

Supplemental information

Genomic and dietary discontinuities during the Mesolithic and Neolithic in Sicily

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Supplementary Data S5: Additional Results, Supplementary Data S5: Additional Results

Text 1. Isotope analyses and radiocarbon dating of specimens from *Grotta dell'Uzzo* (M.Ma)

Number of individuals

Before presenting the palaeodietary information obtained through the stable isotope analysis, we will briefly discuss the results from multiple samples assignable to single individuals. The individuals for which more than one sample has been analysed include: UZZ44 to UZZ46 (UZZ4446: R-EVA 1929, 1930 and 1931) and UZZ50 to UZZ53 (UZZ5054: R-EVA 1934, 1935, 2881 and 2882) that have been assigned to single individuals on genetic grounds and originate respectively from a single stratigraphic spit (F-15) or from two contiguous spits (F-19 and F-20) (Data S1.4).

UZZ44 and UZZ45 are teeth with similar isotopic compositions, the differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ being respectively 0.6‰ and 0.4‰, which is entirely possible for a single individual, given that teeth form at slightly differing times. UZZ46 is a sample of mandible with an intermediate isotopic composition in $\delta^{13}\text{C}$ to UZZ44 and UZZ45, and a lower $\delta^{15}\text{N}$ value respectively by 0.8‰ and 1.2‰, which is entirely possible within the same individual given that the teeth reflect diet in childhood and may partly also reflect diet during breastfeeding, which results in higher nitrogen isotope values in the infant relative to its mother. Samples UZZ50-UZZ53 can also be attributed to a single individual (UZZ5054) on isotopic grounds, given that the four teeth sampled differ isotopically by only 0.2‰ in $\delta^{13}\text{C}$ and 0.3‰ in $\delta^{15}\text{N}$, which is respectively the measurement error or just above it. This implies that altogether we have new isotopic data from a total of 19 additional individuals, which can be added to the 15 individuals analysed by Mannino *et al.* (Mannino *et al.*, 2015) and shown in Table S1.

Radiocarbon dating and cultural attribution of analysed specimens

Most of the dated specimens have yielded radiocarbon dates that accord with their expected chronology relative to the dated sequence of Trench F (Mannino *et al.*, 2015) and with their cultural attribution based on the archaeological context of origin, except for UZZ33 and UZZ96 (Table S2).

Of these, we need to discuss in more detail samples originating from topsoil layers (rim) that may have been potentially more susceptible to mixing. The specimens for the four individuals UZZ79 to UZZ82 (R-EVA 1957, 1958, 1959 and 1960) were recovered from the topmost layer of Trench U, which has been dated to the so-called Mesolithic-Transition phase (now Mesolithic II: Castelnovian *sensu lato*) by Mannino *et al.* (Mannino *et al.*, 2015). The previous date obtained for this context was on a vertebral disc fragment of a Delphinidae for which the calibrated and reservoir corrected age range is 8,560-8,060 cal BP. The new dates from the U-rim all overlap with this previously obtained date and with each other, ranging from 8,650-8,520 cal BP to 8,520-8,160 cal BP. This suggests that the top of Trench U accumulated

during the time when the cave was occupied by Late Mesolithic hunter-gatherers, who were producing Late Mesolithic blade-and-trapeze industries (Castelnovian *sensu lato*).

UZZ74 (R-EVA 1953) was recovered from the top of Trench S and could thus also have been subject to mixing. Nevertheless, the radiocarbon date (UZZ74: 7,280-7,160 cal BP) matches the archaeologically-based chronological/cultural attribution and overlaps with a previously published date on specimen S-EVA 2774 (KIA-36038: 7,240-6,850 cal BP), which confirms that this part of the deposit accumulated in the Neolithic phase II (Stentinello culture).

The three skeletal elements for individual UZZ4446 (R-EVA 1929-1931) were recovered from Trench F Spit 15, which according to the division of the stratigraphic sequence for this trench is the uppermost spit associated with the Mesolithic I, phase II (MESO1/2). The calibrated radiocarbon age range for these specimens, which takes into account the correction for the reservoir effect given their mixed terrestrial/marine isotopic compositions, is 8,420-8,130 cal BP (6,480-6,180 cal BCE). This falls within the radiocarbon age range modelled for the so-called Mesolithic-Neolithic transition by Mannino *et al.* (Mannino *et al.*, 2015) and UZZ4446 can thus be assigned on the grounds of its direct date to what is now called the Mesolithic II phase (MESO2, Castelnovian *sensu lato*). As discussed by Mannino *et al.* (Mannino *et al.*, 2015) in relation to some cetacean bones, it is possible that materials moved post-depositionally down the sequence from the layer immediately above (spits 14-11) into spits 15 and 16. It should also be noted, as discussed below, that UZZ4446 has the same isotope composition as the majority of Mesolithic II individuals.

UZZ69 (R-EVA 1948) was recovered from Trench M spit 3, which on the grounds of the stratigraphy of this part of the deposit would assign it to the Neolithic phase II (Stentinello culture). However, the radiocarbon date of this specimen does not support this contextually-based attribution. In fact, the calibrated and reservoir-corrected age range for this individual is 8,540-8,190 cal BP (6,590-6,240 cal BCE) which places it entirely within the chronological period of the Mesolithic II phase (Castelnovian *sensu lato*) established by Mannino *et al.* (Mannino *et al.*, 2015) to date to around 8,770-7,850 cal BP. It should also be noted that this individual has an isotopic composition that is analogous to most of the other humans from this period, who consumed large amounts of marine protein originating from cetacean meat.

UZZ71 (R-EVA 1950) was recovered in Trench M Spit 10, which is the lowermost stratigraphic spit associated with the Neolithic in that part of the deposit. As described above, Trench M includes the Castelnovian *sensu lato* phase (ex Mesolithic-Neolithic transition) in spits 14 to 11, which are followed by the Neolithic phase I (Impressed Ware culture) in spits 10-6. On purely contextual grounds one would assign UZZ71 to the earliest Neolithic and this is confirmed by the radiocarbon age range obtained for this study (MAMS-48212: 7,960-7,790 cal BP), demonstrating that this individual postdates the start of the Neolithic, which at *Grotta dell'Uzzo* is around 8,050 cal BP (Neolithic I in Trench F: ~8,050-7,400 cal BP (Mannino *et al.*, 2015)). The carbon and nitrogen isotope data for UZZ71 (R-EVA 1950: $\delta^{13}\text{C} = -18.9\text{‰}$ and $\delta^{15}\text{N} = 14.5\text{‰}$) are unlike those of any other individual from *Grotta dell'Uzzo* (as discussed below) or of any published individual for late Upper Palaeolithic, Mesolithic or Early Neolithic

from Italy and the central Mediterranean (Mannino and Richards, 2018). Analogous isotopic compositions have been recorded in Mesolithic humans from the Iron Gates, which were strongly dependent on freshwater resources obtained in and around the Danube river (Bonsall, 2007; Borić and Price, 2013). This kind of diet and the genetic evidence indicate that the individual in question subsisted by hunting and gathering and may have not been local to the area of *Grotta dell'Uzzo*, given that having a diet strongly centred on non-marine aquatic resources could not have been possible in the absence of substantial rivers or other freshwater bodies. This foraging diet and the fact that this individual has a mitogenome haplogroup (U5a2) similar to that of the hunter-gatherers from the Iron Gates, demonstrate along with the radiocarbon date and archaeological context that foragers were still present at *Grotta dell'Uzzo* by the time farmers arrived in NW Sicily.

UZZ77 (R-EVA 3523) was recovered in Trench T Spit 13, which on contextual and stratigraphic grounds is of unclear attribution, as it dates either to the Mesolithic II Castelnovian *sensu lato* or to the Neolithic I Impressed Ware cultural layer. The calibrated radiocarbon age range obtained as part of this study (MAMS-48213: 7,430-7,310 cal BP) is more recent than the transition between these two periods, overlapping marginally with the most recent part of the calibrated age range for the earliest Neolithic phase (~8,050-7,400 cal BP) at *Grotta dell'Uzzo* (Mannino et al., 2015).

UZZ61 (R-EVA 3521) originates from the topsoil layer of Trench H and thus cannot safely be assigned to a cultural period on purely stratigraphic grounds. A date previously obtained on a humerus from this superficial deposit (S-EVA 2777), assigned the dated specimen to the early Mesolithic (MAMS 11084: 10,580-10,290 cal BP (Mannino et al., 2015)). The radiocarbon age range obtained for this study (MAMS-48211: 6,830-6,660 cal BP), shows that the topsoil layer of Trench H was a palimpsest with materials from very different periods. Nevertheless, and importantly for this study, the phalanx specimen UZZ61 clearly dates to the Middle Neolithic, when the local culture was the Stentinello/Trichrome/Serra d'Alto. Moreover, its radiocarbon chronology is fully compatible with its Neolithic ancestry (mitogenome haplogroup: K1a2).

UZZ99 (R-EVA 1974) is a cranial fragment recovered in 1976 within stratigraphic unit D of a small tunnel-like feature (*cunicolo*), running along the walls of *Grotta dell'Uzzo* and outside of the named trenches. This area of the cave is not well-understood stratigraphically, so a chronological attribution on purely archaeological grounds is not possible. The radiocarbon age range obtained for our study (MAMS-40714: 6,000-5,900 cal BP) attributes this specimen to the Middle Neolithic, a time when the Stentinello/Trichrome/Serra d'Alto culture was present in NW Sicily.

UZZ57 (R-EVA 2883) is a phalanx recovered close to the cut of burial Uzzo 5 in Trench G, but despite this its radiocarbon date suggests is not associated to the inhumated Mesolithic individual, given that its calibrated age range (MAMS-40727: 4,150-3,970 cal BP) coincides with the Early Bronze Age (EBA) in Sicily. To the best knowledge of the authors, this is the youngest date obtained on materials from *Grotta dell'Uzzo* and radiocarbon dating proof for the occupation of the site at a time when caves in NW Sicily were still used for funerary purposes, within the remit of the Rodi-Tindari-Vallelunga culture that succeeded the Copper

Age Bell Beaker *facies*. It is not known for what purpose *Grotta dell'Uzzo* was used during the Bronze Age, but its occupation during that period is also attested by the typology of some ceramic finds (Tusa, 1999).

The genetically-typed specimens that were not radiocarbon dated are UZZ33 and UZZ96. For these we discuss here briefly their cultural attribution:

- UZZ33 originates from Trench F Spit 4, for which we have a date from UZZ34 (6351±24 BP). This date is also considered valid for UZZ33, which can be safely attributed to the Neolithic Stentinello culture *sensu lato*.
- UZZ96 is associated to 'Burial Uzzo 8' and can be assigned to the Mesolithic I phase II (~11,110-8,500 cal BP (Mannino et al., 2015)), given that all burials at the site are clearly assignable to that period on stratigraphic grounds. Moreover, this is confirmed by the two burials for which we have radiocarbon dating, 'Burial Uzzo 6' and 'Burial Uzzo X', that have respectively radiocarbon age ranges of 10,700-10,430 cal BP (8,750-8,480 cal BCE) and 10,170-9,760 cal BP (8,220-7,810 cal BCE).

Palaeodietary reconstruction

The palaeodietary reconstructions described here benefit from the understanding gained in the detailed isotopic study published on *Grotta dell'Uzzo* by Mannino *et al.* (Mannino et al., 2015). That research included the analysis of eleven Mesolithic humans (MESO1), five Neolithic humans (NEO 1 & 2) and one individual from the Mesolithic Neolithic Transition (MESO2), the results of which are listed in Table S1. Moreover, the previous isotopic study generated a detailed isotopic baseline for the local trophic web, including all the main prey species consumed by the occupants of *Grotta dell'Uzzo*. The previously published isotopic values for the humans and fauna are also displayed below in Fig. S2.

Before proceeding with the description of the newly acquired data and with their full analysis, it should be highlighted that the kind of isotopic data available for *Grotta dell'Uzzo* are currently not available for any other Mediterranean site from the same time period, as reviewed by Mannino & Richards (Mannino and Richards, 2018). The dearth of evidence from other sites for the different time periods covered by the sequence at *Grotta dell'Uzzo* and particularly for the transitional period between the Mesolithic and the Neolithic is due to many possible causes, of which depopulation in Mesolithic times and hiatuses in sedimentary sequences are just two possible explanations (Biagi, 2003; Biagi and Spataro, 2001). Overall, the previous isotopic study on *Grotta dell'Uzzo* has shown that Mesolithic hunter-gatherers and Neolithic agro-pastoralists had diets centered and dominated by the consumption of terrestrial resources (Mannino et al., 2015). A broadening in the resource base is attested zooarchaeologically (Tagliacozzo, 1993) and isotopically (Mannino et al., 2015) during the so-called Mesolithic-Neolithic transition, when cetaceans were an important resource possibly as an adaptation to increased stranding frequency linked to the so-called 8.2-kyr-BP climatic event.

The Early Mesolithic humans (i.e., from Mesolithic I phases I and II (MESO1/1, MESO1/2; N = 2) have mean $\delta^{13}\text{C}$ values of $-19.6\pm 0.2\text{‰}$ and mean $\delta^{15}\text{N}$ values of $11.0\pm 1.8\text{‰}$, which are

similar values to those of the eleven Mesolithic humans analysed by Mannino *et al.* (Mannino *et al.*, 2015) with respective values of $-19.8 \pm 0.7\text{‰}$ and $10.3 \pm 1.1\text{‰}$. The Late Mesolithic individuals (N = 7), which are those dated to or associable with the so-called Mesolithic-Neolithic transition and which is now the Mesolithic II / Castelnovian *sensu lato* phase, have mean $\delta^{13}\text{C}$ values of $-16.6 \pm 1.8\text{‰}$ and $13.0 \pm 1.0\text{‰}$. These mean values are very similar to the isotope data previously published for the only human individual that could be attributed to this phase of occupation, which is a cranial fragment from Trench F Spit 12 (S-EVA 8010) with values of -16.2‰ for $\delta^{13}\text{C}$ and 12.8‰ for $\delta^{15}\text{N}$ and whose diet included 40-49% of marine-based protein (~32% of which may have originated from cetacean meat consumption). The Neolithic individuals (N = 5), which include both Impressed Ware and Stentinello phase humans, have mean $\delta^{13}\text{C}$ values of $-19.7 \pm 0.4\text{‰}$ and mean $\delta^{15}\text{N}$ values of $8.8 \pm 1.2\text{‰}$. These values are not too dissimilar from those for previously-analysed Neolithic individuals (mean $\delta^{13}\text{C} = -19.2 \pm 0.5\text{‰}$ and mean $\delta^{15}\text{N} = 9.6 \pm 1.1\text{‰}$), the main difference being that they are slightly more depleted both in their carbon and nitrogen isotope compositions. It can, thus, be concluded that increasing the sample numbers of humans analysed from *Grotta dell'Uzzo*, the dietary interpretations proposed by Mannino *et al.* (Mannino *et al.*, 2015) are confirmed.

One specimen (UZZ71 / R-EVA 1950) stands out from the rest of the individuals analysed from *Grotta dell'Uzzo* (Francalacci, 1988; Mannino *et al.*, 2015), as well as from any other later Upper Palaeolithic or Mesolithic site in the Central Mediterranean (Mannino and Richards, 2018). The diet of this individual must have included a large proportion of freshwater protein, similarly to what is recorded for bone collagen from the remains of Mesolithic hunter-gatherers at the Balkanic sites of the Iron Gates (Bonsall, 2007; Boric and Price, 2013). In addition, the ancient DNA analyses suggest that this individual, as other Late Mesolithic individuals, carries the genetic ancestry that may originate from the Balkans, which would be compatible with her isotopic composition and may tentatively indicate that this was a Mesolithic 'immigrant' to NW Sicily (Fig. S2).

We have undertaken statistical testing of the isotopic differences between the different groups of humans whose remains were analysed isotopically both here and by Mannino *et al.* (Mannino *et al.*, 2015), using the data listed in Table S1 and Table S3. It was not possible to undertake meaningful statistics between all the different subgroupings (i.e., MESO1/1, MESO1/2, MESO2, NEO1/1, NEO1/2), because the sample numbers for some groups were too small. However, according to ANOVA analyses with multiple t-tests using Tukey's HSD (95% family-wise confidence level) run in R (ref), all subgroups were statistically significantly different relative to the MESO2 group both for carbon (MESO1/1: p 0.02; MESO1/2: p <0.01; NEO1/1: p 0.02; NEO1/2: p <0.01; following z-transformation to adjust non-normally distributed data) and nitrogen (MESO1/1: p 0.05; MESO1/2: p <0.01; NEO1/1: p <0.01; NEO1/2: p <0.01; data normally distributed) isotope values. Similarly, ANOVA analyses with multiple t-tests were also undertaken on the broader groupings (i.e., MESO1; MESO2 and NEO1), following rank-transformation for normalizing the carbon isotope data, which was not necessary for the nitrogen isotope data that were normally distributed.

The results of this statistical testing are that MESO2 was highly significantly different from MESO1 and NEO1 both in carbon (respectively p <0.01 and p 0.01) and nitrogen

(respectively $p < 0.01$ and $p < 0.01$) isotope composition. The MESO1 and NEO1 were not statistically different in their $\delta^{13}\text{C}$ values ($p = 0.56$), but were significantly different in their $\delta^{15}\text{N}$ values ($p = 0.03$). The statistical testing clearly shows that the diet of the Early Mesolithic, Late Mesolithic and Early Neolithic occupants of *Grotta dell'Uzzo* was significantly different, and that each of these culturally distinct periods was marked by dietary change.

Late Mesolithic (Castelnovian) diets at *Grotta dell'Uzzo*

The main improvement for the overall interpretation of the diets of the occupants of the site is relative to the Late Mesolithic (ex Mesolithic-Neolithic transition phase). In fact, four of the seven newly-analysed individuals from the phase allocated to the *sensu lato* Castelnovian lithic industry have enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values relative to the previously exceptional specimen (S-EVA 8010), which implies that they consumed slightly higher levels of marine protein (*UZZ69*, *UZZ79*, *UZZ80* and *UZZ81*). *UZZ4446* (mandible: $\delta^{13}\text{C} = -16.2\text{‰}$ and $\delta^{15}\text{N} = 12.4\text{‰}$) consumed similar levels of marine protein to S-EVA 8010, whilst *UZZ40* (R-EVA 2880: $\delta^{13}\text{C} = -18.7\text{‰}$ and $\delta^{15}\text{N} = 11.7\text{‰}$) and *UZZ82* (R-EVA 1960: $\delta^{13}\text{C} = -19.7\text{‰}$ and $\delta^{15}\text{N} = 11.6\text{‰}$) had significantly lower carbon and nitrogen isotope compositions. These are typical of the isotopic composition of the Early Mesolithic hunter-gatherers and a reason for their difference from the rest of the Late Mesolithic individuals may lie in their chronology.

UZZ82 is older than the six individuals (including S-EVA 8010 from *Mannino et al.* (Mannino et al., 2015)) who consumed around 40-50% marine protein with a considerable amount from cetacean carcasses, given that its 2σ calibrated range barely overlaps with the calibrated ranges for its contemporaries. This individual may, thus, have preceded the period when cetaceans were readily available for consumption. *UZZ40* has a calibrated age range that overlaps at the lower end with that of the hunter-gatherers who consumed large amounts of cetacean meat. However, the non-corrected age range of this individual is shifted towards a slightly more recent time of the Mesolithic II (MESO2) phase, which may have been a time when stranded cetaceans were no longer readily available. This would be compatible with the hypothesis that anomalous cetacean strandings in NW Sicily concentrated during a narrow interval of time, which may actually be even narrower than the 200-year period hypothesized by *Mannino et al.* (Mannino et al., 2015). As discussed by these authors, and previously highlighted by *Facorellis et al.* (Facorellis et al., 1997), *Siani et al.* (Siani, 2001) and *Daley et al.* (Daley et al., 2011), in the Mediterranean Sea the 8,200 cal BP event coincided with the deposition of Sapropel 1, causing reservoir ages to be higher ($\Delta R = 149 \pm 50$ yrs). Only a detailed evaluation of this issue, which is beyond the scope of this paper, with an *ad hoc* estimation of the exact reservoir age at the time in question will allow us to adequately correct the calibrated ages and to confirm the chronological sequencing between the different individuals from the Mesolithic II phase proposed here.

Text 2: Uniparental marker haplotyping

Mitogenome haplotypes (M.vdL)

We could reconstruct the mitochondrial genomes for 17 individuals (Data S1, 98-100% genome coverage, mean base coverage 7 - 1,034X).

Sicily EM hunter-gatherers

The two oldest HG in our dataset, UZZ5054, UZZ96 carried mitogenome lineages that fall within the U2'3'4'7'8'9 branch, and show a high similarity to the U2'3'4'7'8'9 haplotype that was previously reported for an Epigravettian HG from *Grotta d'Oriente* (I2158 - OrienteC). The three HGs have nine lineage-specific mutations in common and differently relate to each other with regard to three additional private mutations (Data S1.3). U2'3'4'7'8'9 mitogenome lineage is so-far the only lineage found in Sicily during Upper Paleolithic and Early Mesolithic, and have been reported for an Epigravettian-associated individual *ST2* from *San Teodoro*, which is the oldest human settlement found so-far in Sicily (Modi et al., 2021). It has also been found in an Upper Palaeolithic Italian HG associated with the Gravetian (*Paglicci108*), a Magdalenian in France (*Rigney*) and an Azilian in Spain (*Balma Guilanyà*) (Posth et al., 2016; Villalba-Mouco et al., 2019).

Sicily LM hunter-gatherers

We found that all the individuals in the Sicily LM genetic group carried U4a, U5a, and U5b mitogenome haplogroup lineages. All of these are characteristic for West Eurasian Mesolithic HGs (Fu et al., 2012; Posth et al., 2016).

Two Castelnovian-associated HGs carried haplogroup U5b2b and one a more derived variant U5b2b1a (table S3). The individuals who harboured U5b2b (*UZZ69* and *UZZ4446*) shared five private mutations (5585A, 9833C, 12477C, 16311C, 16355T). None of these mutations are typically found on a more derived branch, including U5b2b1a. U5b2b haplotypes were frequently observed among Villabruna cluster individuals high in WHG ancestry (Posth et al., 2016). The oldest individuals found so far to have carried U5b2b are two Italian Epigravettian individuals from *Grotta Paglicci* and *Villabruna*, and two Epipalaeolithic HGs from *Rochedane* and *Aven des Iboussières* in France (Posth et al., 2016). The haplogroup was also found in low frequency among Mesolithic HG from southeastern Europe such as Croatia and Iron Gates fishermen from Serbia (~9,300-8,000 calBP) (Mathieson et al., 2018).

We also found haplogroup U5b3/U5b3d in two Castelnovian-associated HGs and in one individual tentatively contemporaneous to early *Impressa Ware* (Data S1). Notably, these individuals carried only one of the three expected variants that define U5b3d, and had three additional mutations in common (11836G, 16278T, 16385G). The two Castelnovian HGs, a genetic male (*UZZ79*) and female (*UZZ81*) also show a PMMR for autosomal SNP sites that is around 3/4 of that found for unrelated individuals from this time period (Data S1.5). This underlines a first-degree genetic relatedness for these two individuals via at least the maternal side. Interestingly, the U5b3/U5b3d haplogroup has not been reported in European Mesolithic HGs thus far. However, Pala *et al.* (Pala et al., 2009) suggested an origin for U5b3 in the Italian Peninsula based on their analysis on the mitochondrial DNA variation observed among modern individuals. Notably, U5b3 has been found in an early Cardial farmer from the

El Portalon cave at *Sierra de Atapuerca* in Spain, with a high amount of local HG ancestry (Günther et al., 2015). Additional sampling of Sicily Mesolithic HGs should indicate whether this haplogroup can be viewed as a general maternal lineage for the Mesolithic population in Sicily, or whether the individuals sampled here are genetic isolates.

In addition, we found U5a haplogroups in one Castelnovian-associated HG (*UZZ82*) and one individual tentatively contemporaneous to *Impressa Ware* (*UZZ71*) (table S3). *UZZ82* carried U5a1 with three additional private mutations (1007C, 3865G, 9380A). The U5a1 haplogroup has been reported for Mesolithic HGs from Russia and northern Europe (Günther et al., 2018; Mathieson et al., 2015). *UZZ71* harboured U5a2+16294, a basal lineage to U5a2a. The more basal U5a2 haplogroup has been found in two Mesolithic HGs from *Los Closeaux* and *Les Vignolles* in France (Günther et al., 2018; Posth et al., 2016). The more derived haplogroup U5a2a is found in relatively higher frequency among Mesolithic HGs in general, more specifically in those from Ukraine, Serbia and Romania (Mathieson et al., 2018).

Lastly, for one Castelnovian-associated HG, *UZZ40*, we found the rare haplogroup U4a2f without one of the four expected variants (G15172A is missing, Data S1.3). Intriguingly, haplogroup U4a2f has been found also in a Cardial Ware individual from *Cueva de Chaves*, Iberia (GAMBA et al., 2012). U4a haplogroups are mostly found among Mesolithic HGs from northern Europe, the Baltic and Russia (Günther et al., 2018; Mathieson et al., 2018; Mitnik et al., 2018).

Sicily EN farmers

The Sicilian farmers in our study harboured mitogenome haplogroups characteristic for early farmers: U8b1b1 (n=2), K1a2 (n=1), N1a1a1 (n=1), H (n=1), and J1c5 (n=1) (Data S1.1). All these haplogroups have previously been reported in early farmers from the Balkans, and in Neolithic individuals from *Barcın* in north-western Anatolia (Mathieson et al., 2018, 2015). Subsets of these were found among early farmers from all over Europe, albeit in different combinations and frequencies in the Balkans, Central Europe and Iberia (Szécsényi-Nagy et al., 2017).

U8b1b1, found in two of the early Sicilian farmers, has been reported for Starcevo early farmers from Croatia (Mathieson et al., 2018). Haplogroup K1a2 has been reported for early farmers from Romania, Germany LBK and northern Greece (Hofmanová et al., 2016; Lipson et al., 2017b; Mathieson et al., 2018). In addition, K1a2 and the derived K1a2a haplogroup appear frequently among early farmers from Iberia. This includes a ~7,400 cal BP Cardial individual from *Cova Bonica* and a ~7,100 cal BP Epicardial individual from *Cova de Els Trocs* in northeastern Spain, and a ~7,000 cal BP individual from *Cueva del Toro* in southern Spain associated with 'boquique' and 'almagra' technique pottery (Fregel et al., 2018; Haak et al., 2015; Olalde et al., 2015).

The rare haplogroup N1a occurs at a relatively high frequency in LBK early farmers from Central Europe, but is much lower in Iberia (Haak et al., 2010, 2005; Szécsényi-Nagy et al., 2017). The N1a1a1 haplotype that we found in one Sicilian farmer was reported in Germany EN LBK and Hungary EN Starcevo farmers, and for one individual from *Cova de Els Trocs* (Haak et al., 2015; Lipson et al., 2017b; Mathieson et al., 2018). Interestingly, the more basal haplogroup N* was found in three Early Neolithic Cardial farmers from the *Can Sadurní Cave* in Catalonia, northern Spain (GAMBA et al., 2012).

Y-chromosome haplogroups (M.vdL; A.B.R)

We could determine the Y-haplogroup for five males (Data S1.4). The details for allele determination were provided in Data S4. Two Sicilian LM HGs associated with the Castelnovian carried haplogroups I2a1b and I2a1b2, which both are characteristic for Mesolithic HGs from Europe. Haplogroup I2a1b has been previously reported in Mesolithic *Loschbour* from Luxembourg and *Motala* from Sweden, and I2a1b2 in Mesolithic HGs from France (Brunel et al., 2020; Lazaridis et al., 2014; Mathieson et al., 2015).

The two Sicilian early farmers carried haplogroups C1a2b1 and H2. The haplogroup C1a2 has been found in both HGs and early farmers, like ~34k-year-old Sunghir from Russia, ~15k-year-old *Pınarbaşı* HG in Anatolia, Anatolian farmers from *Barcin* and LBK farmers from Austria (Feldman et al., 2019; Mathieson et al., 2018, 2015; Sikora et al., 2017). It was also reported in Middle Neolithic farmers from *Fossato di Stretto Partanna*, Sicily (Fernandes et al., 2020). The haplogroup C1a2b was reported in LBK farmer from Germany and Middle Neolithic farmer from France (Rivollat et al., 2020). The Y-haplogroup H2 that we find in the other Sicilian early farmer has been proposed to be among the more frequent uniparental markers that were introduced by the early farmer populations of the Middle East that were introduced to Europe during the Neolithic transition (Kivisild, 2017).

The Early Bronze Age Sicilian individual carried haplogroup R1b1a1b1a1a2, which belongs to the haplogroup commonly found in Bronze Age Europe, like Germany and France (Brunel et al., 2020; Furtwängler et al., 2020).

Text 3. Mesolithic European population substructure (M.vdL)

Previous studies have shown that the genetic diversity among European HGs after the LGM was shaped by various deeply diverged ancestries (Fu et al., 2016; Jones et al., 2015; Lazaridis et al., 2018; Lipson et al., 2017a; Mathieson et al., 2018; Villalba-Mouco et al., 2019). Individuals of the genetic Villabruna cluster, named after the site name of its oldest representative individual (~14,180-13,880 calBP), and also referred to as Western European Hunter-Gatherers (WHG), appeared ~14,000 calBP throughout continental Europe. During the Mesolithic, WHG replaced most of the diversity found among earlier European HG clusters. This included an ancestry associated with the Upper Palaeolithic Magdalenian techno-complex (El Mirón/Goyet-Q2 cluster), which after ~19,000 calBP only persisted in an admixed form and predominantly among Iberian HGs (Villalba-Mouco et al., 2019). However, in Mesolithic HGs from northern Europe (Scandinavian HGs, SHG), eastern Europe (EHG) and southeastern Europe (~11,500-7,800 calBP), an ancestry related to Upper Palaeolithic Siberians (Ancient North Eurasians, ANE) was found in addition to WHG ancestry (Haak et al., 2015; Lazaridis et al., 2014; Mathieson et al., 2018; Raghavan et al., 2014).

As shown above, the asymmetrical affinities of the Sicily EM and LM HGs to other foragers from western and eastern Europe hint at a genetic change in Sicily and peninsular Italy during the Mesolithic (Fig. 2A). To investigate the genetic differentiation among the Sicily EM and LM HGs, and their relation to other West Eurasian HGs in more detail, we first calculated pairwise genetic distances in the form of $f_3(\text{Mbuti}; \text{HG1}, \text{HG2})$ and visualised the results in a multidimensional scaling (MDS) plot (Fig. S3). The genetic variation among post-LGM European HGs is structured along two previously described clines: 1) a WHG-EHG-ANE cline, confirming the genetic gradient found in Mesolithic HGs from western to eastern Europe and 2) a WHG-GoyetQ2 cline between WHG and Central European Magdalenian-associated individuals on which Iberian HGs take an intermediate position (Fu et al., 2016; Haak et al., 2015; Lazaridis et al., 2016; Villalba-Mouco et al., 2019). As previously reported for *OrienteC* and *Continenza* (R7, R11, R15) (Antonio et al., 2019; Catalano et al., 2020), Sicily EM HGs *UZZ5054* and *UZZ96* fall also at the extreme WHG-end of both ancestry clines, slightly outside the genetic variation of the Villabruna cluster (Fig. S3). In contrast, Sicily LM HGs fall close to the Villabruna cluster along the WHG-EHG-ANE ancestry cline, and in between Sicily EM HGs and Mesolithic Iron Gates HGs (Fig. S3). The extreme position of the Sicily EM HGs in MDS suggests that these individuals are highly drifted and share substantial amounts of genetic drift with *Continenza* individuals from Italy peninsular, respect to other individuals of the Villabruna cluster.

Reduced population genomic diversity in Early Mesolithic Sicilian HGs (M.vdL; A.C)

Based on the observation that Sicily EM individuals share elevated levels of genetic drift with each other (Fig. 1C, Fig. S4), we investigated the extent of genetic drift of the two island groups in comparison with other HGs from peninsular Italy and mainland Europe. Genetic drift causes allele fixation over time, lowering the global nucleotide diversity of the population. Assuming that genetic drift affected the population genomic diversity of the Sicily EM HGs, we expect the global nucleotide diversity levels (π) to be lower than that of the later Sicilian LM HGs and other Villabruna cluster HGs. Indeed, we find a significantly lower nucleotide

diversity (π) for individuals from the Epipalaeolithic/Early Mesolithic time period ($\pi = 0.165$, 95CI = 0.161-0.170), compared to those from the preceding Upper Paleolithic ($\pi = 0.233$, 95CI = 0.227-0.239), and subsequent Late Mesolithic ($\pi = 0.220$, 95CI = 0.217-0.223), Early Neolithic ($\pi = 0.252$, 95CI = 0.248-0.256), Middle Neolithic ($\pi = 0.250$, 95CI = 0.246-0.251) and Early Bronze Age ($\pi = 0.252$, 95CI = 0.244-0.260) (Fig. S4, Data S2.3).

Various demographic processes can result in a reduced population diversity. To distinguish between the effects of recent consanguinity and small effective population size (population bottleneck/founder effect), we investigated runs of homozygosity (ROH) with *hapROH* (Ringbauer and Novembre, 2020). The effects of consanguinity typically result in a larger quantity of long ROH tracts (>20 cM, close-kin unions) whereas, a prolonged period of a small effective population size typically leaves a large quantity of short tracts (4-8cM, background relatedness). In Sicily EM HGs (mean $sROH_{[4-8]} = 360$ cM) we find a larger proportion of 4-8cM ROH segments compared to the later Sicily LM HGs (mean $sROH_{[4-8]} = 57$ cM), *Continenza* HGs (mean $sROH_{[4-8]} = 193$ cM) and *Villabruna* ($sROH_{[4-8]} = 270$ cM) from peninsular Italy, and Late Paleolithic or Mesolithic HGs from other European regions (Fig. S5, Data S2.4). Antonio *et al.* already described a significant reduction in genomic diversity and excess of ROH segments in the *Continenza* HGs compared to non-Italian HGs, and suggested that this may have been the result of a small effective population size on the peninsula (Antonio *et al.*, 2019). Our results confirm the large amount of short ROH segments in the *Continenza* HGs, and additionally show even larger amounts in Sicily EM HGs. This suggests a stronger population bottleneck in the demographic history of the Sicily EM HGs compared to peninsular Italian Mesolithic HGs. The lack of long ROH segments (>20cM) in Sicily EM individuals and *Continenza* also indicates a moderate effect of inbreeding (Antonio *et al.*, 2019), and suggests that the reduced population diversity in both Italian HG groups resulted from a small effective population size, at least in most parts, and not from consanguinity.

Dating of admixture in Late Mesolithic Sicilian HGs (H.Y)

To investigate when the EHG-related ancestry arrived in Sicily, we dated the admixture between Sicily EM HGs and EHG, in Sicily Late Mesolithic HGs (Table S4). When all the LM HGs were grouped, we estimated the admixture to around 20 generations ago, corresponding to ~8,800 yBP. As the two *Impressa* individuals (*UZZ71*, *UZZ88*) were much younger than the LM Castelnovian HGs, we also estimated the admixture in these two groups separately, with Sicily_LM_1 including the seven LM Castelnovian individuals and Sicily_LM_2 including the two *Impressa*-associated individuals carrying HG profile. As expected, the admixture happened recent in Sicily_LM_1 (12 generations) compared to Sicily_LM_2 (45 generations). The admixture dates in these three groups overlapped when the sample ages were taken into consideration, again confirming that the *Impressa*-associated individuals carried a homogeneous genetic profile as older Castelnovian individuals, which was formed in the same admixture event. The estimated admixture date also matched the beginning of the Late Mesolithic at *Grotta dell' Uzzo* (STAR Method), correlating the transition in genetic ancestry with archaeological context.

The affinity to mainland HGs increased in Sicily during the Mesolithic (M.vdL)

Fu *et al.* observed that the Villabruna cluster individuals showed an affinity to the Near East and Feldman *et al.* revealed such affinity between southeastern European HGs and AHG, suggesting a deep connection through a common southeastern European/Near Eastern glacial refugium (Feldman *et al.*, 2019; Fu *et al.*, 2016; Mathieson *et al.*, 2018). However, the details of the spatio-temporal dynamics that resulted in the spread of these ancestries in Europe, as well as their archaeological context, are not well understood. Hence, we systematically modelled the ancestry profiles of West-Eurasian HGs in various temporal windows to obtain a chronological perspective on the underlying population genomic structure in Villabruna cluster individuals. In previous studies, the characterisation of the genomic profile of HG typically was based on *Villabruna* as a proxy for WHG ancestry. However, compared to *Villabruna*, the Sicily EM HGs showed a higher affinity to many WHG individuals (Data S2.2) and also have a more geographically centered position in a southern refugium. In our model we therefore chose Sicily EM HGs rather than *Villabruna* as the proxy for WHG ancestry, and found that it helps to differentiate between Magdalenian, EHG and AHG related ancestries in post-LGM and Mesolithic European HGs (Fig. S6, Data S2.6) (van de Loosdrecht, 2021). We also found that, post-LGM Upper Paleolithic and Early Mesolithic Villabruna cluster individuals had diverse genomic ancestries with influences from EHG, Magdalenian-associated HG, or both, rather than forming a genetically homogenous group (Fig. S6). During the Late Glacial, a WHG/EHG mixture was detected for the first time in *Bichon* (Fig. S6B). This added EHG as an additional dimension to an initial, previously established, Magdalenian-WHG ancestry cline in Iberia and central Europe (Fig. S6A) (Villalba-Mouco *et al.*, 2019). From the start of Holocene, an EHG/AHG ancestry mixture can be found among HGs in northern and (south)eastern Europe (Fig. S6C+D).

Text 4. Investigating the phylogenetic position of the Early Mesolithic Sicilian HGs (H.Y; M.vdL)

Admixture graph models fit allele frequency correlations and allow us to hierarchically build an increasingly complex framework of ancestry streams that fit the genetic diversity observed. Here, we used the *qpGraph* program (Patterson *et al.*, 2012) to construct a phylogeny of ancestry lineages found among Palaeolithic and Mesolithic West Eurasian HGs to further clarify the phylogenetic position of Sicily EM HGs in relation to *Villabruna*, and Magdalenian-associated HGs (e.g. *El Miron* and *GoyetQ2*).

We built the phylogeny models by fitting representative West Eurasian HG ancestry lineages one by one to the phylogeny roughly in order of their respective ¹⁴C dates, and fitted each of them as a branch without admixture or as a two-way admixture between two branches. We selected the model with lowest deviation between observed and fitted *f*-statistics as the best fitting model. When several models fitted equally well, we chose the simplest model with least admixture events, and avoided models with branch lengths of zero (trifurcations).

We started from a skeleton graph of six populations based on a published graphic model in Fu *et al.* 2016, including Mbuti, ~45 kyBP *Ust'Ishim*, ~36 kyBP *Kostenki14*, ~35kyBP

GoyetQ116-1, ~24 kyBP *Mal'ta* and ~14kyBP *Villabruna* (Fu et al., 2016). This graph contained no admixed branches, with *Villabruna* modelled as a sister lineage of *GoyetQ116-1*.

On this skeleton graph, we added Sicily_EM, *El Miron* or *GoyetQ2*, with the latter two being two representatives of Magdalenian-related ancestry. The Sicily_EM group included the two EM HGs reported in this study from *Grotta dell'Uzzo*, and the published individual *OrienteC* (Catalano et al., 2020). We found that Sicily_EM could be fitted as a sister lineage of *Villabruna*, while both *El Miron* and *GoyetQ2* were fitted as the admixture between *GoyetQ116-1*- and *Villabruna*-like lineages. As previous studies reported, *El Miron* carried 85% ancestry from *Villabruna*, much higher than the 12% *Villabruna*-like ancestry estimated in *GoyetQ2* (Fig. S7).

We then tried to add *GoyetQ2*, *El Miron* or a Mesolithic WHG individual *Loschbour* on the best fitted model with Sicily_EM to investigate the relationship between our newly reported Sicily HGs and Magdalenian ancestries or younger Mesolithic western European HGs. The only fitting model we found with *El Miron* and *Loschbour* on the graph with Sicily_EM were the same, suggesting *El Miron* and *Loschbour* as the admixture between *GoyetQ116-1*- and Sicily_EM-like lineages, instead of *Villabruna* (Fig. S8). This confirmed the f_4 -statistics result that Sicilian EM HGs were closer related to both *El Miron* and *Loschbour* than *Villabruna* (Data S2.2).

On the other hand, we found that *GoyetQ2* could be fitted as the admixture between *GoyetQ116-1* and either *Villabruna*, Sicily_EM or the ancestral branch of *Villabruna*/Sicily_EM-like lineages (Fig. S9), with the contribution from Epigravettian-related lineages estimated to 21-25%. The uncertainty in the modelling of *GoyetQ2* could be due to the relatively less contribution, and thus lower resolution from these lineages.

Overall, our qpGraph modelling supported Sicily EM HGs as a sister lineage of *Villabruna*. Compared to *Villabruna*, the Sicily EM HGs could be a better proxy for the modelling of some Magdalenian-related populations and later Mesolithic western European HGs.

Text 5. Tracing early farmer groups through the Mediterranean basin (M.vdL)

The Neolithic expansion routes into the central and western Mediterranean have been long debated by archaeologists and more recently by paleogeneticists. To account for the observed regional complexities, archaeologists proposed different expansion routes (Zilhão, 2014). Given Sicily's central location on both the northern and southern Mediterranean expansion routes, we aimed to investigate the genomic link between the Sicily EN farmers with those from peninsular Italy, and other early farmer groups from the Mediterranean basin in closer detail.

The combination of the HG ancestry proportion and its substructure in the genomes of early farmers has been used to shed light on the Neolithic expansion route in the Mediterranean. In previous studies the European Early Farmer (EEF) ancestry source had been fixed (typically Anatolia EN *Barcin*) to consistently compare differences in HG admixture amount and substructure among early farmers across Europe (Mathieson et al., 2018; Olalde et al., 2019; Rivollat et al., 2020). In a recent study, Rivollat et al. found that the Mediterranean and Danubian/Continental Route of the Neolithic expansion could be differentiated with respect to the contribution of local HG ancestry (Rivollat et al., 2020). Here, we used a different approach, to model the ancestry of EN farmers across Europe with various EEF sources from Italy, the Balkan Peninsula, Aegean and northwestern Anatolia, and a generalised local European HG ancestry, with *qpWave* and *qpAdm*-based methods (Haak et al., 2015). This approach was found to be effective in visualising population substructure among European EN farmer groups (Fig. S10A-C).

Congruent with previous reports, our ancestry models indicate that the HG ancestry proportion is very low or undetectable in the EN farmer groups from the eastern Mediterranean (Hofmanová et al., 2016; Mathieson et al., 2018, 2015). However, despite the general genomic homogeneity, our models could discern some different affinities with regards to the most deeply divergent early farmer groups from the Aegean (*Revenia* in continental Greece and *Diros* in the Peloponnese islands), and western (*Barcin*) and central (*Tepecik Çiftlik*) Anatolia (Fig. S10A). Notably, all EN groups in the eastern Adriatic can be fitted using *Diros* Peloponnese as a source for the EEF ancestry (including *Revenia* and *Barcin*). Contrastingly, *Revenia* and *Barcin* provided adequate fits to the EEF ancestry more sporadically. These differential affinities may reflect substructure that resulted from initial dispersal of the early farmer groups in the eastern Mediterranean, or the involvement of local populations during the process, as has been described in Aegean (Kılınç et al., 2017).

In addition, the ancestry models suggest that the early farmer groups from peninsular Italy and Sicily may relate differently to the EN farmer groups in the eastern Mediterranean (Fig. S10A+B). While Anatolia EN *Barcin* provides a good fit when modelling EEF ancestry in Sicily EN farmers, it is strongly rejected for the EN farmers from *Continenza* and *Ripabianca* (Data S3.5, 3.6). Congruent to the results of Antonio et al., *Continenza* and *Ripabianca* farmers from peninsular Italy can only be adequately approximated by an EN farmer from *Revenia* in northern Greece (or as a mixture of *Diros* Peloponnese and Iran PPN *Ganj Dareh*, see Data S3.6) (Antonio et al., 2019). These subtle differences in genomic ancestry may hence suggest that these groups descended from early farmer groups with different demographic histories, underling the proposed complex mosaic of regional EN horizons in peninsular Italy and Sicily based on archeological evidence (Natali and Forgia, 2018; Radi and Petrinelli Pannocchia, 2018).

Lastly, we find that the EN groups in the western Mediterranean can be approximated by the EEF ancestry as found in either *Barcin*, *Diros* Peloponnese or *Revenia*, as well as the northern Adriatic, central Europe, Sicily and continental Italy (Fig. S10B+C). The observed affinity to the northern Adriatic and central European EN groups is congruent with previous suggestions that the EN farmers from Iberia and farmer groups associated with the Danubian/Continental Route share a common origin in the EN groups of the Balkans (Mathieson et al., 2018; Rivollat et al., 2020). Notably, the Sicily EN farmers, but not the *Continenza* and *Ripabianca* farmers from peninsular Italy, have a similar affinity with the northern Adriatic and central European EN groups associated with the Danubian/Continental Route (Fig. S10B+C). An exception is farmers from Serbia associated with the Starčevo

complex that share a close affinity with *Continenza* EN farmers. These results suggest that the majority of EN farmers from Sicily, as well as Iberia, derived from farmer groups that expanded along a northern Mediterranean route (Mathieson et al., 2018; Rivollat et al., 2020). However, EN farmers from Sicily and Iberia, in particular southern Iberia, also show an affinity to the genomically distinct farmers from *Continenza* (and *Ripabianca*). This raises the possibility that at least two different founder waves from the eastern Adriatic contributed to the genomic diversity of EN farmers in the central and western Mediterranean (Antonio et al., 2019; Mathieson et al., 2018). Currently, the EEF ancestry of the first founder wave may be best approximated by EN farmer ancestry from northern Greece (*Revenia*), whereas that of the second wave appears more closely related to EN farmers from Peloponnese Greece (*Diros*) as well as northwestern Anatolia (*Barcin*). The genomic diversity of early farmer groups in Sicily and Iberia appears to have been shaped by complex interactions between the descendants of both waves. Taken together, these results call for further investigation into a two-stage model of dispersal for farming practises into the Mediterranean (Fiedel and Anthony, 2003).

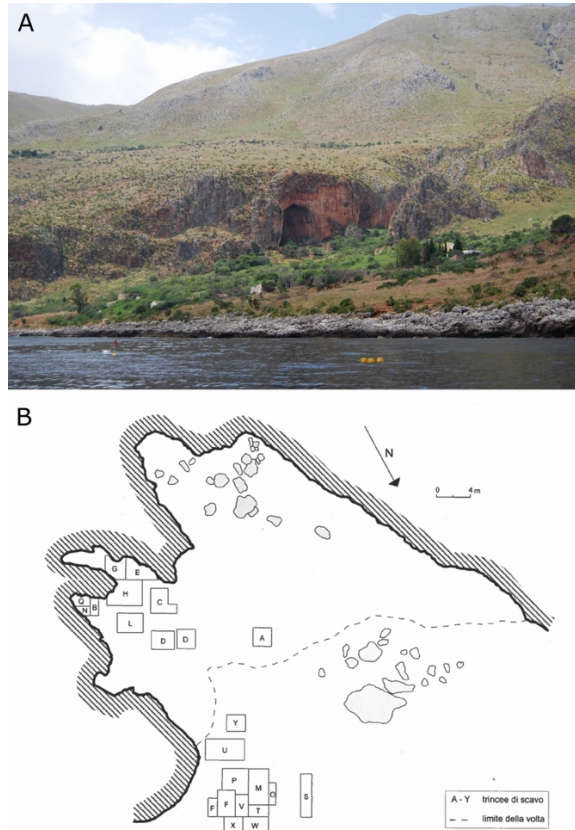


Figure S1. The archaeological site *Grotta dell'Uzzo*, related to STAR Methods. (A) View of *Grotta dell'Uzzo* from the sea (photo by Marcello A. Mannino). (B) Plan of *Grotta dell'Uzzo* with the trenches excavated in the 1970s and 1980s (Tagliacozzo, 1993).

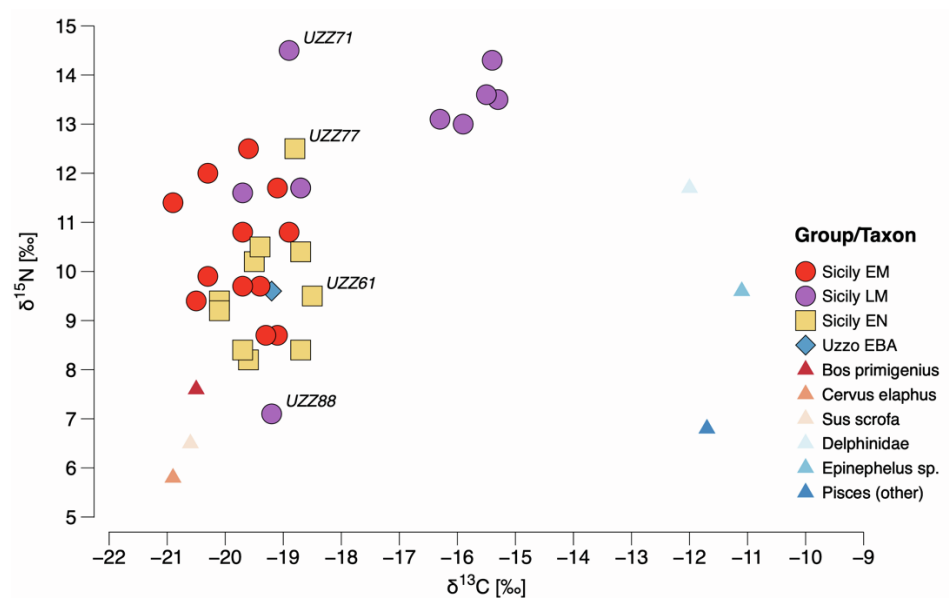


Figure S2. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analysis on human bone collagen, related to Figure 3. Scattergram showing all the isotopic data on humans obtained as part of this study or already published (Mannino et al., 2015). This graph also includes the isotopic compositions for the main faunal taxa previously used to generate the local isotope baseline for *Grotta dell'Uzzo* (Mannino et al., 2015).

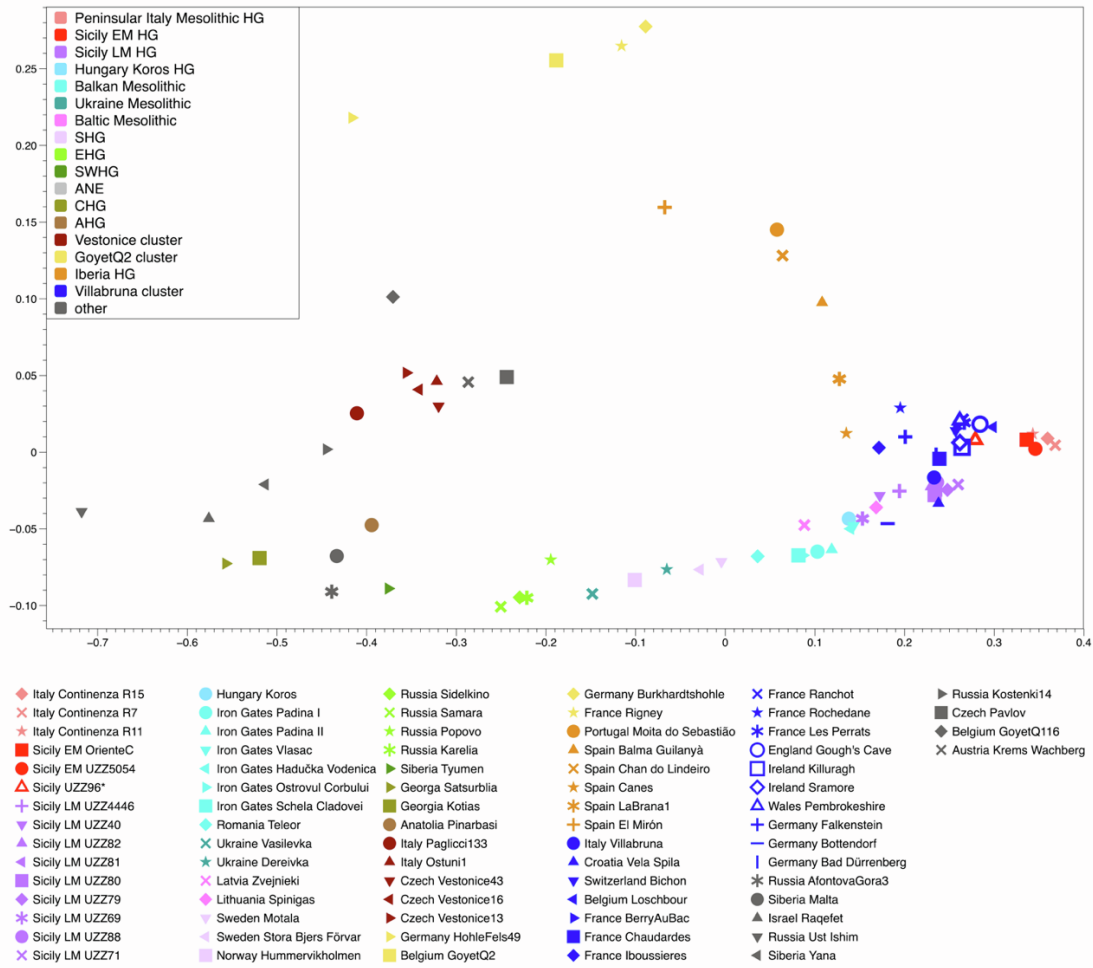


Figure S3. MDS plot showing structure in the genetic variation among West Eurasian HGs, related to Figure 1. Genetic distances are based on pairwise f_3 -outgroup statistics of the form $f_3(Mbuti; HG1, HG2)$. Colours reflect various ancestry clusters or geographical groups.

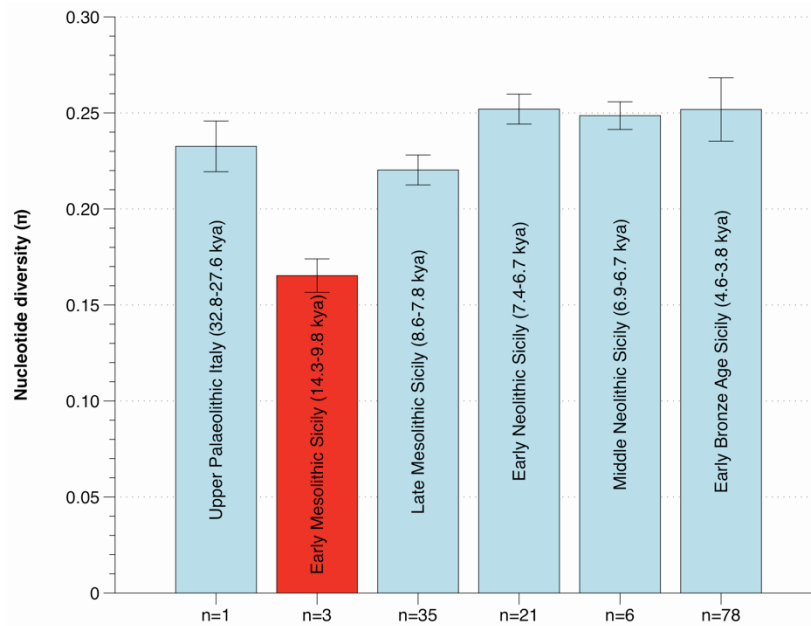


Figure S4. Changes in the nucleotide diversity over time for individuals from peninsular Italy and Sicily, related to Figure 1. The nucleotide diversity (π) is plotted for various transect groups in chronological order. Upper Palaeolithic (Paglicci133, Ostuni1), Early Mesolithic (Sicily EM HGs), Late Mesolithic (Sicily LM HGs), Early Neolithic (Sicily EN farmers), Middle Neolithic (Sicily MN farmers), and Early Bronze Age (Sicily EBA). The number of tests (n) that is used to determine the average for each time period is given. Error bars reflect 3 SEs. Details of the pairwise comparisons are given in Data S2.3.

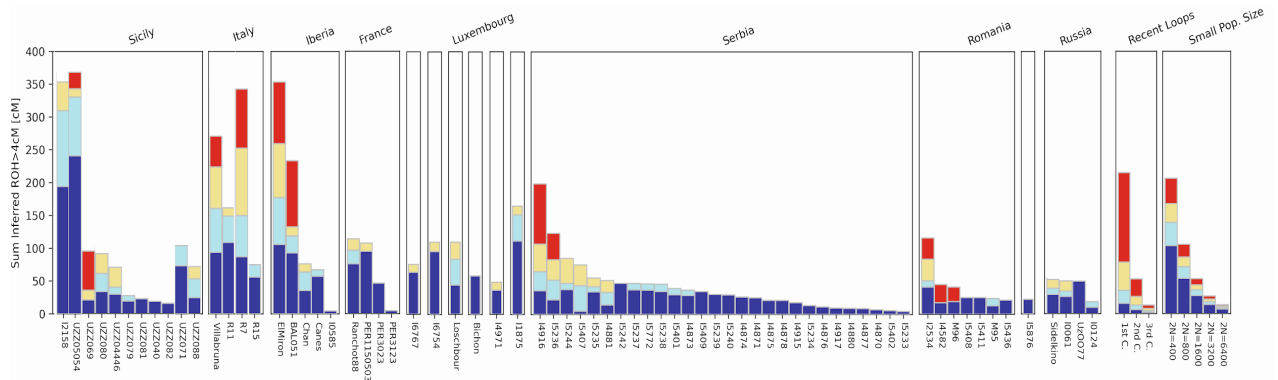


Figure S5. Runs of Homozygosity (ROH) segments for various relevant West-Eurasian HGs, related to Figure 2. ROHs were determined with HapROH (Ringbauer and Novembre, 2020). The length of different colors showed the sum of ROH segments in different length: 4-8 cM, dark blue; 8-12 cM: light blue; 12-20 cM: yellow; 20-300 cM: red. Summary statistics for each individual is given in Data S2.4.

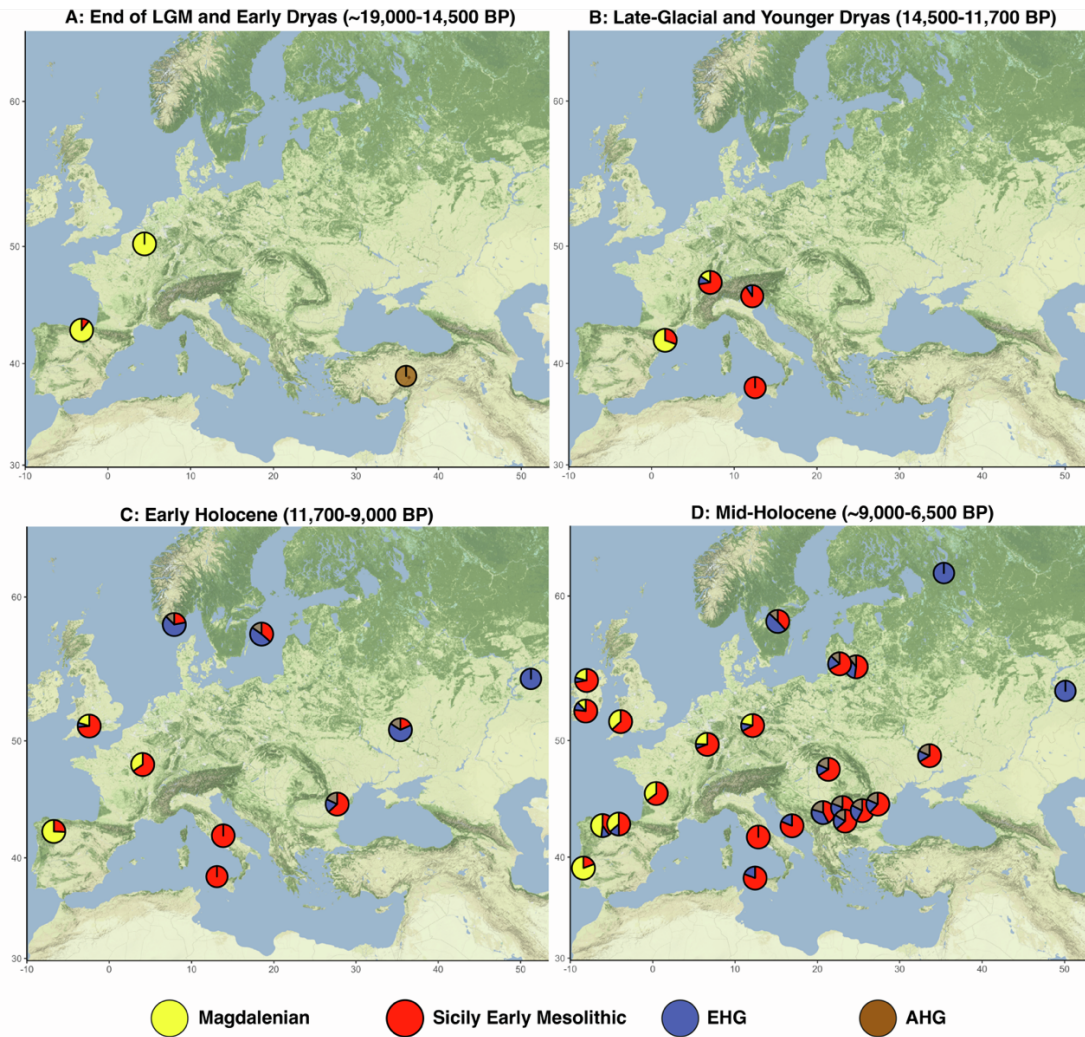


Figure S6. Changes in post-LGM hunter-gatherer genomic structure in Europe over time, related to Figure 2. Magdalenian ancestry (yellow) approximated by *GoyetQ2*; Sicily EM HG (WHG) ancestry approximated by *UZZ5054* and *OrienteC*; EHG (blue): Eastern hunter-gatherers from Russia; AHG (brown): Anatolia hunter-gatherer Pinarbasi. Details for the modelling are provided in Data S2.6 and Data S2.7.

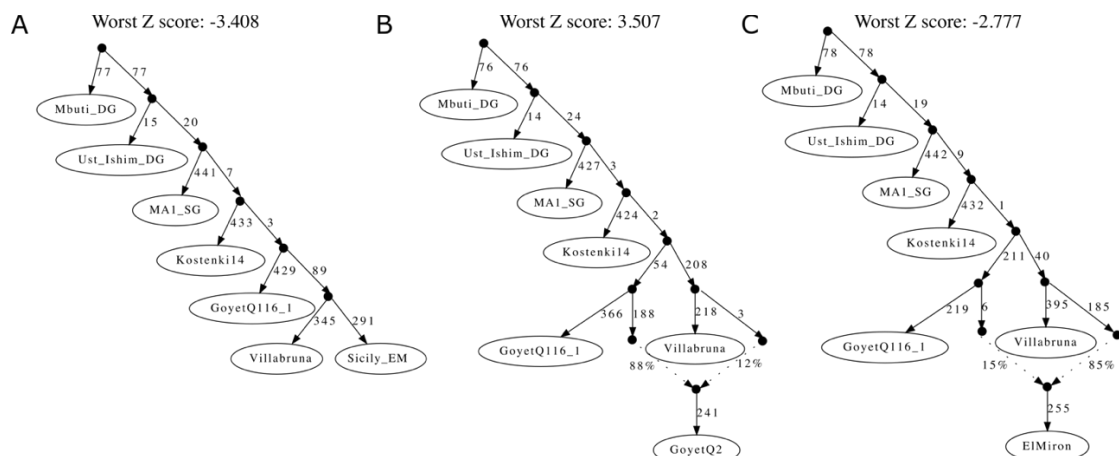


Figure S7. Admixture graph fitting Sicily_EM, GoyetQ2 and EI Miron on the 6pop skeleton graph, related to Figure 2. (A) Sicily_EM was fitted as a sister lineage of Villabruna. (B) GoyetQ2 and (C) EI Miron were fitted as admixture between GoyetQ116-1 and Villabruna lineages.

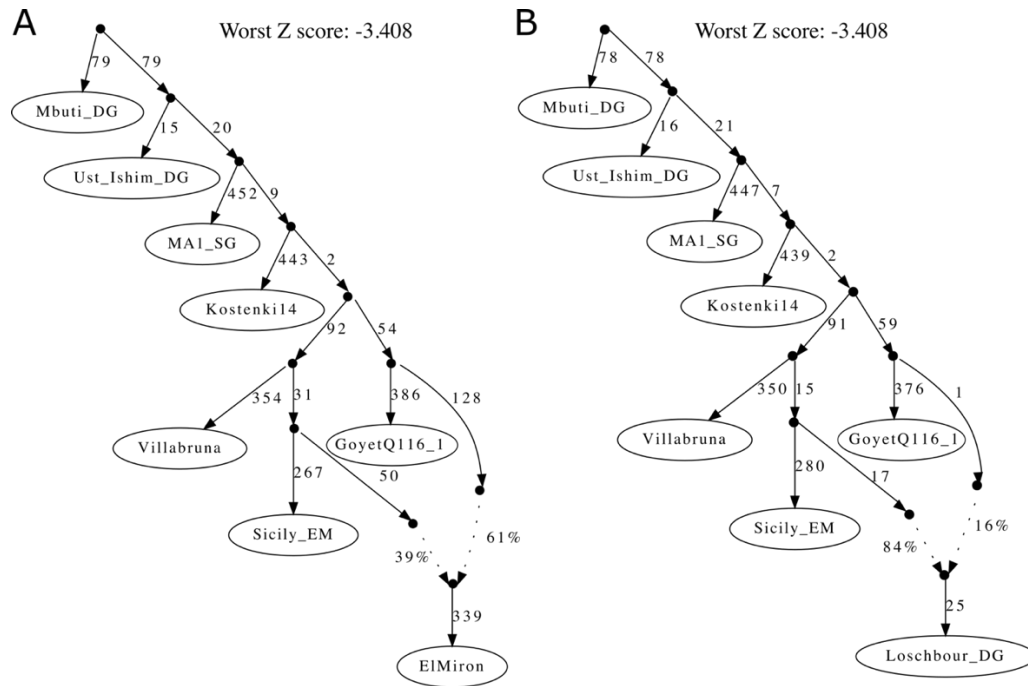


Figure S8. Admixture graph with both Sicily_EM and *El Miron* or *Loschbour*, related to Figure 2.

Both (A) *El Miron* and (B) *Loschbour* were fitted as the admixture between Sicily_EM and *GoyetQ116-1*, with the contribution from Sicily_EM estimated to 39% and 84%, respectively.

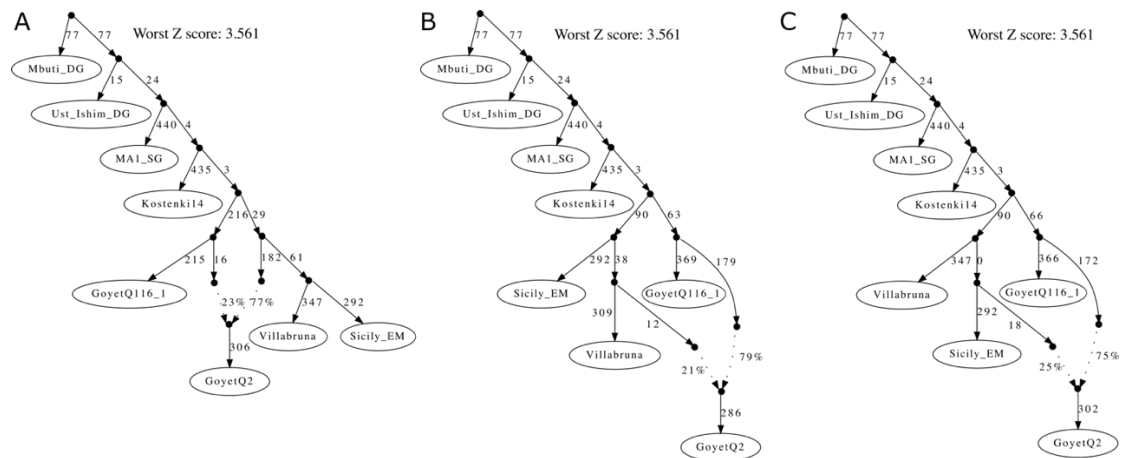


Figure S9. Admixture graph with both Sicily_EM and *GoyetQ2*, related to Figure 2.

GoyetQ2 could be fitted as the admixture between *GoyetQ116-1* and either (A) the ancestral lineage of Villabruna/Sicily_EM, (B) Villabruna or (C) Sicily_EM. The contribution from Epigravettian-related lineage was estimated to 21-25%.

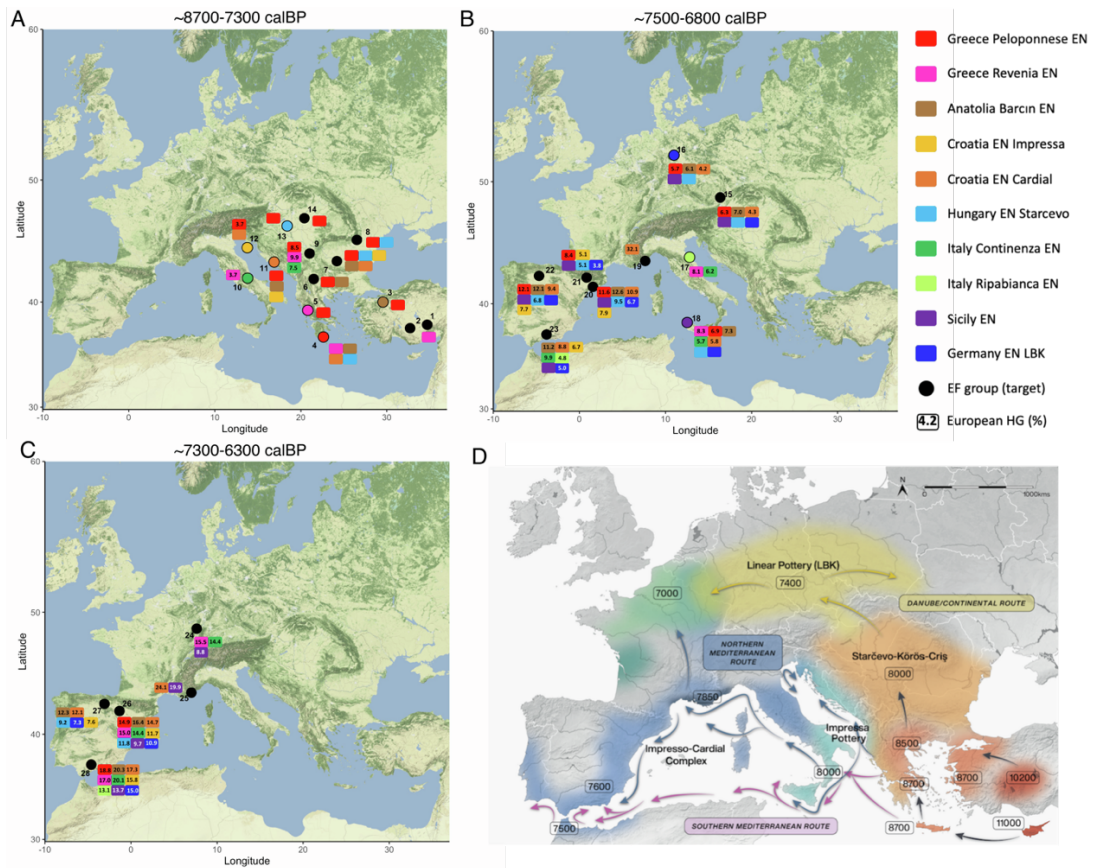


Figure S10. Tracing Early Neolithic farmer groups through the Mediterranean for various time frames, related to Figure 3. (A-C) Ancestry profiles were determined with *qpWave* and *qpAdm*-based models, using various farmer groups from the East and Central Mediterranean as proxies for EEF ancestry. Two-way admixture models with additional general European Mesolithic HG ancestry are given when a sole EEF source did not provide an adequate fit to the target Early Farmer (EF) group for (P-value threshold: 0.1 see Data S3.5 for SEs and P-values). **(D)** Proposed routes for the expansion of farming practises in Europe and northwestern Africa. Dates are in calBP. The map is based on Figure-S11 in *Rivollat et al., 2020* (with permission) after maps in (Rivollat et al., 2020)(Gronenborn et al., 2020)(Manen et al., 2004). Arrows indicate broad directions of the distribution of archaeological features and not discrete routes human expansion. The date for the arrival of the ceramic Early Neolithic in Morocco is from *Cortés Sánchez et al., 2012* and *Martínez-Sánchez et al., 2018* (Martínez-Sánchez et al., 2018; Sánchez et al., 2012).

Table S1. Isotopic, elemental and yield data on the bone collagen extracted from the genetically-typed human individuals from *Grotta dell'Uzzo* related to Figure 3.

Individual ID	Sample ID	R-EVA	Trench Spit	Phase	Skeletal element	$\delta^{13}\text{C}$ [‰]	$\delta^{15}\text{N}$ [‰]	%C	%N	C/N	% yield
UZZ26.cont	UZZ26	1918	A-8	MESO1/1	cranium	-19.4	9.7	40.2	14.9	3.2	12.6
	UZZ50	1934	F-19	MESO1/2	tooth	-19.7	12.3	37.9	13.8	3.2	3.9
	UZZ51	1935	F-19	MESO1/2	tooth	-19.7	12.3	43.7	16.0	3.2	7.6
UZZ5054	UZZ52	2881	F-19	MESO1/2	tooth	-19.6	12.6	41.6	15.2	3.2	2.3
	UZZ53	2882	F-20	MESO1/2	tooth	-19.5	12.6	46.0	16.6	3.2	7.3
			Average			-19.6	12.5	42.3	15.4	3.2	5.3
UZZ40	UZZ40	2880	F-13	MESO2	tooth	-18.7	11.7	46.7	17.0	3.2	9.4
	UZZ44	1929	F-15	MESO2	tooth	-16.0	13.2	47.5	17.6	3.2	10.0
UZZ4446	UZZ45	1930	F-15	MESO2	tooth	-16.6	13.6	47.0	17.3	3.2	10.4
	UZZ46	1931	F-15	MESO2	mandible	-16.2	12.4	45.7	16.6	3.2	5.0
				Average			-16.3	13.1	46.7	17.0	3.2
UZZ69	UZZ69	1948	M-3	MESO2	mandible	-15.3	13.5	40.0	14.4	3.2	5.1
UZZ79	UZZ79	1957	U-rim	MESO2	temporal	-15.4	14.3	45.5	17.0	3.1	12.8
UZZ80	UZZ80	1958	U-rim	MESO2	temporal	-15.4	13.6	43.3	16.1	3.1	12.6
UZZ81	UZZ81	1959	U-rim	MESO2	temporal	-15.9	13.0	44.3	16.6	3.1	10.9
UZZ82	UZZ82	1960	U-rim	MESO2	temporal	-19.7	11.6	45.5	17.0	3.1	8.3
UZZ71	UZZ71	1950	M-10	NEO1/1	tooth	-18.9	14.5	43.0	15.7	3.2	6.7
UZZ88	UZZ88	1965	W-14	NEO1/1	phalanx	-19.2	7.1	42.8	15.5	3.2	12.4
UZZ77	UZZ77	3523	T-13	NEO1/1	tooth	-18.8	12.5	34.6	12.5	3.2	3.7
UZZ34	UZZ34	2879	F-4	NEO1/2	tooth	-20.1	9.4	48.1	17.7	3.2	12.3
UZZ74	UZZ74	1953	S-rim	NEO1/2	femur	-19.6	8.2	43.9	16.3	3.2	3.3
UZZ75	UZZ75	1954	S-5	NEO1/2	temporal	-20.1	9.2	44.6	16.2	3.2	2.8
UZZ87	UZZ87	1964	W-2	NEO1/2	humerus	-19.5	10.2	45.0	16.7	3.2	4.0
UZZ61	UZZ61	3521	H-rim	MID/NEO	phalanx	-18.5	9.5	43.1	15.6	3.2	7.1
UZZ99	UZZ99	1974	n.a.**	M-L/NEO	cranium	-19.6	10.0	36.8	13.4	3.2	5.5
UZZ57	UZZ57	2883	G-Uzzo 5***	EBA	phalanx	-19.2	9.6	46.5	16.9	3.2	5.2

The abbreviations of the different cultural phases in this and in the following tables stand for: Mesolithic 1 phase 1 (MESO1/1); Mesolithic 1 phase 2 (MESO1/2); Mesolithic (MESO); Mesolithic 2: Castelnovian *sensu lato* phase (MESO2); Neolithic 1 phase 1: Impressed Ware culture (NEO1/1) and Neolithic 1 phase 2: Stentinello culture (NEO1/2); Middle Neolithic: Stentinello/Trichrome/Serra d'Alto culture (MID/NEO); Middle / Late Neolithic transition: Stentinello/Trichrome/ Serra'Alto or Diana culture (M-L/NEO). These last two specimens fall outside of the chronological range originally proposed by Tagliacozzo (Tagliacozzo, 1993). Specimens UZZ44, UZZ45 and UZZ46 have been shown genetically to all belong to the same individual (UZZ4446). Specimens UZZ50, UZZ51, UZZ52 and UZZ53 have been shown genetically to all belong to the same individual (UZZ5054). (*) UZZ71 has been analyzed a second time, as part of an attempt to radiocarbon date powder from this sample; the results from the second elemental and isotopic analyses (%C: 28.7, %N: 10.4, $\delta^{13}\text{C}$: -19.0‰, $\delta^{15}\text{N}$: 14.0‰) are similar to the first analysis, but not considered further here; the reason for this is that the extract from the powder, albeit falling within what is acceptable according to the quality criteria established by van Klinken (van Klinken, 1999) had a considerably lower yield (1.1%) than that from the fragment of UZZ71 used for the first analysis (6.7%), as well as lower %C and %N. (**) This cranial fragment with *cribra orbitalia* was recovered during the 1976 campaign in stratigraphic unit D within a small tunnel-like feature (*cunicolo*) excavated along the walls of *Grotta dell'Uzzo*, outside the named trenches. (***) UZZ57 is a phalanx recovered close to the cut of burial Uzzo 5 in Trench G and as its radiocarbon date suggests is not associated to the inhumated individual, given that its calibrated age range coincides with the Early Bronze Age (EBA) in Sicily (see Table S2).

Table S2. Radiocarbon dates, calibrated and corrected ages of humans from *Grotta dell'Uzzo*, related to Figure 1.

Individual ID	R-EVA	MAMS	Trench-Spit	Phase	¹⁴ C date BP	Calibrated age BP (2σ)	Calibrated age BCE (2σ)
<i>UZZ26.cont</i>	1918	40708	A-8	MESO1/1	9,436±36	10,990-10,560	9,040-8,620
<i>UZZ5054</i>	1935	40710	F-19	MESO1/2	9,436±29	10,750-10,580	8,800-8,620
<i>UZZ82</i>	1960	40722	U-rim	MESO2	7,809±26	8,650-8,520	6,700-6,570
<i>UZZ69</i>	1948	40711	M-3	MESO2	7,848±26	8,540-8,190	6,590-6,240
<i>UZZ81</i>	1959	40721	U-rim	MESO2	7,807±26	8,520-8,180	6,570-6,230
<i>UZZ79</i>	1957	40719	U-rim	MESO2	7,809±26	8,520-8,160	6,570-6,210
<i>UZZ80</i>	1958	40720	U-rim	MESO2	7,809±26	8,520-8,160	6,570-6,210
<i>UZZ4446</i>	1930	40709	F-15	MESO2	7,713±26	8,420-8,130	6,480-6,180
<i>UZZ40</i>	2880	40726	F-13	MESO2	7,471±26	8,370-8,190	6,420-6,240
<i>UZZ71</i>	3522	48212	M-10	NEO1/1	7,051±27	7,960-7,790	6,010-5,840
<i>UZZ88</i>	1965	40712	W-14	NEO1/1	7,036±25	7,940-7,790	6,000-5,840
<i>UZZ77</i>	3523	48213	T-13	NEO1/1	6,446±25	7,430-7,310	5,480-5,360
<i>UZZ34</i>	2879	40725	F-4	NEO1/2	6,351±24	7,330-7,160	5,380-5,210
<i>UZZ74</i>	1953	40716	S-rim	NEO1/2	6,310±23	7,280-7,160	5,330-5,210
<i>UZZ75</i>	1954	40717	S-5	NEO1/2	6,310±23	7,280-7,160	5,330-5,210
<i>UZZ87</i>	1964	40723	W-2	NEO1/2	6,286±24	7,260-7,160	5,320-5,210
<i>UZZ61</i>	3521	48211	H-rim	MID/NEO	5,923±25	6,830-6,660	4,880-4,710
<i>UZZ99.cont</i>	1974	40714	n.a.*	M/L NEO	5,185±31	6,000-5,900	4,050-3,950
<i>UZZ57*</i>	2883	40727	G Uzzo 5 fossa	EBA	3,708±19	4,150-3,970	2,200-2,030

The AMS radiocarbon dates reported in this table were all performed at the Klaus Tschira Laboratory of the Curt-Engelhorn-Zentrum Archaeometrie in Mannheim (MAMS). Dates were calibrated with the OxCal 4.4 software (Bronk Ramsey, 2009) using the IntCal20 curve (Reimer et al., 2020) and, in addition, the Marine20 curve (Heaton et al., 2020) for individuals that had clearly consumed marine protein. The estimation of the amount of marine protein consumed is based on calculations made for specimen S-EVA 8010 (40±10% marine) by Mannino *et al.* (Mannino et al., 2015). The specimens for which a correction was necessary are *UZZ44* and *UZZ45* (40±10% marine), *UZZ81* (45±10% marine), *UZZ69*, *UZZ79* and *UZZ80* (50±10% marine). Corrections were made using the reservoir correction estimated for the Mediterranean Basin by Reimer and McCormac (Reimer and McCormac, 2002), which is $\Delta R = 58 \pm 85$ ¹⁴C yr. (*) This specimen was recovered during excavations in 1976 in stratigraphic unit D within a small tunnel-like feature (*cunicolo*) excavated along the walls of *Grotta dell'Uzzo*, outside the named trenches.

Table S3. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values of bone collagen from human remains recovered at *Grotta dell'Uzzo* and previously published (Mannino et al., 2015), related to Figure 3.

S-EVA	Trench-Spit	Phase	Skeletal element	$\delta^{13}\text{C}$ [‰]	$\delta^{15}\text{N}$ [‰]	%C	%N	C/N	% yield
2777	H-rim	MESO1/1	humerus	-18.9	10.8	43.4	15.2	3.3	1.8
7991	Burial 1A	MESO1/2	rib	-20.3	9.9	43.1	14.1	3.6	2.1
15135	Burial 1B	MESO1/2	rib	-20.5	9.4	11.1	3.7	3.5	1
7998	Burial 5	MESO1/2	rib	-20.9	11.4	45.2	15	3.5	5.8
2758	Burial 7	MESO1/2	phalanx	-20.3	12	42.6	14.2	3.5	0.5
8000	Burial 8	MESO1/2	rib	-19.3	8.7	34.3	12.9	3.1	1.1
2771	Burial 11	MESO1/2	rib	-19.1	11.7	42.7	14.8	3.4	0.5
8014	Burial X	MESO1/2	ulna	-19.7	10.8	41.4	14.7	3.3	0.6
8773	Y-3	MESO1/2	cranium	-19.1	8.7	45.8	16.3	3.3	1
8012	F-16	MESO1/2	cranium	-19.7	9.7	38.8	13.8	3.3	0.8
8010	F-12	MESO2	cranium	-16.2	12.8	40.7	15.4	3.1	1.4
8013	F-9	NEO1/1	cranium	-18.7	8.4	43.4	15.9	3.2	3.2
8772	X-21	NEO1/1	metatarsal	-18.7	10.4	38.6	14	3.2	1.3
2778	W-2	NEO1/2	humerus	-19.4	10.5	41.1	14.1	3.4	1.3
2774	S-1	NEO1/2	femur	-19.7	8.4	43.1	15.2	3.3	1.3

Table S4. The admixture dates in Sicilian LM HGs, related to Figure 3.

Testpop	Overall (gen)	Jmean (gen)	SD (gen)	Average Age (yBP)	Age SD (year)	Admix date (yBP)	SD (year)	Note
Sicily_LM	19.8	19.6	4.9	8253	860	8822	1003	All LM HGs
Sicily_LM_1	12.1	12.0	3.8	8362	460	8704	570	LM Castelnovian
Sicily_LM_2	45.3	43.0	6.9	7870	170	9118	370	EN Impressa

The admixture date is calculated with a generation time of 29 years, and standard error is the sum of dating estimation and sample age SDs.