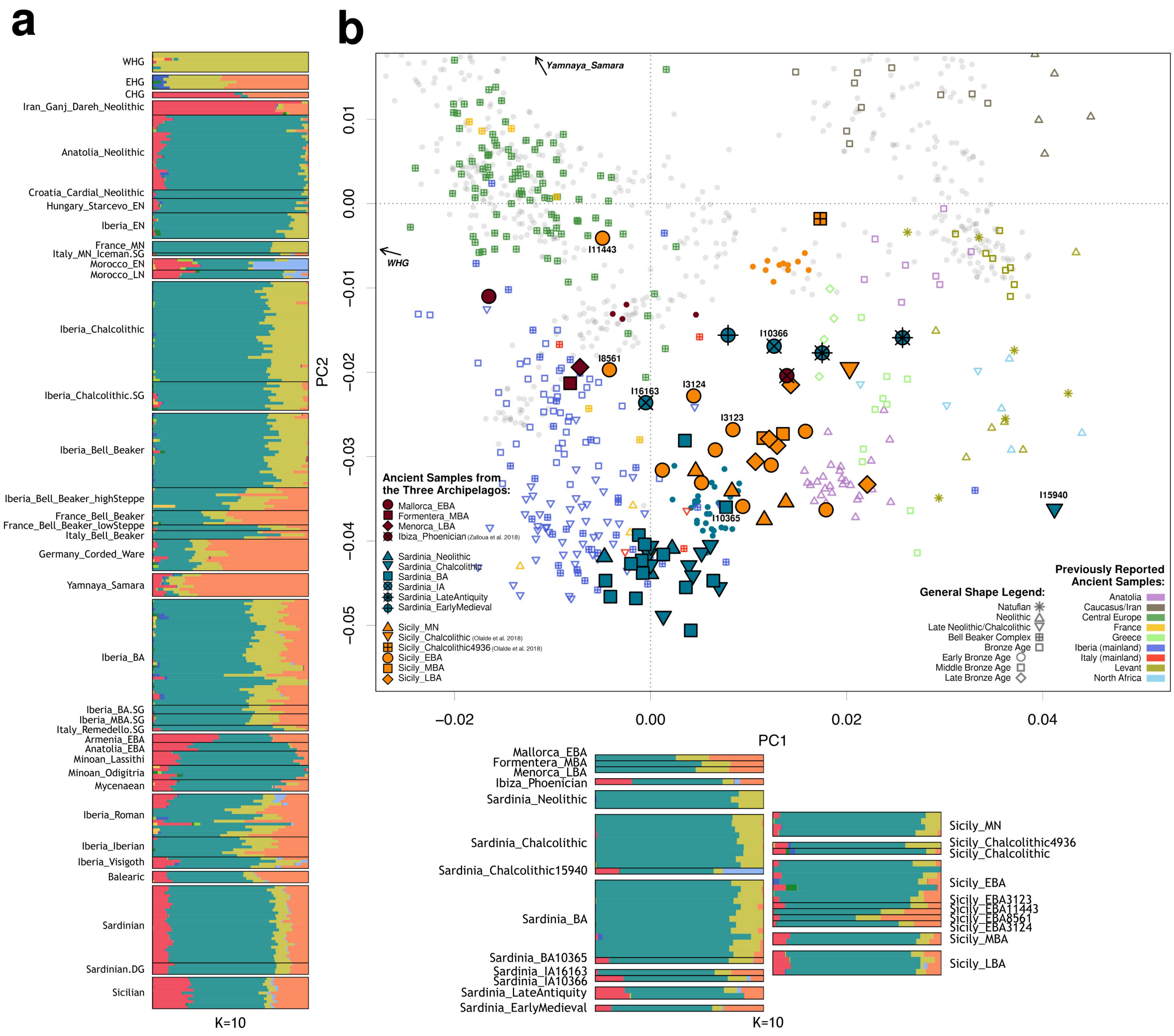
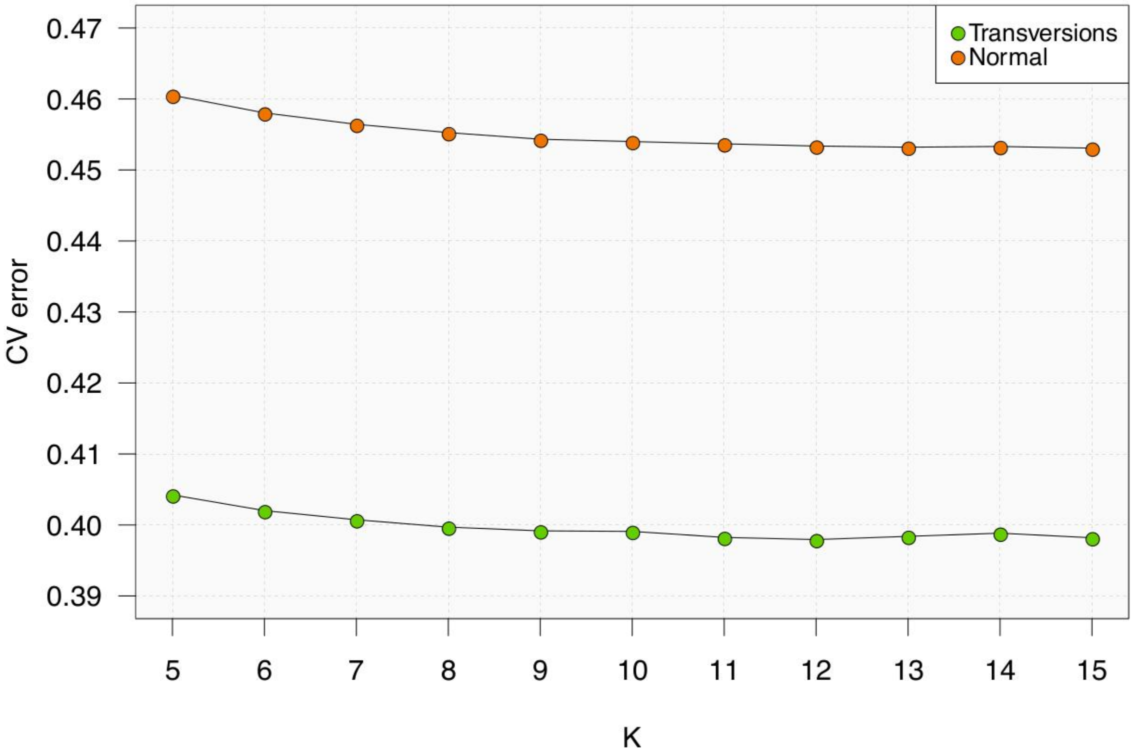
Supplementary Information

The Spread of Steppe and Iranian Related Ancestry in the Islands of the Western Mediterranean

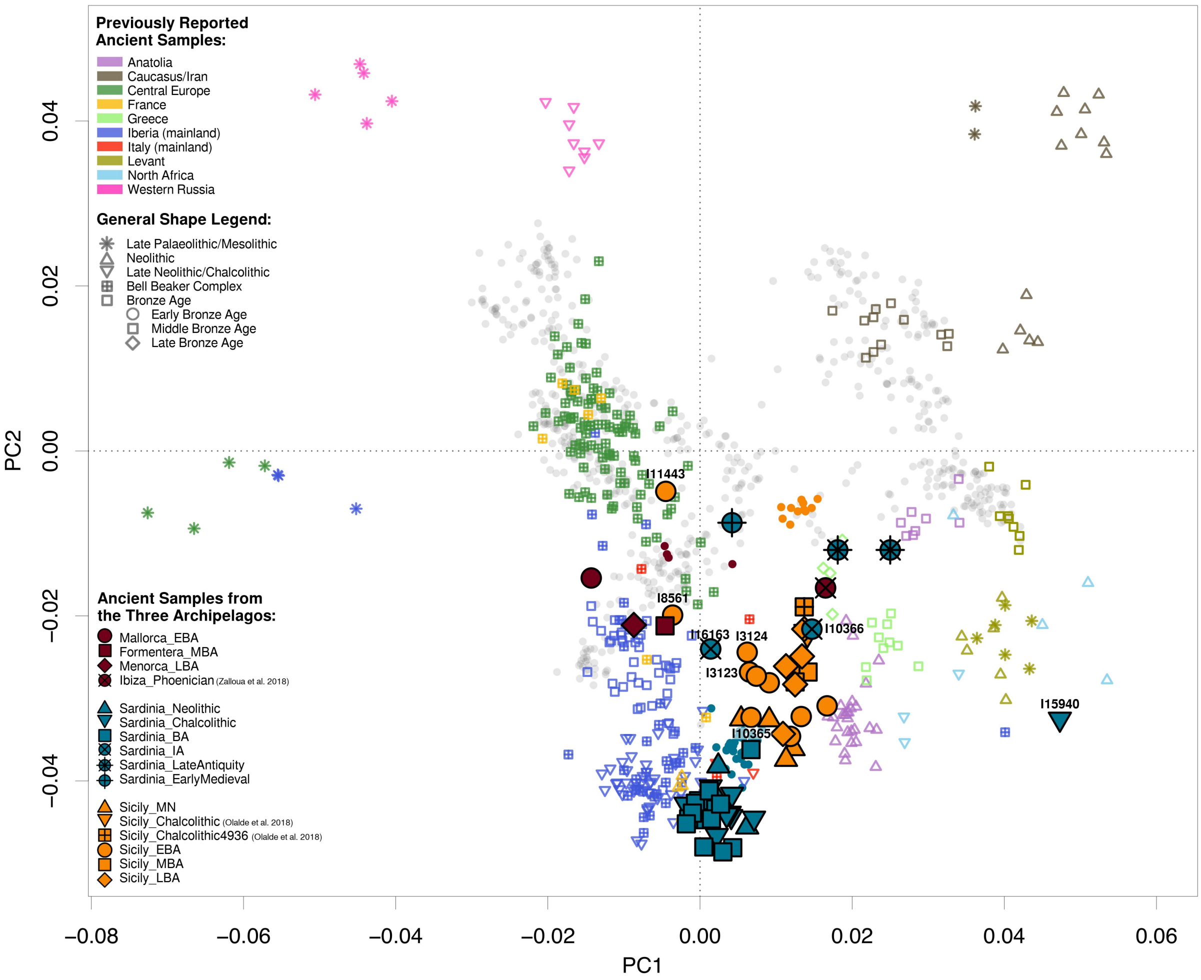
SUPPLEMENTARY FIGURES



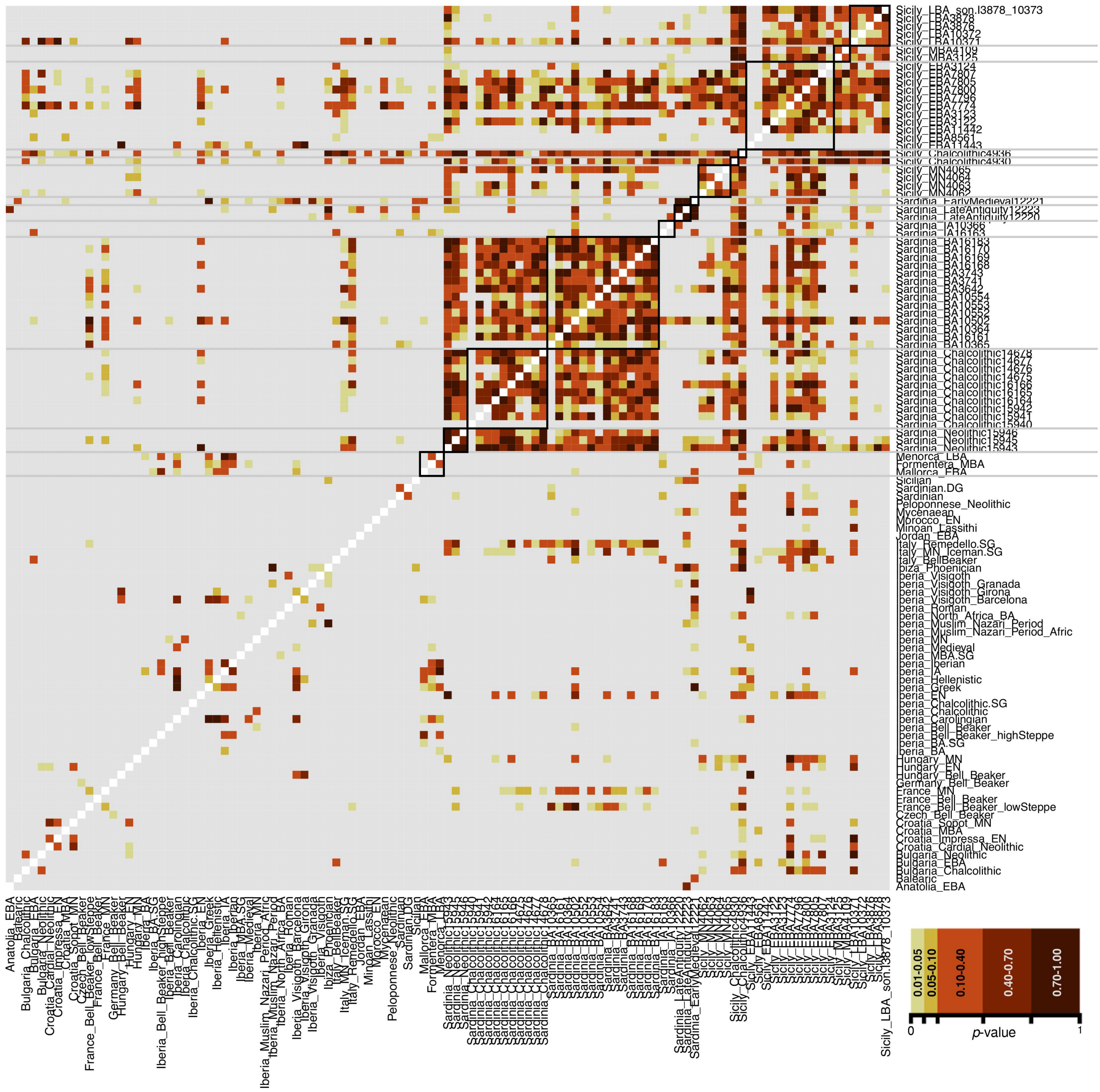
Supplementary Fig. 1: Qualitative relationships between ancient Sardinians, Sicilians and Balearic islanders and other ancient and present-day populations using a dataset restricted to only transversion SNPs (total of 98,631) according to a) unsupervised ADMIXTURE analysis with K=10 clusters; and b) PCA with previously published ancient individuals (non-filled symbols), projected onto variation from present-day populations shown in solid-color circles without outlines (maroon for Balearic, orange for Sicilians, blue for Sardinians, and gray for all others).



Supplementary Fig. 2: Lowest ADMIXTURE cross-validation errors (K=5 to15) for the full dataset and the one restricted to transversion SNPs only.



Supplementary Fig. 3: Non-truncated version of PCA of Fig. 2b.



Supplementary Fig. 4: Enlarged set of pairwise *qpWave* tests to group individuals. Black lines represent the initial clusters of individuals from this study by location and/or period. Gray-colored models have a P-value below 0.01 and are rejected.

SUPPLEMENTARY DATA LEGENDS

(as Excel file) Supplementary Data 1. Sample information, sequencing, and contamination results for the newly reported individuals in this study.

(as Excel file) Supplementary Data 2. New direct radiocarbon dates presented in this study.

(as Excel file) Supplementary Data 3. Information about literature samples used in this study.

(as Excel file) Supplementary Data 4. Uniparental haplogroup calls and identified markers.

(as Excel file) Supplementary Data 5. Symmetry f_4-statistics after separating the *Sardinia_BA* individuals by genotyping pipeline. No statistically significant differences are observed between the two pipelines.

(as Excel file) Supplementary Data 6. Testing for artifactual attraction of samples processed by Pipeline 1 and samples processed by Pipeline 2. We give ABBA and BABA counts and associated P-values for differences in the rates of these counts for the set of samples (individual1_pipeline1, individual1_pipeline2, individual2_pipeline1, individual2_pipeline2). For example, a BABA count corresponds to a case where the sequences match for the two individuals processed using Pipeline 1, and mismatch the sequences at the same position for the two individuals processed using Pipeline 2 (a BABA count is the direction expected for a bias, and hence we expect to observe an excess of BABA over ABBA counts if there is a bias). On the left we show the individual ABBA and BABA counts, and on the right we should a grid of p-values from a one-sided binomial test for an excess of BABA counts under the conservative assumption that all counts are independent. The lowest p-vaue of 0.022 is not significant after Bonferroni correction for the 190 tests performed (if we restrict to cases where we have at least 5 BABA and ABBA counts which is the minimum number needed to provide power to detect a signal at $P<0.05$ nominal significance, there are 89 tests performed which is still not significant after multiple hypothesis testing correction).

SUPPLEMENTARY NOTES

1 - Archaeological descriptions and contexts of newly reported samples

In what follows we list the latitude and longitude after the name of each site.

<u>*Cova des Moro, Mallorca, Spain (⊕ 39.505, 3.302)*</u>

Cova des Moro is a natural cave in East Mallorca (municipality of Manacor) with both paleontological and archaeological deposits. It is found in a Miocene coastal cliff and formed by a large hall (about 60 by 30 m) divided into different spaces by stalagmite formations[1]. Archaeological excavations took place between 1995 and 2002[2,3], identifying three main periods of occupation. The earliest was dated to the second half of the 3rd millennium BCE. The material evidence (pottery and animal bones) indicates that in this period it was used as a temporary shelter. The second period, dated to the Late Bronze Age (mid to late 2nd millennium BCE), has been interpreted as a ritual use of the cave, during which a cyclopean corridor at the entrance of the cave was probably built. Lastly, the cave was used as refuge by Muslims during the Catalan conquest of Mallorca (1229-1232 CE).

Far from the entrance, several human bones were found in a shallow sinkhole in the central part of the cave. Radiocarbon dates were determined on two human samples, UtC-7878: uncal3840±60 BP[4] (2470-2139 calBCE), and KIA-30020: uncal3900±30 BP[5] (2395-2316 calBCE), relating these human bones to the early occupation period of the cave. The following sample from this site was used (same format used for all sites below):

CDMDR (*Mallorca_EBA*, I4329). Various skull and mandible fragments that probably belonged to the same individual, of which a tooth was used for DNA analysis. C14 dated to 2395-2316 calBCE (3900±30 BP, KIA-30020)

<u>*Cova 127, Formentera, Spain (⊕ 38.670, 1.584)*</u>

Cova 127 is a cave located in the cliffs at La Mola (Formentera) and was excavated from 2014 to 2016. The cave entrance connects to a gallery that leads to a main hall. A burial with two skeletons was found at the junction of both areas, consisting of fragmented remains from two individuals[6]: one middle-aged adult around 35 years old at death (used in this study) and one juvenile of 10-15 years old. Some bone buttons were recovered (one with a V-shaped perforation) and ceramic fragments were uncovered along with the human remains. The ascription to the Bronze Age period is supported not only by the direct radiocarbon dating of the human bones, but also by cultural similarities with the early occupation layers of the nearby Cap de Barbaria II settlement[6].

Cova127, 2015, Ni 1077; No. Estracio 5209 (*Formentera_MBA*, I4420_all). Petrous bone, from an assemblage of fragmented human bones (n=159) and isolated teeth from two individuals from Cova 127. We newly report two C14 dates for I4420 - a skull piece dated to 1879-1691 calBCE (3454±26 BP, D-AMS018425), and a maxillary piece dated to 1881-1701 calBCE (3473±20 BP, MAMS-22647). We use the union of these date ranges, 1881-1961 calBCE for I4420, to represent I4420.

Naveta des Tudons, Menorca, Spain (⊕ 40.003, 3.892)

This funerary Cyclopean building in West Menorca (municipality of Ciutadella) has a maximum length of 13.6 m, maximum width of 6.4 m, and height of 4.5 m. During the excavations 1959-60 while it was being restored, human remains were recovered from a collective burial. Four radiocarbon dates were determined on the human samples - IRPA-1178: uncal2740±40 BP, IRPA-1181: uncal2780±35 BP, IRPA-1184: uncal2690±35 BP, uncalIRPA-1179: uncal2820±40 BP[7]. These dates all fall in the range of ca. 1100-800 calBCE, and cluster in the first half of the 9th century calBCE. This chronology, as well as the materials recovered, place this individual into a Talaiotic context. “Naveta” is a Catalan word which means small boat, and is locally used to name these buildings because of their similarity with inverted boats. The burial naveta, as a type of funerary monument, is generally considered to be a local development (only documented in Menorca) derived from the dolmens of the Early Bronze Age[8,9]. The elongated and stylized construction of Naveta des Tudons probably represents the final evolution of this type of burial structure.

NT14 (*Menorca_LBA*, I3315). Petrous bone, recovered from an assemblage of at least 100 individuals in a collective inhumation[10]. C14 dated to 904-817 calBCE (2715±20 BP, PSUAMS-3717).

Stretto Partanna, Sicily, Italy (⊕ 37.724, 12.916)

The excavation at the ditch-trench of Stretto Partanna (Trapani) took place in 1989. The archaeological materials consist predominantly of trichromatic ceramics, and radiocarbon dates attribute the occupation period to the Middle Neolithic (5800-4900 BCE)[11]. The ditch-trench, over 13 metres deep, was characterized by the presence of artifacts along with skeletal remains of domesticated fauna. The human skeletal remains consist of at least 7 secondary burials that were not anatomically articulated[11]. The data in this study came from 4 petrous bones:

FS2 (*Sicily_MN*, I4062). Petrous bone, C14 dated to 4946-4787 calBCE (5980±30 BP, PSUAMS-1950).

FS3 (*Sicily_MN*, I4063). Petrous bone, C14 dated to 4963-4795 calBCE (5995±30 BP, PSUAMS-2263).

FS4 (*Sicily_MN*, I4064). Petrous bone, C14 dated to 4837-4713 calBCE (5900±30 BP, PSUAMS-2266).

FS5 (*Sicily_MN,* I4065). Petrous bone, C14 dated to 4975-4781 calBCE (5980±35 BP, PSUAMS-1951).

Buffa Cave II, Sicily, Italy (⊕ 37.908, 13.478)

The human skeletal remains from the cave of Buffa II (near Villafrati, a commune in the Province of Palermo, located about 25 km southeast of Palermo), were recovered on behalf of Ferdinand Freiherr von Andrian-Werburg (1835-1913) in the winter of 1876/77. Andrian-Werburg entrusted Domenico Heina, servant in the Geologic Cabinet of the University of Palermo and collector of natural produce, to carry out the excavations. Besides a mix of different archaeological objects and animal remains, he recovered human cranial and postcranial remains of a minimum of 21 individuals. Andrian-Werburg published the results in 1878 in the article "Prähistorische Studien aus Sicilien" in a Supplement booklet of volume X of the Berliner Zeitschrift für Ethnologie. His descriptions are divided into two parts: the first chapter deals with Palaeolithic findings, and the second with Neolithic/Chalcolithic[12].

More recent work on Buffa Cave II identified an occupation extending into the Early Bronze Age, as evidenced by the finding of pottery belonging to the Bell Beaker and Capo Graziano cultures[13]. Von Andrian-Werburg's study analyzed the human remains initially attributed to the Neolithic/Chalcolithic. The cranial and postcranial remains were investigated by Emil Zuckerkandl, prosector at the Institute of Anatomy, and then handed over as a "personal present" to the newly founded Anthropological Ethnographical Department at the Natural History Museum Vienna. The cranial and postcranial elements were inventoried 13th December 1890 by C. Heinzel und J. Szombathy with the numbers 3029 till 3062 (a total of 179 bone pieces). The remains belong most likely to the Neolithic ("Neolithische Schichte"), although Andrian-Werburg recognised cultural differences with phases that he could not clearly separate[12]. Direct radiocarbon analysis of three of the individuals identifies the remains as belonging to the Early and Middle Bronze Age, closer to what is described in [13].

The following details are extracted from the handwritten notes (Inventory register no. 3, pages 00027-00029) written by Zuckerkandl[14], who concentrated in his study on the anatomical and morphological features of the specimens from Buffa cave near Villafrati.

BU32, grave 3032 (*Sicily_EBA11443,* I11443). Petrous bone, C14 dated to 2279-2102 calBCE (4090±60 BP, OxA-32773).

BU30, grave 3030, cranium II (*Sicily_EBA,* I3122). Skull, with portions of the cranial base missing, slightly asymmetric, with worn teeth and some cavities. The skull sutures are open and the age at death is estimated at ca. 25-30 years. Morphologically the individual is inferred to be a male which is contradicted by the genetic sex determination of female. Petrous bone C14 dated to 2266-2032 calBCE (3730±30 BP, PSUAMS-1985).

BU36C, grave 3036 (*Sicily_EBA*, I11442). One of four temporal bones preserved from grave 3036 (2 from the left and 2 from the right side). C14 dated to 2281-2047 calBCE based on a union of two radiocarbon dates [2273-2047 calBCE (3750±20 BP, PSUAMS-4547), 2281-2062 calBCE (3765±20 BP, PSUAMS-4620)].

BU31, grave 3031, cranium III (*Sicily_EBA3123*, I3123). Skull, with the left portion of the frontal bone missing, extremely brachycephalic ("planoccipital"). The skull sutures are open. Morphologically the individual is inferred to be a male. Petrous bone C14 dated to 2287-2044 calBCE (3760±30 BP, PSUAMS-3892).

BU36, grave 3036 (*Sicily_EBA3124*, I3124). One of four temporal bones preserved from grave 3036 (2 from the left and 2 from the right side). Petrous bone C14 dated to 1948-1777 calBCE (3545±20 BP, PSUAMS-4317).

BU36A, grave 3036 (*Sicily_MBA*, I3125). One of four temporal bones preserved from grave 3036 (2 from the left and 2 from the right side). Petrous bone C14 dated to 1612-1506 calBCE (3275±20 BP, PSUAMS-4318).

BU36B, grave 3036 (*Sicily_MBA*, I4109). One of four temporal bones preserved from grave 3036 (2 from the left and 2 from the right side). Petrous bone C14 dated to 1631-1509 calBCE (3300±25 BP, PSUAMS-1949).

Marcita, Sicily, Italy (⊕ 37.680, 12.789)

The Marcita necropolis (Castelvetrano - Trapani) was dated between the Early and the Middle Bronze Age. The excavations took place in 1984, when three rock-cut tombs were revealed: A, B and C. Tombs A and B included archaeological finds attributed to the Bell Beaker culture, while Tomb C had human remains but almost no archaeological assemblage (only an ivory comb)[15]. It was composed of about 70 individuals in secondary deposition and not in anatomical connection. The individuals seem to have been placed randomly and all together, perhaps related to a violent event[15].

MA4 (*Sicily_LBA*, I10373). Petrous bone, dated to C14 dated to 1450-1150 BCE based on being the son of I3878 at 1379-1196 calBCE (3015±20 BP, PSUAMS-3995)].

MA8 (*Sicily_LBA*, I3878). Petrous bone, C14 dated to 1379-1196 calBCE (3015±20 BP, PSUAMS-3995).

MA1 (*Sicily_LBA*, I10371). Petrous bone, context dated to 1400-900 BCE based on C14 dates from other individuals at the same site.

MA2 (*Sicily_LBA*, I10372). Petrous bone, C14 dated to 1374-1131 calBCE (3005±20 BP, PSUAMS-5891).

MA3 (*Sicily_LBA*, I3876). Petrous bone, C14 dated to 1071-902 calBCE (PSUAMS-1986).

Serra Crabiles, Sardinia, Italy (⊕ 40.79, 8.59)

The Serra Crabiles necropolis was excavated in 1981 by the Superintendency for Archaeological Heritage for the Provinces of Sassari and Nuoro, and is associated with the Monte Claro culture, Copper Age, between 2700-2000 BCE, due to the presence of vases of the Monte Claro and Bell Beaker cultures, millstones, grinders, obsidian, and small idols[16]. The tombs of the *domus de janas* type contained 5 graves and a minimum of 6 individuals[16].

1267, tomb III; Cella B (*Sardinia_Chalcolithic*, I14675). Petrous bone, C14 dated to 2141-1976 calBCE (3680±25 BP, PSUAMS-6689).

1268, tomb II; Cella A (*Sardinia_Chalcolithic*, I14676). Petrous bone, C14 dated to 2199-2032 calBCE (3715±25 BP, PSUAMS-6690).

1269, tomb III; Cella A (*Sardinia_Chalcolithic*, I14677). Petrous bone, C14 dated to 2465-2297 calBCE (3890±25 BP, PSUAMS-6691).

1270, tomb III; Cella A (*Sardinia_Chalcolithic*, I14678). Petrous bone, C14 dated to 2456-2203 calBCE (3840±25 BP, PSUAMS-6692).

Anghelu Ruju, Sardinia, Italy (⊕ 40.63, 8.32)

The necropolis of Anghelu Ruju was discovered in 1903, near Alghero, in the province of Sassari, and excavated in three phases in 1904[17], 1936[18], and 1967[19]. A minimum of 64 individuals were identified in 38 collective *domus de janas* burial tombs, belonging to primary and secondary depositions, some of which were reused[20]. The skeletons of the first excavation are the only ones to have been studied in a systematic way from an anthropological point of view[21], while a more general study of all the graves has been made in more recent times[22]. Only the skeletons of 4 of the 38 excavated tombs (E, F, VI and XX) have been preserved. Due to the high degree of heterogeneity of the grave goods, 5 individuals have been dated at the "CIRCE" Synchrotron (Center for Isotopic Research on Cultural and Environmental Heritage) in Caserta. This analysis established that the necropolis was used from the Late Neolithic to the Late Bronze. Individuals 12 (3996-3764 calBCE, 5101±57 BP, DSH4758) and 20 (3545-3364 calBCE, 4697±67 BP, DSH3646) from tomb E were dated to the Late Neolithic. Individual 10 from tomb F was dated to the Copper Age (2469-2332 calBCE, 3902±28 BP, DSH3643). Individual 2 from tomb XX (1749-1526 calBCE, 3356±46 BP, DSH7383) and 6 from tomb VI (1316-1049 calBCE, 2967±41 BP, DSH4121) were dated to the Middle and Late Bronze Age. These datings have been made on maxillary and mandibular portions.

In this study we present 7 new radiocarbon dates, from three of the graves examined (VI, E and F). These datings were carried out directly on the petrous samples that had given DNA results; only in the cases of tombs VI we dated all analyzed individuals (**Supplementary Data 2**). For this tomb the dates are quite consistent with those obtained previously. For Tomb E, 4 individuals have been dated, of which 3 have given dates referable to the period of Bell Beaker Culture: 2345-2146 calBCE (3815±25 BP, PSUAMS-6697); 2340-2145 calBCE (3810±25 BP, PSUAMS-6693); 2462-2213 calBCE (3865±25 BP, PSUAMS-6694), and one dated to the Bronze Age: 1451-1301 calBCE (3125±25 BP, PSUAMS-6696). For Tomb E we therefore have later dates than those obtained previously. However, we have the presence of three individuals from the Bell Beaker Cultures, as evidenced also by archaeological data[23], and for the analyzes presented in this study we labeled, in a conservative way, the individuals without a direct C14 date by tomb and period, to the later phase of the Bronze Age. Although we understand this may introduce temporal bias in the attribution of specific individuals to specific periods, all non-directly-dated individuals form a clade with each other in the various analyzes presented below, demonstrating their similar genetic composition and consistent with our cultural assignments (see section 2 and 3 of this document). For tomb F we have a dating from the recent Neolithic period: 4052-3959 calBCE (5205±30 BP, PSUAMS-6699), in accordance with archaeological data[24], and we have attributed the other 4 individuals to the recent Neolithic. Tomb XX, according to previous dating and archaeological archaeological data[23,24], is attributed to the Bronze Age.

2476, tomb XX (*Sardinia_BA_contam*, I15939). Petrous bone, context dated to 1749-1526 calBCE (3356±46 BP, DSH-7383).

2479, tomb E (*Sardinia_Chalcolithic15940*, I15940). Petrous bone, C14 dated to 2345-2146 calBCE (3815±25 BP, PSUAMS-6697).

2480, tomb E (*Sardinia_Chalcolithic*, I15941). Petrous bone, C14 dated to 2340-2145 calBCE (3810±25 BP, PSUAMS-6693).

2481, tomb E (*Sardinia_Chalcolithic*, I15942). Petrous bone, C14 dated to 2462-2213 calBCE (3865±25 BP, PSUAMS-6694).

2482, tomb F (*Sardinia_Neolithic*, I15943). Petrous bone, context dated to 4100-3900 based on a C14 date of 4052-3959 calBCE (5205±30 BP, PSUAMS-6699) from the same tomb.

2483, tomb F (*Sardinia_Neolithic_contam*, I15944). Petrous bone, context dated to 4100-3900 based on a C14 date of 4052-3959 calBCE (5205±30 BP, PSUAMS-6699) from the same tomb.

2484, tomb F (*Sardinia_Neolithic*, I15945). Petrous bone, context dated to 4100-3900 based on a C14 date of 4052-3959 calBCE (5205±30 BP, PSUAMS-6699) from the same tomb.

2485, tomb F (*Sardinia_Neolithic*, I15946). Petrous bone, C14 dated to 4052-3959 calBCE (5205±30 BP, PSUAMS-6699).

2486, tomb F (*Sardinia_Neolithic_contam*, I15947). Petrous bone, context dated to 4100-3900 based on a C14 date of 4052-3959 calBCE (5205±30 BP, PSUAMS-6699) from the same tomb.

2461, tomb VI (*Sardinia_BA*, I16161). Petrous bone, C14 dated to 1606-1433 calBCE (3230±25 BP, PSUAMS-6681).

2462, tomb VI (*Sardinia_IA16163*, I16163). Petrous bone, C14 dated to 762-434 calBCE (2465±20 BP, PSUAMS-6682).

2467, tomb E (*Sardinia_BA*, I16168). Petrous bone, context dated to 1500-1300 based on a C14 date of 1451-1301 calBCE (3125±25 BP, PSUAMS-6696) from the same tomb.

2468, tomb E (*Sardinia_BA*, I16169). Petrous bone, context dated to 1500-1300 based on a C14 date of 1451-1301 calBCE (3125±25 BP, PSUAMS-6696) from the same tomb.

2469, tomb E (*Sardinia_BA*, I16170). Petrous bone, C14 dated to 1451-1301 calBCE (3125±25 BP, PSUAMS-6696).

2526, tomb E (*Sardinia_BA*, I16183). Petrous bone, context dated to 1500-1300 based on a C14 date of 1451-1301 calBCE (3125±25 BP, PSUAMS-6696) from the same tomb.

Sa Ucca de su Tintirriolu, Sardinia, Italy (⊕ 40.41, 8.64)

In 1970, the Middle Neolithic cave site of Sa Ucca de su Tintirriolu, in the municipality of Mara, Sassari, was excavated by the Superintendency for Archaeological Heritage for the Provinces of Sassari and Nuoro[25,26]. The earliest layers found at this cave necropolis are attributed to the Middle Neolithic Bonu Ighinu culture, with more recent layers containing vases of the Ozieri and Monte Claro cultures, hatchets, arrows, male and female figurines, a vase with a square mouth, and a menhir[25,27]. Evidence of cultivation of wheat, barley and legumes was found, alongside with numerous remains of sea shells and land snails[25,27]. Although there is debate if this site should be attributed to the Neolithic[28] or the Chalcolithic[29,30], we present a new C14 date of 2570-2346 calBCE (3955±30 BP, PSUAMS-6695), which along with a previously published date of 2904-2631 calBCE (4200±BP, XXXX) agrees with a Chalcolithic attribution.

2463, G1 (*Sardinia_Chalcolithic*, I16164). Petrous bone, C14 dated to 2570-2346 calBCE (3955±30 BP, PSUAMS-6695).

2464, Trincea B, Livello 40 (*Sardinia_Chalcolithic*, I16165). Petrous bone, context dated to 2600-2300 based on a C14 date of 2570-2346 calBCE (3955±30 BP, PSUAMS-6695) from the same site.

2465, Trincea I, Livello 2 (*Sardinia_Chalcolithic*, I16166). Petrous bone, context dated to 2600-2300 based on a C14 date of 2570-2346 calBCE (3955±30 BP, PSUAMS-6695) from the same site.

Anulù, Seui, Sardinia, Italy (⊕ 39.85, 9.38)

The municipality of Seui, in central-western Sardinia, is located along the southern slopes of the Gennargentu massif. The area, though characterized by a particularly rugged terrain, extremely abrupt changes in altitude, and temperatures that are unusually cold for Sardinia, appears to have been densely settled in the Nuragic period[31]. A case example is the archaeological complex of Anulù: a site located within the forest of Montarbu that includes a nuraghe (at an altitude of 996 m above sea level), one of the so-called *tomba di giganti* or "giants' tombs" and an ensemble of huts whose structures are, in part, well preserved[32,33]. Architectural elements and archaeological analyzes indicate the main occupational phase of the site dates to the recent and late Bronze age (ca. 1400-1100 BCE). However, surface surveys indicate that the site was not abandoned and was used to some extent in later times.

In 2017-2019 the Italian Ministry for Cultural Heritage (Superintendency of Archaeology, Fine Arts and Landscape for the city of Cagliari and the provinces of Oristano and South Sardinia), proceeded with the excavation of the *tomba di giganti* from Anulù: a collective burial site typical of the Nuragic civilization that shows a number of characteristic features such as a posteriorly apsed, rectangular-shaped funerary chamber fronted by a semi-circular wall that delimited an *esedra* that was supposedly used for ritual purposes.

The excavation uncovered a stratigraphic deposit that was partially disturbed. The lower layers, however, appear to have been left untouched since ancient times, as is also suggested by the lack of intrusive pottery dated to later periods. The presence along the margins of the funerary chamber of skeletal elements that, though unarticulated, tend to maintain a certain proximity to one another (mandibles close to associated crania, infant bones close to others of presumably the same individual etc.) appear to indicate that, in ancient times and once the bodies had decomposed, the bones were moved to the sides so as to leave space for the next inhumation.

The sample used in the present study was selected from one of the layers that do not show any intrusive pottery and that can be ascribed archaeologically to Nuragic times. The portion selected for analysis was a buccal root of the upper left second molar from an unarticulated mandible that was discovered near the northeastern margin of the funerary chamber, which is from an adult whose age at death can be estimated at 50 ore more according to dental wear[34].

FC_CGL_1 (*Sardinia_BA*, I10502). Tooth, C14 dated to 1277-1121 calBCE (2980±25 BP, PSUAMS-6688).

Alghero, Sardinia, Italy (⊕ 40.55, 8.31)

Lu Maccioni (Alghero, Province of Sassari) is a natural cave located in Northern Sardinia, precisely in the historical-geographical region of Nurra, at sea level (Province of Sassari). Pieces of dark ceramic and a cylinder in white marble have been found in the cave. The 19 skulls and the 57 long bones recovered allowed the estimation of a minimum number of 40 individuals. Teeth showed presence of caries and traces of trauma have been observed in the skulls.

Relative dating and radiocarbon analysis traced back the site to final Neolithic. The site originates during the Ozieri culture (late Neolithic), but it was reused during the Nuragic III (Bronze Age) and the Phoenician period (Iron Age).

The osteological collection of Lu Maccioni is housed in the Sardinian Museum of Anthropology and Ethnography of the University of Cagliari[35].

Lu Maccioni_Ind.A (*Sardinia_BA*, I10364). Petrous bone, context dated to 1150-800 BCE based on a C14 date of 1126-825 calBCE (2810±60 BP, Beta-82329) from the same layer.

Lu Maccioni_31 Neo-Iron_23 (*Sardinia_BA*, I3642). Tooth, C14 dated to 1117-976 calBCE (2870±20 BP, PSUAMS-2387).

Perdasdefogu, Sardinia, Italy (⊕ 39.683, 9.433)

Perdasdefogu cave is a natural cave located in the historical-geographical region of Quirra, central eastern Sardinia, at 535 m above sea level. It consists of a single corridor, about 605 m long and 0.9 m wide, and with a height varying from 0.7-2.0 m.

The excavations took place in 1963. The cave had been damaged by human action. No funerary utensils, ornaments, or other archaeological materials were found. Due to the collective burial ritual, no articulated skeletons were found, but several human bones, including 36 skulls and 58 long bones, were collected and attributed to a minimum number of 50 individuals.

The ascription to the final Bronze Age period is supported by radiocarbon dating.

The osteological collection of Perdasdefogu cave is housed in the Sardinian Museum of Anthropology and Ethnography of the University of Cagliari[35].

Perda-1 (*Sardinia_BA*, I10552). Petrous bone, C14 dated to 1384-1213 calBCE (3025±20 BP, PSUAMS-4875).

Perda-2 (*Sardinia_BA*, I10553). Petrous bone, C14 dated to 1226-1056 calBCE (2950±20 BP, PSUAMS-4876).

Perda-3 (*Sardinia_BA*, I10554). Petrous bone, C14 dated to 1260-1112 calBCE (2960±20 BP, PSUAMS-4877).

PERDASDEFOGU 15R (*Sardinia_BA*, I3741). Tooth, C14 dated to 1219-1049 calBCE (2935±25 BP, PSUAMS-2118).

Seulo, Sardinia, Italy (⊕ 39.867, 9.233)

Stampu Erdi (Seulo) is a natural cave located in central southern Sardinia, in the historical-geographical region of Barbagia of Seulo, at 740 m above sea level. Archaeological excavation took place in 1956. Besides human cranial and postcranial remains, pottery and objects in obsidian have been found. The site was dated to the Bronze age (Nuragic II period) through both relative and radiocarbon analysis[35].

The osteological collection of Stampu Erdi is housed in the Sardinian Museum of Anthropology and Ethnography of the University of Cagliari.

SEULO 59 (*Sardinia_BA*, I3743). Tooth, C14 dated to 2134-1947 calBCE (3655±25 BP, PSUAMS-2076).

Seulo_Ind.B (*Sardinia_BA10365*, I10365). Petrous bone, C14 dated to 1643-1263 calBCE (3190±80 BP, Beta-37705).

Usellus, Sardinia, Italy (⊕ 39.81, 8.84)

Motrox 'e Bois (Usellus) is a collective burial monument typical of the so-called “giants’ tombs” of Nuragic Sardinia. It is located in the historical-geographical region of Arborea, central western Sardinia, at 289 m above sea level. The excavations took place in 1957. Based on the study of mandibles and isolated teeth, a minimum number of 42 individuals was estimated.

The skeletal material has been dated back to the Final Bronze Age by radiocarbon analysis. The osteological collection of Motrox 'e Bois burial is housed in the Sardinian Museum of Anthropology and Ethnography of the University of Cagliari[35].

MSAE 8784 (Motrox 'e Bois) (*Sardinia_IA10366*, I10366). Petrous bone, C14 dated to 391-209 calBCE (2250±20 BP, PSUAMS-4874).

Grotta Colombi, Sardinia, Italy (⊕ 39.182, 9.163)

Sant’Elia Cape (Cagliari, Southern Sicily) harbors extensive archaeological material in the caves of its Miocene limestone cliff. It includes the well-preserved Cala Mosca stratigraphic section, which represents the type locality of the so-called Tyrrhenian plain[36]. At Marine Isotope State (MIS) 5.5, high sea levels caused the Sant'Elia hill to be an island separated from the Cagliari coast by about 1.5 km of sea. During successive glacial periods when sea levels were lower, the marine shelf surrounding the Sant'Elia promontory was largely exposed. For the last 7000-5000 years, the coastline has followed a similar course to the present one.

The Grotta dei Colombi (Pigeon’s Cave) is one of the five natural caves located on the slope of the promontory, deriving its name from the many wild pigeons that have nested there in the past. This large cave has been known since ancient times, and opens today just above sea level.

The cave entrance, triangular in shape, can only be accessed from the sea. The internal hall, approximately circular in shape, is about 30 m wide and 20 m high.

During the Late Neolithic (if not earlier) the cave was either temporarily inhabited or visited for funeral use by people reaching it by boats, as documented by the presence of a human skeleton and fragments of pre-Nuragic ceramic pottery (Ozieri Culture, 3200-2800 BCE) in the lowermost levels of the sedimentary sequence filling the cave. The uppermost levels contain a variety of Nuragic culture associated pottery dated to the 8th century BCE[17,37-39].

The cave was still occasionally visited by fishermen and hunters even in recent times.

The human remains analyzed here were collected during a geological survey by the Italian researcher A. Malatest at different points on the surface floor of the Grotta dei Colombi cave together with some fragments of ceramic pottery and few fragments of bird (mainly *Columba livia*) and mammal bones (*Capra hircus, Ovis aries*, medium-sized deer).

GC3 (*Sardinia_LateAntiquity*, I12220). Long bone, C14 dated to 566-640 calCE (1465±15 BP, PSUAMS-5283).

GC1 (*Sardinia_LateAntiquity*, I12223). Tooth, C14 dated to 256-403 calCE (1700±25 BP, PSUAMS-5427).

GC4 (*Sardinia_EarlyMedieval*, I12221). Bone, C14 dated to 892-990 calCE (1100±20 BP, PSUAMS-6157).

Contrada Paolina, Castellucciana, Sicily, Italy (⊕ 36.883, 14.571)

The skeletal remains come from the district of Paolina, in the hinterland of Camarina in Ragusa Province, Southern Sicily, Italy. The complex, consisting of three tombs, is located on a low hill between the last slope of the Ragusa plateau down towards the sea. The excavation was conducted between April and May 1977 by the Archaeological Superintendence of Syracuse. Three artificial tombs are present, excavated in slightly coherent limestone. The human remains come from Tomb 2 with a diameter of 2.40 and height of 1.32 m, a “grotticella” tomb of the "oven type” in the common typology of the Castelluccian tombs[40]. In previous studies, the presence of at least 76 individuals has been estimated, including adults, and infants of both sexes (determined anthropologically)[41-45]. The multiple depositions were grouped along the tomb chamber walls, interpreted as subsequent burials in different times probably by family or clan (common rite in coeval sites[40]), and a single primary undisturbed burial was found in the middle of the room.

PP G (*Sicily_EBA*, I7774_d). Petrous bone, context dated to 2200-1600 BCE based on other dates from the same site.

PP K (*Sicily_EBA*, I7796). Petrous bone, C14 dated to 1879-1691 calBCE (3455±25 BP, PSUAMS-6465)

PP O (*Sicily_EBA*, I7800). Petrous bone, C14 dated to 2121-1917 calBCE (3630±25 BP, PSUAMS-6466)

PP V (*Sicily_EBA*, *I7807*). Petrous bone, C14 dated to 2190-2143 calBCE (3700±20 BP, PSUAMS-6468).

PP T (*Sicily_EBA*, *I7805*). Petrous bone, C14 dated to 2014-1781 calBCE (3565±25 BP, PSUAMS-6467).

Isnello, Sicily, Italy (⊕ 37.95, 14.083)

The Cave Abisso del Vento, of karstic origin, is located in the locality "Ficuzedde" in the Municipality of Isnello (Palermo), and is one of the most complex cave systems in Sicily in the mountain districts of Pizzo Dipilo (1385 m above sea level) and Pizzo Carbonara (1979 m above sea level) in the eastern Madonie area. The cave has yielded anthropological materials since the nineteenth century, which can be classified as falling between the Eneolithic and Early Bronze Age and are currently kept at the Minà Palumbo Museum in Castelbuono. The sample we analyzed derives from a multiple burial just below the entrance of the cave. The anthropological sex determination agreed with the genetic determination.

Abisso del Vento 2017 (Grotto) (*Sicily_EBA8561*, I8561). Petrous bone, C14 dated to 2346-2199 calBCE (3825±20 BP, PSUAMS-4873).

Vallone Inferno, Sicily, Italy (⊕ 37.872, 13.935)

Vallone Inferno rock-shelter is located in the Madonie mountain range in Sicily[46]. Archaeological excavation has provided a long prehistoric and historic sequence from the Neolithic to the Medieval period. Of the four stratigraphic complexes, complex 3 has provided almost all the archaeological remains. 14C AMS dates, obtained from five samples place the human activities between the Middle Neolithic to the Medieval period[47]. A persistent use of the shelter for pastoral (herding) activities has been shown from macrofaunal and plant remains. The aridification and the opening of the landscape from the base to the top of the sequence has been documented through the analysis of environmental data obtained from microvertebrate and archaeobotanical remains.

Within the archaeological layers of the Early Bronze Age, the main cultural attribution of the ceramic is to the Castelluccian culture, with the sporadic presence (one ceramic fragment) of Bell Beaker associated pottery.

Several human remains were found within the Early Bronze Age layers, with a preponderance of dental remains (76%)[46].

***VALINF (Sicily_EBA_lowcov,* I4383).** Long bone, context dated to 2600-2300 BCE based on its relationship to a radiocarbon date on bone in layer 3.4.g of 2601-2309 calBCE (3948±35 BP, DSH-1976), and a radiocarbon date on seed in layer 3.4.b of 1643-1411 calBCE (3244±42 BP, DSH-2815).

2 - *qpWave* analysis to group individuals of similar contexts into analysis clusters

We used *qpWave*[48] from ADMIXTOOLS[49] to cluster individuals from similar archaeological contexts. We used a p>0.01 threshold as a criterion for clustering, *allsnps:YES*, and the following "Right" populations (a set we call "B14"):

Mbuti.DG, Ust_Ishim, CHG, EHG, ElMiron, Vestonice16, MA1, Israel_Natufian, Jordan_PPNB, Anatolia_Neolithic, WHG, Iran_Ganj_Dareh_Neolithic, Yamnaya_Samara, Morocco_LN

We present a small subset of the results of this analysis in **Fig. 3**, focusing on our newly analyzed individuals and some relevant previously published data. The results for the full set of tests are presented in **Supplementary Fig. 4**, and are summarized below. Pairs of individuals that passed this *qpWave* analysis at the p>0.01 threshold and were from the same region and chronological period were grouped for analysis, with a few exceptions.

After identifying all pairs of chronologically/geographically grouped individuals that gave evidence of being different in ancestry at the p<0.01 level, we further tested them using the same method for a difference in ancestry compared to other individuals in the grouping.

Bronze Age Balearic Islanders:

Two pairs of individuals were detected as genetically inhomogeneous by *qpWave*: *Mallorca_EBA-Menorca_LBA* (p=0.001) and *Mallorca_EBA-Formentera_MBA* (p=0.001). (**Supplementary Table 1**). While *Formentera_MBA* and *Menorca_LBA* were consistent with being a clade with each other, based on the chronological and geographical differences among the three Balearic island samples we analyzed them individually.

Supplementary Table 1: P-values for the *qpWave* models between the Balearic individuals. (In parentheses we give P-values based on using a more sensitive outgroup set adding in more closely related groups; that is, we add in *Iberia_Bell_Beaker*, *Iberia_Bell_Beaker_highSteppe*, *Sardinia_Chalcolithic*, *Sardinia_BA* and *Sicily_EBA*.)

	Mallorca_EBA	Formentera_MBA	Menorca_LBA
Mallorca_EBA		0.001 (0.006)	0.001 (0.002)
Formentera_MBA	0.001 (0.006)		0.380 (0.083)
Menorca_LBA	0.001 (0.002)	0.380 (0.083)	

The Iron Age Phoenician individual from Ibiza from [50] did not form a clade with any of the three preceding individual from our study (*Mallorca_EBA* $p=1.53\times10^{-11}$, *Formentera_MBA* $p=2.50\times10^{-13}$, *Menorca_LBA* $p<10^{-12}$), but was consistent with forming a clade with the Late Antiquity Sardinian individuals (P-value always above 0.318) that possessed large amounts of Iranian-related ancestry (**Fig. 3**), showing the contribution of ancestries not evident earlier in the Balearic Islands.

When we examined modern Balearic individuals they were consistent with forming a clade only with *Sardinia_EarlyMedieval* (p=0.357) (**Fig. 3**).

Early Bronze Age Sicilians:

Among the Sicilian Early Bronze individuals, I11443 from Buffa Cave (*Sicily_EBA11443*) and individual I8561 from Isnello (*Sicily_EBA8561*) were not consistent with forming a clade with the remaining Early Bronze Age Sicilians in the great majority of tests. We therefore treated them as outliers for analysis (**Supplementary Table 2**).

Supplementary Table 2: P-values for the *qpWave* models between Early Bronze Age Sicilians. (In parentheses we give P-values based on using a more sensitive outgroup set adding in more closely related groups; that is, we add *Italy_MN_Iceman.SG, Italy_Remedello.SG, Iberia_Bell_Beaker, Iberia_Bell_Beaker_highSteppe, Sardinia_Chalcolithic, Sardinia_BA, Iberia_MN, Iberia_Chalcolithic, France_Bell_Beaker, France_Bell_Beaker_lowSteppe, Anatolia_EBA.*)

	I11443	**I8561**	**I11442**	**I3122**	**I3123**	**I7774**	**I7796**	**I7800**	**I7805**	**I7807**	**I3124**
I11443		1.35E-06 (2.72E-07)	7.06E-32 (2.79E-28)	8.50E-38 (1.45E-36)	2.84E-16 (1.35E-14)	9.58E-08 (1.56E-07)	1.65E-21 (6.01E-18)	6.69E-07 (6.33E-06)	1.83E-11 (3.31E-10)	1.68E-25 (6.30E-23)	6.12E-21 (6.79E-18)
I8561	1.35E-06 (2.72E-07)		3.09E-11 (5.75E-09)	6.19E-11 (7.76E-12)	0.002 (0.000)	0.056 (0.125)	2.00E-07 (1.16E-07)	0.004 (0.012)	0.002 (0.003)	4.32E-08 (1.22E-06)	2.27E-04 (0.003)
I11442	7.06E-32 (2.79E-28)	3.09E-11 (5.75E-09)		0.766 (0.413)	0.127 (0.256)	0.385 (0.239)	0.977 (0.768)	0.434 (0.479)	0.149 (0.305)	0.011 (0.042)	0.079 (0.351)
I3122	8.50E-38 (1.45E-36)	6.19E-11 (7.76E-12)	0.766 (0.413)		0.020 (0.013)	0.312 (0.398)	0.586 (0.239)	0.518 (0.604)	0.027 (0.003)	0.392 (0.210)	0.001 (0.003)
I3123	2.84E-16 (1.35E-14)	0.002 (0.000)	0.127 (0.256)	0.020 (0.013)		0.073 (0.088)	0.053 (0.021)	0.892 (0.891)	0.477 (0.631)	0.006 (0.060)	0.279 (0.283)
I7774	9.58E-08 (1.56E-07)	0.056 (0.125)	0.385 (0.239)	0.312 (0.398)	0.073 (0.088)		0.268 (0.395)	0.070 (0.120)	0.515 (0.924)	0.397 (0.629)	0.223 (0.082)
I7796	1.65E-21 (6.01E-18)	2.00E-07 (1.16E-07)	0.977 (0.768)	0.586 (0.239)	0.053 (0.021)	0.268 (0.395)		0.521 (0.561)	0.385 (0.572)	0.879 (0.277)	0.030 (0.055)
I7800	6.69E-07 (6.33E-06)	0.004 (0.012)	0.434 (0.479)	0.518 (0.604)	0.892 (0.891)	0.070 (0.120)	0.521 (0.561)		0.358 (0.349)	0.398 (0.599)	0.029 (0.128)
I7805	1.83E-11 (3.31E-10)	0.002 (0.003)	0.149 (0.305)	0.027 (0.003)	0.477 (0.631)	0.515 (0.924)	0.385 (0.572)	0.358 (0.349)		0.964 (0.642)	0.027 (0.013)
I7807	1.68E-25 (6.30E-23)	4.32E-08 (1.22E-06)	0.011 (0.042)	0.392 (0.210)	0.006 (0.060)	0.397 (0.629)	0.879 (0.277)	0.398 (0.599)	0.964 (0.642)		2.16E-04 (0.001)
I3124	6.12E-21 (6.79E-18)	2.27E-04 (0.003)	0.079 (0.351)	0.001 (0.003)	0.279 (0.283)	0.223 (0.082)	0.030 (0.055)	0.029 (0.128)	0.027 (0.013)	2.16E-04 (0.001)	

Individuals I3123, I3124, and I7807 were involved in other models that produced p<0.01 so we compared them against the group that gave no evidence of outliers (**Supplementary Table 3**). Only individuals I3123 and I3124 were not consistent with forming a clade with the main grouping (p=0.005 and p=0.001, respectively) so we treated them as outliers (*Sicily_EBA3123, Sicily_EBA3124*). We grouped the remaining seven Sicilian Early Bronze Age individuals as *Sicily_EBA* for our main analyzes.

Supplementary Table 3: P-values for the *qpWave* models between Early Bronze Age Sicilian individuals with some evidence of ancestry heterogeneity, against all other individuals.

	I11442, I3122, I7774, I7796, I7800, I7805
3123	0.005
3124	0.001
11443	1.27E-48
I8561	1.09E-16
I7807	0.052

Middle Bronze Age Sicilians:

The pair of Middle Bronze Age individuals I3125-I4109 formed a clade (p=0.379) (**Supplementary Table 4**) and so were analyzed as a group (*Sicily_MBA*).

Supplementary Table 4: P-values for *qpWave* models between Middle Bronze Age Sicilians. (In parentheses we give P-values based on using a more sensitive outgroup set adding in more closely related groups; that is, we add *Italy_MN_Iceman.SG*, *Italy_Remedello.SG*, *Iberia_Bell_Beaker*, *Iberia_Bell_Beaker_highSteppe*, *Sardinia_Chalcolithic*, *Sardinia_BA*, *Iberia_MN*, *Iberia_Chalcolithic*, *France_Bell_Beaker*, *France_Bell_Beaker_lowSteppe*, *Anatolia_EBA*, *Sicily_EBA, Sicily_EBA3123, Sicily_EBA3124, Sicily_EBA8561* and *Sicily_EBA11443.*)

	I3125	**I4109**
I3125		0.379 (0.294)
I4109	0.379 (0.294)	

Late Bronze Age Sicilians:

All Late Bronze Age individuals were consistent with forming a clade with each other (**Supplementary Table 5**), and so we pooled them as *Sicily_LBA*.

Supplementary Table 5: P-values for *qpWave* models between Late Bronze Age Sicilians. (In parentheses we give P-values based on using a more sensitive outgroup set adding in more closely related groups; that is, we add *Italy_MN_Iceman.SG, Sicily_EBA, Italy_Remedello.SG, Sicily_EBA3123, Sicily_EBA3124, Iberia_Bell_Beaker, Sicily_EBA8561, Iberia_Bell_Beaker_highSteppe, Sicily_EBA11443, Sardinia_Chalcolithic, Sardinia_BA, Sicily_MBA, Iberia_MN, Iberia_Chalcolithic, France_Bell_Beaker, France_Bell_Beaker_lowSteppe, Anatolia_EBA.*)

	I10371	**I10372**	**I3876**	**I3878**	**I10373**
I10371		0.012 (0.224)	0.109 (0.429)	0.102 (0.606)	0.198 (0.493)
I10372	0.012 (0.224)		0.010 (0.006)	0.022 (0.041)	0.094 (0.137)
I3876	0.109 (0.429)	0.010 (0.006)		0.279 (0.111)	0.261 (0.597)
I3878	0.102 (0.606)	0.022 (0.041)	0.279 (0.111)		0.943 (0.815)
I10373	0.198 (0.493)	0.094 (0.137)	0.261 (0.597)	0.943 (0.815)	

We found that modern Sicilians were consistent with forming a clade with *Ibiza_Phoenician* (p=0.060), as well as with the *Sardinia_LateAntiquity* individuals (*Sardinia_LateAntiquity12220*, p=0.056; *Sardinia_LateAntiquity12223*, p=0.186), and *Sardinia_EarlyMedieval* (p=0.200).

Neolithic Sardinians:

All pairs of the three individuals were consistent with forming a clade with each other, suggesting homogeneity among the Neolithic Sardinians (**Supplementary Table 6**).

Supplementary Table 6: P-values for the *qpWave* models between the Neolithic Sardinian individuals. (In parentheses we give P-values based on using a more sensitive outgroup set adding in more closely related groups; that is, we add in *Italy_MN_Iceman.SG*, *Italy_Remedello.SG*, *Iberia_Bell_Beaker*, *Iberia_Bell_Beaker_highSteppe*, *Sicily_MN*, *Sicily_EBA*, *Iberia_MN*, *Iberia_Chalcolithic*, *France_Bell_Beaker*, *France_Bell_Beaker_lowSteppe*, and *Anatolia_EBA*.)

	I15943	**I15945**	**I15946**
I15943		0.722 (0.966)	0.740 (0.809)
I15945	0.722 (0.966)		0.603 (0.359)
I15946	0.740 (0.809)	0.603 (0.359)	

Chalcolithic Sardinians:

Individual I15940 was not consistent with forming a clade with the remaining *Sardinia_Chalcolithic* individuals ($p<10^{-12}$). Similarly, individuals I14676, I15941 and 15942 also produced pairwise models with a P-value below the 0.01 threshold (**Supplementary Table 7**) and therefore we analyzed these three individuals against the group of the remaining individuals that were consistent with forming a clade.

Supplementary Table 7: P-values for the *qpWave* models between the Chalcolithic Sardinian individuals. (In parentheses we give P-values based on using a more sensitive outgroup set adding in more closely related groups; that is, we add in *Italy_MN_Iceman.SG*, *Italy_Remedello.SG*, *Iberia_Bell_Beaker*, *Iberia_Bell_Beaker_highSteppe*, *Sicily_MN*, *Sicily_EBA*, *Iberia_MN*, *Iberia_Chalcolithic*, *France_Bell_Beaker*, *France_Bell_Beaker_lowSteppe*, and *Anatolia_EBA*.)

	I14675	**I14676**	**I14677**	**I14678**	**I15940**	**I15941**	**I15942**	**I16164**	**I16165**	**I16166**
I14675		0.402 (0.128)	0.052 (0.055)	0.190 (0.330)	5.70E-170 (3.23E-195)	0.152 (0.123)	0.005 (0.003)	0.015 (0.078)	0.245 (0.451)	0.668 (0.554)
I14676	0.402 (0.128)		0.025 (0.022)	0.270 (0.034)	2.97E-149 (3.09E-189)	0.002 (0.001)	4.74E-04 (1.84E-04)	0.007 (0.027)	0.365 (0.035)	0.632 (0.158)
I14677	0.052 (0.055)	0.025 (0.022)		0.451 (0.110)	8.45E-141 (7.65E-158)	0.228 (0.079)	0.084 (0.014)	0.989 (0.721)	0.492 (0.286)	0.709 (0.663)
I14678	0.190 (0.330)	0.270 (0.034)	0.451 (0.110)		1.47E-145 (2.00E-174)	0.361 (0.429)	0.163 (0.104)	0.604 (0.900)	0.789 (0.393)	0.415 (0.381)
I15940	5.70E-170 (3.23E-195)	2.97E-149 (3.09E-189)	8.45E-141 (7.65E-158)	1.47E-145 (2.00E-174)		2.23E-169 (5.59E-182)	1.37E-128 (3.36E-140)	2.67E-150 (2.19E-173)	6.79E-144 (5.52E-177)	3.93E-63 (3.60E-69)
I15941	0.152 (0.123)	0.002 (0.001)	0.228 (0.079)	0.361 (0.429)	2.23E-169 (5.59E-182)		0.006 (0.043)	0.434 (0.635)	0.657 (0.151)	0.015 (0.166)
I15942	0.005 (0.003)	4.47E-04 (1.84E-04)	0.084 (0.014)	0.163 (0.104)	1.37E-128 (3.36E-140)	0.006 (0.043)		0.635 (0.712)	0.55 (0.024)	0.477 (0.312)
I16164	0.015 (0.078)	0.007 (0.027)	0.989 (0.721)	0.604 (0.900)	2.67E-150 (2.19E-173)	0.434 (0.635)	0.635 (0.712)		0.878 (0.774)	0.392 (0.826)
I16165	0.245 (0.451)	0.365 (0.035)	0.492 (0.286)	0.789 (0.393)	6.79E-144 (5.52E-177)	0.657 (0.151)	0.550 (0.024)	0.878 (0.774)		0.115 (0.136)
I16166	0.668 (0.554)	0.632 (0.158)	0.709 (0.663)	0.415 (0.381)	3.93E-63 (3.605E-69)	0.015 (0.166)	0.477 (0.312)	0.392 (0.826)	0.115 (0.136)	

Only the outlier I15940 remained as not forming a clade with the grouped *Sardinia_Chalcolithic*, therefore only this individual was treated as an outlier for our main analyses and the remaining 9 individuals were grouped together (**Supplementary Table 8**).

All individuals associated with a Bell Beaker cultural context (I14675, I14676, I14677, I14678, I15940, I15941, I15942) were consistent with forming a clade with all remaining individuals from the same chronological period, excluding the outlier I15940. This demonstrates that Sardinia, like Sicily and Iberia, was a location where the expansion of the Bell Beaker complex did not necessarily involve admixture and the spread of Steppe related ancestry[51].

Supplementary Table 8: P-values for the *qpWave* models between the Neolithic Sardinian individuals with some evidence of ancestry heterogeneity, against all other individuals.

	I14675+I14677+I14678+I16164+I16165+I16166
I14676	0.112
I15940	1.97E-213
I15941	0.290
I15942	0.034

Bronze Age Sardinians:

Individual I10365 was not consistent with forming a clade with some of the other Bronze Age Sardinian individuals from our dataset, and individuals I3741, I10554 and 10364 also produced P-values below the threshold of 0.010 (**Supplementary Table 9**).

Supplementary Table 9: P-values for *qpWave* between the Bronze Age Sardinian individuals. (In parentheses we give P-values based on using a more sensitive outgroup set adding in more closely related groups; that is, we add in *Italy_MN_Iceman.SG*, *Italy_Remedello.SG*, *Iberia_Bell_Beaker*, *Iberia_Bell_Beaker_highSteppe*, *Sicily_MN*, *Sicily_EBA*, *Iberia_MN*, *Iberia_Chalcolithic*, *France_Bell_Beaker*, *France_Bell_Beaker_lowSteppe*, and *Anatolia_EBA*.)

	I16161	I10365	I10364	10502	I10552	I10553	I10554	I3642	I3741	I3743	I16168	I16169	I16170	I16183
I16161		0.058 (7.16E-04)	0.855 (0.483)	0.414 (0.744)	0.665 (0.468)	0.688 (0.124)	0.084 (0.030)	0.788 (0.433)	0.510 (0.056)	0.816 (0.291)	0.012 (0.014)	0.424 (0.167)	0.689 (0.264)	0.501 (0.127)
I10365	0.058 (7.16E-04)		0.097 (0.085)	0.079 (0.051)	2.13E-06 (7.83E-07)	2.57E-04 (2.43E-05)	0.029 (1.25E-04)	0.038 (0.232)	0.034 (1.77E-03)	0.010 (0.011)	0.002 (8.35E-03)	0.001 (3.88E-03)	0.013 (1.28E-03)	0.048 (0.197)
I10364	0.855 (0.483)	0.097 (0.085)		0.183 (0.061)	0.074 (0.288)	0.162 (0.015)	0.445 (0.304)	0.552 (0.631)	0.168 (0.189)	0.836 (0.783)	0.008 (0.114)	0.346 (0.147)	0.788 (0.356)	0.149 (0.085)
10502	0.414 (0.744)	0.079 (0.051)	0.183 (0.061)		0.073 (0.033)	0.837 (0.844)	0.431 (0.647)	0.500 (0.517)	0.170 (0.038)	0.370 (0.431)	0.750 (0.517)	0.576 (0.158)	0.711 (0.837)	0.756 (0.251)
I10552	0.665 (0.468)	2.13E-06 (7.83E-07)	0.074 (0.288)	0.073 (0.033)		0.047 (0.069)	0.060 (0.161)	0.612 (0.448)	0.371 (0.013)	0.123 (0.361)	0.024 (0.078)	0.597 (0.587)	0.123 (0.108)	0.102 (0.234)
I10553	0.688 (0.124)	2.57E-04 (2.43E-05)	0.162 (0.015)	0.837 (0.844)	0.047 (0.069)		0.134 (0.036)	0.381 (0.353)	0.199 (0.010)	0.457 (0.708)	0.280 (0.656)	0.414 (0.368)	0.877 (0.757)	0.069 (0.044)
I10554	0.084 (0.030)	0.029 (1.25E-04)	0.445 (0.304)	0.431 (0.647)	0.060 (0.161)	0.134 (0.036)		0.453 (0.495)	0.005 (2.16E-04)	0.043 (0.037)	0.192 (0.300)	0.072 (0.057)	0.067 (0.072)	0.097 (0.270)
I3642	0.788 (0.433)	0.038 (0.232)	0.552 (0.631)	0.500 (0.517)	0.612 (0.448)	0.381 (0.353)	0.453 (0.495)		0.677 (0.124)	0.702 (0.748)	0.916 (0.882)	0.158 (0.054)	0.631 (0.617)	0.541 (0.763)
I3741	0.510 (0.056)	0.034 (1.77E-03)	0.168 (0.189)	0.170 (0.038)	0.371 (0.013)	0.199 (0.010)	0.005 (2.16E-04)	0.677 (0.124)		0.229 (0.078)	0.005 (9.66E-04)	0.054 (0.024)	0.450 (0.316)	0.128 (0.039)
I3743	0.816 (0.291)	0.010 (0.011)	0.836 (0.783)	0.370 (0.431)	0.123 (0.361)	0.457 (0.708)	0.043 (0.037)	0.702 (0.748)	0.229 (0.078)		0.120 (0.141)	0.702 (0.755)	0.742 (0.763)	0.756 (0.577)
I16168	0.012 (0.014)	0.002 (8.35E-03)	0.008 (0.114)	0.750 (0.517)	0.024 (0.078)	0.280 (0.656)	0.192 (0.300)	0.916 (0.882)	0.005 (9.66E-04)	0.120 (0.141)		0.176 (0.526)	0.271 (0.381)	0.614 (0.501)
I16169	0.424 (0.167)	0.001 (3.88E-03)	0.346 (0.147)	0.576 (0.158)	0.597 (0.587)	0.414 (0.368)	0.072 (0.057)	0.158 (0.054)	0.054 (0.024)	0.702 (0.755)	0.176 (0.526)		0.409 (0.389)	0.868 (0.773)
I16170	0.689 (0.264)	0.013 (1.28E-03)	0.788 (0.356)	0.711 (0.837)	0.123 (0.108)	0.877 (0.757)	0.067 (0.072)	0.631 (0.617)	0.450 (0.316)	0.742 (0.763)	0.271 (0.381)	0.409 (0.389)		0.956 (0.922)
I16183	0.501 (0.127)	0.048 (0.197)	0.149 (0.085)	0.756 (0.251)	0.102 (0.234)	0.069 (0.044)	0.097 (0.270)	0.541 (0.763)	0.128 (0.039)	0.756 (0.577)	0.614 (0.501)	0.868 (0.773)	0.956 (0.922)	

We further tested the four individuals that were involved in these p<0.01 outcomes against the pool of Bronze Age individuals that were all mutually consistent with forming a clade. Only individual I10365 was not consistent with forming a clade with the grouped individuals at p<0.01. We therefore treated this individual as outlier in subsequent analysis (**Supplementary Table 10**).

Supplementary Table 10: P-values for the *qpWave* models between the Bronze Age Sardinian individuals with some evidence of ancestry heterogeneity, against all other individuals.

	I16161+I16168+I16169+I16170+I16183+I3642+I3743 +I10502+I10552+I10553
I10365	2.39E-05
I10364	0.368
I10554	0.018
I3741	0.119

Iron Age Sardinians:

The two Iron Age individuals from Sardinia (I10366: 391-209 calBCE and I16163: 762-434 calBCE) were not consistent with forming a clade with each other (**Supplementary Table 11**) or any of the individuals from the Neolithic, Chalcolithic, or Bronze Age Sardinian groups. However, the former was consistent with forming a clade with one of the two individuals from Late Antiquity that was modeled with Iranian-related ancestry (I12220, p=0.146), and the latter with *Sardinia_EarlyMedieval* who had Steppe ancestry (I12221, p=0.258) (see **Fig. 2** or **Supplementary Fig. 1**). This suggests arrival in Sardinia of new ancestry types at least by the Iron Age, potentially related to the period of Phoenician or Greek settlement. We analyzed I10366 and 16163 separately.

Supplementary Table 11: P-values for the *qpWave* models between the Iron Age Sardinians. (In parentheses we give P-values based on using a more sensitive outgroup set adding in more closely related groups; that is, we add *Sardinia_Neolithic*, *Sardinia_Chalcolithic15940*, *Sardinia_Chalcolithic*, *Sardinia_BA* and *Sardinia_BA10365*.)

	I10366	**I16163**
I10366		8.48E-04 (4.31E-05)
I16163	8.48E-04 (4.31E-05)	

Late Antiquity Sardinians:

The two individuals from this period were consistent with forming a clade based on *qpWave* (**Supplementary Table 12**), and so were analyzed as a group.

Supplementary Table 12: P-values for the *qpWave* models between the Late Antiquity Sardinians. (In parentheses we give P-values based on using a more sensitive outgroup set adding in more closely related groups; that is, we add *Sardinia_Neolithic*, *Sardinia_Chalcolithic15940*, *Sardinia_Chalcolithic*, *Sardinia_BA*, *Sardinia_BA10365*, *Sardinia_IA16163* and *Sardinia_IA10366*.)

	I12220	**I12223**
I12220		0.723 (0.559)
I12223	0.723 (0.559)	

Early Medieval Sardinian (I12221):

The most recent individual in our dataset (892-990 calCE) was consistent with forming a clade with the two Late Antiquity Sardinians, and 6 other individuals from the other ancient Sardinian groups (**Supplementary Table 13**). However, these results must be viewed with caution because of the low coverage of some of the individuals involved leading to small numbers of SNPs covered in both individuals being compared (the test individual, I12221, has 78437 SNP covered at least once) (**Supplementary Data 1**). Similar limitations due to small SNP counts are reflected in **Fig. 3** of the main manuscript for the low-coverage *Sicily_Chalcolithic4936* (23600 SNPs) and *Sicily_Chalcolithic* (40252 SNPs) individuals, whose evidence of forming a clade with the majority of the other analyzed individuals plausibly just reflects limited data and thus limited statistical resolution.

Supplementary Table 13: P-values for the significant *qpWave* models between the Early Medieval Sardinian individual and other ancient Sardinians.

	Sardinia_ LateAntiquity I12220	**Sardinia_ LateAntiquity I12223**	**Sardinia_BA I3642**	**Sardinia_BA I10502**	**Sardinia_IA I16163**	**Sardinia_ Neolithic I15943**	**Sardinia_ Neolithic I15945**	**Sardinia_ Chalcolithic I16166**
I12221	0.733	0.970	0.748	0.037	0.277	0.416	0.069	0.062

We finally analyzed modern Sardinians (*Sardinian*) and found that they were not consistent with forming a clade with any of the individuals from the main Neolithic, Chalcolithic, and Bronze Age Sardinian clusters. However, they were consistent with forming a clade with *Sardinia_BA10365* (p=0.050) and *Sardinia_IA16163* (p=0.058) (**Fig. 3, Supplementary Fig. 4**). Thus, modern Sardinian individuals cannot have been direct descendants without admixture of the main populations of which most of the Neolithic, Chalcolithic, and Bronze Age individuals analyzed in our study were a part.

3 - *qpAdm* modeling

We used *qpAdm* (https://github.com/DReichLab)[48] to estimate proportions of ancestry in various test populations using as surrogates both very distantly related populations ("distal modeling") and closely related populations ("proximal modeling").

qpAdm works by computing all possible statistics of the form $f_4(Left_i,\ Left_j;\ Right_k,\ Right_l)$ relating a *Test* population and a set of groups proposed to be clades with the sources of ancestry in the *Test* population (together, these constitute the "Left" populations in *qpAdm*), and a set of outgroups chosen to be differentially related to the "Left" populations ("Right" populations). The method produces a P-value for the fit of the model, as well as admixture proportions[48,52].

We used the following 9 populations/individuals as our base "Right" set (or "B9"):

Mbuti.DG, Ust_Ishim, CHG, EHG, ElMiron, Vestonice16, MA1, Israel_Natufian, Jordan_PPNB

For distal modeling, following the strategy of [53], we began by investigating the ancestry of each *Test* individual/population as derived from a set of five geographically and chronologically distal populations in order to identify and quantify the influence of five ancestries known to have contributed to Europeans. We used a pool of *Anatolia_Neolithic* individuals to represent the ancestry brought into Europe by western Anatolian farmers around 8500 years ago. We represented *WHG* (Western European hunter-gatherers) by 7 individuals from Spain (*Chan.SG*, *Canes.SG* and *I0585*), Hungary (I1507 and I4971), Luxembourg (*Loschbour.DG*), and Switzerland (*Bichon.SG*). We used *Yamnaya_Samara* individuals as a proxy for the ancestry brought into Europe by Steppe pastoralist groups after around 3000 BCE, and *Iran_Ganj_Dareh_Neolithic* as a proxy for the Western Zagros herder-related ancestry (Iranian-related ancestry) that has been documented to have contributed to Middle to Late Bronze Age Aegean populations by at least ~2000 BCE[53]. Lastly we used *Morocco_LN* as a proxy for North African ancestry.

In our distal modeling, we tried 2, 3, 4, and 5-way models for the possible combinations of these 5 sources. When more than one model from the most parsimonious rank fit we used a "model competition" approach, moving the other potential source population(s) into the "Right" set of populations[52,54]. In a few exceptional situations where the models with *Morocco_LN* produced very large standard errors or did not produce valid models, possibly due to the presence of European admixture in Morocco_LN[53] we tried replacing it with the temporally preceding sample *Morocco_EN*.

To increase statistical resolution, we set a threshold of 100,000 SNPs when modeling with single individuals. All groups of individuals have coverage of at least one individual on >100,000 SNPs so we did not apply any such threshold to groups.

3.1 - Results of Distal Modeling

We ran *qpAdm* with the groupings from the previous section (**Supplementary Table 14**). In the Balearic Islands, we observe a significant decrease in *Yamnaya_Samara*-related ancestry from the Early to the Late Bronze Age individuals (38.1 ± 4.4% to 20.8 ± 3.6%), along with a concomitant increase in *Anatolia_Neolithic*-related ancestry. We do not need to model any Iranian-related ancestry to fit the Balearic individuals.

Supplementary Table 14: Admixture proportions for the most parsimonious *qpAdm* models of the populations/individuals from this study fit as derived from groups related to five sources - *Anatolia_Neolithic* (1), *WHG* (2), *Iran_Ganj_Dareh_Neolithic* (3), Y*amnaya_Samara* (4), and *Morocco_LN* (5). Asterisks identify models where *Morocco_EN* was used as (5). "B9" outgroup set. These results are plotted in **Fig. 4b**.

Test	P-value	Admixture Sources and Proportions					Standard Error				
		1	2	3	4	5	SE1	SE2	SE3	SE4	SE5
Mallorca_EBA	0.623	0.436	0.183	-	0.381	-	0.035	0.033	-	0.044	-
Formentera_MBA	0.399	0.646	0.150	-	0.204	-	0.029	0.024	-	0.034	-
Menorca_LBA	0.118	0.612	0.180	-	0.208	-	0.028	0.027	-	0.036	-
Ibiza_Phoenician	0.468	-	-	-	0.108	0.892	-	-	-	0.027	0.027
Balearic (modern)	0.135	0.261	0.074	0.099	0.259	0.306	0.063	0.020	0.049	0.022	0.110
Sardinia_Neolithic	0.028	0.860	0.140	-	-	-	0.018	0.018	-	-	-
Sardinia_Chalcolithic	0.699	0.854	0.146	-	-	-	0.010	0.010	-	-	-
*Sardinia_Chalcolithic15940**	0.321	0.227	-	-	-	0.773*	0.024	-	-	-	0.024*
Sardinia_BA	0.380	0.829	0.171	-	-	-	0.009	0.009	-	-	-
Sardinia_BA10365	0.120	0.856	0.144	-	-	-	0.022	0.022	-	-	-
Sardinia_IA16163	0.208	0.715	0.061	-	0.225	-	0.030	0.027	-	0.036	-
Sardinia_IA10366	0.070	0.738	0.135	0.127	-	-	0.038	0.022	0.035	-	-
Sardinia_LateAntiquity	0.119	0.656	0.051	0.293	-	-	0.046	0.025	0.041	-	-
Sardinian.DG (modern)	0.967	0.646	0.127	0.227	-	-	0.023	0.013	0.021	-	-
Sardinian (modern)*	0.366	0.625	0.097	0.139	0.106	0.034*	0.014	0.010	0.021	0.017	0.013
Sicily_MN	0.338	0.895	0.105	-	-	-	0.012	0.012	-	-	-
Sicily_EBA (fit 1)	0.087	0.791	0.116	0.093	-	-	0.026	0.014	0.023	-	-
Sicily_EBA (fit 2)	0.219	0.830	0.071	-	0.099	-	0.019	0.017	-	0.022	-
Sicily_EBA3123 (fit 1)	0.068	0.721	0.163	0.115	-	-	0.039	0.021	0.034	-	-
Sicily_EBA3123 (fit 2)	0.666	0.756	0.103	-	0.141	-	0.029	0.024	-	0.034	-
Sicily_EBA11443	0.760	0.506	0.103	-	0.390	-	0.030	0.027	-	0.035	-
Sicily_EBA8561	0.070	0.611	0.168	-	0.221	-	0.029	0.026	-	0.036	-
Sicily_EBA3124	0.379	0.788	0.077	-	0.135	-	0.028	0.027	-	0.034	-
Sicily_MBA	0.060	0.775	0.067	0.157	-	-	0.028	0.016	0.026	-	-
Sicily_LBA	0.648	0.815	0.059	-	0.127	-	0.018	0.016	-	0.021	-
Sicilian (modern)	0.522	0.232	-	0.199	0.100	0.469	0.042	-	0.014	0.026	0.056

Note: For the analysis of the present-day Sardinians, we not only analyzed the 4 individuals with shotgun data (*Sardinian.DG*) but also 27 individuals with genotyping data on the Human Origins SNP array (*Sardinian*) where we have data at about half the positions but many more samples. For both the *Sardinian.D*G and the *Sardinian* analyzes, the models only fit with *Iran_Ganj_Dareh_Neolithic* included as a source although with the larger number of samples that is available for the genotyping data we also have resolution to detect additional ancestries.

We tried to model the Phoenician individual from [50] using the same 5 distal sources and obtained the simplest valid models for *Morocco_LN* (89.1 ± 2.7%) and *Yamnaya_Samara* (10.9 ± 2.7%)

(p=0.713) and for *Morocco_LN* (93.4 ± 1.9%) and *WHG* (6.6 ± 1.9%) (p=0.713). However, after model competition only the first model remained valid (p=0.468).

Modern Balearic islanders (HG01613, HG01615, HG01625, and HG01626) fit a 5-way model (p=0.135) with 26.1 ± 6.3% *Anatolia_Neolithic*, 7.4 ± 2.0% *WHG*, 9.9 ± 4.9% *Iran_Ganj_Dareh_Neolithic*, 25.9 ± 2.2% *Yamnaya_Samara*, and 30.6 ± 11.0% *Morocco_LN* (**Supplementary Table 14**).

In Neolithic Sardinia, the cluster of three individuals is consistent with a simple mixture of *Anatolia_Neolithic* and *WHG* ancestries (the estimated proportion of *Anatolia_Neolithic* is 86.0 ± 1.8%) (**Supplementary Table 14**). Of all possible 1, 2, 3, 4, and 5-way models tested this was the only valid model with a P-value above the 0.01 threshold albeit a marginal fit (p=0.028).

Modern Sardinians harbor ancestry that is more closely related to the main *Sardinia_Neolithic* cluster than to *Anatolia_Neolithic,* as when we add modern Sardinians (*Sardinian*) to the outgroup set in distal *qpAdm* modeling of *Sardinia_Neolithic*, we reject the model (p=1.54E-4). This is consistent with some degree of continuity between Neolithic and modern Sardinians.

The *Sardinia_Chalcolithic* group can be modeled similarly to *Sardinia_Neolithic*, as 85.4 ± 1.0% *Anatolia_Neolithic*, and 14.6 ± 1.0% *WHG (*p=0.699) (**Supplementary Table 14**).

A striking finding related to the Chalcolithic outlier individual I15940, who in PCA and ADMIXTURE (**Fig. 2**) shows clear evidence of North African ancestry, is that he produced no valid models using *Morocco_LN* as the source of such ancestry (all models with p<0.05), but a good fit when we use *Morocco_EN* instead, and exclude *WHG*, with 22.7 ± 2.4% *Anatolia_Neolithic* and 77.3 ± 2.4% *Morocco_EN* (p=0.321). This individual from Anghelu Ruju who is dated to 2345-2146 calBCE provides the earliest ancient DNA evidence in Sardinia (after its Neolithic peopling) for ancestry not derived from a mixture of Anatolian farmers and *WHG*. Specifically, this finding documents gene flow from North Africa into Sardinia far earlier than the average admixture date of ~630 CE inferred by Hellenthal and colleagues[56] through the analysis of present-day Sardinians suggests that this earlier gene flow is unlikely to be the primary source of North African-related ancestry in Sardinia. An approximately contemporary example of North African-related gene flow into southern Europe millennia before the time of the Phoenicians has been documented in Iberia[57], where individual I4246 from Camino de las Yeseras dating to 2473-2030 calBCE had large proportions of North African-related ancestry and the same typically North African uniparental markers as I15940 (mtDNA M1a1b1 and Y chromosome E1b1b1a). A second, later, individual (I7162) from Loma del Puerco dating to 1932-1697 calBCE also harbored a substantial fraction of North African-related ancestry. These three clear examples of North African gene flow into southern Europe from the 3rd through the 2nd millennia BCE provide clear evidence showing that the cultural exchanges across the Mediterranean in this time were also accompanied by movements of people in large enough numbers to be detected in the ancient DNA available from this time from western Mediterranean Europe. To quantify this, we counted the number of individuals in our analysis dataset from the period in question (point estimates 5000-3000 years ago) from Mediterranean Europe (which we define as all individuals from the present countries of Italy, Greece, Croatia, France, Spain and France) (using all the individuals listed in the union of **Supplementary Data** 1 and 3). This number is 191, and thus we observe a fairly substantial proportion of analyzed individuals (3 of 191, or 1.6%) that harbor North African ancestry likely due to migration within the last few generations prior to the time we sampled them. A caveat is that the burials we analyzed are unlikely to be statistically representative of the entire population of Mediterranean Europe in this period--both with respect to geographic distribution, and with respect to their social status--and so the true proportion of individuals affected by recent North African admixture across the region is likely to be different.

We were able to model the Bronze Age Sardinians in a similar way as the two previous main groups, here as 82.9 ± 0.9% *Anatolia_Neolithic* and 17.1 ± 0.9% *WHG* (p=0.380) (**Supplementary Table 14**). *qpAdm* provided no insights as to why I10365 was an outlier in *qpWave* analysis, as *Sardinia_BA10365* also produced a valid P-value for this 2-way model (p=0.120), with 85.6 ± 2.2% *Anatolia_Neolithic* and 14.4 ± 2.2% *WHG*.

The Sardinian Iron Age individual I16163 fit a model with *Anatolia_Neolithic*, *WHG*, and *Yamnaya_Samara* (p=0.208), demonstrating the presence of non-Anatolian Neolithic or *WHG*-related ancestry in Iron Age Sardinia. However, Sardinian Iron Age individual I10366 can only fit if we model Iranian-related ancestry (p=0.070; the fit is p=0.0008 for the 2-way model that works for Neolithic, Chalcolithic, and Bronze Age individuals, and p=0.009 for the 3-way model that uses *Yamnaya_Samara* instead of *Iran_Ganj_Dareh_Neolithic* as a source) (**Supplementary Table 14**). This evidence of Steppe and Iranian-related ancestries is the earliest genome-wide ancient DNA evidence for these ancestries in Sardinia. The radiocarbon date of 762-434 calBCE for I16163, which is during the period of Phoenician or Greek influence in Sardinia makes these cultures a plausible source for these ancestries. This scenario is also supported by previously reported mitochondrial ancient DNA evidence that documents Phoenician-associated mitochondrial DNA haplogroups in Sardinia[55]. The addition of modern Sardinians (*Sardinian*) to the “Right” outgroup set does not make the distal models fail, providing no evidence that either *Sardinia_IA10366* (p=0.062) or *Sardinia_IA16163* (p=0.083) contributed to modern Sardinians.

For the Sardinian Late Antiquity individuals (~200-700 CE) we found a working 3-way model with *Anatolia_Neolithic* (65.6 ± 4.6%), *WHG* (5.1 ± 2.5%) and *Iran_Ganj_Dareh_Neolithic* (29.3 ± 4.1%) that yields a P-value of 0.119, thus confirming the presence of Iranian-related ancestry in Sardinia already documented in the Iron Age individual (**Supplementary Table 14**).

Our dataset of 4 modern Sardinians (*Sardinian.DG*) can only be modeled (p=0.967) with additional *Iran_Ganj_Dareh_Neolithic*-related ancestry (the estimated proportion of *Anatolia_Neolithic* is 64.6 ± 2.3% and the estimated proportion of *Iran_Ganj_Dareh_Neolithic* is 22.7% ± 2.1%) (**Supplementary Table 14**). This is a surprising result in light of the literature, which has not previously inferred Iranian-related ancestry in present-day Sardinians, and hence we repeated the analysis using a set of 27 Sardinians genotyped on the Affymetrix Human Origins SNP array (where we have data at only about half the SNPs but where the larger sample size compensates in statistical power) (*Sardinian*)[49]. In this analysis, the same 3-way mixture model did not fit (p=3.40E-05), but the 4-way model with *Anatolia_Neolithic*, *WHG*, *Yamnaya_Samara*, and *Morocco_LN* did (p=0.536) (**Supplementary Table 15**). To investigate if we could detect the Iranian-related ancestry seen in *Sardinian.DG* we added *Iran_Ganj_Dareh_Neolithic* to the "Right" of the working 4-way model for Sardinian and the model failed (p=0.005) suggesting the presence of some level of Iranian-related ancestry. As seen in **Supplementary Table 15**, the inclusion of Neolithic Iranians for a full 5-way model provides a model with a P-value of 0.516, but with the standard errors larger than the ancestry proportion estimates for *Iran_Ganj_Dareh_Neolithic* and *Morocco_LN*. Considering that when *Morocco_LN* is moved from the sources into the "Right" the 4-way model produced is not valid (p=3.83E-11), we used *Morocco_EN* in order to reduce the standard errors from 12.8 to 1.3%. The full 5-way model with *Morocco_EN* provides a good fit (p=0.366) with errors no higher than 2.1% (**Supplementary Table 15**). Although this finding of North African-related ancestry in modern Sardinians could reflect admixture events dating as far back as the Chalcolithic (as suggested by the *Sardinia_Chalcolithic15940* outlier with North African-related ancestry), we found no such ancestry in any of the ancient Neolithic, Chalcolithic, or Bronze Age Sardinian individuals that formed the main clusters. A plausible scenario is that this finding may be related to the observation of North African admixture in the ancestry of present-day Sardinians by Hellenthal and colleagues which was inferred to have an average admixture date of ~630 CE[56], as well as to findings of sub-Saharan African admixture which could have been mediated by North African admixture into Sardinia[58-60].

Supplementary Table 15: Distal *qpAdm* results for *Sardinian.DG* and *Sardinian* using *Anatolia_Neolithic* (1), *WHG* (2), *Iran_Ganj_Dareh_Neolithic* (3), *Yamnaya_Samara* (4), and Morocco_LN (5) as sources. Asterisks identify models where *Morocco_EN* was used as (5).

Test	Right	P-value	Admixture Sources and Proportions					Standard Error				
			1	2	3	4	5	SE1	SE2	SE3	SE4	SE5
Sardinian.DG	B9	0.967	0.646	0.127	0.227	-	-	0.023	0.013	0.021	-	-
Sardinian	B9	3.40E-05	0.649	0.134	0.217	-	-	0.019	0.010	0.018	-	-
Sardinian	B9	0.536	0.551	0.055	-	0.189	0.205	0.059	0.022	-	0.026	0.060
Sardinian	B9+Iran_Ganj_Dareh_Neolithic	0.005	0.348	0.047	-	0.154	0.451	0.042	0.009	-	0.012	0.045
Sardinian	B9	0.516	0.573	0.079	0.089	0.139	0.120	0.062	0.038	0.112	0.067	0.128
Sardinian	B9+Morocco_EN	1.30E-07	0.655	0.096	0.164	0.084	-	0.013	0.010	0.018	0.016	-
Sardinian	B9+Morocco_LN	3.83E-11	0.677	0.088	0.150	0.085	-	0.013	0.010	0.018	0.017	-
Sardinian	**B9**	**0.366**	**0.625**	**0.097**	**0.139**	**0.106**	**0.034***	**0.014**	**0.010**	**0.021**	**0.017**	**0.013***

In Sicily, the Middle Neolithic individuals can be modeled as 2-way mixtures of *Anatolia_Neolithic* (89.5 ± 1.2%) and *WHG* (10.5 ± 1.2%) (p=0.338) (**Supplementary Table 14**). By the Early Bronze Age, however, we detect the presence of an additional ancestry type, but our model was unable to distinguish between Yamnaya or Iranian-related ancestries, in both *Sicily_EBA* (p=0.219 and 0.087, respectively) and *Sicily_EBA3123* (p=0.666 and 0.068, respectively), even with model competition. In **Supplementary Table 14** and **Fig. 4** we present both models, but make an argument that the model for *Yamnaya_Samara* is the most likely one in our proximal modeling analysis below. The outliers *Sicily_EBA11443*, *Sicily_EBA8561*, and *Sicily_EBA3124* possessed large proportions of *Yamnaya_Samara*-related ancestry (39.0 ± 3.5, 22.1 ± 3.6%, and 13.5 ± 3.4% respectively), showing this ancestry was already in Sicily at least in outlier individuals by this time.

The two Middle Bronze Age Sicilian individuals can only be fit parsimoniously with the model requiring Iranian-related ancestry (15.7 ± 2.6%, p=0.060) while the model that used only *Yamnaya_Samara* ancestry as a source does not fit (p=2.21E-04) (**Supplementary Table 14**).

In Late Bronze Age Sicily at the site of Marcita, our *qpAdm* models required only the presence of *Anatolia_Neolithic* (81.5 ± 1.8%), *WHG* (5.9 ± 1.6%), and *Yamnaya_Samara* (12.7 ± 2.1%).

We were able to model modern Sicilians with a 4-way model that included 23.2 ± 4.2% *Anatolia_Neolithic*, 19.9 ± 1.4% *Yamnaya_Samara*, 10.0 ± 2.6% *Iran_Ganj_Dareh_Neolithic*, and 46.9 ± 5.6% *Morocco_LN* (p=0.522) (**Supplementary Table 14**).

In **Supplementary Table 16** (and **Fig. 4a**) we present these analyzes on a by-individual basis. In what follows we selectively highlight some notable features of these analyzes where they reveal patterns that are not clearly evident in the grouped analysis.

For *Sardinia_BA*, individual I10554 produced no valid models after model competition for p>0.05. The most parsimonious fitting model for p>0.01 was the model with 75.2 ± 3.6% *Anatolia_Neolithic*, 17.0 ± 2.1% *WHG*, and 7.7 ± 3.3% *Iran_Ganj_Dareh_Neolithic* (p=0.040). The 2-way model with *Anatolia_Neolithic* and *WHG*, seen in the other Bronze Age individuals, is weakly rejected at p=0.009, suggesting the possibility of a small proportion of Iranian-related ancestry in this individual. We view this inference with caution, however, as this individual lumps with the other *Sardinia_BA* individuals in *qpWave*, so any analysis of this individual alone is compromised by concerns about multiple hypothesis testing.

For the *Sicily_EBA* cluster, 3 out of 4 individuals produced valid 2-way models with *Anatolia_Neolithic* and *WHG*, while I11442 fits a single 3-way model (p=0.297) with 72.5 ± 3.9% *Anatolia_Neolithic*, 11.0 ± 2.3% *WHG*, and 16.5 ± 3.5% *Iran_Ganj_Dareh_Neolithic* (*qpAdm* weakly rejects the model of only *Yamnaya_Samara*-related ancestry for this individual with p=0.040). We view this signal with caution as the p=0.040 rejection of the model with *Yamnaya_Samara* is only weak, and the *qpWave* clustering lumped this individual with the main *Sicily_EBA* group. Furthermore, the statistic f_4(*Sicily_EBA11442*, *SIcily_EBA3122/7796/7807*; *Iran_Ganj_Dareh_Neolithic*, *Yamnaya_Samara/Anatolia_Neolithic*) always produce non-significant values, with Z between -0.849 and 0.625), providing no evidence that *Iran_Ganj_Dareh_Neolithic* shares significantly more alleles with I11442 than other high-coverage individuals from *Sicily_EBA*. Thus, the weakly significant signal in this by-individual analysis may be an artifact of multiple hypothesis testing. The fact that none of the models for the *Sicily_EBA* individuals analyzed by themselves requires *Yamnaya_Samara* ancestry in order to fit, despite the fact that the analysis of the pool of all samples in **Supplementary Table 14** includes *Yamnaya_Samara*, is also notable. It highlights the statistical power that comes from pooling samples.

Supplementary Table 16: Admixture proportions for the most parsimonious *qpAdm* models of the all ancient individuals from this study fit as derived from groups related to five sources - *Anatolia_Neolithic* (1), *WHG* (2), *Iran_Ganj_Dareh_Neolithic* (3), *Yamnaya_Samara* (4), and *Morocco_LN* (5). Asterisks identify models where *Morocco_EN* was used as (5). “B9” outgroup set. These data are used to produce **Fig. 4a**.

Test	P-value	Admixture Sources and Proportions					Standard Error				
		1	2	3	4	5	SE1	SE2	SE3	SE4	SE5
Mallorca_EBA	0.623	0.436	0.183	-	0.381	-	0.035	0.033	-	0.044	-
Formentera_MBA	0.399	0.646	0.150	-	0.204	-	0.029	0.024	-	0.034	-
Menorca_LBA	0.118	0.612	0.180	-	0.208	-	0.028	0.027	-	0.036	-
Sardinia_Neolithic15943	0.507	0.841	0.159	-	-	-	0.049	0.049	-	-	-
Sardinia_Neolithic15945	0.602	0.930	0.070	-	-	-	0.042	0.042	-	-	-
Sardinia_Neolithic15946	0.045	0.830	0.170	-	-	-	0.030	0.030	-	-	-
Sardinia_Chalcolithic15941	0.867	0.829	0.171	-	-	-	0.027	0.027	-	-	-
Sardinia_Chalcolithic15942	0.584	0.953	0.047	-	-	-	0.027	0.027	-	-	-
Sardinia_Chalcolithic16164	0.302	0.877	0.123	-	-	-	0.027	0.027	-	-	-
Sardinia_Chalcolithic16165	0.644	0.861	0.139	-	-	-	0.027	0.027	-	-	-
Sardinia_Chalcolithic16166	0.360	0.935	0.065	-	-	-	0.047	0.047	-	-	-
*Sardinia_Chalcolithic15940**	0.321	0.227	-	-	-	0.773*	0.024	-	-	-	0.024*
Sardinia_Chalcolithic14675	0.760	0.812	0.188	-	-	-	0.027	0.027	-	-	-
Sardinia_Chalcolithic14676	0.942	0.823	0.177	-	-	-	0.027	0.027	-	-	-
Sardinia_Chalcolithic14677	0.320	0.892	0.108	-	-	-	0.028	0.028	-	-	-
Sardinia_Chalcolithic14678	0.873	0.882	0.118	-	-	-	0.027	0.027	-	-	-
Sardinia_BA16161	0.424	0.863	0.137	-	-	-	0.026	0.026	-	-	-
Sardinia_BA16168	0.902	0.932	0.068	-	-	-	0.003	0.030	-	-	-
Sardinia_BA16169	0.393	0.848	0.152	-	-	-	0.031	0.031	-	-	-
Sardinia_BA16170	0.619	0.813	0.187	-	-	-	0.029	0.029	-	-	-
Sardinia_BA16183	0.743	0.815	0.185	-	-	-	0.044	0.044	-	-	-
Sardinia_BA10364	0.493	0.840	0.160	-	-	-	0.027	0.027	-	-	-
Sardinia_BA10552	0.207	0.840	0.160	-	-	-	0.028	0.028	-	-	-
Sardinia_BA10553	0.288	0.823	0.177	-	-	-	0.026	0.026	-	-	-
Sardinia_BA10554	0.041	0.752	0.17	0.077	-	-	0.036	0.021	0.033	-	-
Sardinia_BA3642	0.200	0.846	0.154	-	-	-	0.052	0.052	-	-	-
Sardinia_BA3741	0.171	0.843	0.157	-	-	-	0.028	0.028	-	-	-
Sardinia_BA3743	0.679	0.862	0.138	-	-	-	0.028	0.028	-	-	-
Sardinia_BA10365	0.120	0.856	0.144	-	-	-	0.022	0.022	-	-	-
Sardinia_IA10366	0.070	0.738	0.135	0.127	-	-	0.038	0.022	0.035	-	-
Sardinia_IA16163	0.208	0.715	0.061	-	0.225	-	0.030	0.027	-	0.036	-
Sardinia_LateAntiquity12220	0.070	0.704	-	0.296	-	-	0.045	-	0.045	-	-
Sicily_MN4062	0.338	0.895	0.105	-	-	-	0.012	0.012	-	-	-
Sicily_MN4063	0.468	0.929	0.071	-	-	-	0.028	0.028	-	-	-
Sicily_MN4064	0.706	0.949	0.051	-	-	-	0.027	0.027	-	-	-
Sicily_MN4065	0.455	0.930	0.070	-	-	-	0.026	0.026	-	-	-
Sicily_EBA11442	0.297	0.725	0.110	0.165	-	-	0.039	0.023	0.035	-	-
Sicily_EBA3122	0.1936	0.863	0.137	-	-	-	0.022	0.022	-	-	-
Sicily_EBA7796	0.681	0.925	0.075	-	-	-	0.030	0.030	-	-	-
Sicily_EBA7807	0.488	0.903	0.097	-	-	-	0.030	0.030	-	-	-
Sicily_EBA3123 (fit 1)	0.068	0.721	0.163	0.115	-	-	0.039	0.021	0.034	-	-
Sicily_EBA3123 (fit 2)	0.666	0.756	0.103	-	0.141	-	0.029	0.024	-	0.034	-
Sicily_EBA11443	0.760	0.506	0.103	-	0.390	-	0.030	0.027	-	0.035	-
Sicily_EBA8561	0.070	0.611	0.168	-	0.221	-	0.029	0.026	-	0.036	-
Sicily_EBA3124	0.379	0.788	0.077	-	0.135	-	0.028	0.027	-	0.034	-
Sicily_MBA3125	0.433	0.772	0.053	0.175	-	-	0.037	0.022	0.034	-	-
Sicily_MBA4109	0.217	0.752	0.088	0.160	-	-	0.040	0.023	0.036	-	-
Sicily_LBA10372	0.176	0.873	0.127	-	-	-	0.028	0.028	-	-	-
Sicily_LBA3876	0.121	0.811	-	-	0.189	-	0.029	-	-	0.029	-
Sicily_LBA3878	0.118	0.828	-	-	0.172	-	0.026	-	-	0.026	-
Sicily_LBA_son.I3878_10373	0.233	0.830	-	-	0.170	-	0.028	-	-	0.028	-

In Late Bronze Age Sicily we identified valid models without *WHG* ancestry for 3 of the 4 individuals analyzed (**Supplementary Table 16**), modeled as *Anatolia_Neolithic* and *Yamnaya_Samara*. On the other hand, the fourth individual, I10372, fit for *Anatolia_Neolithic* and *WHG* (p=0.176). The absence of a statistical signal of *WHG* ancestry in these models does not necessarily imply to a total absence of *WHG*, as both *Anatolia_Neolithic* and *Yamnaya_Samara* carry some *WHG* ancestry themselves in admixed form.

3.2 - Results of Proximal Modeling

The objective of proximal *qpAdm* modeling is to identify possible sources of admixture that are more closely related in geography and time to a *Test* population. As a base "Right" set for the proximal modeling we used the same "Right" populations that were used for the distal modeling, but we now also add in the five sources of the distal modeling (or "B14").

Mbuti.DG, Ust_Ishim, CHG, EHG, ElMiron, Vestonice16, MA1, Israel_Natufian, Jordan_PPNB, Anatolia_Neolithic, WHG, Iran_Ganj_Dareh_Neolithic, Yamnaya_Samara, Morocco_LN

We tested different relevant sets of sources for each region and followed a similar approach as in [52,54]. We first identified all valid models with the base "Right" set, and then we re-evaluated these by adding all the remaining unused sources to the "Right" set of populations ("model competition"). The results presented are after "model competition". We considered models as valid if their fit was p>0.05.

Balearic Bronze Age Proximal Modeling

To investigate the origins and ancestry of the three Balearic Bronze Age individuals we tested the following populations as sources, examining all possible combinations of 1-, 2-, and 3-way models, and highlighting the parsimonious ones:

Iberia_Bell_Beaker, Iberia_Bell_Beaker_highSteppe, Iberia_Chalcolithic, Iberia_Chalcolithic.SG, France_Bell_Beaker, France_Bell_Beaker_lowSteppe, Sardinia_Chalcolithic, Sardinia_Neolithic, Italy_MN_Iceman.SG, Italy_Remedello.SG, Italy_Bell_Beaker, Iberia_MBA.SG, Iberia_BA.SG, Iberia_BA

Mallorca_EBA: After model competition, we found a single valid 1-way model with *Iberia_Bell_Beaker_highSteppe*, a group of outliers from Iberia buried in a Bell Beaker mortuary context who unlike most individuals from this context in that region had high proportions of Steppe ancestry (p=0.413)[51].

<u>*Formentera_MBA*</u>: We added *Mallorca_EBA* as a possible source to test for local continuity, and found several valid 2-way models that require both Steppe and Anatolian farmer-related ancestries (**Supplementary Table 17**). Although *qpWave* results showed that *Formentera_MBA* did not form a clade with *Mallorca_EBA*, it is possible that some of her ancestry is derived from local sources such as Mallorca_EBA as some models of this type fit. Nevertheless, at least 3 models without *Mallorca_EBA* or *Iberia_Bell_Beaker_highSteppe* are also a fit.

Supplementary Table 17: Valid 2-way *qpAdm* models for *Formentera_MBA*.

Admixture Sources		P-value	Mixture Proportion		Standard Error	
A	B		Anc_A	Anc_B	SE_A	SE_B
France_Bell_Beaker	*France_Bell_Beaker_lowSteppe*	0.231	0.299	0.701	0.046	0.046
France_Bell_Beaker	*Sardinia_Chalcolithic*	0.313	0.469	0.531	0.032	0.032
France_Bell_Beaker	*Sardinia_Neolithic*	0.330	0.425	0.575	0.034	0.034
Iberia_Bell_Beaker_highSteppe	*Italy_Bell_Beaker*	0.130	0.578	0.422	0.115	0.115
Iberia_Bell_Beaker_highSteppe	*Sardinia_Neolithic*	0.079	0.745	0.255	0.068	0.068
Italy_Bell_Beaker	*Mallorca_EBA*	0.382	0.376	0.624	0.098	0.098
Italy_MN_Iceman.SG	*Mallorca_EBA*	0.254	0.195	0.805	0.048	0.048
Italy_Remedello.SG	*Mallorca_EBA*	0.260	0.224	0.776	0.056	0.056
Sardinia_Chalcolithic	*Mallorca_EBA*	0.263	0.222	0.778	0.050	0.050
Sardinia_Neolithic	*Mallorca_EBA*	0.649	0.276	0.724	0.050	0.050
France_Bell_Beaker_lowSteppe	*Mallorca_EBA*	0.508	0.388	0.612	0.052	0.052

<u>*Menorca_LBA*</u>: We added *Mallorca_EBA, Formentera_MBA, Sardinia_BA10365* and *Sardinia_BA* as possible sources. The only parsimonious 1-way model was *Formentera_MBA* (p=0.172). We tested for evidence of a putative Talaiotic-Nuragic connection between Sardinia and the Balearic Islands by examining the valid 2-way models for *Menorca_LBA* but found no statistically significant evidence for a Sardinian-specific input, as models with non-Sardinian populations such as *France_Bell_Beaker_lowSteppe*, *Italy_Bell_Beaker* and *Italy_Remedello.SG* also fit, even with Neolithic, Chalcolithic, and Bronze Age Sardinians on the "Right" (**Supplementary Table 18**).

Supplementary Table 18: Valid 2-way *qpAdm* models for *Menorca_LBA*.

Admixture Sources		P-value	Mixture Proportion		Standard Error	
A	B		Anc_A	Anc_B	SE_A	SE_B
Italy_MN_Iceman.SG	*Mallorca_EBA*	0.061	0.087	0.913	0.045	0.045
Italy_Remedello.SG	*Mallorca_EBA*	0.085	0.121	0.879	0.056	0.056
Sardinia_Neolithic	*Mallorca_EBA*	0.511	0.240	0.760	0.059	0.059
Sardinia_Chalcolithic	*Mallorca_EBA*	0.390	0.240	0.076	0.059	0.059
Sardinia_BA	*Mallorca_EBA*	0.277	0.167	0.833	0.051	0.051
Sardinia_BA10365	*Mallorca_EBA*	0.350	0.312	0.688	0.089	0.089
France_Bell_Beaker_lowSteppe	*Mallorca_EBA*	0.151	0.257	0.743	0.092	0.092
Italy_Bell_Beaker	*Mallorca_EBA*	0.088	0.210	0.790	0.096	0.096

<u>*Ibiza_Phoenician*</u>: We investigated if the published Phoenician individual from Ibiza[50] was consistent with inheriting some ancestry from the populations of which the earlier individuals from

the Balearic Islands were a part, so we used the same proximal sources as for *Menorca_LBA* but then added *Menorca_LBA*, *Mycenaean*, *Sardinia_IA10366*, *Sardinia_IA16163*, *Sicily_MBA*, *Morocco_LN*, and *Jordan_EBA*. As the distal modeling showed us that this individual possessed North African-related ancestry, we moved *Morocco_LN* from the "Right" to the "Left" to use it as a chronologically more proximate source of ancestry. Only models with two sources of admixture produced valid results, and all of them required *Morocco_LN* as one of those sources plus another source with Steppe-related ancestry (**Supplementary Table 19**). Even though we used model competition to try to reduce the number of working models by adding the unused sources to the "Right" (including the Bronze Age Balearic individuals), none of the initially working models failed and we do not see a clear favoring of Balearic or non-Balearic sources for the Steppe-related ancestry. Considering that in *qpWave* this individual did not form a clade with the other Balearic individuals, it is possible that these models represent an unsampled group. These results clearly demonstrate a link to North African ancestry in the Phoenician settlement of the Balearic Islands.

Supplementary Table 19: Most parsimonious *qpAdm* models for *Ibiza_Phoenician*.

Admixture Sources		P-value	Mixture Proportion		Standard Error	
A	B		Anc_A	Anc_B	SE_A	SE_B
France_Bell_Beaker	*Morocco_LN*	0.935	0.170	0.830	0.031	0.031
Iberia_BA	*Morocco_LN*	0.410	0.142	0.858	0.036	0.036
Iberia_BA.SG	*Morocco_LN*	0.227	0.126	0.874	0.043	0.043
Iberia_Bell_Beaker_highSteppe	*Morocco_LN*	0.695	0.169	0.831	0.037	0.037
Sardinia_IA16163	*Morocco_LN*	0.731	0.298	0.702	0.070	0.070
Mallorca_EBA	*Morocco_LN*	0.856	0.348	0.652	0.066	0.066
Formentera_MBA	*Morocco_LN*	0.649	0.204	0.796	0.048	0.048
Menorca_LBA	*Morocco_LN*	0.716	0.188	0.812	0.043	0.043

Sicilian Bronze Age Proximal Modeling

<u>Early Bronze Age</u>: We used the following populations as possible sources for *Sicily_EBA*:

Iberia_Bell_Beaker, Iberia_Bell_Beaker_highSteppe, France_Bell_Beaker, France_Bell_Beaker_lowSteppe, Sicily_MN, Mallorca_EBA, Minoan_Lassithi, Anatolia_EBA, Italy_Bell_Beaker, Italy_Remedello.SG, Sardinia_Chalcolithic

Since the great majority of individuals from the *Sicily_EBA* group formed a clade with the individuals from *Sicily_MN* in the *qpWave* analysis, we modeled *Sicily_EBA* as *Sicily_MN* and any combination of the other sources (**Supplementary Table 20**). The valid fits had as a second source of ancestry *Iberia_Bell_Beaker_highSteppe, Italy_Bell_Beaker, or Mallorca_EBA*, which shows some agreement with the Y chromosome evidence of an Iberian affinity of some of the Early Bronze Age Sicilians. These are the most parsimonious models and do not require any source of Iranian-related ancestry to model *Sicily_EBA*, despite such ancestry being part of a valid model in the distal analysis. So, despite the higher rank 3-way models that also provide valid fits with the addition of a source of that ancestry, we favor the models with Steppe-related ancestry because 1) in the distal modeling they consistently gave the highest P-value, and 2) the simplest proximal models require only a source of that ancestry.

Supplementary Table 20: Two and 3-way *qpAdm* models for the Early Bronze Age Sicilians.

Test	**Admixture Sources**			**P-value**	**Mixture Proportion**			**Standard Error**		
	A	**B**	**C**		**Anc_A**	**Anc_B**	**Anc_C**	**SE_A**	**SE_B**	**SE_C**
Sicily_EBA	*Sicily_MN*	*Mallorca_EBA*	-	0.131	0.752	0.248	-	0.045	0.045	-
Sicily_EBA	*Sicily_MN*	*Italy_Bell_Beaker*	-	0.053	0.638	0.362	-	0.081	0.081	-
Sicily_EBA	*Sicily_MN*	*Iberia_Bell_Beaker_highSteppe*	-	0.065	0.849	0.151	-	0.035	0.035	-
Sicily_EBA	*Sicily_MN*	*Minoan_Lassithi*	*Italy_Bell_Beaker*	0.066	0.568	0.047	0.385	0.109	0.066	0.080
Sicily_EBA	*Sicily_MN*	*Anatolia_EBA*	*Sardinia_Chalcolithic*	0.407	0.273	0.225	0.502	0.136	0.042	0.110
Sicily_EBA	*Sicily_MN*	*Mallorca_EBA*	*Anatolia_EBA*	0.106	0.731	0.231	0.039	0.050	0.050	0.042
Sicily_EBA	*Sicily_MN*	*Mallorca_EBA*	*Minoan_Lassithi*	0.134	0.666	0.251	0.083	0.081	0.044	0.063
Sicily_EBA	*Sicily_MN*	*Iberia_Bell_Beaker*	*Anatolia_EBA*	0.074	0.611	0.240	0.149	0.071	0.051	0.040
Sicily_EBA	*Sicily_MN*	*Iberia_Bell_Beaker_highSteppe*	*Minoan_Lassithi*	0.084	0.744	0.164	0.093	0.082	0.034	0.070
Sicily_EBA	*Sicily_MN*	*Iberia_Bell_Beaker_highSteppe*	*Anatolia_EBA*	0.097	0.792	0.131	0.077	0.046	0.036	0.043
Sicily_EBA	*Sicily_MN*	*Iberia_Bell_Beaker*	*Minoan_Lassithi*	0.202	0.345	0.351	0.305	0.119	0.057	0.076
Sicily_EBA8561	*France_Bell_Beaker*	*Italy_Bell_Beaker*	-	0.192	0.183	0.817	-	0.049	0.049	-
Sicily_EBA8561	*France_Bell_Beaker*	*Sicily_MN*	-	0.672	0.438	0.562	-	0.031	0.031	-
Sicily_EBA8561	*Iberia_Bell_Beaker_highSteppe*	*Italy_Bell_Beaker*	-	0.034	0.281	0.719	-	0.139	0.139	-
Sicily_EBA8561	*Iberia_Bell_Beaker_highSteppe*	*Minoan_Lassithi*	-	0.173	0.686	0.314	-	0.043	0.043	-
Sicily_EBA8561	*Mallorca_EBA*	*Italy_Bell_Beaker*	-	0.035	0.297	0.703	-	0.157	0.157	-

Our modeling of the two main outliers, I8561 and I11443, identifies that the latter fit a 1-way model with *Mallorca_EBA* (p=0.245), strengthening the claim of a western connection with Sicily, while I8561 shows 2-way models with sources with Steppe and Anatolian-farmer related ancestries, with only 1 out of 5 models requiring *Sicily_MN* (**Supplementary Table 20**). Both individuals I3123 and I3124 formed a clade with *Italy_Bell_Beaker* (p=0.137 and 0.312, respectively). This result finds support in the *qpWave* analysis where I3123 and I3124 do not form a clade with any of the Middle Neolithic Sicilian individuals (**Fig. 3, Supplementary Fig. 4**) and could be explained by a geographical origin in another region of the Italian peninsula.

Middle Bronze Age: For the Middle Bronze Age Sicilians we further added *Sicily_EBA* and the four outliers from the same period as sources. *Sicily_MBA* did not necessarily require a source of ancestry from Sicily and was well modeled as a mixture of *Anatolia_EBA* or *Minoan_Lassithi* and a Chalcolithic or Bell Beaker-associated population from the north/west, with low proportions of Steppe-related ancestry, or local Sicilians. *Sicily_MBA* therefore shows a clear need for Iranian-related ancestry (**Supplementary Table 21**).

Supplementary Table 21: *qpAdm* models for Middle Bronze Age Sicilians.

Admixture Sources		P-Value	Mixture Proportions		Standard Error	
A	B		Anc_A	Anc_B	SE_A	SE_B
Sardinia_Chalcolithic	*Anatolia_EBA*	0.785	0.527	0.473	0.035	0.035
Sicily_EBA	*Anatolia_EBA*	0.593	0.723	0.277	0.047	0.047
France_Bell_Beaker_lowSteppe	*Minoan_Lassithi*	0.075	0.458	0.542	0.050	0.050
Iberia_Bell_Beaker	*Minoan_Lassithi*	0.136	0.343	0.657	0.034	0.034
Italy_Bell_Beaker	*Minoan_Lassithi*	0.199	0.535	0.465	0.056	0.056
Sicily_EBA	*Minoan_Lassithi*	0.210	0.669	0.331	0.069	0.069
Sicily_EBA3124	*Minoan_Lassithi*	0.216	0.624	0.376	0.083	0.083

Late Bronze Age: For the last Sicilian group we used the same sources as before but now with the addition of *Sicily_MBA*. We find that they require as one of the sources an outlier individual from Early Bronze Age Sicily, and then a source of Iranian-related ancestry as either *Sicily_MBA* or *Minoan_Lassithi* (**Supplementary Table 27**). These results seem to contradict the distal modeling results for *Sicily_LBA*, where only Steppe-related ancestry was required (**Supplementary Table 22**). However, as seen in the by-individual modeling (**Supplementary Table 15**) some of the individuals were modeled without *WHG*, as *Anatolia_Neolithic* and *Yamnaya_Samara*, which could be the result of a strong connection to Aegean populations, perhaps related to *Anatolia_EBA* or *Minoan_Lassithi*, which had a stronger connection to *Anatolia_Neolithic* than central and western European populations, and small proportions of Iranian-related ancestry that were perhaps under the limit of resolution of detection in our distal modeling analysis.

Supplementary Table 22: *qpAdm* models for Late Bronze Age Sicilians.

Admixture Sources		P-Value	Mixture Proportions		Standard Error	
A	B		Anc_A	Anc_B	SE_A	SE_B
Sicily_EBA3124	*Minoan_Lassithi*	0.445	0.789	0.211	0.076	0.076
Sicily_EBA3124	*Sicily_MBA*	0.309	0.523	0.477	0.379	0.379
Sicily_EBA8561	*Sicily_MBA*	0.258	0.204	0.796	0.073	0.073

Sardinian Proximal Modeling

Sardinia_Neolithic: We investigated the origins of the *Sardinia_Neolithic* group with the following Neolithic sources:

Iberia_Chalcolithic, Sicily_MN, Iberia_EN, Iberia_MN, Croatia_Cardial_Neolithic, France_MN, Hungary_EN, LBK_EN, Italy_MN_Iceman.SG, Croatia_Impressa_EN

After model competition only two models remained valid, defining *Sardinia_Neolithic* as 77.2 ± 4.5% *France_MN* and 22.8 ± 4.5% *Croatia_Cardial_Impressa_EN* (p=0.052), or 74.0 ± 5.7% *France_MN* and 26.0 ± 5.7% *Hungary_EN* (p=0.051). While *Sardinia_Neolithic* could in theory be the result of admixture between groups related to these two populations, a more parsimonious source is unsampled intermediate groups such as Early Neolithic populations from the Italian peninsula.

Sardinia_Chalcolithic: We modeled *Sardinia_Chalcolithic* with *Sardinia_Neolithic* and other Middle/Late Neolithic sources:

Iberia_Chalcolithic, Sicily_MN, Iberia_MN, France_MN, Italy_MN_Iceman.SG, Croatia_Sopot_MN, Hungary_MN, Sardinia_Neolithic

The models that worked for Neolithic Sardinians did not provide valid fits for Chalcolithic Sardinians, but the working model after model competition was a 1-way fit with *Sardinia_Neolithic* (p=0.285).

Sardinia_Chalcolithic15940: For the Chalcolithic Sardinian outlier with North African ancestry we used mostly a set of Neolithic sources, but added some Bronze Age populations to serve as proxy for specific ancestries. To be able to model North African ancestry we moved *Morocco_LN* from the "Right" to the "Left" to use it as a source, and also included the Iberian Bell Beaker individual with North African ancestry from Camino de las Yeseras, near Madrid (Iberia_Bell_Beaker_o, I4246)[57].

Iberia_Chalcolithic, Sicily_MN, Iberia_MN, France_MN, Italy_MN_Iceman.SG, Croatia_Sopot_MN, Hungary_MN, Sardinia_Neolithic, Mallorca_EBA, Anatolia_EBA, Morocco_LN, Jordan_EBA, Iberia_Bell_Beaker_o

Even before model competition we found no valid fits for p>0.05. The 1-way model with I4246, however, gave p=0.034, suggesting a clade with this individual at a more permissive P-value threshold. We obtained a single good fit when we replaced *Morocco_LN* by *Morocco_EN* and after model competition were able to model *Sardinia_Chalcolithic15940* as 74.2 ± 5.1% Iberia_Bell_Beaker_o and 25.8 ± 5.1% *Morocco_EN* (p=0.802).

Sardinia_BA: Although the individuals within this group date to a broad temporal range from 2134-1947 calBCE (I3743) to 1116-824 calBCE (I10364), they all form a clade with similar genetic composition. To model them we used sources from the Mediterranean Chalcolithic and Bronze Age, and also added two possibly relevant Middle Neolithic groups from Sicily and Italy:

Iberia_Bell_Beaker, Iberia_Bell_Beaker_highSteppe, Iberia_Chalcolithic, Iberia_Chalcolithic.SG, France_Bell_Beaker, France_Bell_Beaker_lowSteppe, Sicily_MN, Mallorca_EBA, Minoan_Lassithi, Italy_Bell_Beaker, Italy_MN_Iceman.SG, Italy_Remedello.SG, Sardinia_Chalcolithic, Iberia_BA.SG, Iberia_MBA.SG, Iberia_BA

After model competition we found that the 1-way model for *Sardinia_Chalcolithic* was a good fit (p=0.173).

Sardinia_BA10365: For the Bronze Age outlier I10365 (1643-1263 calBCE) we added *Sicily_EBA*, *Sardinia_BA, Anatolia_EBA*, *Jordan_EBA*, and *Mycenaean* as additional possible sources to the ones also tested for the larger *Sardinia_BA* grouping. The results, shown in **Supplementary Table 23**, reveal eight parsimonious 2-way admixture models that always require *Sardinia_BA* or *Sardinia_Chalcolithic* (suggesting some degree of local continuity that agrees with *qpWave*) and either a source of Iranian-related ancestry (*Jordan_EBA* or *Mycenaean*), or Steppe-related ancestry (*France_Bell_Beaker, Italy_Bell_Beaker or Mallorca_EBA*). The inference of eastern ancestry in this individual is different from the finding in the distal modeling where a model of just *Anatolia_Neolithic* and *WHG* passes (p=0.120), although this is in no way a contradiction because the proximal modeling potentially has more statistical power. As documented above, modern Sardinians definitively do have eastern admixture, which may explain why present-day Sardinians are consistent in our *qpWave* analysis with forming a clade *with Sardinia_BA10365* but not with the main *Sardinia_BA* cluster.

Supplementary Table 23: Most parsimonious *qpAdm* models for *Sardinia_BA10365*.

Admixture Sources		P-value	Mixture Proportion		Standard Error	
A	B		Anc_A	Anc_B	SE_A	SE_B
Mallorca_EBA	*Sardinia_Chalcolithic*	0.059	0.417	0.583	0.066	0.066
France_Bell_Beaker	*Sardinia_Chalcolithic*	0.297	0.214	0.786	0.030	0.030
Mallorca_EBA	*Sardinia_BA*	0.101	0.408	0.592	0.072	0.072
Jordan_EBA	*Sardinia_BA*	0.315	0.214	0.786	0.031	0.031
Mycenaean	*Sardinia_BA*	0.368	0.439	0.561	0.059	0.059
France_Bell_Beaker	*Sardinia_BA*	0.628	0.210	0.790	0.031	0.031
Italy_Bell_Beaker	*Sardinia_BA*	0.694	0.568	0.432	0.090	0.090

Sardinia_IA: For the Iron Age individuals we used a set of potential sources from Bell Beaker-associated contexts, the Aegean and Levantine Bronze Age, and some of our ancient Balearic and Sicilian groups:

Iberia_Bell_Beaker, Iberia_Bell_Beaker_highSteppe, France_Bell_Beaker, France_Bell_Beaker_lowSteppe, Mallorca_EBA, Formentera_MBA, Anatolia_EBA, Italy_Bell_Beaker, Italy_Remedello.SG, Jordan_EBA, Sardinia_BA, Sardinia_BA10365, Sardinia_Chalcolithic15940, Minoan_Lassithi, Mycenaean, Iberia_BA.SG, Iberia_MBA.SG, Iberia_BA, Sicily_EBA, Sicily_EBA3123, Sicily_EBA3124, Sicily_MBA

For *Sardinia_IA16163* who showed signs of Steppe-related ancestry in both the PCA and ADMIXTURE (Fig. 2) we were left with two working 1-way fits for *Italy_Bell_Beaker* (p=0.711) and *Formentera_MBA* (p=0.574), which is in agreement with *qpWave*. For *Sardinia_IA10366* we found a few 2-way valid models that in most cases require a source with Steppe-related ancestry, and then a source of Iranian-related ancestry (**Supplementary Table 24**).

Supplementary Table 24: Most parsimonious *qpAdm* models for *Sardinia_IA10366*.

	Admixture Sources		P-value	Mixture Proportion		Standard Error	
	A	B		Anc_A	Anc_B	SE_A	SE_B
Sardinia_IA10366	*Anatolia_EBA*	*Sicily_EBA3123*	0.143	0.231	0.769	0.089	0.089
Sardinia_IA10366	*Mycenaean*	*Sicily_EBA3123*	0.081	0.660	0.340	0.316	0.316
Sardinia_IA10366	*Sardinia_Chalcolithic15940*	*Sicily_EBA3123*	0.120	0.089	0.911	0.034	0.034
Sardinia_IA10366	*Jordan_EBA*	*Sicily_EBA3123*	0.283	0.209	0.791	0.056	0.056
Sardinia_IA10366	*Jordan_EBA*	*Sicily_EBA3124*	0.227	0.212	0.788	0.055	0.055
Sardinia_IA10366	*France_Bell_Beaker_lowSteppe*	*Mycenaean*	0.075	0.125	0.875	0.063	0.063
Sardinia_IA10366	*Iberia_Bell_Beaker*	*Mycenaean*	0.111	0.098	0.902	0.038	0.038
Sardinia_IA10366	*Italy_Remedello.SG*	*Mycenaean*	0.113	0.172	0.828	0.062	0.062
Sardinia_IA10366	*Mallorca_EBA*	*Minoan_Lassithi*	0.227	0.470	0.530	0.046	0.046
Sardinia_IA10366	*Formentera_MBA*	*Minoan_Lassithi*	0.059	0.465	0.535	0.054	0.054

Notably, these models also fit with both *Sardinia_BA* and *Sardinia_BA10365* in the outgroup set, and thus our analysis suggests little if any contribution of Neolithic/Bronze Age Sardinians to these Iron Age Sardinian individuals.

<u>*Sardinia_LateAntiquity*</u>: For the group of 2 *Sardinia_LateAntiquity* individuals we explored the following as possible sources:

Iberia_Bell_Beaker, Iberia_Bell_Beaker_highSteppe, France_Bell_Beaker, France_Bell_Beaker_lowSteppe, Anatolia_IA.SG, Italy_Bell_Beaker, Jordan_EBA, Sardinia_BA, Sardinia_BA10365, Sardinia_Chalcolithic15940, Sardinia_IA16163, Sardinia_IA10366, Minoan_Lassithi, Mycenaean, Iberia_BA.SG, Iberia_MBA.SG, Iberia_BA, Sicily_EBA, Sicily_EBA3124, Sicily_MBA, Sicily_LBA, Bulgaria_IA, Croatia_Early_IA, Ibiza_Phoenician, Germany_Early_Medieval.SG, Hungary_Langobard, Iberia_Iberian, Iberia_Visigoth, Iberia_Roman

After model competition we obtained good fit for the 1-way model with *Ibiza_Phoenician* (p=0.238) (**Supplementary Table 25**). Notably, this fit works with *Sardinia_BA* and *Sardinia_BA10365* among the outgroups, suggesting that there is no evidence of these Late Antiquity individuals inheriting ancestry from Bronze Age Sardinians. Even if 2-way models are considered, from the total of 21 working models for *Sardinia_LateAntiquity*, only one accepts the main Bronze Age Sardinian group as a source, and then with only a modest contribution of 9.4 ± 4.6% (p=0.204).

Supplementary Table 25: Working *qpAdm* models for *Sardinia_LateAntiquity*.

Admixture Sources		P-value	Mixture Proportion		Standard Error	
A	B		Anc_A	Anc_B	SE_A	SE_B
Ibiza_Phoenician	-	0.238	1.000	-	0.000	-
Minoan_Lassithi	*Iberia_Visigoth*	0.058	0.387	0.613	0.057	0.057
Sardinia_IA10366	*Iberia_Visigoth*	0.072	0.829	0.171	0.163	0.163
Jordan_EBA	*Iberia_MBA.SG*	0.095	0.563	0.437	0.045	0.045
Jordan_EBA	*Iberia_BA.SG*	0.102	0.585	0.415	0.043	0.043
Anatolia_IA.SG	*Sicily_EBA*	0.104	0.602	0.398	0.047	0.047
Anatolia_IA.SG	*Sicily_MBA*	0.194	0.543	0.457	0.056	0.056
Sardinia_Chalcolithic15940	*Sardinia_IA10366*	0.128	0.154	0.846	0.046	0.046
Anatolia_IA.SG	*Sardinia_IA10366*	0.641	0.361	0.639	0.075	0.075
Jordan_EBA	*Sardinia_IA10366*	0.227	0.248	0.752	0.086	0.086
Jordan_EBA	*Sardinia_IA16163*	0.051	0.431	0.569	0.063	0.063
Ibiza_Phoenician	*Sardinia_Chalcolithic*	0.227	0.934	0.066	0.041	0.041
Ibiza_Phoenician	*France_Bell_Beaker_lowSteppe*	0.232	0.918	0.082	0.068	0.068
Ibiza_Phoenician	*Mycenaean*	0.286	0.734	0.266	0.345	0.345
Ibiza_Phoenician	*Sardinia_BA*	0.302	0.903	0.097	0.040	0.040
Ibiza_Phoenician	*Sicily_LBA*	0.372	0.871	0.129	0.072	0.072
Ibiza_Phoenician	*Sicily_EBA*	0.393	0.857	0.143	0.058	0.058
Ibiza_Phoenician	*Minoan_Lassithi*	0.410	0.860	0.140	0.061	0.061
Ibiza_Phoenician	*Sardinia_BA10365*	0.415	0.851	0.149	0.071	0.071
Ibiza_Phoenician	*Sicily_MBA*	0.443	0.825	0.175	0.073	0.073
Ibiza_Phoenician	*Sardinia_IA10366*	0.791	0.698	0.302	0.152	0.152

Sardinian: We evaluated the contribution of Neolithic and Bronze Age Sardinians to the genomes of modern Sardinians using a combination of proximal and distal modeling. Knowing that modern Sardinians can be modeled as a 5-way admixture with our full distal model, and that they are the modern European population that is more closely related to Early Neolithic groups, we replaced *Anatolia_Neolithic+WHG* in our modeling by *Sardinia_Neolithic*, *Sardinia_Chalcolithic*, or *Sardinia_BA*. As opposed to the distal model where we used *Morocco_EN* as the source of North African ancestry, here we use *Morocco_LN*, who have an European component similar to that present in ancient Sardinians and therefore should allow a higher resolution distinction of the local ancestry. The results presented in **Supplementary Table 26** give us an estimate of between 56.3 ± 8.1% and 62.2 ± 6.6% of ancient Sardinian ancestry in modern Sardinians. As all three ancient Sardinian groups could be modeled as *Anatolia_Neolithic+WHG*, we investigated if these values could be strictly derived from these ancient Sardinians or if another source of *Anatolia_Neolithic* ancestry was required. For the models involving Neolithic and Chalcolithic Sardinians the estimated proportion of *Anatolia_Neolithic* ancestry required on top of the local ancestry is a negative estimate, and in the case of *Sardinia_Neolithic* has a very large standard error (**Supplementary Table 27**), demonstrating that no extra *Anatolia_Neolithic* ancestry is required to model modern Sardinians. For the model with *Sardinia_BA*, an estimate of 8.0 ± 5.8% of *Anatolia_Neolithic* ancestry is accepted, which could have been brought to the island along with the other ancestries seen in modern Sardinians, as those are estimated with lower proportions than in the other two models.

Supplementary Table 26: Modeling modern Sardinians with distal sources and local ancient Sardinians with *qpAdm*.

Test	P-value	Admixture Sources and Proportions				Standard Error			
		Test (1)	Iran_Ganj_Dareh_Neolithic (2)	Yamnaya_Samara (3)	Morocco_LN (4)	SE1	SE2	SE3	SE4
Sardinia_Neolithic	0.386	0.563	0.117	0.094	0.227	0.081	0.041	0.023	0.099
Sardinia_Chalcolithic	0.969	0.568	0.092	0.113	0.227	0.054	0.028	0.015	0.068
Sardinia_BA	0.524	0.622	0.139	0.069	0.171	0.066	0.032	0.019	0.080

Supplementary Table 27: Evaluating how much *Anatolia_Neolithic* ancestry is required on top of the local ancestry to model modern Sardinians with *qpAdm*.

Test	P-value	Admixture Sources and Proportions					Standard Error				
		Test (1)	Anatolia_Neolithic (2)	Iran_Ganj_Dareh_Neolithic (3)	Yamnaya_Samara (4)	Morocco_LN (5)	SE1	SE2	SE3	SE4	SE5
Sardinia_Neolithic	0.621	0.856	-0.271	0.172	0.045	0.198	0.301	0.243	0.077	0.057	0.133
Sardinia_Chalcolithic	0.913	0.584	-0.019	0.094	0.111	0.230	0.097	0.079	0.029	0.019	0.067
Sardinia_BA	0.631	0.533	0.080	0.123	0.086	0.179	0.085	0.058	0.032	0.021	0.072

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