fig. 3 and Supplementary Dataset 4), and they may represent early instances of the shift towards eastern Mediterranean ancestries characteristic of the succeeding Roman imperial period⁸. 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. 2 and Supplementary Fig. 3). In contrast, the appearance of steppe-related ancestry during the Bronze Age in central Italy originally occurred in small amounts, ubiquitously in the population¹², suggesting that this ancestry may have spread gradually through small local interactions over many generations.

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

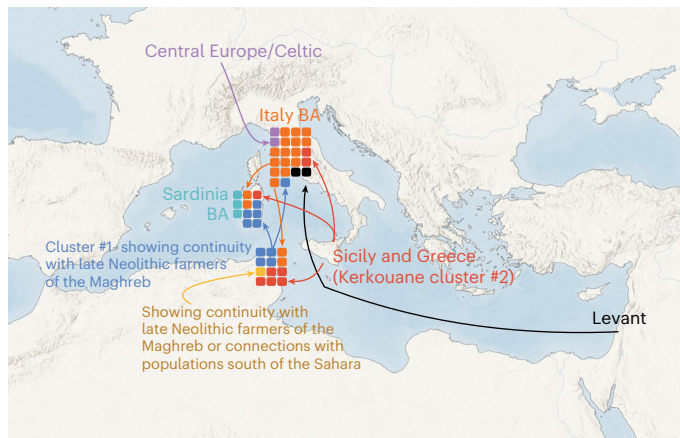


Fig. 5 | Visualization of qpWave clustering. Map showing the memberships of central Mediterranean Iron Age individuals in different qpWave clusters, organized by site. Regional labels and arrows show likely sources of ancestries (the arrows are not intended to indicate specific routes). Colours indicate ancestry clusters as identified by qpWave. The map was generated using the World_Basemap_v2 from the ArcGIS online platform⁶⁴.

of individuals with ancestry from other parts of the Mediterranean world and beyond. While the high heterogeneity at these coastal cities is striking, we were curious whether the mobility indicated thereby was limited to port cities or extended into the hinterlands as well. While there is no available ancient data from the rural contexts surrounding these ports, modern data offer insights into whether the ancestries introduced in the first millennium BCE were transient (and thus isolated to the port cities) or permanently impacted the population structure of these regions, indicating that this mobility extended beyond the ports, albeit perhaps at lower rates. In qpAdm and ADMIXTURE modelling (Fig. 3 and Supplementary Fig. 4), three ancestral components were generally sufficient for modelling the pre-Iron Age populations in North Africa, Sardinia and Italy, but in the Iron Age, a minimum of five components are needed to model the ancestry profiles of individuals at the sites studied. The Morocco late Neolithic component, which was predominantly found in North Africa before 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 Carthage as a trading hub and maritime power in the region. This mobility seems to have contributed to the present-day genetic structure of these populations. Similar to contemporaneous populations in the eastern Mediterranean and Southwest Asia⁴⁶, increasing interconnectivity was also paired with increasing long-distance gene flow patterned by the historical, geopolitical and even environmental conditions of the time, maintaining population structure in the region⁴⁷.

Discussion

By integrating our research questions across the populations of the central Mediterranean, we describe here how 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 Etruscan-speaking city-states, both through trade and also, at times, as allies facing common adversaries, especially Greek and Roman Imperial expansion, such as at the Battle of Alalia around 535 BCE to oppose Greek expansion in the central Mediterranean. In particular, our findings support and add temporal resolution to previous suggestions based on modern and historical data^{45,47–49} that the Iron Age was a key time for trans-Mediterranean mobility and connectivity between the regions we call today as northern Africa and southern

Europe. Supporting this, the presence of several individuals similar to contemporaneous Italian and Greek populations at Kerkouane suggests a bidirectional movement of people, especially within the central Mediterranean. At both Kerkouane and Tarquinia, we observe that individuals buried together have diverse and geographically distant ancestries (Table 1). Non-local ancestry does not seem to have necessarily resulted in differential treatment in funerary celebrations.

Not only do we see evidence for increasing mobility and interactions across the Mediterranean, but we also see possible indications of interactions across the Saharan desert. The sub-Saharan ancestry we observe at Kerkouane may result either from direct contact or indirect contact through the nomadic populations of the Sahara. 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^{50,51}. Herodotus noted the coexistence of sedentary peoples and nomadic peoples in northern Africa in the fifth century BCE⁵². In addition to overland networks, these connections to sub-Saharan Africa also occurred by sea. Herodotus described Phoenician trade routes as extending far beyond the Mediterranean to Western Africa via the Atlantic coast and even that a Phoenician and Egyptian expedition had circumnavigated Africa the previous century^{1,2}. The Iron Age may have been a key period for gene flow across the Sahara as well.

Kerkouane was highly cosmopolitan, reflecting the diverse material culture of the city. We observe two groups of individuals who show genetic continuity with the preceding populations of the Maghreb, suggesting standing population structure in coastal North Africa in this period, as well as individuals with non-local ancestries. The contribution of autochthonous populations in the region is obscured by the use of terms such as ‘Western Phoenicians’ and even to an extent, ‘Punic’, in the literature, as it implies a primarily colonial population in North Africa and diminishes local involvement in Iron Age North Africa and the rise of Carthage. As a result, the role of autochthonous populations has been largely overlooked in studies of the Carthaginian world. 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^{1,53}. Surprisingly, we did not detect individuals with large amounts of Levantine ancestry at Kerkouane. Due to the historically attested connection between the Levant and Iron Age North Africa, we had anticipated to see individuals with ancestries similar to Phoenician individuals, such as those published in ref. 13. 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 might have been based on trade relationships rather than occupation⁵⁴. Alternatively, this could potentially be due to differential burial practices between local and diasporic communities, such as differential cremation practices (although it is thought that Phoenician burial practices had shifted from cremations to interments in the central and western Mediterranean around 650 BCE²⁸, predating the individuals in the study), or to a disruption in connections between Carthaginian territories and the eastern Mediterranean after the annexation of the Phoenician city-states into the Achaemenid Empire.

While 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, the genomic data from Sardinia during the Iron Age show a rapid increase in heterogeneity in ways that mirror the affiliation of Sardinia as an important part of the Carthaginian Mediterranean and later of the Roman ‘mare nostrum’. In the early- and mid-Iron Age, many individual genomes form a clade in pairwise qpWave with 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.

Table 1 | Burial context and biological relatedness information for individuals in this study, with qpWave ancestry groups from Fig. 4 indicated

Pian Sultano, Crepaccio II - Italy Bronze Age			Kerkouane - Tomb 1			Kerkouane - Tomb 4		
ID	qpWave group	Biological kindred?	ID	qpWave group	Biological kindred?	ID	qpWave group	Biological kindred?
R11102	Bronze Age Italy		R11746	Cluster #1		R11776	Cluster #2	
R11104	Bronze Age Italy	1st degree with R11105	R11749	Cluster #2		R11778	Cluster #1	
R11105	Bronze Age Italy	1st degree with R11104	R11751	Cluster #2		R11780	Cluster #2	
R11107	Bronze Age Italy							
Tarquinia, Tomb 6176 - Italy Iron Age			Kerkouane - Tomb 2			Kerkouane - Tomb 15		
ID	qpWave group	Biological kindred?	ID	qpWave group	Biological kindred?	ID	qpWave group	Biological kindred?
R10337	Levant		R11753	Cluster #2		R11790	Cluster #1	
R10338	Bronze Age Italy		R11755	Cluster #1		R11791	Cluster #2	
R10339	Central Europe/Celtic		R11759	R11759		R11793	Cluster #2	
R10340	Bronze Age Italy							
R10341	Levant							
R10342	Central Europe/Celtic							
R10343	Bronze Age Italy							

In contrast, in Italy, the majority of studied individuals cluster genetically with the Bronze Age populations of central Italy, indicating a continuity of populations, consistent with the recent findings in ref. 9. Alongside this, we observe an increase in heterogeneity, with nearly 40% of the population best modelled with non-local ancestry. Thus, continuity is paired with gene flow patterned by the historical, geopolitical and even environmental conditions of the time.

The Iron Age in the Mediterranean was characterized by leaps in the ease of seafaring and, consequently, mobility. We see that these technological changes were accompanied by an increase in gene flow and genetic mobility across the Mediterranean, which shaped 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 technological shift was accompanied by a parallel increase in genetic heterogeneity, in comparison with preceding populations. We observe large amounts of local continuity, with mobility and accompanying gene flow patterned by historical and environmental factors. While further research is needed to make claims about cultural practices, we see indications of admixture between local and diasporic populations, as well as two instances of consanguinity within the diasporic population at Kerkouane. We also suggest that there is a connection between the trend of increasing local heterogeneity and shifts towards modern Mediterranean population structure, indicating that the genetic impacts of mobility were not isolated to port cities, but extended, at least to some degree, to populations inland from the coasts. 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". Ancient DNA adds a new approach to examining these webs in the central Mediterranean during the Iron Age.

Methods

Site descriptions

See Supplementary Information for extended descriptions.

Kerkouane. Kerkouane is an exceptionally well-preserved town located on Tunisia's Cap Bon Peninsula and provides one of the best-surviving

windows into daily life in this period^{25,26,28,30}. Originally inhabited from 650–250 BCE, the population of Kerkouane is thought to have been ~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 Tyrian purple dye derived from locally harvested *Murex* sp. shells²⁹. Kerkouane was abandoned after the First Punic War between Carthage and Rome and was never re-inhabited or rebuilt by the Romans, resulting in the excellent preservation of the original architecture of the town.

Sant'Imbenia. Sant'Imbenia was a port town in northwest Sardinia (present-day Alghero). It is thought that the town had a diverse population, including local autochthonous people, as well as short and long-term residents from the Levant and North Africa. The material culture at the site also shows extensive trade contacts with the Etruscan 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 suggests it might have been a major centre 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 artefacts indicating that Pian Sultano was a farming community that also drew heavily on marine resources. Many ceramics feature design motifs characteristic of the central Italian 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 4 central Mediterranean island sources: Lipari, Pantelleria, Sardinia or Sicily⁵⁵.

Ancient DNA library preparation

We cleaned, isolated and powdered the cochlear portion of the petrous bone in dedicated cleanrooms following previously described

protocols^{56,57}. After a 30 min uracil–DNA–glycosylase treatment, double-stranded library preparation followed a modified version of a previously described protocol⁵⁸ (Supplementary Information and 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) the damage patterns at the terminal ends of reads and (3) contamination analyses using Schmutzi⁵⁹.

Dataset

In total, 30 individual genomes passed endogenous preservation and quality-control thresholds (Fig. 1 and Supplementary Dataset 1). 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). For the analyses in this paper, we merged the newly generated data reported here with the Allen Ancient DNA Resource 1240K panel (v.44)^{60,61} using PLINK v.1.9059. We also added recently published data from Bronze Age Italy to the reference dataset^{9,12,18}. We performed all subsequent analyses on autosomal data.

Population genetic analyses

Individual biological sex was inferred on the basis of the ratio of reads from sex chromosomes and autosome coverages, and kinship analysis was performed using READ (Relationship Estimation from Ancient DNA)⁶². We carried out qpAdm and qpWave analyses using ADMIXTOOLS2 (ref. 63). For modelling the distal ancestries (Fig. 3), we used Mbuti.DG, Russia_Ust_Ishim.DG, CHG, Russia_EHG, Iberia_EIMiron, Czech_Vestonice1, Russia_MA1_HG.SG, Israel_Natufian and Jordan_PPNB as outgroup populations. All individuals in these analyses and the curation of the group labels for these runs can be found in Supplementary Dataset 2.

Permits to work with archaeological materials

The material reported in this study represents 4 archaeological sites. We have provided the names of the sites, the time periods of occupation, the exact coordinates of the sites and information about each in Supplementary Text 1 (Site descriptions). For each site, we worked with the appropriate governmental authorities and permitting bodies to obtain permissions and permits to work on these materials. For Pian Sultano and Tarquinia, both located in the Lazio Administrative Region of Italy, we received permissions and permits from Daniela De Angelis, the Director of the National Etruscan Museum of Tarquinia (operated by the Direzione Generale Musei Lazio, which is part of the Italian Government's Ministry of culture) where all material for both Pian Sultano and Tarquinia are housed, on 22 December 2018. We also received permits to visit and conduct research in the collection in March and July of 2019. For the material from Sant'Imbenia, Sardinia, we received permissions from Dr Gabriella Gasperetti, the Archaeological Superintendent of the province of Sassari e Nuoro (Soprintendenza Archeologia, belle arti e paesaggio per le province di Sassari e Nuoro; Sassari, Italy) who oversees these collections, on 1 November 2019. For the material from Kerkouane, Tunisia, we received a permit and signed a partnership agreement with the National Heritage Institute of the Tunisian Government's Ministry of Culture. These were signed and approved by the Director General, Dr Faouzi Mahfoud on 4 April 2020.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

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 Information. Alignment files for the DNA sequences for all newly reported

individual genomes are available at the European Nucleotide Archive (ENA) database under project accession number [PRJEB49419](https://doi.org/10.1038/s41559-023-02143-4).

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Author contributions

R.P., J.K.P., A.C. and M.F. designed research; R.P., S.S., V.O., E.P., O.C., K.T.O., L.D., H.M.M. and D.F. performed and supervised laboratory work; A.C. and M.F. designed the collection strategy for archaeological material; M.F., A.C., M.L., F.L.P., F.G., F.C., D.D.A., G.G., H.M.M., Y.M.S.C. and S.A. assembled skeletal material and provided archaeological background; H.M.M., M.A., S.S., J.P.S., V.O., C.L.W., E.P., B.Z. and Z.G. curated and analysed data with input from J.K.P., R.P., A.C., D.F. and Y.M.S.C.; H.M.M., J.K.P., R.P., J.P.S., M.A., S.S., C.L.W., Y.M.S.C. and Z.G. wrote the paper with input from all collaborators.

Competing interests

The authors declare no competing interests.

Additional information

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Software and code

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Data collection

We used the following open source tools to process the reads returns from sequencing and screen the libraries for preservation and authenticity. Libraries were sequenced on an Illumina NovaSeq 6000 sequencing platform to generate whole-genome shotgun data. Adapters were removed from libraries using Cutadapt (v1.14). Reads were filtered for minimum length of 30, then aligned to hg19 using bwa (0.7.15-r1140). For each library, aligned reads were sorted by coordinate using Picard's SortSam (version 2.9.0-1-gf5b9f50-SNAPSHOT) and read groups were added using Picard's AddOrReplaceReadGroups (version 2.9.0-1-gf5b9f50-SNAPSHOT). Reads with mapping quality < 25 (including unaligned reads) were filtered out. Duplicates were removed using samtools rmdup (<http://www.htslib.org/doc/samtools.html>). Genome-wide and chromosomal coverage were assessed using depth-cover (version 1.0.3, <https://github.com/jalvz/depth-cover>). Samples were screened and selected using the following criteria: 1) >10% reads aligned to the hg19 build of the human genome; 2) a C>T mismatch rate at the 5'-end and G>A at the 3'-end of the sequencing read of 5% or above (characterized with mapDamage v2.0.8) (21); 3) with a contamination level <= 3%. Contamination rates were estimated with three methods: 1) damage pattern and polymorphism in mitochondrial DNA with Schmutzi, 2) atypical ratios of coverages of X and Y chromosomes to autosomes calculated with ANGSD and 3) for male samples, high heterozygosity on non-pseudo-autosomal region of the X chromosome with the "contamination" tool in ANGSD (version 0.936/0.937).

Data analysis

Pseudohaploid genotypes for individuals in this study were called using the pipeline and tool created by Stephan Schiffels found here: <https://github.com/stschiff/sequenceTools>. Samtools mpileup was used to generate read coverage of a select SNP. Pseudohaploid genotypes were called by randomly choosing one allele from each site where there was read coverage, using pileupCaller. For the analyses in the paper, we merged the newly generated data reported here with the Allen Ancient DNA Resource v44 using PLINK v1.9059.

We used the contDeam tool in schmutzi (v1.5.6) with the following parameters: length of expected deamination set to 2 (--lengthDeam 2) and library type set to double strand (--library double). Haplogroups for the endogenous mitochondrial genomes were called using the command line version of Haplogrep (v2.1.20).

Kinship analysis was performed using READ (Relationship Estimation from Ancient DNA).

We generated a Principal Component Analysis (PCA) reference space using the tool smartpca from the EIGENSOFT package version 8.0.0. We used the parameter “numoutlieriter:0” to retain all outlier individuals in the projections, and “shrinkmode: YES” to correct for shrinkage towards the origin when estimating PC scores.

We carried out qpAdm and qpWave analyses using ADMIXTOOLS version 2.0.0. Since we are using pseudohaploid data, the program functions similar to the Admixtools, Inbreed=YES option. We used ADMIXTURE (version 1.3.0) modeling to interpret the study individuals as a mixture of source populations.

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Sample size	The total size for the study was 50 samples (i.e. libraries created from the petrous portion of 50 ancient individuals). We created and screened libraries from 23 Etruscan individuals with 11 libraries (all from Tarquinia) passing endogenous preservation and quality control measures. From Kerkouane, we created libraries for 20 individuals, with 12 successfully passing preservation and quality control standards. All libraries created for Sant’Imbenia (n=3) and Pain Sultano (n=4) successfully passed our screening thresholds.
Data exclusions	From the original 50, 30 libraries passed screening. 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 the contamination estimate tool Schmutzi.
Replication	All tools and data needed to reproduce and evaluate the conclusions in this paper are presented in the main text and the Supplementary Materials. All data presented in the study are being made available on the European Nucleotide Archive under the accession number Project PRJEB49419. (www.ebi.ac.uk/ena/browser/view/PRJEB49419).
Randomization	Randomization was not relevant to the study since the samples in the study were grouped by the archaeological site at which they were excavated.
Blinding	Blinding was also not necessary, since our goal is to describe and better understand the population of these ancient sites (i.e. the grouping used), rather than to test difference between the sites.

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Materials & experimental systems

- n/a Involved in the study
- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Human research participants
- Clinical data
- Dual use research of concern

Methods

- n/a Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Palaeontology and Archaeology

Specimen provenance

The material reported in this study represents 4 archaeological sites (3 in Italy and 1 in Tunisia). We have provided the names of the sites, the time periods of occupation, the exact coordinates of the sites, and information about each individual and burial in the supplementary information. For each site, we worked with the appropriate governmental authorities and permitting bodies to obtain permissions and permits to work on this material. We also visited each location, involved local collaborators in the research, and presented in the study findings to the local communities.

For Pian Sultano and Tarquinia, both located in Lazio Administrative Region of Italy, we received permissions and permits from Daniela De Angelis, the Director of the National Etruscan Museum of Tarquinia (operated by the Direzione Generale Musei Lazio, which is part of the Italian Government's Ministry of culture) where all material for both Pian Sultano and Tarquinia are housed on December 22, 2018. We also received permits to visit and conduct research in the collection in March and July of 2019.

For the material from Sant'Imbenia, Sardinia, we received permissions from Dr. Gabriella Gasperetti, the Archaeological Superintendent of the province of Sassari e Nuoro (Soprintendenza Archeologia, belle arti e paesaggio per le province di Sassari e Nuoro; Sassari, Italy) who oversees these collections on November 1, 2019.

For the material from Kerkouane, Tunisia, we received a permit and signed a partnership agreement with the National Heritage Institute of the Tunisian government's Ministry of Culture. There were signed and approved by the Director General, Dr. Faouzi Mahfoud on April 4, 2020.

Specimen deposition

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). We have uploaded the data and are in the process of making in public in the next few weeks so that it available for public use.

Dating methods

To determine the chronological dates for the individuals in the study, AMS radiocarbon dating was conducted on 60% of the individuals. This includes all three individuals from Sant'Imbenia, 2 individuals from Pian Sultano, 6 individuals from Kerkaoune, and 7 individuals from Tarquinia. We made sure to send an aliquot from at least 1 individual per tomb so that we could obtain an estimate for the use dates of each tomb.

The aliquots sampled consisted of 1 gram of dense petrous bone. These were sent to the W.M. Keck Carbon Cycle Accelerator Mass Spectrometer Lab at the University of California at Irvine. The results were calibrated using the intCal20 calibration curve using the OxCal interface (<https://c14.arch.ox.ac.uk/oxcal/OxCal.html>). We report these results in Dataset S3.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Each of the 5 core institutions was involved in the ethical oversight of the project. This includes the UNESCO World Heritage Site at Kerkouane, the UNESCO World Heritage Site at Tarquinia, Sapienza University in Rome, the University of Vienna, and Stanford University. Two of the authors, Hannah Moots and Jonathan Pritchard, have been involved in creating an interdisciplinary review board at Stanford University to provide guidance on ethical practices in ancient DNA research. While this body does not have official paperwork yet, we did discuss this and other projects with this group to ensure ethical best practices were followed.

Note that full information on the approval of the study protocol must also be provided in the manuscript.