Article

High continuity of forager ancestry in the Neolithic period of the eastern Maghreb

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Ancient DNA from the Mediterranean region has revealed long-range connections and population transformations associated with the spread of food-producing economies[1–6]. However, in contrast to Europe, genetic data from this key transition in northern Africa are limited, and have only been available from the far western Maghreb (Morocco)[1–3]. Here we present genome-wide data for nine individuals from the Later Stone Age through the Neolithic period from Algeria and Tunisia. The earliest individuals cluster with pre-Neolithic people of the western Maghreb (around 15,000–7,600 years before present (BP)), showing that this 'Maghrebi' ancestry profile had a substantial geographic and temporal extent. At least one individual from Djebba (Tunisia), dating to around 8,000 years BP, harboured ancestry from European hunter–gatherers, probably reflecting movement in the Early Holocene across the Strait of Sicily. Later Neolithic people from the eastern Maghreb retained largely local forager ancestry, together with smaller contributions from European farmers (by around 7,000 years BP) and Levantine groups (by around 6,800 years BP), and were thus far less impacted by external gene flow than were populations in other parts of the Neolithic Mediterranean.

The genetic history of human populations in the Mediterranean basin includes contributions from many groups in both historic and prehistoric times[7,8]. Extensive ancient DNA research in Southern Europe has revealed a detailed chronicle of population transformations through time, but similar work in northern Africa has been much more limited. A key transformation was the beginning of food production during the Neolithic period[9]. On the European side of the Mediterranean, farmers with roots in Anatolia first expanded rapidly along the coast as far as Iberia around 7,500 years before present (BP)[10], absorbing 0–30% Western European hunter–gatherer (WHG) ancestry along the way[4–6]. Characteristic 'Cardial' pottery linked to Iberia and southern France has been found in the western Maghreb (Morocco), together with evidence of domestic plants and animals and other associated materials, but the demographic impact of European farmers in northern Africa has been debated[9,11]. Some have also argued that farmers could have crossed the Mediterranean from Sicily to the eastern Maghreb (Tunisia and northeastern Algeria) and expanded westward from there[12], although it is now more widely believed that Neolithic domesticates found in the western Maghreb were introduced from Iberia[2,13,14].

The first genome-wide ancient DNA data from Neolithic northern Africa came from the approximately 7,000-years-BP site of Ifri n'Amr o'Moussa (IAM) in the western Maghreb[1], where individuals were found to have ancestry derived from a 'Maghrebi' gene pool related to much earlier (15,000–14,000 years BP) Late Stone Age (LSA) individuals from the site of Taforalt (TAF) (Iberomaurusian culture)[3]. However, European farmer migrants had a large impact on closely neighbouring and nearly contemporaneous populations, contributing around 80% to the ancestry of individuals from the approximately 7,200-years-BP site of Kaf Taht el-Ghar (KTG)[2]. Within another millennium, a new component, related to Neolithic populations from the Levant and hypothesized to have been derived from an expansion of early pastoralist societies from southwestern Asia, also appeared, constituting as much as around 50% of the ancestry of individuals from the site of Skhirat-Rouazi (SKH) (around 6,400 years BP)[2]. All three components (Maghrebi, European

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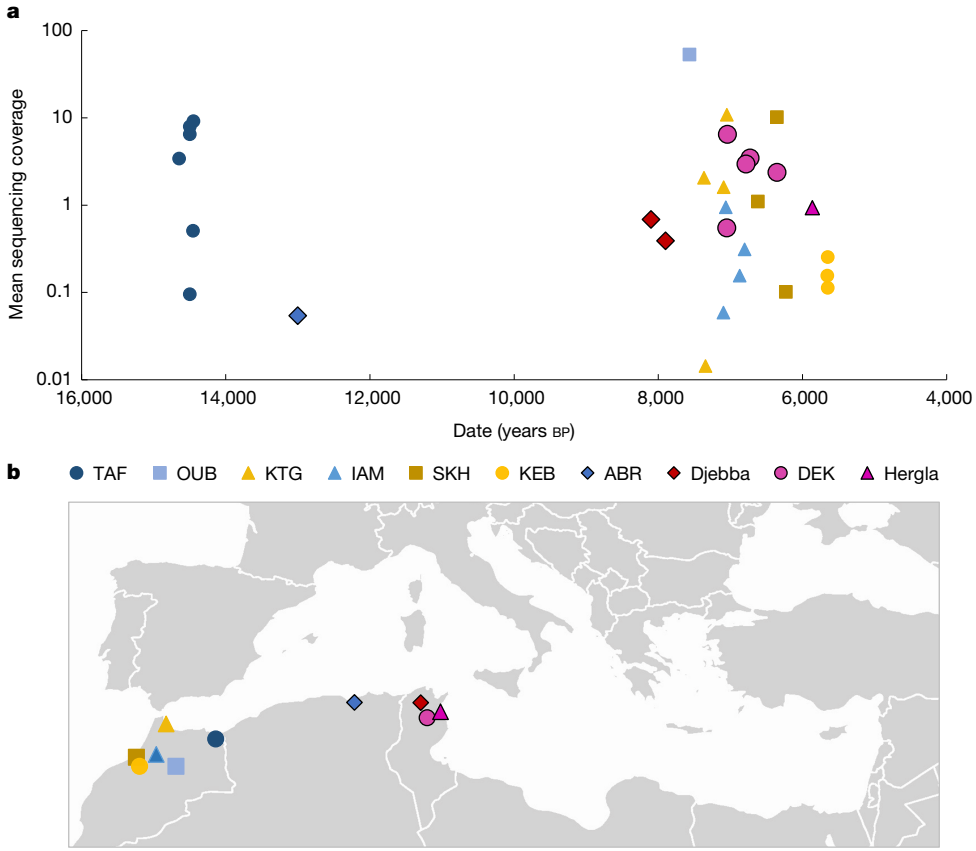

Fig. 1 | Summary of the dataset. a, Radiocarbon dates and sequencing coverage (see also Extended Data Table 1) (two undated individuals are estimates only) for previously published and newly reported (outlined in black) ancient individuals. Note the log scale on the *y* axis. Site abbreviations are defined in the text. **b**, Locations of the sites in present-day Tunisia, Algeria and Morocco. The map image was adapted from https://freevectormaps.com/.

farmer-related and Levantine) contributed to individuals from the Late Neolithic site of Kehf el Baroud (KEB) (around 5,700 years BP)[1,2].

An open question is whether the eastern and western Maghreb followed similar genetic trajectories during the Neolithic. In the east, archaeological evidence documents a distinct Early Holocene (pre-Neolithic) cultural tradition, the Capsian[15,16], following the Iberomaurusian. Capsian communities occupied large, open-air, shell-midden sites, with fewer rock shelters, and practiced hunting and gathering, focusing on terrestrial molluscs, large herbivores and wild plants. Cultural connections extended westward, eastward and even northward[9,13,15,17–20]. Although the subsequent Neolithization process incorporated domesticated animals (of probable Levantine origin), the people otherwise retained many elements of the Capsian economy[13,18]. At Doukanet el Khoutifa (DEK, Tunisia), for example, bones from domesticated caprines and (in lower numbers) cattle are present, especially after around 7,000 years BP, but domesticated plants found farther east and west in northern Africa are absent, and several elements reminiscent of the Capsian period remain (lithic technologies, ornaments, tooth avulsion and consumption of terrestrial molluscs)[9,13,18,21]. The pottery styles are mixed, with some but not all reminiscent of impressed designs found in other parts of the Mediterranean[9]. An analysis of dental morphological data using a mean measure of divergence has indicated that Neolithic Maghreb populations were most similar overall to contemporaneous groups from the northern Mediterranean coast, although some from the eastern Maghreb showed a high degree of affinity with either earlier Iberomaurusian populations or Natufian populations from the Levant[22].

We generated high-quality genome-wide data for eight individuals (seven with direct radiocarbon dates) from three sites in present-day Tunisia and one from Afalou Bou Rhummel (ABR) in Algeria (around 15,000–11,000 years BP), an Iberomaurusian site that mitochondrial DNA analysis showed had maternal affinity to the TAF people[23] (Fig. 1, Extended Data Figs. 1–3, Extended Data Tables 1–3 and Supplementary Table 1) (Methods). Authenticity metrics indicated minimal contamination (Supplementary Table 1), except for individual I13901 (ABR), for whom we restricted analysis to molecules with evidence of damage characteristic of ancient DNA (Methods). The sequencing coverage ranged from around 0.4 to 6.4×, measured at approximately 1.15 million single nucleotide polymorphisms (SNPs) on chromosomes 1–22 targeted for in-solution enrichment (0.05× for I13901 after damage restriction). Both individuals from Djebba (Tunisia) have earlier dates (around 8,000 years BP) than expected for a site assigned to the Neolithic[24,25]. We refer to these here as Late Capsian because they fall near the temporal boundary of the Capsian and the Neolithic (Methods). From DEK, we obtained dates for four individuals, ranging from around 7,000 to around 6,350 years BP. The one individual from Hergla (Tunisia) yielded a surprisingly recent date (around 5,900 years BP) for a site only known to be occupied during the Capsian (Methods)[26]. In fact, it appears that Hergla continued to be used by mid-Holocene groups (at least to bury their dead), a practice attested to at other Capsian sites[13,27]. We assessed the newly reported individuals together with published data to study the population history of the central portion of northern Africa before and during the Neolithic period and to trace connections to broader trends in the Mediterranean region at this time.

We carried out a principal component analysis (PCA), as described in ref. 2, using 33 present-day individuals from 16 populations[28] from the greater Mediterranean region to compute the axes and project the ancient individuals (Fig. 2). The newly reported ancient individuals fall within the same triangular region defined by previously published ancient individuals from the western Maghreb. The three

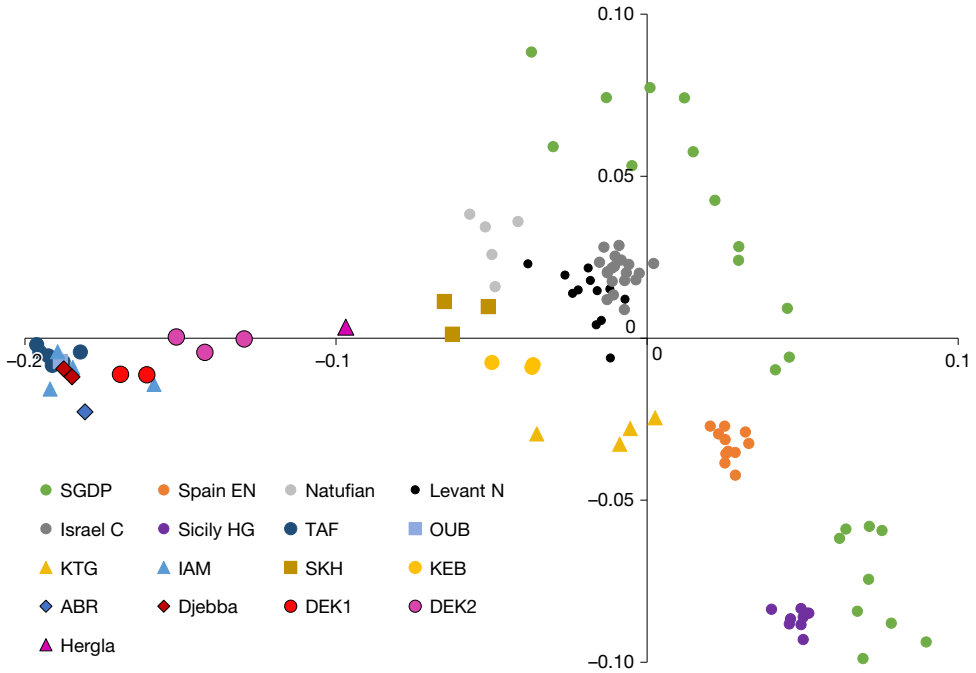

Fig. 2 | PCA results. PC1 is on the *x* axis and PC2 on the *y* axis. The symbols for the northern African ancient individuals follow those in Fig. 1 (with new data from this study outlined in black). Some present-day (Simons Genome Diversity Project) individuals fell outside the displayed axis range and were omitted for the sake of readability. N, Neolithic; EN, Early Neolithic; C, Chalcolithic; HG, hunter–gatherers. The other abbreviations are defined in the text.

earliest eastern Maghreb individuals—from ABR (I13901, older than 10,000 years BP) and Djebba (I20824 and I20825, from around 8,000 years BP)—clustered with people of the pre-Neolithic western Maghreb (TAF and Ifri Ouberrid (OUB)). By contrast, the later individuals from the eastern Maghreb lie on a cline between this early cluster and groups from the ancient Levant and the Neolithic western Maghreb sites of SKH and KEB.

We tested models of ancestry using qpAdm software[4]. We began by reanalysing published data from the western Maghreb (Fig. 3a and Supplementary Table 2), using individuals with no apparent recent admixture (either TAF or later individuals) as a proxy for Maghrebi ancestry, Early Neolithic individuals from Spain as a proxy for European farmer ancestry, Chalcolithic-period individuals from Israel as a proxy for Levantine ancestry, and a set of outgroups chosen to distinguish among these three components (Methods). Our results are similar to those previously reported[1,2], with the primary exception that we found evidence for a small amount of European farmer ancestry (although the power was not sufficient to rule out a different source) among the Early Neolithic individuals from IAM, who were previously described as having 100% Maghrebi ancestry. We inferred 9.6 ± 2.5% European farmer ancestry for IAM.7 (the farthest right of the four IAM individuals in the PCA) (Fig. 2) and 4.7 ± 2.0% for IAM004. These two individuals were slightly more recent (by around 200 years) than the others published from the site. Additionally, whereas the Middle Neolithic individuals from SKH were previously modelled as a two-way mixture of Maghrebi and Levantine ancestry, we found that such a model was insufficient ($P < 0.05$) (Methods), with signals of excess ancestry related to European farmers or to European hunter–gatherers (using reference individuals from Spain and Serbia, who derived most of their ancestry from the WHG gene pool). In fact, even a three-way model with Maghrebi, European farmer and Levantine ancestry failed to fit ($P = 0.006$). The results in Fig. 3a are based on a model in which we used Middle Neolithic farmers from Sardinia, who had more WHG ancestry than the Early Neolithic individuals from Spain (around 20% versus around 10%), as a proxy for European farmer ancestry ($P = 0.15$). Although we report a three-way model for KEB as well (as in previous work), we found weaker evidence for Levantine ancestry ($P = 0.044$ for a model with only Maghrebi and European farmer ancestry).

For the eastern Maghreb, we tested similar two- and three-way admixture models (Fig. 3b and Supplementary Table 2). As a proxy for Maghrebi ancestry, we used the OUB individual together with one from IAM (IAM.4.5). Individual I13901 (ABR, Algeria) fit with 100% Maghrebi ancestry ($P > 0.18$). We also repeated the analysis with the full (contaminated) data and obtained a nearly successful fit ($P = 0.023$) with Maghrebi ancestry plus a component representing contamination from a present-day European source (1000 Genomes Northern Europeans from Utah (CEU) as a proxy, proportion approximately 28%).

The two early individuals from Djebba clustered with ABR, TAF and related groups in the PCA. For both Djebba and ABR, we observed (through means of F_4 statistics) distinctive allele-sharing with Natufians, as previously reported for TAF[3], confirming a high degree of long-term genetic continuity leading up to the Neolithic (Supplementary Table 3). However, while one of the Djebba individuals (I20824, around 8,100 years BP) could be fit with 100% Maghrebi ancestry in qpAdm ($P = 0.1$), the second (I20825, around 7,900 years BP) had excess affinity with WHGs. The direct F_4-statistic allele-sharing tests were significantly non-zero at up to $Z = 5$ (Supplementary Table 3). This signal cannot be explained by adding European farmer ancestry (which includes a WHG-related component) in qpAdm ($P = 0.0002$ or 0.0001 using Spain EN or Sardinia N as a proxy). However, we were able to fit a model for I20825 with Maghrebi plus 5.7 ± 1.1% WHG-related ancestry ($P = 0.36$). For the two individuals as a pair (Fig. 3b), the proportion was lower (3.1 ± 0.8%, $P = 0.8$), and the evidence against models without WHG-related ancestry was weaker (although still marginally significant at $P = 0.02$–0.04).

We searched for allele-sharing signals that might indicate the possible source of the European hunter–gatherer-related ancestry at Djebba (Methods). Specifically, we computed statistics to detect differential relatedness to hunter–gatherers from Sicily (Epigravettian and Mesolithic[6,29]), Germany and the Netherlands ('northern WHG'[29]), Spain[29–33], Serbia[34] and Russia[29,31]. For I20825, we observed (marginally) significant signals of differentiation when comparing the Sicily hunter–gatherers

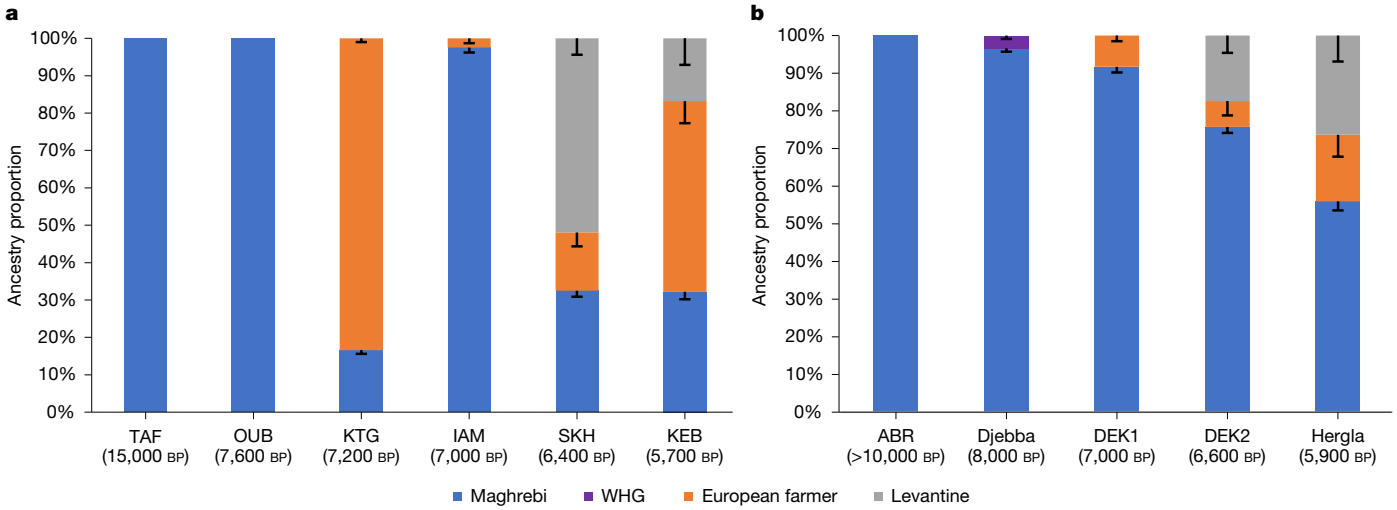

Fig. 3 | Inferred ancestry proportions from qpAdm. a,**b**, Results are shown for previously published ancient groups from the western Maghreb (**a**) and newly reported groups from the eastern Maghreb (**b**), with approximate average dates for each site. The values represent best-fitting model estimates, with bars showing one standard error for each ancestry component, based on a jackknife procedure over the $n = 1{,}150{,}452$ targeted autosomal SNP loci (Methods). For DEK2 and Hergla, the models were fitted with DEK1 or DEK2 (respectively) as one proxy source, but we show standard errors obtained from a direct three-way model with Maghrebi, European farmer and Levantine ancestry. Numbers of individuals pooled together per site (from left to right) are 1, 3, 4, 2 and 3 (**a**) and 1, 2, 2, 3 and 1 (**b**).

to those from Spain and Russia ($Z = 2.1$ and 2.3), lower differentiation from Serbia ($Z = 1.6$) and none between Sicily and the northern WHG ($Z = -0.1$) (Supplementary Table 3). By contrast, all statistics are consistent with zero ($|Z| < 1$) for I20824, despite lower standard errors. Given what is known about genetic structure in Mesolithic Europe[29]—including similar WHG ancestry across a wide geographic area—these observations were as expected for a very small proportion of WHG ancestry in I20825. We also used subsets of the European hunter–gatherer data to test for the possible presence of Maghrebi ancestry in Sicily, but did not observe any significant signals (maximum $Z < 1.7$ out of 24 statistics computed) (Supplementary Table 3).

Outside of Djebba, the highest proportion of Maghrebi ancestry in any individual was approximately 92% in I22580 (around 7,000 years BP from DEK), who could be fit with a mixture of Maghrebi and European farmer ancestry ($P = 0.18$), but not Maghrebi and Levantine ($P = 0.0046$) (Fig. 3b and Supplementary Table 2). The individual closest to I22580 in the PCA was I22862 (undated from DEK; I22580 and I22862 are labelled DEK1 in Fig. 2). Individual I22862 also had a high proportion of Maghrebi ancestry, but with a slightly higher affinity to WHG (best fit with approximately 88% Maghrebi, 10% farmer-related and 3% WHG ancestry, $P = 0.093$) (Supplementary Table 2). When we pooled I22580 and I22862 as DEK1, a two-way model with Maghrebi and European farmer ancestry was successful (respectively, approximately 92% and 8%, $P = 0.16$).

The other four Neolithic eastern Maghreb individuals—three from DEK (DEK2 in Fig. 2) and one from Hergla—fell farther to the right in the PCA. Individual I22866 (DEK) could be fit with Maghrebi plus either Levantine ($P = 0.76$) or European farmer ($P = 0.07$) ancestry (or both), while I22867 (DEK) could be fit with all three sources ($P = 0.6$) and nearly fit with only Maghrebi and European farmer ancestry ($P = 0.04$). Individual I22577 (DEK) fit best with all three sources, but remained sub-threshold ($P = 0.01$), and we observe a similar pattern for I22852 (Hergla) ($P = 0.0004$). We pooled together all three DEK2 individuals, resulting in successful fits with Maghrebi plus Levantine ancestry (approximately 76% and 24%, respectively, and $P = 0.14$), with all three sources (including approximately 9% European farmer ancestry, $P = 0.26$), or in a two-way model using the DEK1 subgroup as one source plus Levantine ancestry (approximately 83% and 17%, respectively, and $P = 0.23$), which is the model shown in Fig. 3b. For I22852 (Hergla), we also tested models with either the DEK1 or DEK2 subgroup as one source plus European farmer or Levantine ancestry. Several combinations had better fit quality than the three-way model above but were still slightly sub-threshold ($P \approx 0.01$–0.02), for example, DEK2 as one source plus additional European farmer and Levantine ancestry (approximately 74%, 13% and 14%, respectively) (Fig. 3b and Supplementary Table 2).

As with the WHG ancestry signal at Djebba, we performed additional analyses to investigate refinements to the qpAdm models for DEK and Hergla. First, we used F_4 statistics to compare allele-sharing between the Tunisia individuals and early farmers from Spain and Italy[6,35–38]. None of the statistics detected asymmetry (maximum $|Z| = 1.5$) (Supplementary Table 3). Second, for DEK2, we tested alternative models using earlier ancient Levantine groups (either Natufian[39,40] or Neolithic Levant[39–41]) as proxies in the DEK1-plus-Levantine model. Both had lower P values than the Chalcolithic Israel proxy source ($P = 0.048$ and 0.032 versus $P = 0.23$), despite less power to reject the model (that is, larger standard errors) (Supplementary Table 2). Third, we searched for signals of additional ancestry from the Saharan region using qpAdm experiments with present-day Fulani, Laka and Bulala populations[42] as outgroups, finding no significant violations of the baseline models (Methods) (Supplementary Table 4).

Uniparental markers are consistent with our genome-wide results in indicating a majority of Maghrebi ancestry among the newly reported individuals, with more admixture from other sources later in the transect. Of the five individuals assigned male at birth, four could be assigned to Y-chromosome haplogroup E1b1b1a1, which is characteristic of northern Africa, particularly in ancient individuals with Maghrebi ancestry[3,43]. The exception was I22852 (Hergla), who carried T1a1a, associated with Levantine farmers[39]. For mtDNA, the individuals from ABR and Djebba, as well as both individuals from the DEK1 subgroup and one from DEK2, carried subclades of U6, also known primarily from ancient northern Africa[3,44,45]. Haplogroup L3f1b + 16292 (I22867, DEK2) belongs to a clade hypothesized to have originated in eastern Africa and spread to other parts of the continent[46], while R0a2 (I22852, Hergla) has a wide distribution, but has also been observed in the Neolithic Levant[39,40]. Finally, individual I22866 (DEK2) carried mtDNA haplogroup U5b2b1, which is characteristic of pre-Neolithic Europe[47], and is probably derived from European hunter–gatherers, either directly (hunter–gatherers crossing the Strait of Sicily) or by means of WHG ancestry in European farmers.

We inferred admixture dates using admixture-induced linkage disequilibrium for evolutionary relationships (ALDER)[48] and distribution of ancestry tracts of evolutionary signals (DATES)[49]. As reference populations, we used TAF plus Early Neolithic individuals from Spain, Chalcolithic-period individuals from Israel or Mesolithic hunter–gatherers from Serbia (Methods). For IAM (western Maghreb), we inferred significant signals of admixture approximately 8–13 generations in the past (Supplementary Table 5), showing that our inference of European farmer ancestry in individual IAM.7 was not due to contamination (which would not generate a signal of admixture linkage disequilibrium). For Djebba (Tunisia), I20825 had a significant admixture signal (using the Serbia Mesolithic reference), with a date of 18.2 ± 3.0 generations before the individual lived from ALDER and 13.9 ± 6.5 from DATES. We obtained a weaker, but still significant, signal for I20824 with ALDER (16.3 ± 6.4 generations, amplitude $10 \pm 5.0 \times 10^{-4}$, compared to $28 \pm 5.7 \times 10^{-4}$ for I20825), raising the possibility that both individuals from Djebba had a small proportion of WHG-related ancestry. When we used the Spain farmer reference for the Djebba individuals, the signals became weaker (19.3 ± 6.5 generations, amplitude $17 \pm 3.9 \times 10^{-4}$ for I20825 with ALDER, not significant for I20824, and not significant for either individual with DATES, although we obtained a date of 20.2 ± 6.2 generations for I20825 using the Israel reference), consistent with a WHG-related, rather than European farmer-related, source. We obtained relatively recent dates for the five individuals from DEK (approximately 5–25 generations), indicating that admixture was occurring within a couple of hundred years of the time the individuals lived.

We used hapROH[50] software to infer runs of homozygosity (ROH) for the seven individuals for whom we had sufficient data (all except I13901 from ABR, and I20825 from Djebba) (Fig. 4a and Supplementary Table 6). The presence of ROH can reflect both small local population sizes and the close relatedness of an individual's parents. The longest ROH and largest ROH totals were found in the two DEK1 individuals (I22580 and I22862), whose distributions suggested second-cousin parents. The next-longest segments and next-largest total belonged to I20824 (Djebba), with a distribution consistent with a relatively small recent effective population size (N_e = 95%, confidence interval (CI) = 337–1,401). Among the three DEK2 individuals, we inferred only one ROH longer than four centimorgans (cM), yielding an estimated N_e of at least approximately 3,500. Individual I22852 (Hergla) had a small, but non-zero, ROH, with two segments between 4 and 5 cM (N_e = 95%, CI = 828–15,540). Overall, the ROH totals in the eastern Maghreb were lower than in the west (Extended Data Fig. 4), where Neolithic and pre-Neolithic individuals had extensive ROH and low ancestral population sizes, similar to Mesolithic Europe[2,29,50].

Finally, we used the software ancIBD[51] to search for segments of chromosomes shared identical by descent (IBD) due to recent shared ancestry between the ancient eastern Maghreb individuals (four with sufficient sequencing coverage, all from DEK) and other ancient individuals (Fig. 4b and Supplementary Table 7). The only long (>20 cM) segment was shared between I22580 from DEK1 and I22866 from DEK2, indicating at least some direct continuity at the site. We also found three medium-long segments (12–20 cM), indicating shared ancestry within around 2,000 years—I22867 and I22577 from DEK2 each shared one such segment with skh002 from SKH, and I22867 shared one with a Neolithic individual from France (who also shares a shorter segment with I22866). Of 45 shorter IBD segments (8–12 cM), all involved sharing between the DEK individuals and either ancient northern Africans (eight between DEK and skh002, one shared with TAF, and three shared within DEK) or ancient European farmers (around 8,000–4,000 years BP) (Fig. 4b and Supplementary Table 7).

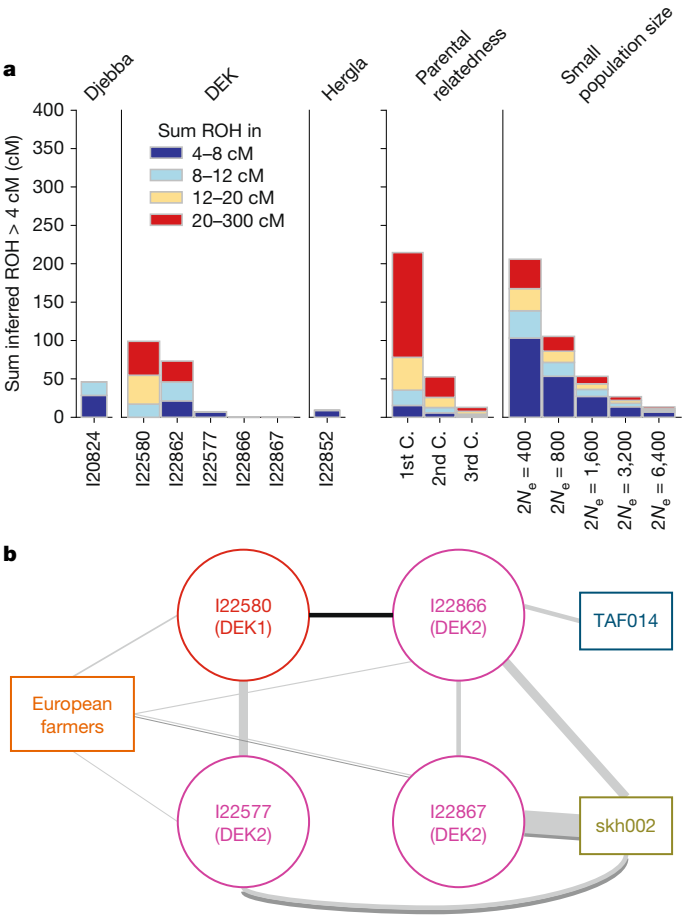

Fig. 4 | ROH and IBD results. a, Inferred ROH, with the left side showing the ROH for seven eastern Maghreb individuals, while the right shows expected distributions under different scenarios. C, cousin. **b**, Graph of inferred inter-individual IBD sharing for four of the eastern Maghreb individuals. The European farmer node represents an aggregate of 33 individuals. Darker lines indicate longer segments (black, >20 cM; dark grey, 12–20 cM; light grey, 8–12 cM), with the line thicknesses being proportional to the number of segments (normalized by a factor of 33 for sharing with European farmers).

Discussion

We have shown that while the eastern Maghreb experienced multiple instances of admixture during (and even before) the Neolithic, the proportions of ancestry involved were relatively small and populations maintained a high degree of local genetic continuity. These findings align with evidence from the eastern Maghreb also showing a distinctively high degree of continuity from an archaeological perspective[13].

The two sampled individuals from Djebba (Tunisia, Late Capsian) and one from ABR (Algeria, Iberomaurusian) share a similar genetic composition as contemporaneous groups from the western Maghreb[1–3], revealing that this 'Maghrebi' ancestry had a wide geographic and temporal extent. Unlike in the west, however, in the eastern Maghreb, we found evidence of admixture from Western European hunter–gatherers at Djebba, with the geographic proximity to Sicily suggesting a likely route. Shared technical innovations in material culture between these two regions, such as the pressure technique[13,20] that started in the eastern Maghreb from at least around 8,500–8,400 calibrated (cal.) years BP, and the movements of raw materials, such as the Pantellerian obsidian found in the eastern Maghreb from around 8,000 cal. years BP[9,13], document extensive seafaring across the Strait of Sicily during this period. These cultural interactions appear to have been accompanied by the movement of hunter–gatherers, at least from north to south, and possibly in both directions.

In the Neolithic, we observed at least a small proportion of European farmer-related ancestry at every site in both the western and

eastern Maghreb, including those where it was previously inferred to be absent[1,2]. However, whereas some individuals in the west had high proportions of farmer-related ancestry (specifically from KTG in the Early Neolithic, with more than 80%, and KEB in the Late Neolithic, with more than 50%), in the east, the maximum was less than 20%. These results are consistent with archaeological evidence for a greater influence from European farmers in the western than in the eastern Maghreb (where no Cardial pottery has been found, and the farming of domesticated crops only appears much later)[13,14,18]. In fact, from the perspective of the archaeological evidence, the presence of European farmer ancestry in the eastern Maghreb (based on both admixture modelling and IBD sharing) is perhaps more surprising than its low proportions relative to the west. Overall, our results support Iberia as the primary source for the migration of farmers from Europe to the Maghreb[2,13,14], although further research will be necessary to determine whether the farmer-related ancestry we observe in the eastern Maghreb arrived through northern Africa from the west or possibly through a separate crossing from Sicily in the north.

The second, later widespread admixture event documented by our analysis involved Levantine-related populations. In the western Maghreb, the first appearance of Levantine ancestry was at SKH (around 6,400 years BP, oldest single individual 6,730–6,500 cal. years BP)[2], while we found it slightly earlier in the eastern Maghreb, through I22867 and I22866 from DEK (6,888–6,678 and 6,828–6,662 cal. years BP, respectively). In the case of DEK, it is tempting to associate the (limited) European farmer admixture in the DEK1 genetic cluster with the earlier stage of occupation at the site (around 7,400–7,000 years BP) and Levantine admixture in the DEK2 genetic cluster with the later stage (around 7,000–6,300 years BP, with increased reliance on domesticated animals and more usage of typically 'Neolithic' pottery and stone tools)[9,13]. Although we did not observe any IBD segments shared between eastern Maghreb individuals and ancient individuals from the Levant, their absence from our dataset is plausibly due to a combination of both limited sampling in the Levant and larger effective population sizes in this region compared to European farmers, resulting in less extensive IBD. The archaeological record provides a possible correlate for the movement of people from the Levant in the form of domestic caprines (of probable Levantine origin), which were first recorded in the eastern portion of northern Africa by around 8,200 years BP and then spread westward[13,17,52,53]. Our observation of multiple shared IBD segments between individuals from DEK and SKH suggests that people carrying Levantine ancestry passed through the eastern Maghreb before reaching the western Maghreb. Future ancient DNA research could conceivably reveal that some individuals analysed here carried small proportions of ancestry related to as-yet-unsampled populations (that thereby might help refine models of the spread of any of their ancestry components). However, the good fits of our qpAdm models (with a range of African and Eurasian outgroups) (Methods) indicate that the formulations of these models are probably nearly complete.

Our results show that regional demographic trajectories were highly variable in Europe and northern Africa during the Neolithic transition. In Europe, virtually all populations traced most of their ancestry to early migrants from Anatolia, with smaller contributions from local hunter–gatherers, and similar admixture trajectories in different regions[35]. By contrast, data from the western[1,2] and eastern Maghreb show that northern Africa featured both more heterogeneity and more continuity of autochthonous ancestry after the arrival of migrants and the adoption of new lifestyles and technologies. Indeed, in the eastern Maghreb, the archaeological record of the Neolithic transition is more consistent with local continuity than in Europe[9,13]. Possible explanations for the more limited admixture in the eastern Maghreb are that local hunter–gatherer populations remained more stable and resilient than those across the Mediterranean during the approximately 8,200 years BP climatic cooling event, and also that the density of migrating farmers was lower than in Europe and the western Maghreb, perhaps because the region was less suitable for agriculture (which did not develop in the eastern Maghreb until much later)[13]. Larger population sizes in the eastern Maghreb are tentatively suggested by our ROH analysis and, combined with low numbers of migrants, could account for a less substantial dilution of local ancestry than in Europe or the western Maghreb. Overall, the insights from this study of a time and place previously unsampled in the ancient DNA literature highlight how much remains to be learned through interdisciplinary study of the human past.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-025-08699-4.

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Methods

Site descriptions

Afalou Bou Rhummel (Algeria). The ABR shelter is part of a vast karstic network located in the coastal region of the Babors Massif. This extends from east to west, starting from the Soummam Valley to the town of Jijel. The southern slope of the massif slopes down to the high plains of the Sétif region, while the northern slope, facing the sea, forms the cliffs that today make up the Kabyle corniche. These cliffs rise to a height of between 500 and 800 m. It is on this coastal slope, along the semicircle formed by the Gulf of Bédjaïa, that the ABR shelter opens.

The shelter overlooks the road, which runs between the massif and the sea, at a height of 40 m. It faces north-northeast and is 22 m wide and 10–12 m deep. The ceiling is convex and is pierced in the middle by a natural chimney that rises 10 m, its diameter reaching 3 m. This naturally formed chimney illuminates and ventilates the shelter.

The site was discovered by the geologist A. Ehermann in 1920, and in 1928, he began to excavate the site with Boule, Vaufrey, Reygasse and Arambourg. The survey revealed the presence of prehistoric industry, faunal remains and numerous human remains. Arambourg conducted three excavation campaigns (1928, 1929 and 1930)[54]. This research led to the discovery of a large Iberomaurusian (LSA) occupation.

A team from the Centre National de Recherches Préhistoriques, Anthropologiques et Historiques (CNRPAH), directed by Hachi, resumed research at the site and carried out several excavation campaigns between 1983 and 1993. They discovered abundant archaeological material and new burials in the same levels as those at Arambourg. From the different levels, a series of dates was obtained (Extended Data Table 2)[55–58].

Because the field archives from Arambourg were never found, the stratigraphic relationship between the two excavations has not been established with certainty. However, it has been speculated that the old level I is related to layer IV of the new (Hachi) stratigraphy and the old level III to the new layer X[55–58] (Extended Data Tables 2 and 3).

The funerary complex at ABR comprises two levels of burials (Extended Data Table 3). In the upper level, two multiple burials were discovered. The first, which we refer to as A and which includes the individual from which we successfully extracted DNA, housed 49 individuals, of which 39 were determined to be adults, one adolescent and nine younger children (Arambourg excavations 1928–1932)[59]. The second, which we refer to as B, functioned as a true collective burial, with an empty space and a collective grave (Hachi excavation 1983–1993). It housed eight individuals[58]. The lower level also yielded two burials. The first, labelled C (Arambourg excavations 1928–1932), housed primary individual H 28 (adult) together with individual H 16 (immature). The second, designated D/E, is a sepulchral unit containing two primary and individual deposits, H IX and H X[59,60].

The entire ABR collection from the Arambourg excavations is kept at the Institut de Paléontologie Humaine in Paris, except for a craniofacial block that remains at the CNRPAH in Algiers (Algeria). The material is in a very good general state of preservation, with little fragmentation. Most of the human remains are numbered according to the order in which they were found.

Djebba (Tunisia). The shelter at Djebba is located on the Goraa Plateau (900 m above sea level) in the Haut Tell (northwestern Tunisia) (Extended Data Fig. 1). The Goraa Plateau overhangs the perched village of Djebba, which is 6 km south of the city of Thibar (Beja, northwestern Tunisia), and overlooks the Medjerda plain. The huge shelter of Djebba, cut into the Eocene limestone of Goraa Mountain, is 150 m in length and 35 m in width.

The site was discovered by R. Vaufrey in 1927, who opened three trenches from the top to the bottom of the 'rammadiya' (ashy mound) deposits. Despite the poor archaeological material, Vaufrey categorized the shelter as Neolithic[24]. In 1978, J. Zoughlami (INP, Tunisia) opened a test trench in the middle of the shelter[61]. The material exhumed, mainly composed of lithic artifacts, includes microlithic elements and geometrics, among which trapezes are the most frequent[62,63].

In 2018, new fieldwork was undertaken at the site, led by co-author N. Aouadi (INP, Tunisia), and in the dolmen of the Goraa Plateau to understand differences and continuity in the occupations of the Goraa Mountain[64]. In the shelter of Djebba, five test trenches—SDJ1, SDJ2, SDJ3, SDJ4 and SDJ5—were opened in different areas. The site is a typical rammadiya deposit, consisting of a mound of ashes, burnt stones, land-snail shells, faunal remains, lithic artifacts, *Unio* shells, pottery and ostrich beads. The faunal remains belong to wild taxa, such as gazelles and aurochs, and domestic taxa, such as caprines and cattle.

In SDJ2, human remains from two individuals were uncovered. Skeleton 2 (I20824), from the lower part of the sequence (SU6b), showed avulsion of both maxillary and mandibular central incisors, as well as one of the two mandibular lateral incisors. This individual has been dated to approximately 8,200–8,000 cal. years BP (Extended Data Table 1 and Supplementary Table 1). Skeleton 1 (I20825), from SU4, was dated to 8,000–7,800 cal. years BP. Given the lack of a confirmed stratigraphic association between the two individuals and the domestic faunal remains, it is plausible that they belong to an earlier phase of occupation, potentially dating to the Late Capsian period or situated near the transitional boundary between the Capsian and Neolithic.

Doukanet el Khoutifa (Tunisia). Doukanet el Khoutifa is a Neolithic site located on a series of superposed terraces about 700 m above sea level along the El Garia crest of the Tunisian Ridge. The upper and main terraces occupy a platform of approximately 2,200 m^2.

All the occupation layers have been attributed to the Neolithic. The first series of radiocarbon dates assigned the sequence to a period ranging from around 7,500–6,700 years BP. New dating, done directly on the skeletons analysed in the present research, confirms this previous data and places the cemetery in the same time frame (Extended Data Table 1).

The site was mentioned for the first time by L. Balout in 1955[65], and J. Zoughlami later explored it in 1972–1973 and 1976[61,62], excavating four trenches, which revealed the presence of a structured village and a cemetery organized around a massive rock at the centre of the terrace. The resumption of the archaeological investigations in 2013[66] saw the creation of a new topographical plan (Extended Data Fig. 2), the opening of a survey in the eastern quadrant of the site of approximately 8 × 5 m, realized to understand the structures and spatial layout of the site (sondage 1), and further investigations in the area identified as the cemetery (sondages 2–3–4).

Field activities at the site were carried out in 2013, 2018 and 2022, with the excavation of new trenches, to better clarify the chrono-cultural sequence and occupational patterns, and verify the spatial organization between the living and burial areas. Analysis of faunal and floral remains confirmed the presence of domestic animals, among which were cattle and caprines, but did not reveal the presence of domesticated plants[9].

From the original excavations in 1972–1973 and 1976[61,62] in the area corresponding to sector 2 of the excavation, the minimum number (calculated on the basis of teeth) of individuals buried is 18, with the post-cranial bones attributed with certainty to 10 of these[62,67]. It was possible to distinguish two infants, three juveniles, 12 adults and one older adult individual. Determination of the sex of the morphological adults is more complex due to the fragmentary nature of skeletal remains. However, 60% of the skeletal remains have been attributed to individuals assigned male at birth and 30% to individuals assigned female at birth[62]. The positions of the heads were generally towards the north. In some cases, a circle of stone was documented around the deposition, which could be interpreted as part of the burial[62,67]. A further four individuals were identified and partially studied during the 2013 excavations, and then fully exhumed during the 2018 and 2022 excavations.

Hergla (Tunisia). Hergla (SHM-1) is a Late Capsian open-air site located in the Hammamet Gulf, on the eastern Tunisian coast[26]. It occupies an Early Holocene hydro-aeolian dune formed during an arid episode, on the western edge of the Halk el Menjel sabkha–lagoon, about 3 m above the current sabkha (salt flat).

The site was discovered by E.G. Gobert in 1954, and was first excavated between 1969 and 1971 by M. Harbi-Riahi and J. Zoughlami[68]. Seven new trenches, covering a total of 110 m^2, were excavated between 2002 and 2007 within the framework of a joint Italian–Tunisian scientific agreement (University of Bologna, Istituto Italiano per l'Africa e l'Oriente of Rome and Institut National du Patrimoine of Tunis).

The geomorphological and pollen analysis carried out on the sediments of the site and around the sabkha allowed reconstruction of the climatic evolution and local environmental changes. The sabkha–lagoon system was fed by an impressive river supply and characterized by the presence of abundant fishing resources.

The micromorphological analysis of the sediments highlighted an uninterrupted anthropogenic sequence, confirming the continuity of the occupations and the sedentary lifestyle of the groups living in the area.

The site consists of seven occupation layers that can be divided into two main phases. The first phase, from layers 1 to 4, is dated to around 9,000–8,000 years BP, and the second, from layers 5 to 7, is dated to around 8,000–7,500 years BP. Numerous remains of buildings are present in all the occupation layers—storage structures, cooking features, fireplaces, remnants of walls and possibly post holes.

Archaeological analysis has suggested that the economy was largely based on hunting, fishing and gathering activities. No remains of domestic fauna were recognized, but a significant variability of exploited species were observed, including gazelle, giraffe, rhinoceros, wild boar, small mammals and birds. During the second phase of occupation, there is evidence of the intensive exploitation of bovids, including *Bos primigenius*, *Alcelaphus buselaphus*, *Gazella dorcas* and *Gazella cuvieri*. Domestic plants are absent. The site also revealed the presence of pottery starting after around 8,000 years BP. The decorative motifs recall the impressed ceramics that developed at the same time in the central Mediterranean. In the second phase of occupation, the site also yielded a number of artifacts manufactured with obsidian from Pantelleria.

Two primary burials have been identified at the site—burial 1 (lacking a skull) and burial 2 (skull present), as well as fragments bringing the total to at least four individuals—a 4–6-year-old child, a 1–2-year-old child, a rather robust adult (burial 1) and a rather frail adult (burial 2)[69,70]. The grave filling of burial 2—the individual successfully sampled for ancient DNA for this study (Extended Data Fig. 3)—is dated on a mollusc (*Cerastoderma glaucum*) as 8,269–7,762 cal. years BP (7,595 ± 80 years BP, Pa-2471). However, the direct dating of the petrous bone used for ancient DNA analysis is 5,985–5,754 cal. years BP (5,130 ± 25 years BP, PSUAMS-9396), a difference too marked to be a marine reservoir effect. The genetic analysis also indicates admixture with people having European farmer- and Levantine-related ancestry, providing independent support for a later date. These results suggest an alternative scenario in which the site continued to be used in post-Capsian times, at least as a place for Neolithic people to bury their dead. This practice is attested at other Capsian sites in the eastern Maghreb[27]. Earlier exploration of the site had already hinted at possible activity during the mid-Holocene. A carbon-14 (^{14}C) date obtained from a sample of *Cardium* shell yielded a date of 6,400–5,750 cal. years BP (5,320 ± 150 years BP), close to the one obtained from the burial 2 individual[71].

Ancient DNA data preparation

Ancient human skeletal samples (either cochlear portions of petrous bone or teeth, the cochleae isolated from the petrous bone by sandblasting, followed by milling) (Supplementary Table 1) were drilled for powder in dedicated clean rooms in Vienna, Austria, with standard protocols to minimize possible contamination. We extracted DNA in Boston, MA, USA using a robotic procedure with silica beads[72], also in clean-room facilities. From the extracts, we built sequencing libraries in the presence of uracil-DNA glycosylase (UDG) to reduce errors induced by DNA degradation, with either a double-stranded (partial UDG)[73] or single-stranded (USER)[74] preparation (Supplementary Table 1). We used in-solution hybridization to enrich for molecules overlapping a set of approximately 1.2 million targeted SNPs in the nuclear genome, together with the mitochondrial genome[75]. We added 7- or 8-bp indexing barcodes[76] and sequenced the libraries in pools on either NextSeq 500 or HiSeq X10 sequencing platforms with 76- or 101-bp paired-end reads.

We assigned the raw sequences to individual libraries based on their barcodes and indices, allowing a maximum of one mismatch. We also merged sequences overlapping by at least 15 bases (with at most one mismatch) using a modified version of SeqPrep v.1.1 (https://github.com/jstjohn/SeqPrep), retaining the allele call from the base with the higher-quality score. After trimming the barcodes and adaptors, we aligned the reads to the mitochondrial reference genome Reconstructed Sapiens Reference Sequence[77] and the human reference genome (version hg19), using the samse command in the Burrows–Wheeler Aligner[78]. We removed duplicate molecules as well as sequences with mapping quality less than 10 (for nuclear DNA) or 30 (for mitochondrial DNA). Finally, we trimmed two bases on each end of aligned sequences to further reduce potential damage artifacts.

For the data used in the analyses, we called 'pseudo-haploid' genotypes by selecting one allele at random from the sequences covering each targeted SNP (with a base quality score of at least 20, sites with no data being marked as missing). We determined the genetic sex of each individual based on counts of sequences aligning to the X and Y chromosomes[79]. We determined Y-chromosome haplogroups using a previously published method[40], based on the YFull YTree phylogeny v.8.09 (https://www.yfull.com/tree/), with SNPs from the International Society of Genetic Genealogy's generic genome browser YBrowse (https://ybrowse.org/). Finally, we determined mitochondrial DNA haplogroups using HaploGrep v.2.1.1 (ref. 80), based on all aligned sequences.

Quality control

We used several methods to search for possible evidence of contamination in the ancient DNA data (Supplementary Table 1). First, we computed the rate of apparent cytosine-to-thymine substitutions in the last position of sequenced molecules (before trimming), with authentic ancient DNA expected to show evidence of such substitutions due to deamination damage. Next, we ensured that all individuals had appropriate sex chromosome sequence ratios (proportion of Y chromosome either less than 0.03 for female-assigned individuals or more than 0.35 for male-assigned individuals). Finally, we used two approaches based on observed variation at haploid genome sites (where there should in fact be no variation within a single individual): (1) for mitochondrial DNA, we evaluated mismatch rates using contamMix v.1.0.1051 (ref. 81); and (2) for the X chromosome (in male-assigned individuals only), we evaluated mismatch rates using analysis of next generation sequencing data (ANGSD) software[82]. For individual I13901 (ABR), both contamMix and ANGSD indicated the presence of substantial contamination, and thus we only used data for our main analyses from molecules with ancient DNA damage. The contamination estimate reported here for the damage-restricted data is based on mismatch rates from contamMix (coverage was too low to apply ANGSD).

From the published western Maghreb data, we excluded one individual from SKH (skh003) from our qpAdm analysis because of evidence of contamination, and one each from KTG (ktg001) and IAM (IAM.3) from all analyses because of low coverage (less than 0.02×).

Radiocarbon dating

We sent samples of the same skeletal elements used for the ancient DNA analysis for accelerator mass spectrometry (AMS) radiocarbon dating

(using standard methods) at either the Pennsylvania State University (PSU) Radiocarbon Laboratory[83,84] or the University of Georgia (UG) Center for Applied Isotope Studies Radiocarbon Dating Laboratory[85]. We calibrated the dates using OxCal v.4.4 (ref. 86) and the IntCal20 calibration curve[87]. Two dates were discarded due to suspicion of surface contamination on the samples (no ultrafiltration was applied in the UGAMS laboratory). First, a date of 5,030 ± 35 years BP (uncalibrated) (UGAMS-72041) for I22852 (Hergla) was (modestly) inconsistent with our date of 5,130 ± 25 years BP (PSUAMS-9396) ($P = 0.02$), although this did not challenge our assignment of this individual to the Neolithic. Second, a date of 5,317–4,970 cal. years BP (4,500 ± 55 years BP, UGAMS-72040) for I22862 (DEK) was more recent than any known occupation at the site, and the uncertainty (standard error) of the measurement was unusually large. Genetic analysis also did not provide any reason to expect such a recent date.

Statistical analyses

We performed PCA using smartpca v.18270 software[88], with the options lsqproject and shrinkmode for projecting ancient individuals onto the axes determined from present-day individuals. Allele-sharing statistics (F_4 statistics) (Supplementary Table 3) were computed using ADMIXTOOLS software (qpDstat v.1152) with the f4mode option[89]. Standard errors were from a jackknife procedure, with units consisting of blocks of length 5 cM. For statistics testing the relative allele-sharing between Djebba and hunter–gatherers from different parts of Europe, we computed F_4(X, OUB + IAM; Sicily HG, Y), where X is one of the two Djebba individuals and Y is another hunter–gatherer group. To test for signals of Maghrebi ancestry in Sicily, we computed F_4(Sicily HG, Northern WHG; Maghrebi, Outgroup), where 'Maghrebi' was either Djebba or OUB + IAM (one individual from each of OUB and IAM with approximately 100% Maghrebi ancestry) and Sicily HG was either six individuals from Epigravettian contexts, three individuals from Mesolithic contexts or all nine individuals. Finally, to compare possible sources of European farmer ancestry, we used statistics of the form F_4(Tunisia, OUB + IAM; Spain EN, Italy N), for five different groupings of the ancient Tunisia individuals and three different Neolithic farmer groups from Italy (Sicily EN[6], Sicily MN[6,37] and Sardinia MN[37,38]).

The qpAdm software[4] estimates ancestry proportions for an admixed test individual or group of individuals, based on an input set of proxy sources and outgroups (the test population plus sources make up the 'left' set, while the outgroups are the 'right' set). The underlying model does not assume that the proxy sources are the exact source populations for the ancestry in the test group, but rather that each component of ancestry in the test group forms a clade with its corresponding proxy source with respect to the outgroups provided. The software returns the inferred proportions of ancestry related to each of the proxy sources, with standard errors (computed as for F_4 statistics, above), as well as a P value for overall model fit. Low P values indicate that the model is violated, typically because one (or more) of the left populations has some un-modelled ancestry related to one or more of the outgroup populations.

Our left population list consisted of the test individual(s) plus proxy sources—either two or three from the set of TAF, OUB + IAM (see above), Spain Early Neolithic[31,35,36], Sicily hunter–gatherers[29], Spain Mesolithic[30,31,33], Sardinia Middle Neolithic[37,38], Israel Chalcolithic[90], and other Neolithic groups from northern Africa. As right outgroups, we used all of the populations from the following set, provided that they were not present in the left list for a given analysis: Spain Early Neolithic; Spain Mesolithic; Sardinia Middle Neolithic; Israel Chalcolithic; Serbia Mesolithic[34]; Turkey Neolithic[31,40]; Iran Neolithic[39,91]; Kenya Pastoral Neolithic[92]; and Cameroon Stone-to-Metal Age[93]. We used a threshold of $P > 0.05$ for considering a model to be successful. We chose this set of outgroups with the goal of helping to constrain the tested models of Maghrebi plus western Eurasian-related ancestry, as well as to avoid potential confounding from different methods of data generation by using only UDG-treated target-capture data in the outgroup set. As noted above, the success ($P > 0.05$) of a given model does not mean that the proxy sources should be considered as exact representatives for the respective ancestry components in the test group, only that the proxy sources are more closely related (to within our statistical power) to the true sources than are the outgroups.

We performed one special set of qpAdm analyses in which we used present-day Fulani, Laka and Bulala populations as outgroups to search for possible hints of shared ancestry between the ancient Tunisia individuals (DEK and Hergla) and groups from farther south. Our strategy was to compare baseline qpAdm models with our standard outgroup sets to augmented models to which we added any one of the three present-day populations. To reduce batch effect artifacts (given the addition of shotgun-sequenced present-day genomes among the outgroups, as mentioned above), we used Djebba as our proxy source for Maghrebi ancestry (thus making the data types uniform for the left population set), with the full baseline models being: (1) Maghrebi plus European farmer ancestry for DEK1; (2) Maghrebi plus Levantine ancestry for DEK2; and (3) all three sources for Hergla.

We estimated dates of admixture using both ALDER[48] and DATES[49]. At least two individuals in the test population were required for ALDER, so to obtain dates for single individuals, we formed pairs consisting of the individual of interest plus the Moroccan OUB individual (who, with approximately 100% Maghrebi ancestry, should not have recent admixture linkage disequilibrium). For DATES, we ran the program in both affine and non-affine modes (allowing or not allowing a non-zero asymptote for the decay of admixture linkage disequilibrium with distance). We report the results in units of generations in the past. For ALDER, we also report the inferred amplitude of the decay curve. We consider results to be significant at a threshold of $P < 0.05$—that is, $|Z| > 1.96$ (for ALDER, including both the date of admixture and the amplitude).

ROH and IBD detection

We used the software hapROH[50] to infer ROH from the genome-wide data, with default settings, using the 1000 Genomes haplotype reference panel. As a minimum coverage level, we applied the recommended threshold of more than 400,000 SNPs covered from the core approximately 1.15 million target set, and we inferred ROH with a minimum length of 4 cM. To convert ROH distributions to estimates of effective population size (in the absence of apparent familial relatedness between parents), we used a maximum likelihood approach[94], based on inferred ROH segments between 4 and 8 cM. The reported 95% CIs were based on a grid of log-likelihood scores. For DEK2, the upper bound was effectively infinite.

To infer segments of shared IBD between individuals, we used the software ancIBD[51], again with default settings and the 1000 Genomes haplotype reference panel. Genotype probabilities were imputed from genotype likelihoods imputation and phasing (GLIMPSE)[95], as previously described[51]. We set a minimum of 8 cM for IBD detection, with the recommended individual-level quality threshold (at least 70% of imputed SNPs on chromosome 3 imputed with maximum genotype probability greater than 0.99), together with a minimum coverage threshold of 400,000 SNPs. We tested for sharing between the (four) individuals from this study meeting the thresholds, and all of the published ancient individuals from the Allen Ancient DNA Resource v.54.1 (ref. 96), augmented with additional published western Maghreb individuals[2].

Ethics, inclusion and permissions

We honour the ancient individuals whose remains we study, the present-day people from Tunisia and Algeria whose cultural heritage these remains are, and people of North Africa, including co-authors, who have contributed in key ways to this work. Human remains from

Djebba, Tunisia, were excavated under INP authorization (2018), directed by N.A. Human remains from DEK and Hergla were collected within the framework of the Northern Tunisia Archaeological Project, under three scientific cooperation agreements: (1) between INP, CNR-ISPC and ISMEO (the International Association for Mediterranean and Oriental Studies) (2021–2024), co-direction by N.A., L.A., L.B., A.C. and G.L.; (2) between INP, Sapienza University of Rome and the University of Bologna (2012–2018), co-directed by L.B., A.C. and S. Mulazzani; and (3) between INP and the University of Bologna (2002–2007), co-directed by S. Mulazzani and R. Boussoffara. The two individuals (H1 and H2) from ABR sampled for this study were held at the Institut de Paléontologie Humaine (IPH) in Paris. Sampling was conducted at the IPH in 2016 by R.P., with permission provided by IPH Director H.d.L.

Reporting summary

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

Data availability

The aligned sequences for the newly reported individuals are available through the European Nucleotide Archive under accession number PRJEB83667. Genotype data used in the analysis of the newly reported individuals are available through Dataverse (https://doi.org/10.7910/DVN/ILWB3K). The previously published data used in our analyses are available at the following: Allen Ancient DNA Resource (https://doi.org/10.7910/DVN/FFIDCW); western Maghreb ancient DNA data[2], European Nucleotide Archive accession number PRJEB59008); 1000 Genomes haplotype reference panel (http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/); human reference genome hg19 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.13/); mitochondrial reference genome RSRS (https://doi.org/10.1016/j.ajhg.2012.03.002); YFull YTree phylogeny (https://www.yfull.com/tree/); and ISOGG Y-chromosome SNPs (https://ybrowse.org/). Open-science principles require making all data used to support the conclusions of a study maximally available, and we support these principles here by making fully publicly available not only the digital copies of the molecules (the uploaded sequences), but also the molecular copies (the ancient DNA libraries themselves, which constitute molecular data storage). Those researchers who wish to carry out deeper sequencing of libraries published in this study should make a request to corresponding author D.R. We commit to granting reasonable requests as long as the libraries remain preserved in our laboratories, with no requirement that we be included as collaborators or co-authors on any resulting publications.

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Author contributions A.C., R.P. and D.R. designed the study. O.C., S.M., N.R., R.P. and D.R. generated the ancient DNA data. G.L., N.A., L.A., L.B., A.-R.D., F.G., F.L.P, M. Lucci, H.d.L.,

A.N., A.C. and R.P. were responsible for archaeological and bioanthropological analysis. G.L., N.A., L.A., A.-R.D., F.G., F.L.P., N.M. and F.T. excavated the sites. M. Lipson and H.R. conducted the formal analysis. M. Lipson, H.R., G.L. and A.C. wrote the original manuscript. M. Lipson, G.L., A.C., R.P. and D.R. reviewed and edited the paper. G.L., S.M., N.R., A.C., R.P. and D.R. supervised the study. G.L., A.C., R.P. and D.R. were responsible for funding acquisition.

Competing interests The authors declare no competing interests.

Additional information

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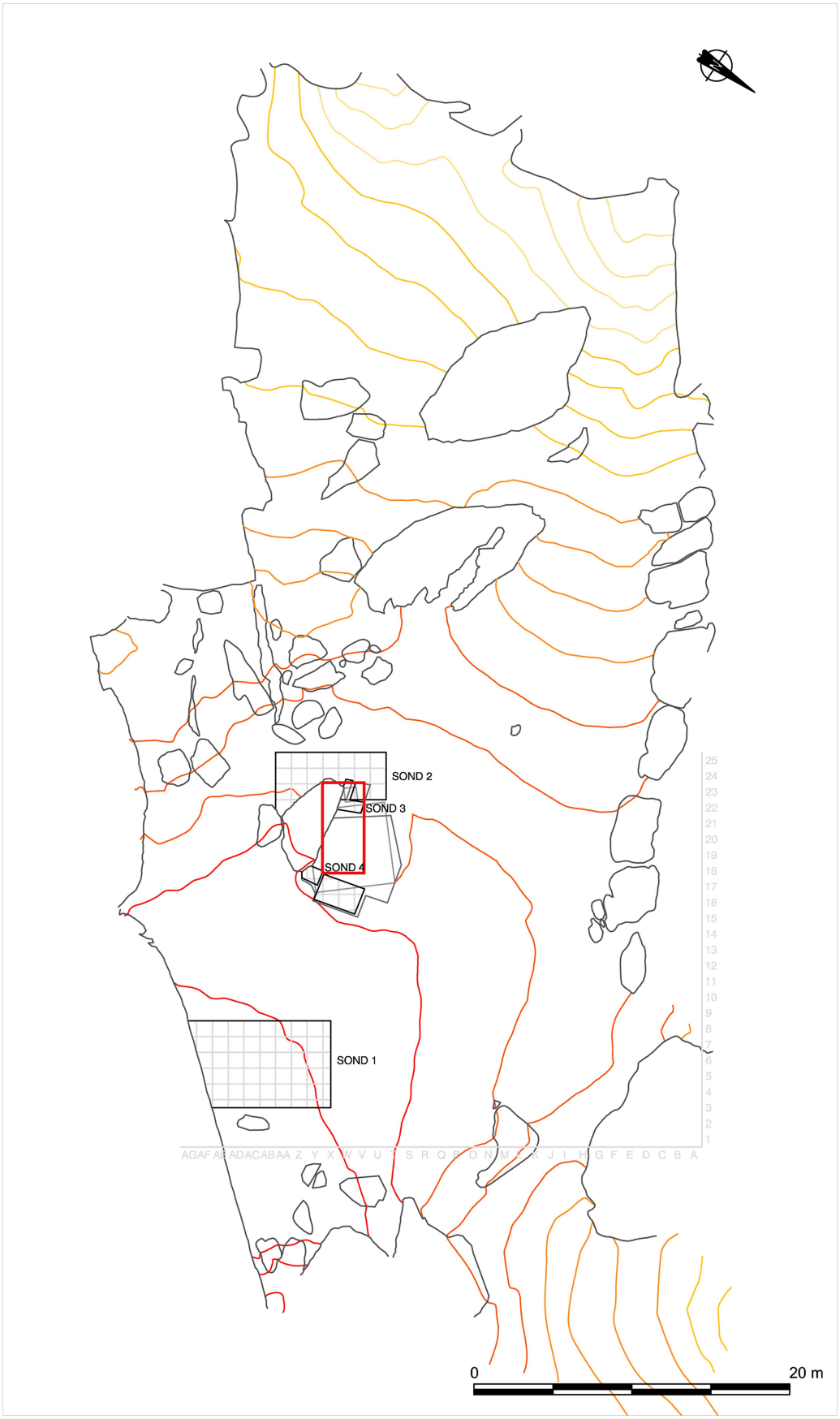
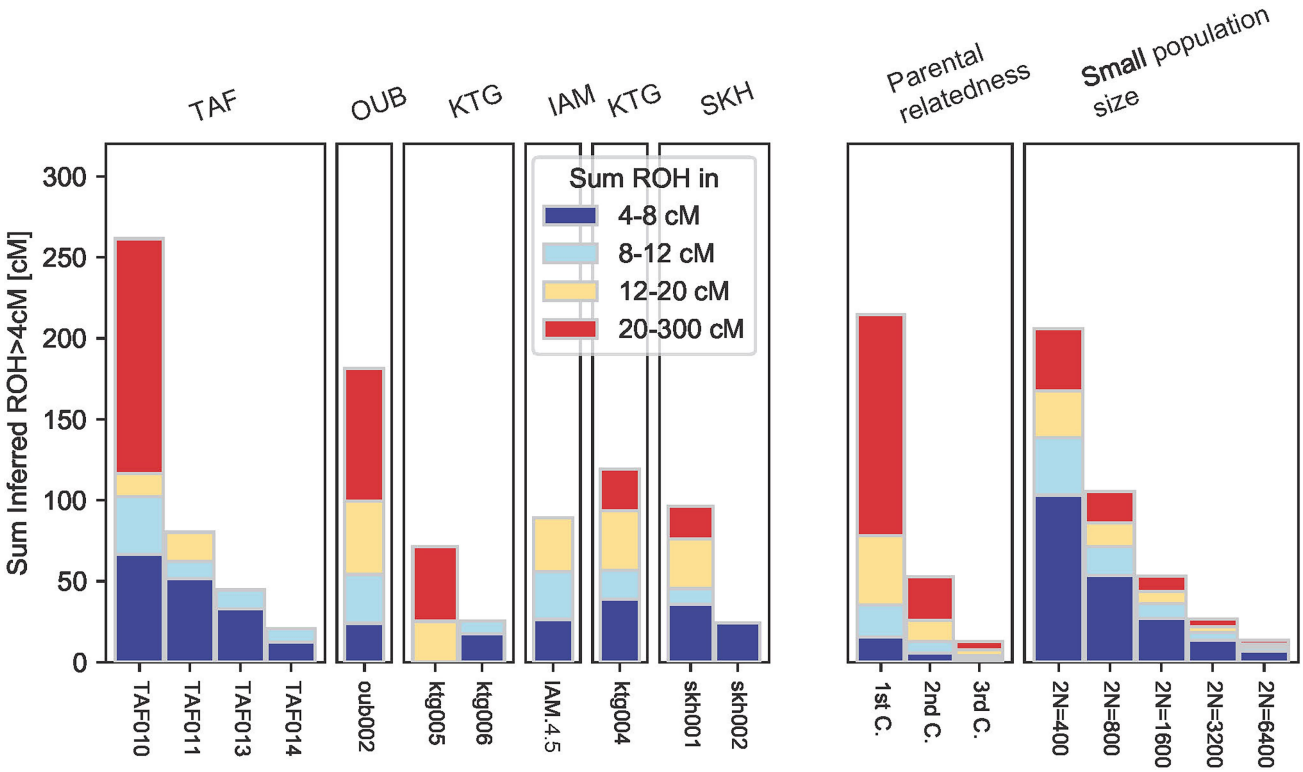
Extended Data Fig. 1 | View of the Djebba Shelter. Shown is an exterior view of the site of the excavations at Djebba (Tunisia).



Extended Data Fig. 2 | Site plan from Doukanet el Khoutifa (DEK). Shown is a diagram of the excavations from 2013, with the cemetery area indicated with a red rectangle.

Extended Data Fig. 3 | Burial 2 from Hergla (SHM-1). Shown is the skeleton of the individual sampled for ancient DNA in this study.



Extended Data Fig. 4 | Comparative ROH results for the western Maghreb. Left side, inferred runs of homozygosity (ROH) for ancient individuals from the western Maghreb; right side, expected distributions under different scenarios. Three individuals have signatures of likely parental relatedness: TAF010 and oub002 (first-cousin parents), and ktg005 (second-cousin parents).

Extended Data Table 1 | Radiocarbon dates and uniparental markers for newly reported ancient individuals

IID	Site	C14 Date	mtDNA	Y Haplogr.	Seq. Cov.
I13901	ABR	N/A (>10000 BP)	U6a6	E1b1b1a1	0.05
I20824	Djebba	8178-8026 cal BP	U6a3+185	Female	0.68
I20825	Djebba	7971-7800 cal BP	U6a	E1b1b1a1	0.39
I22580	DEK (1)	7161-6906 cal BP	U6d	Female	6.43
I22862	DEK (1)	N/A (~7050 BP)	U6d	Female	0.55
I22867	DEK (2)	6888-6678 cal BP	L3f1b+16292	E1b1b1a1	2.94
I22866	DEK (2)	6828-6662 cal BP	U5b2b1	Female	3.44
I22577	DEK (2)	6400-6305 cal BP	U6b	E1b1b1a1	2.36
I22852	Hergla	5985-5754 cal BP	R0a2	T1a1a	0.93

DEK subgroups 1 and 2 are defined from genetic analysis (see main text). For two undated individuals ("N/A"), we use estimates based on genetics and archaeological context in comparison to existing dates. mtDNA, mitochondrial DNA; Haplogr., haplogroup; Seq. Cov., mean sequencing coverage (on autosomal target sites; note coverage for I13901 is after damage-restriction).

Extended Data Table 2 | Radiocarbon dating of the different levels of the Afalou Bou Rhummel shelter

Arambourg stratigraphic level	Hachi stratigraphic layer	Lab code	Date (C14 BP)	Date (Cal BP)
I	III	Ly 3227	11450 ± 230	13580-13106
I	IV	Gif 6532	12020 ± 170	14093-13675
		Ly 3228	12400 ± 230	14907-14097
		Alger 0008	13120 ± 370	16532-15223
III	X	Gif 9637	14910 ± 180	18496-17960

Results are from refs. 55,57, with hypothesized correspondences added between layers from the Arambourg and Hachi excavations (see Methods).

Extended Data Table 3 | Distribution and types of burials at the Afalou Bou Rhummel site

Type of burial	Number of individuals	Excavation	Level of appearance	New denomination
Plural burials	49	Arambourg, (1928-1930)	Level I (Arambourg)	Burial A
Collective burials	8	Hachi (1983-1993)	Level I (Arambourg), layer IV (Hachi)	Burial B
Double burials (H 28, H 16)	2	Arambourg, (1928-1930)	level III (Arambourg)	Burial C
Individual burial (H IX)	1	Hachi (1983-1993)	Layer X (Hachi), level III (Arambourg)	Burial D
Individual burial (H X)	1	Hachi (1983-1993)	Layer X (Hachi), level III (Arambourg)	Burial E

Hypothesized correspondences are added between layers from the Arambourg and Hachi excavations (see Methods).

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Data collection	BWA v0.6.1, HaploGrep v2.1.1, SeqPrep v. 1.1, contamMix v1.0.1051, ANGSD v0.923, OxCal v4.4
Data analysis	ADMIXTOOLS (qpDstat v. 1152), smartpca v. 18270, qpAdm v. 2050, ALDER v. 1.0.3, DATES v. 4555, hapROH v. 1.0, ancIBD v. 1.0, GLIMPSE v. 1.1.1

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The aligned sequences for the newly reported individuals are available through the European Nucleotide Archive under accession number PRJEB83667. Genotype data used in analysis for the newly reported individuals are available through Dataverse (doi: 10.7910/DVN/ILWB3K). Previously published data used in our analyses are available as follows: Allen Ancient DNA Resource (doi: 10.7910/DVN/FFIDCW), western Maghreb ancient DNA data (ref. [2]; European Nucleotide Archive

accession number PRJEB59008), 1000 Genomes haplotype reference panel (http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/), human reference genome hg19 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.13/), mitochondrial reference genome RSRS (doi: 10.1016/j.ajhg.2012.03.002), YFull YTree phylogeny (https://www.yfull.com/tree/), and ISOGG Y-chromosome SNPs (https://ybrowse.org/).

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Genetic analyses were performed on genomic data obtained from ancient human skeletons. Statistical models from population genetics were fit to the data to learn about the individuals' ancestral relationships to other human groups.
Research sample	New genomic data were obtained from nine ancient individuals from the eastern Maghreb (what are now Algeria and Tunisia) who lived from roughly 6000 years ago to more than 10,000 years ago. We analyzed the new data together with a large number of previously published ancient and present-day genomes from across the Mediterranean region and beyond.
Sampling strategy	We attempted to collect data from a total of 22 skeletal samples from the four archaeological sites, obtaining working data from nine unique individuals (12 samples, three from duplicate individuals). We targeted DNA sequences covering a set of approximately 1.2 million genome-wide SNPs, which effectively cover almost all independent loci in the genome (due to linkage disequilibrium) and provide good power in population history analyses.
Data collection	DNA from the ancient remains was extracted, sequenced, and processed into SNP genotype calls (final data overseen by S.M., N.R., R.P., and D.R.)
Timing and spatial scale	Ancient individuals were sampled from the sites of Afalou Bou Rhummel (Algeria), Djebba, Doukanet el Khoutifa, and Hergla (all Tunisia).
Data exclusions	Ten of the skeletal samples did not yield working data as assessed by pre-established ancient DNA quality criteria. One individual yielded working data but with evidence of contamination, so we restricted to sequences containing damage patterns indicative of an authentic ancient origin.
Reproducibility	All attempts to reproduce were successful.
Randomization	Individuals were grouped by site, and in one case (Doukanet el Khoutifa) into two subgroups based on genome-wide ancestry.
Blinding	Analyses were performed both on group level and individual level; other sample-specific features were not relevant to results.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

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

Methods

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

Palaeontology and Archaeology

Specimen provenance	Human remains from Djebba, Tunisia, were excavated under authorization (2018; directed by co-author NA). Human remains from Doukanet el Khoutifa and Hergla were collected within the framework of the Northern Tunisia Archaeological Project (NOTAP), under two scientific cooperation agreements: one between INP, CNR-ISPC, and ISMEO (2021-2024; co-direction by co-authors NA, LB, AC, and GL), and another between INP and the University of Bologna (2002-2007; co-direction: Simone Mulazzani and Ridha Boussoffara). We are grateful to the current and former INP General Directors, Tarek Baccouche and Faouzi Mahfoudh, for their support, and Adelaide Marsilio for his key role in the excavation of Doukanet el Khoutifa in 2022.
Specimen deposition	The specimens are available from the permitting authorities, and samples will not be retained at the ancient DNA laboratories.
Dating methods	We sent samples of the same skeletal elements used for ancient DNA analysis for accelerator mass spectrometry (AMS) radiocarbon dating at the Pennsylvania State University Radiocarbon Laboratory, using standard methods. We calibrated the dates using OxCal (v4.4), and the IntCal20 calibration curve.

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

Ethics oversight	This study followed the ethical protocol described in Alpaslan-Roodenberg et al. 2022 Ethics of DNA research on human remains: five globally applicable guidelines (Nature 599, 41-6), and its ethical approach was additionally reviewed as part of the requests that led to the sampling of skeletal remains from the four sites reported on here.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*