










# Genomic ancestry and social dynamics of the last hunter-gatherers of Atlantic France

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Since the early Holocene, western and central Europe was inhabited by a genetically distinct group of Western Hunter-Gatherers (WHGs). This group was eventually replaced and assimilated by the incoming Neolithic farmers. The western Atlantic façade was home to some of the last Mesolithic sites of mainland Europe, represented by the iconic open-air sites at Hoedic and Tévéc in southern Brittany, France. These sites are known for the unusually well-preserved and rich burials. Genomic studies of Mesolithic European hunter-gatherers have been limited to single or a few individuals per site and our understanding of the social dynamics of the last Mesolithic hunter-gatherers of Europe and their interactions with incoming farmers is limited. We sequenced and analyzed the complete genomes of 10 individuals from the Late Mesolithic sites of Hoedic, Tévéc, and Champigny, in France, four of which sequenced to between 23- and 8-times genome coverage. The analysis of genomic, chronological and dietary data revealed that the Late Mesolithic populations in Brittany maintained distinct social units within a network of exchanging mates. This resulted in low intra-group biological relatedness that prevented consanguineous mating, despite the small population size of the Late Mesolithic groups. We found no genetic ancestry from Neolithic farmers in the analyzed hunter-gatherers, even though some of them may have coexisted with the first farming groups in neighboring regions. Hence, contrary to previous conclusions based on stable isotope data from the same sites, the Late Mesolithic forager community was limited in mate-exchange to neighboring hunter-gatherer groups, to the exclusion of Neolithic farmers.

Mesolithic | genomics | palaeogenomics | foragers

The onset of the Holocene, ca. 11,700 y ago (1) brought amenable climate conditions that impacted the foragers of that time in Europe. This period is characterized by significant changes in socio-cultural practices, as evidenced by new settlement patterns, technology, subsistence, mortuary practices, and worldviews, which define the transition from the Paleolithic to the Mesolithic in archaeological terms (2). Paleogenomic studies of ancient human remains have shown that several genetically distinct groups existed across Europe during the Paleolithic (3, 4). In western Europe, an ancestry group associated with the Upper Paleolithic Magdalenian culture (ca. 20,000 to 14,000 cal B.P., e.g., represented by individual Goyet Q2 (3, 5), and referred to as the “Magdalenian” ancestry group) was predominant during the Last Glacial Maximum. This group was largely replaced by the so-called “Western European hunter-gatherer” (WHG) ancestry group (4, 6–8), during the early Holocene, except in the Iberian Peninsula and to some degree in southwestern France (4, 5, 9, 10).

For several millennia, the WHGs were the most common group across most of Europe, until the arrival of the Neolithic farmers (6–8). The period of coexistence of WHGs and Neolithic farmers is narrow in western Europe and the interactions between hunter-gatherers (HG) and incoming farming populations have been difficult to decipher, partly because the precise chronology of the last HGs has been challenging (11–13). In southern Brittany, the Mesolithic way of life seems to have ended around 6,750 cal B.P. (14, 15) with the earliest Neolithic sites in Brittany dating between 6,950 and 6,650 cal B.P. (16). Neolithization of northern Brittany of the region started ca. 6,850 cal B.P. (17), which is ca. 200 y later than in other regions in France, such as at the neighboring Normandy or in the Paris Basin (18).

It is evident in the archaeological record that the arrival of Neolithic populations changed long-established HG socio-cultural practices throughout Europe. In genetic terms, it is now clear that Neolithic populations assimilated HGs to some extent (10, 19–21). How this process occurred is unknown, partly because genetic data from some of the key Late Mesolithic sites are still missing. There is increasing evidence of regional or even local nuances of contact and mixture (21–23). For instance, in Sicily, at Grotta dell’Uzzo, there

## Significance

Since the early Holocene, western and central Europe was inhabited by a genetically distinct group of hunter-gatherers. We generated different types of biomolecular data, including deep coverage complete genome sequencing, from human skeletal remains buried in the iconic sites of Tévéc and Hoedic in Brittany, representing some of the last hunter-gatherers of western Europe. The data show that these last foragers were part of a network of people who maintained exogamic practices. These socio-cultural dynamics contributed to avoiding inbreeding. Some of the forager individuals overlapped in time with the arrival of Neolithic farmers to neighboring regions. However, we did not find any farmer-associated ancestry in the analyzed hunter-gatherers and the mate-exchanging networks appear to be exclusive for the foraging group.

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are indications of interactions between farmers and HGs based on diet patterns (24). However, it remains unclear whether gene flow also occurred from farmers into extant Late Mesolithic populations (which has not yet been reported), a question that can only be answered by investigating the genomes of HGs living contemporarily with farmers.

Genomic studies of ancient HGs have mainly focused on the demographic processes that shaped their patterns of genetic diversity (e.g. refs. 4–7 and 10). Only a few studies have generated genomic datasets of HG social groups, for which multiple individuals with confirmed chronological and spatial coexistence are analyzed. Such datasets provide unique opportunities to study the social dynamics of HG societies (25). The shell middens of Hoedic and Tévéc in southern Brittany, France, at the Atlantic façade of Europe, are among the most significant Mesolithic sites in France, due to the rich and unusually well-preserved large number of human burials which remain unparalleled in the region (26, 27). Together with the Late Mesolithic burial grounds of Portugal and southern Scandinavia (28, 29), Hoedic and Tévéc bear important evidence about the life and death of the last hunter-gatherers in western Europe up to the Neolithic transition (11). To investigate the genetic ancestries and social dynamics of some of the last Mesolithic HGs in western Europe, we sequenced and analyzed the genomes of 10 Late Mesolithic individuals from Tévéc and Hoedic in North-West France and Mont Saint-Pierre, Champigny in the North-East (Fig. 1), and integrated the new genomic evidence with previously published dietary data and new high-resolution chronological analysis.

## Results

**Chronology of the Burial Activity.** We obtained reliable radiocarbon ( $^{14}\text{C}$ ) and stable isotope data of carbon ( $^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}$ ) for four of the sequenced individuals from Hoedic (hoe002, 004-006) and used previously published measurements for Tévéc (tev001, 003), Hoedic (hoe001), and Champigny (spt001) (Table 1 and Dataset S1). Two sequenced individuals (tev002, hoe003) remain undated due to difficulties in obtaining well-preserved collagen (SI Appendix, Supplementary Note 2 and Datasets S1 and S2). For a comprehensive chronological analysis, we integrated the dated individuals from the sequence dataset (Table 1) with all published radiocarbon dates from individuals not sampled for genetic analyses. Given the high trophic level of the studied individuals, indicating a substantial consumption of seafood, we corrected the C14 dates for a marine reservoir effect (SI Appendix, Supplementary Note 2.1). The revised chronology of the burial activity at Tévéc and Hoedic (SI Appendix, Figs. S8 and S9) is in line with what was already known (11, 15) but further demonstrates that the later phases of burial activity at Hoedic [e.g., J(11)-hoe005, J(7)-hoe004, C-2(2)-hoe001, C-3-hoe002, B(1)], ca. 7,200/7,100 to 6,650 cal B.P., may have overlapped in time with Early Neolithic farmers that settled in neighboring regions, and possibly in Brittany (Fig. 1 and SI Appendix, Fig. S9).

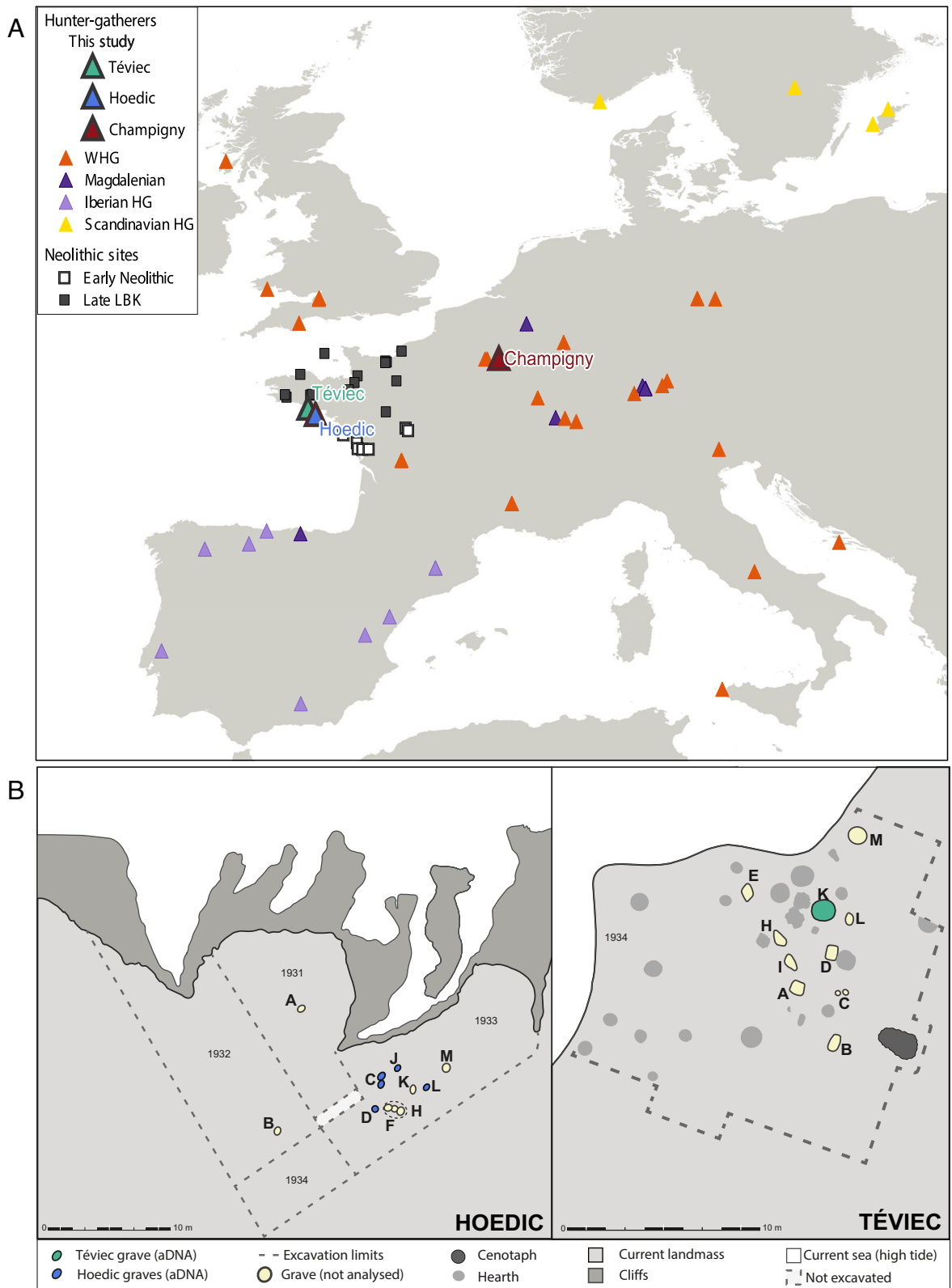
Graves with multiple burials are relatively common in Tévéc and Hoedic. All three individuals from Tévéc that were genomically analyzed were buried in the same grave (K) at various depths (SI Appendix, Supplementary Note 1). At Hoedic, we investigated grave J which contained one adult and a child [J(7)hoe004, J(11)-hoe005], as well as grave C with the remains of several children (including C3-hoe002) and an adult [C2(2)-hoe001]. Radiocarbon data and each grave's stratigraphic relationships indicate that individuals buried in the same grave either coexisted in time or are from consecutive generations (SI Appendix, Supplementary Notes 1 and 2).

**Marine Protein in Diet.** The people buried in both Hoedic and Tévéc show substantial consumption of seafood. Most of their protein intake was obtained from high trophic marine foods such as large fish, relative to low trophic level food, such as shellfish (SI Appendix, Supplementary Note 2). Specifically, new and previously published data from Hoedic show that the human collagen samples display stable isotope values ranging between  $-15.1\text{‰}$  and  $-13.0\text{‰}$  ( $-13.9 \pm 0.7\text{‰}$ , mean  $\pm$  SD,  $n = 10$ ) for carbon, and  $13.9$  to  $15.5\text{‰}$  ( $14.6 \pm 0.5\text{‰}$ ,  $n = 7$ ) for nitrogen. These values indicate that the individuals buried at Hoedic obtained an exceptionally high proportion of their protein from fished marine foods ( $57 \pm 9\%$  to  $78 \pm 9\%$ ), higher than most reported trophic levels for historically recorded/prehistoric HGs (30). The individuals buried at Tévéc also show high consumption of marine foods ( $38 \pm 9\%$  to  $60 \pm 13\%$ ) but with a considerably higher consumption of protein from terrestrial sources relative to Hoedic. Available measurements of carbon isotope values range between  $-16.6\text{‰}$  and  $-14.6\text{‰}$  ( $-15.5 \pm 0.6\text{‰}$ ,  $n = 8$ ), and  $11.7$  to  $15.2\text{‰}$  ( $13.4 \pm 1.8\text{‰}$ ,  $n = 3$ ) for nitrogen, indicating the consumption of a mixture of marine and terrestrial foods at Tévéc.

At both sites, the  $\delta^{13}\text{C}$  (Fig. 2) and  $\delta^{15}\text{N}$  values fluctuate over time and do not follow any particular tendency (Dataset S1). While there are no apparent chronological or age and sex biases in relation to the sources of protein consumed, we observe some intra-site variation. Notably, at Hoedic, the woman and the 4- to 7-y-old girl buried together in grave J (hoe004 and hoe005) display a more balanced consumption of marine and terrestrial foods (ca.  $56 \pm 9\%$ ) in contrast with the predominantly marine diets of other individuals buried at the site, including those buried around the same time period in grave C (hoe001 and hoe002, ca.  $69 \pm 9\%$ ).

**Ancient DNA Data.** We generated whole-genome sequencing data from uracil-DNA glycosylase (UDG) treated libraries for 10 individuals from three Late Mesolithic sites in modern-day France (Fig. 1, Table 1 and SI Appendix, Table S1), after initial investigation of post-mortem damage on non-UDG treated libraries and fragmentation typical of ancient DNA (aDNA). Mitochondrial and X chromosome contamination estimates were consistently very low ( $<3\%$ , Table 1). DNA preservation was exceptional for most individuals, enabling an average (across 10 individuals) genomic sequencing depth of  $8.25\times$  (ranging from  $0.03\times$  to  $22.88\times$ , Table 1). We compared the generated genome sequences with previously published genetic data from relevant ancient individuals (Dataset S5) as well as present-day west Eurasian populations (from the Simons Genome Diversity Project, SGDP) (31) and the Human Origins panel (32).

**Genetic Similarities to Contemporaneous Groups.** The Late Mesolithic HGs from modern-day France were genetically very similar to other WHGs (Figs. 3A and 4), suggesting a long-term and geographically stable population. The mitochondrial and Y chromosome haplogroups are, respectively, U5 and I2a1 (Table 1), which are typical for Late Mesolithic WHGs (8, 11, 12). Moreover, the individuals buried at Tévéc and Hoedic are at the northern end of the cline of two late-Pleistocene lineages—Magdalenian-associated and WHG Villabruna-related ancestry—previously observed for other West Atlantic European Mesolithic HGs (SI Appendix, Supplementary Note 4). Despite being contemporary to the first farmers in northwestern France, none of the individuals of Tévéc and Hoedic show evidence of admixture with Neolithic groups (Fig. 3A and SI Appendix, Supplementary Note 5). The individuals at Tévéc and Hoedic show greater genetic similarity among themselves compared to other WHGs from modern-day France, such as Champigny (stp001, Fig. 4).



**Fig. 1.** Location of individuals and graves discussed in the text. (A) Map indicating the location of Paleolithic and Mesolithic sites used for comparative genetic analysis, highlighting the location of the sites investigated in this study and the Neolithic occupation, for which there is no genetic data available. (B) Schematic representation of the excavated graves in the shell middens of Téviéc and Hoedic based on the field map by M. Péquart and S.-J. Péquart (26, 27).

Phenotypically, we find some diversity during the Late Mesolithic in France. We observe that while most individuals carry the dark skin and blue eyes characteristic of WHGs, D(4)-hoe003 and J(11)-hoe005 likely had pale to intermediate skin pigmentation (*SI Appendix, Table S11*).

**Social Structure and Biological Relatedness.** To gain insight into effective population sizes and levels of consanguinity, we computed runs of homozygosity (RoH) for the four higher-coverage Late Mesolithic HGs (stp001, hoe003, hoe005, and tev003) and a panel of comparative ancient and modern-day individuals. We observe

**Table 1. Mesolithic individuals sequenced in this study**

aDNA lab ID	Burial ID	Archaeological site	Age	<sup>14</sup> C cal B.P. (95.4%)	δ13C (‰)	δ15 N (‰)	Genome coverage	Biol. sex	mt haplo-group	Y chr. haplo-group	Contamination (X chr.)	Contamination (mt)
stp001	F528	Mont S:t Pierre Champigny	Adult	8,300 to 8,015	n/a	n/a	17.53	XY	U5b2	I2a1 (L460)	0.009	0.001
tev001	K1(8)	Téviéc	Adult	7,320 to 7,065	-15.6 AMS	n/a	2.16	XY	U5b	I2a1 (L460)	0.011	0.002
tev002	K3 (9)	Téviéc	Adult	n/a	n/a	n/a	0.21	XX	U5b	-	-	0.019
tev003	K6(16)	Téviéc	Adult	7,425 to 7,180	-15.4	13.4	22.43	XY	U5b1	I2a1 (L460)	0.009	0.0001
hoe001	C-2 (2)	Hoedic	Adult	7,160 to 6,760	-14.0	14.2	0.20	XX	U5b	-	-	0.009
hoe002	C-3	Hoedic	Child 2 to 7 y	7,155 to 6,790	-13.6	14.7	3.82	XY	U5b	I2a1 (L460)	0.007	0.0007
hoe003	D (4)	Hoedic	Adult	n/a	n/a	n/a	22.87	XY	U5b2b	I2a1 (L460)	0.026	0.002
hoe004	J (7)	Hoedic	Adult	7,240 to 6,885	-15.1	15.5	4.92	XX	U5b1	-	-	0.0006
hoe005	J (11)	Hoedic	Child 3 to 7 y	7,260 to 6,950	-14.9	14.6	8.32	XX	U5a2	-	-	0.001
hoe006	L (10)	Hoedic	Adult	7,910 to 7,575	-13.0	15.1	0.03	XX	U5a2	-	-	0.023

Samples were collected from the bone remains of 10 individuals from three archaeological sites in France. See *SI Appendix* for detailed information on each sample and measurements. The following abbreviations are used in the table; Years (y), mitochondria (mt), chromosome (chr.), biological (biol.).

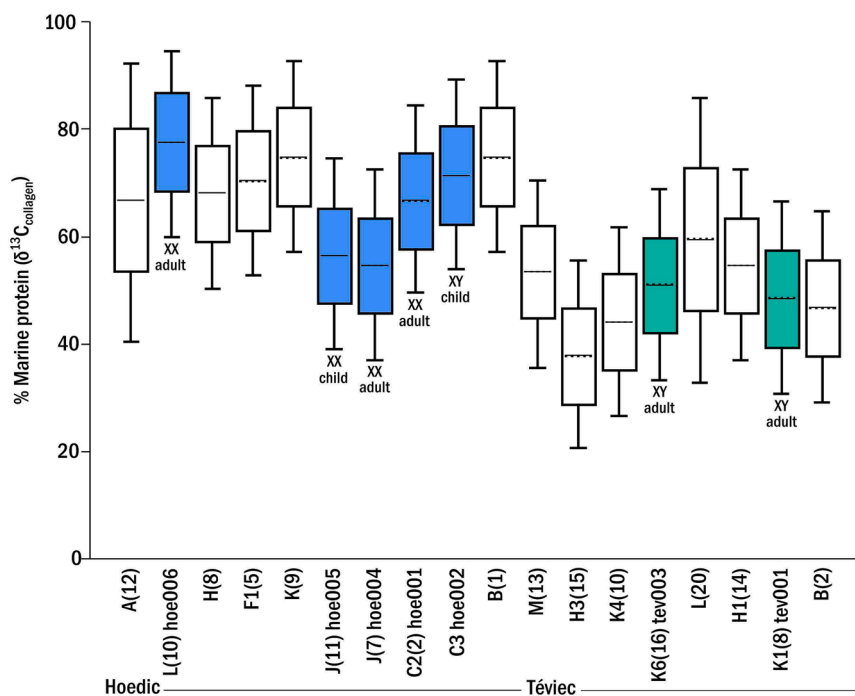
long and frequent RoH in Brittany's HGs and stp001, consistent with a small effective population size. Contrary to the expectations from their geographical and temporal isolation, the Late Mesolithic French HGs show similar, but slightly less RoH than those observed in other high coverage genomes from WHGs, such as Loschbour [ca. 8,000 y old, Luxembourg (6)] and SRA62 [ca. 8,000 y old, Ireland (33)]. Interestingly, we find no evidence of consanguinity in Mesolithic Brittany [such as an excess of long RoH fragments instead of a proportional increase in the total length of the sum of RoH and the number of RoH segments (Fig. 3*B*)].

Given their broad contemporaneity (*SI Appendix*, Figs. S8 and S9), we investigated the degree of biological relatedness among the Téviéc and Hoedic individuals, with methods that allow relationship inference up to the second and fourth degree for aDNA data (34–36). The individuals of Mesolithic Brittany are predominantly unrelated, and first-degree kinship was not identified (Fig. 5 and *SI Appendix*,

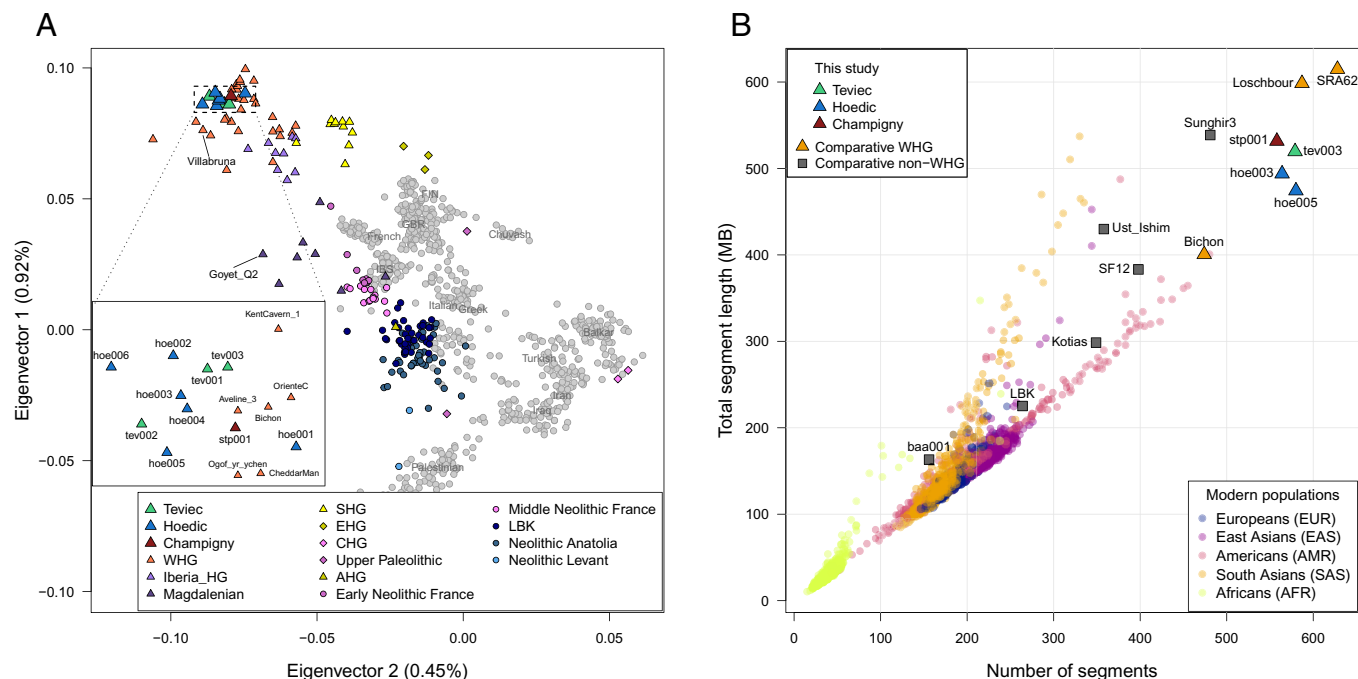
*Supplementary Note 6*). Within Téviéc, where all analyzed individuals come from the same grave, two pairs of individuals (tev001-tev003 and tev002-tev003) were inferred to be second- or third-degree relatives (Fig. 5 and *SI Appendix*, *Supplementary Note 6*). These familial relationships can also be seen in the tighter genetic clustering within the site of Téviéc (Fig. 4). All other pairs of individuals have third-degree or higher kin relationships, even those buried together. Notably, the adult female (hoe004) and 4- to 7-y-old girl (hoe005) buried together in grave J were biologically unrelated, in line with the different phenotypic appearances.

## Discussion

The dynamics of social interactions between past HG populations are poorly studied genetically, in part due to the scarcity of human remains and, consequently, DNA sequence data. To address this,



**Fig. 2.** Marine protein estimate based on all available  $\delta^{13}\text{C}_{\text{collagen}}$  measured on human bone samples from Hoedic and Téviéc, modeled using FRUITS v. 3.1. Individuals are sorted chronologically within each site (older on the left side). Samples with genomic data produced in this study are shaded in blue (Hoedic) and green (Téviéc). The other individuals were not sampled for genetic analysis, but their previously published isotopes are used here for comparative background (30).

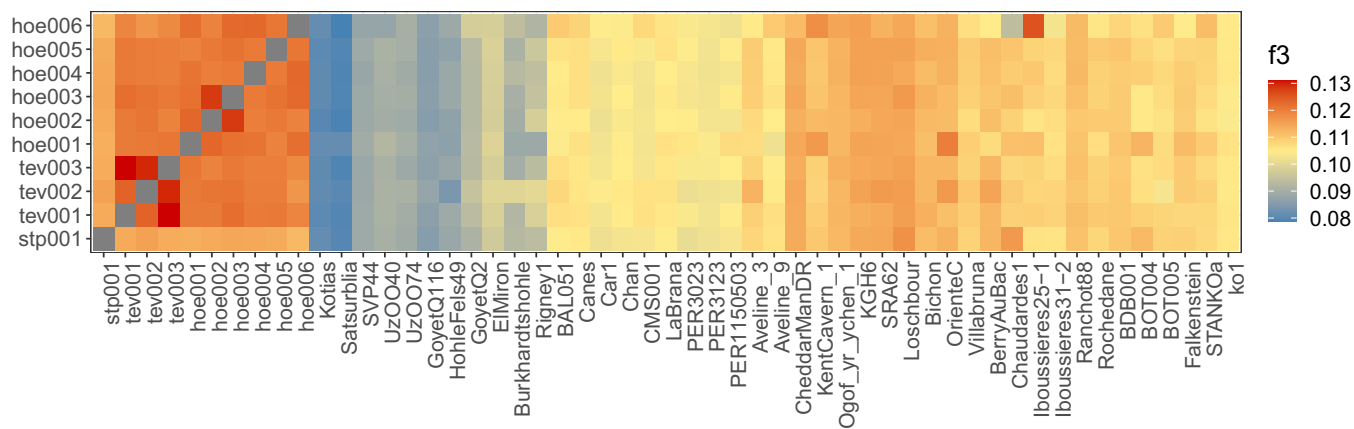


**Fig. 3.** (A) Principal component analysis (PCA) of present-day West Eurasians genotyped on the Human Origins dataset (gray dots), and ancient Upper Paleolithic and Mesolithic hunter-gatherers and Neolithic populations (Dataset S5) from West Eurasia projected onto the axes defined by modern variation. (B) Runs of homozygosity for 13 high coverage ancient individuals, including four newly reported Mesolithic hunter-gatherers from France and a total of nine European Mesolithic hunter-gatherers, and modern populations from the 1000 Genome Panel.

we generated whole-genome data of Late Mesolithic HGs and confirmed with new calibrated radiocarbon dates on human bone collagen that the individuals buried in Téviéc and Hoedic were not only in spatial proximity but were also largely contemporaneous, forming a biological population, possibly substructured into different groups or clans. This provided an unprecedented opportunity to investigate Mesolithic HG demographic structure and socio-cultural dynamics, by integrating genomic, radiocarbon, stable isotopes, and archaeological data, even if we consider archaeological preservation biases and possible selection of individuals for burial.

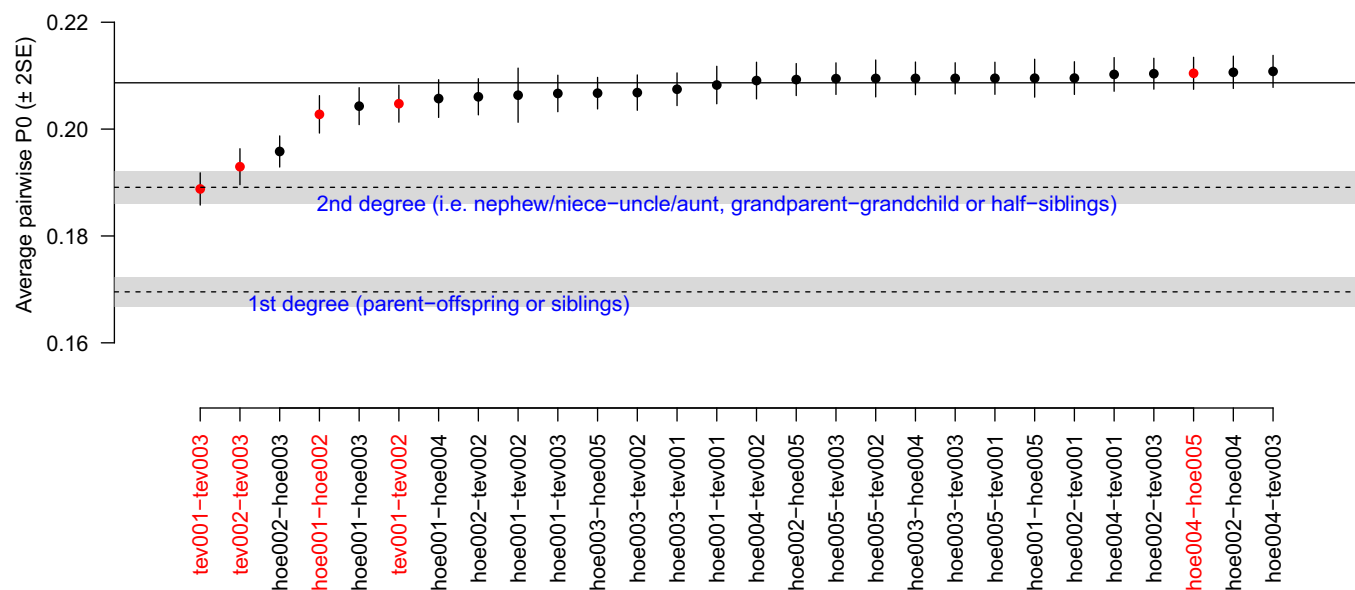
While our genetic analysis confirms that the people buried in Téviéc and Hoedic were genetically more related to each other than to other WHGs, stable isotopes show relatively distinct subsistence strategies at each site [Fig. 2, (11)]. The high consumption of seafood at both sites indicates that both groups relied heavily

on exploiting marine resources. This is not surprising given that both sites are located within a small region on the coast and that HG diets are systematically related to environmental conditions (30), which were comparable in Téviéc and Hoedic, implying similar food resource availability (14). However, each group had different resource exploitation preferences. In the Late Mesolithic, the sea levels were 5 to 15 m lower than today, and while the present-day island of Hoedic is the result of the splitting up of a larger island, Téviéc was on the seashore (37, 38). This possibly granted easier access to terrestrial foods to the foragers of Téviéc. Dietary data on historically recorded HG populations indicate that very few groups worldwide depend on marine resources for more than 50% of their diet (e.g., Alsea, Haida, Makah, and Inuit), possibly due to the technological up-front cost associated with the intensive use of aquatic resources, including boats, nets, traps, hooks, and lines (30). The extremely high intake of marine



**Fig. 4.** Heatmap of the genetic distances between Mesolithic HG individuals from modern-day France reported here (Y axis) and Mesolithic/Lower Upper Paleolithic European HGs (X axis, >20,000 SNPs), measured by  $f_3$ -outgroup statistics of the form  $f_3(\text{France HG 1, Eurasian HG 2; Mbuti})$ . Please see *S1 Appendix, Supplementary Note 3* for more information on the comparative individuals. Warmer colors correspond to higher  $f_3$  values and indicate a higher shared genetic drift between pairs of ancient individuals.

## READ kinship estimates



**Fig. 5.** Biological kinship analysis results using READ (34). Individuals buried in the same grave or in very close proximity are highlighted in red (all individuals sampled from Tévéc were buried in the same grave; hoe004 and hoe005 correspond to the simultaneous burial of an adult female and a child placed on top of the adult).

protein noted among those buried in Hoedic indicates that fishing was their main subsistence activity, while the individuals buried in Tévéc probably spent relatively more time hunting and gathering terrestrial foods (11). The specific dietary patterns of each site indicate that they lived as separate social units.

We observe low intra-site genetic relatedness, suggesting that HGs from the Mesolithic in Brittany had social systems in place that would avoid inbreeding among immediate relatives, similar to what has been observed some Late Upper Paleolithic HGs (25). This pattern is in line with historically recorded HG populations, for which residential groups have less than 10% primary kin relatives (39, 40). Interestingly, and contrary to expectation, individuals buried together did not have close biological kin relationships. An exception to this pattern is the individual buried at the bottom of grave K in Tévéc [K6(16)-tev003], who had closer biological kin links to at least two (sampled of a total of five) of the individuals buried above him, while these were not closely related with each other. This finding corroborates the importance and singularity that K6(16)-tev003 could have had based on the arrangement of the grave and associated archaeological material. Moreover, osteological analysis revealed two microlithic armatures, likely from a projectile weapon, punched in the sixth and eleventh dorsal vertebrae, the first of which may have resulted in immediate death by severing the aorta (1). His mandible also bore an old, well-healed fracture, which has been suggested as evidence of a lifestyle marked by a certain violence (26).

In line with the biological relatedness analysis, which shows that the social units of Tévéc and Hoedic were broadly not based on close biological relatives, the RoH patterns show increased background relatedness due to small population sizes instead of direct consanguinity (41, 42). This observation is similar to other Late Mesolithic populations in western Europe and the phenomenon seems to be more pronounced in western Europe [Fig. 3, (4)] compared to eastern, northern, and southern Europe [Fig. 3, (4, 24, 43)]. In modern-day populations, inbreeding is more prevalent in areas where consanguinity is favored culturally, such as

parts of West and South Asia, but it also occurs as a consequence of small population size and endogamy, even if there is random mating (41). The people of Tévéc and Hoedic seem to have implemented strategies to avoid consanguineous mating, such as exchanges between groups. The wider Mesolithic landscape in Brittany could have facilitated mobility, contact, and exchange of mates between small HG groups.

Exogamic practices have been previously proposed for the Late Mesolithic sites in Brittany based on dietary isotopes. Schulting and Richards' (11) isotopic analysis of a large number of individuals indicated that the diet of younger women buried in Tévéc and Hoedic tended to be more reliant on terrestrial protein than the sites' average, shifting toward the group's marine-terrestrial values at older ages (11). This dietary shift suggested a patrilocal exogamic behavior, where women from other more inland groups migrated into these coastal communities. The possible chronological overlap between these last HG communities and the first Neolithic groups in the neighboring regions gave rise to the idea that young women at Hoedic and Tévéc, given their more inland diet, were moving in from Neolithic farmer groups (11). We now confirm the chronological overlap at the later phases of burial activity in Hoedic and show that these females (illustrated in this study by hoe004 and hoe005) did not come from Neolithic populations, as they are within the HG genetic variation and show no traces of Neolithic farmer-related ancestry (Figs. 3 and 4). While the dietary profile of this woman and child buried in grave J at Hoedic differs from the average observed at the site and aligns with that of Tévéc, their origin remains unknown, but a possible common foreign origin could explain their joint burial without a kin relationship. Ethnographic data show that among human societies and especially foragers, child caretaking is often multiple. The density within foragers' settlements is frequently higher than in farmers', and thus multiple members of the group participate in the upbringing of children (44). Biological unrelatedness in graves with adult female-child is not uncommon in other contexts and

periods (45) and while these issues are not universal across societies, our findings support that social bonds (kinship) were established beyond biological relatedness and that some social significance was attributed to such links, postmortem.

The lack of farmer-related ancestry in the HGs analyzed, particularly those from the later phase at Hoedic, is elucidating of the dynamics of interaction between these populations and ultimately the fate of Mesolithic HG populations. Across Europe, the directional gene flow from HG to farmer populations has been demonstrated by the consistent finding of ancestry associated with HGs in farming contexts. In France, it has been shown that Early Neolithic farmers carried Late Pleistocene hunter-gatherer lineages, suggesting multiple events of admixture, before and after the arrival of Neolithic groups in France (10, 21). Moreover, evidence of delayed HG-farmer admixture in Southern France, occurring only several generations after the initial arrival of farming together with the development of local pottery traditions, has been interpreted as local adoption of aspects of the Neolithic package by HG groups (21, 46, 47). In Sicily, an individual with HG ancestry showed a diet-signal similar to Sicilian Early Neolithic farmers, raising the idea of interactions between groups (24). However, genetic data of HGs that coexisted with farmers remains rare. Here, we confirm that some of the last burial events at Hoedic (*SI Appendix, Fig. S9*) are contemporary with neighboring farmer sites in Brittany, such as Pluvignon, Kervouric, and Kervouyec (*SI Appendix, Supplementary Note 5.1*). We observe that this HG population remained unadmixed throughout a time transect that overlaps the arrival of Neolithic people to the region. In spite of the lack of genetic data from early farmers in Brittany, farmers carried HG-related ancestry in other regions of France (10, 21). Taken together, these results show that gene flow between foragers and farmers was typically unidirectional and resulted from individuals with HG ancestry joining farmer groups and not the other way around. By sampling HG groups that overlap chronologically with early farmers instead of HG groups that predated the arrival of Neolithic farmers, we exclude sampling bias as an explanation for the absence of admixed farmer-related ancestry. While such pattern of interaction between HG and farmer groups is clear in Brittany, gene-flow in the opposite direction could have happened in other contexts and areas.

## Conclusion

Our revision of the chronology of the burial activity at Tévéc and Hoedic provides a more robust interpretation for the end of the Mesolithic in Brittany, placing Hoedic as the last known Mesolithic site in France. The individuals of Tévéc and Hoedic lived on the very edge of the geographical and chronological distribution of the western European Mesolithic HG groups. These circumstances could have cornered these groups into severe genetic drift due to extremely small population size, leaving no alternative to consanguinity and its deleterious consequences. By integrating genetic, radiocarbon and stable isotopes results, we observe that these groups implemented strategies to avoid consanguinity, pointing to the maintenance of intermarriage networks between different HG subgroups. These practices were likely active all the way till the end of the existence of these HG groups, when they were finally assimilated or replaced by the Neolithic farmers. Such strategies could be rooted in HG practices since the Early Upper Paleolithic, when exogamy and regular exchanges between groups seem to have avoided consanguinity (25). By combining genetic and dietary analyses, we uncover the complexity of hunter-gatherer socio-cultural systems, which are also expressed in their funerary practices.

## Materials and Methods

**Archaeological Material.** The Tévéc and Hoedic individuals were sampled in accordance with a permit (HDL.EF.2017.201) issued by Henry de Lumley, director of the Institut de Paléontologie Humaine, Fondation Albert I<sup>er</sup>, Prince de Monaco, in Paris. Mont Saint-Pierre, Champigny was sampled in agreement with the Service archéologique de la Communauté Urbaine du Grand Reims, as authorized by Nicolas Garmond, responsible for the archaeological excavation conducted in 2017.

**Radiocarbon and Stable Isotopes of Carbon and Nitrogen.** We used previously published and newly produced measurements of <sup>14</sup>C and stable isotope of carbon (<sup>13</sup>C) and nitrogen (<sup>15</sup>N) on human bone collagen and carried out a comprehensive evaluation of all measurements to evaluate reliability, resulting in the rejection of several measurements mostly due to poor preservation of bone collagen. In some cases, we tested the reliability of the measurements by processing multiple samples from the same bone in different radiocarbon laboratories (Uppsala, Oxford) (*SI Appendix, Supplementary Note 2 and Tables S1 and S2*).

We employed a Bayesian chronological model implemented within the software package OxCal v.4.4 to estimate the lifetime of each investigated individual which included a dietary correction for marine radiocarbon reservoir effect using FRUITS software (48, 49) (*SI Appendix, Supplementary Note 2*).

Calendar ages are reported as "cal B.P." (B.P., where Present is 1950 CE). All calibrated ranges are given at 95.4% probability and rounded to the nearest 5 y, since the modeled results vary from run to run.

**Ancient DNA.** Genomic data were generated in dedicated aDNA facilities at the Human Evolution Laboratory, Uppsala University following a similar procedure as in ref. 50. DNA was extracted, using adapted versions of refs. 51 and 52, from bone pieces from the inner part of the petrous bone and from tooth roots (53–55). DNA extracts were then converted to double-stranded Illumina libraries. A portion of each sample's first extract was used to build sequencing libraries to observe deamination and fragment size typical of post-mortem DNA damage (56). The following double-stranded libraries were built using USER enzyme to remove deamination. Libraries were amplified with a unique indexed primer (57). After quality control, libraries were pooled and whole-genome shotgun sequenced on Illumina HiSeq X (Tévéc and Hoedic samples) or on NovaSeq 6000 at the SNP & SEQ Technology Platform in Uppsala.

After sequence demultiplexing, forward and reverse paired-end reads were trimmed and merged when an overlap of at least 11 bp was found. Merged reads were mapped against the human reference genome using BWA aln 0.7.13 (58). Fragments with identical start and end positions were collapsed into consensus sequences. All reads shorter than 35 bp, with >10% mismatches to the reference genome or a mapping quality <30 were removed. For each library, we merged bam files resulting from all resequencing rounds using samtools merge v1.5. Data from all USER-treated libraries were merged per individual.

We used the method described in ref. 59 for biological sex determination. Contamination was estimated based on contradicting signals in the mitochondria (60) and for individuals identified as males, in the X chromosome (61). Mitochondrial haplogroup was assigned using Haplogrep v. 2.1.16 (62) and Phylotree 17. Y chromosome haplogroup was ascertained on informative SNP positions from Phylotree (version 9 Mar 2016) or ISOGG (v.11, April 2016) with samtools mpileup v1.3.

Two pseudo-haploid datasets were generated by randomly drawing one read per SNP from the newly generated ancient data and previously published ancient individuals from relevant populations: the 1,240 k SNP panel (3), merged with modern populations from the Simons Genome Diversity Panel (31) and the Human Origins [HO (32)] panel. For high coverage sequenced individuals, we performed diploid calls of transversion sites that are enriched in the Yoruba population of the 1000 Genome Panel (KGP), phase 3 data.

PCA was performed using smartPCA from the EIGENSOFT package v. 7.2.1 (63) by projecting the ancient individuals over the principal components computed on modern West Eurasian individuals from HO dataset using "lsqproject." *f*-statistics were performed with POPSTATS (64) and SEs calculated with weighted block jack-knife. We explored one- and two-source qpAdm models using Admixtools2, with Loschbour and GoyetQ2 as sources and with the following reference set: Mota, Ust\_Ishim, MA1, ko1, GoyetQ116, MbutiPygmy, Papuan, Onge, Han, Karitiana, Natufian (and Anatolia Neolithic for farmer admixture modeling). Genetic kinship

between all pseudo-haplotized Tévéc-Hoëdic samples, except hoe006 (excluded due to too low coverage), was analyzed with READ (34), using default normalization method, KIN (36), and correctKin (35).

Runs of homozygosity were estimated for a diploid dataset, for which ancient sample VCF files were subset to transversion sites enriched in the Yoruba population of the 1000 Genome Panel (KGP), phase 3 data, with the `--homozyg` command in PLINK (65). Pigmentation phenotypes for high-coverage ancient samples were estimated with the HlrisPlex-S system (66). Detailed descriptions of the ancient DNA wet lab, bioinformatics, and Population genetic analysis procedures available in *SI Appendix, Supplementary Note 3*.

**Data, Materials, and Software Availability.** Genome Sequence data from prehistoric human remains data have been deposited in European Nucleotide Archive (<https://www.ebi.ac.uk/ena/browser/home>, PRJEB1770) (67).

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1. M. Walker *et al.*, Formal definition and dating of the GSSP (Global Stratotype Section and Point) for the base of the Holocene using the Greenland NGRIP ice core, and selected auxiliary records. *J. Quaternary Sci.* **24**, 3–17 (2009).
2. M. Otte, "The paleolithic-mesolithic transition" in *Sourcebook of Paleolithic Transitions: Methods, Theories and Interpretations*, M. Camps, P. Chauhan, Eds. (Springer, 2009), pp. 537–553.
3. Q. Fu *et al.*, The genetic history of ice age Europe. *Nature* **534**, 200–205 (2016).
4. C. Posth *et al.*, Palaeogenomics of Upper Palaeolithic to Neolithic European hunter-gatherers. *Nature* **615**, 117–126 (2023).
5. V. Villalba-Mouco *et al.*, Survival of late pleistocene hunter-gatherer ancestry in the Iberian Peninsula. *Curr. Biol.* **29**, 1169–1177.e7 (2019).
6. I. Lazaridis *et al.*, Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* **513**, 409–413 (2014).
7. W. Haak *et al.*, Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* **522**, 207–211 (2015).
8. I. Olalde *et al.*, Derived immune and ancestral pigmentation alleles in a 7,000-year-old Mesolithic European. *Nature* **507**, 225–228 (2014).
9. I. Olalde *et al.*, The genomic history of the Iberian Peninsula over the past 8000 years. *Science* **363**, 1230–1234 (2019).
10. S. Brunel *et al.*, Ancient genomes from present-day France unveil 7,000 years of its demographic history. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 12791–12798 (2020).
11. R. J. Schulting, M. P. Richards, Dating women and becoming farmers: New palaeodietary and AMS dating evidence from the Breton Mesolithic cemeteries of Tévéc and Hoëdic. *J. Anthropol. Archaeol.* **20**, 314–344 (2001).
12. R. Peyroteo-Stjerna, Chronology of the burial activity of the last hunter-gatherers in the Southwestern Iberian Peninsula, Portugal. *Radiocarbon* **63**, 265–299 (2021).
13. G. Marchand, R. J. Schulting, Chronologie du second Mésolithique dans le Nord-Ouest de la France (2019).
14. C. Dupont *et al.*, Harvesting the seashores in the Late Mesolithic of Northwestern Europe: A view from Brittany. *J. World Prehistory* **22**, 93–111 (2009).
15. G. Marchand, R. Schulting, "Chronologie du second Mésolithique dans le Nord-Ouest de la France" in *Le Second Mésolithique Des Alpes à l'Atlantique (7-5 Millénaire)*, Table Ronde Internationale, Strasbourg, Les 3 et 4 Novembre 2015, R.-M. Arbogast, S. Griselein, C. Jeunesse, F. Séra, Eds. (Mémoires d'Archéologie du Grand-Est, 2019), pp. 109–125.
16. J.-Y. Tinézat *et al.*, Les vestiges d'habitat du Néolithique ancien de Quimper, Kervoueyec (Finistère). *Bull. Société préhistorique française* **112**, 269–316 (2015).
17. C. Marcigny, E. Ghesquiere, L. Juhel, F. Charraud, "Entre Néolithique ancien et Néolithique moyen en Normandie et dans les îles anglo-normandes. Parcours chronologique" in *Premiers Néolithiques de l'Ouest. Cultures, réseaux, échanges des premières sociétés néolithiques à leur expansion*, C. Billard, M. Legris, Eds. (2010), pp. 117–162.
18. M. V. Linden, "To tame a land: Archaeological cultures and the spread of the Neolithic in western Europe" in *Investigating Archaeological Cultures: Material Culture, Variability, and Transmission*, B. W. Roberts, M. Vander Linden, Eds. (Springer, 2011), pp. 289–319.
19. T. Günther *et al.*, Ancient genomes link early farmers from Atapuerca in Spain to modern-day Basques. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 11917–11922 (2015).
20. I. Olalde *et al.*, A Common genetic origin for early farmers from mediterranean cardial and central european LBK cultures. *Mol. Biol. Evol.* **32**, 3132–3142 (2015).
21. M. Rivollat *et al.*, Ancient genome-wide DNA from France highlights the complexity of interactions between Mesolithic hunter-gatherers and Neolithic farmers. *Sci. Adv.* **6**, eaaz5344 (2020).
22. M. Lipson *et al.*, Parallel palaeogenomic transects reveal complex genetic history of early European farmers. *Nature* **551**, 368–372 (2017).
23. I. Mathieson *et al.*, The genomic history of southeastern Europe. *Nature* **555**, 197–203 (2018).
24. H. Yu *et al.*, Genomic and dietary discontinuities during the Mesolithic and Neolithic in Sicily. *iScience* **25**, 104244 (2022).
25. M. Sikora *et al.*, Ancient genomes show social and reproductive behavior of early Upper Paleolithic foragers. *Science* **358**, 659–662 (2017).
26. M. Péquart, S.-J. Péquart, M. Boule, H.-V. Vallois, *Tévéc, station-nécropole mésolithique du Morbihan* (Masson et Cie, 1937).
27. M. L. N. C. Péquart, S.-J. V. Péquart, *Hoëdic, deuxième station-nécropole du Mésolithique côtier Armoricaïn* (de Sikkell, 1954).

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28. R. Peyroteo Stjerna, "On death in the Mesolithic: Or the mortuary practices of the last hunter-gatherers of the South-Western Iberian Peninsula, 7th–6th Millennium BCE" (Uppsala University, Uppsala, 2016).
29. L. Nilsson Stutz, *Embodied Rituals & Ritualized Bodies: Tracing Ritual Practices in Late Mesolithic Burials* (Almqvist & Wiksell International, 2003).
30. R. L. Kelly, *The Lifeways of Hunter-Gatherers: The Foraging Spectrum* (Cambridge University Press, 2013).
31. S. Mallick *et al.*, The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* **538**, 201–206 (2016).
32. I. Lazaridis *et al.*, Genomic insights into the origin of farming in the ancient Near East. *Nature* **536**, 419–424 (2016).
33. L. M. Cassidy *et al.*, A dynastic elite in monumental Neolithic society. *Nature* **582**, 384–388 (2020).
34. J. M. Monroy Kuhn, M. Jakobsson, T. Günther, Estimating genetic kin relationships in prehistoric populations. *PLoS One* **13**, e0195491 (2018).
35. E. Nyerki *et al.*, correctKin: An optimized method to infer relatedness up to the 4th degree from low-coverage ancient human genomes. *Genome Biol.* **24**, 1–21 (2023).
36. D. Popli, S. Peyrégne, B. M. Peter, KIN: A method to infer relatedness from low-coverage ancient DNA. *Genome Biol.* **24**, 10 (2023).
37. A. García-Artola *et al.*, Holocene sea-level database from the Atlantic coast of Europe. *Q. Sci. Rev.* **196**, 177–192 (2018).
38. P. Stéphane, Evolutions morphologiques et indices d'occupation humaine au Pléistocène et à l'Holocène le long des côtes françaises de la Manche et de l'Atlantique. *Les Nouvelles de l'archéologie* **156**, 53–59 (2019).
39. K. R. Hill *et al.*, Co-residence patterns in hunter-gatherer societies show unique human social structure. *Science* **331**, 1286–1289 (2011).
40. M. Dyble *et al.*, Sex equality can explain the unique social structure of hunter-gatherer bands. *Science* **348**, 796–798 (2015).
41. F. C. Ceballos *et al.*, Human inbreeding has decreased in time through the Holocene. *Cur. Biol.* **31**, 3925–3934.e8 (2021).
42. H. Ringbauer, J. Novembre, M. Steinrücken, Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nat. Commun.* **12**, 5425 (2021).
43. T. Günther *et al.*, Population genomics of Mesolithic Scandinavia: Investigating early postglacial migration routes and high-latitude adaptation. *PLoS Biol.* **16**, e2003703 (2018).
44. B. S. Hewlett, Demography and childcare in preindustrial societies. *J. Anthropol. Res.* **47**, 1–37 (1991).
45. K. Rebay-Salisbury *et al.*, Motherhood at early bronze age Unterhautzenthall, lower Austria. *Archaeol. Austriaca* **102**, 71–134 (2018).
46. L. Gomart *et al.*, Spiralled patchwork in pottery manufacture and the introduction of farming to Southern Europe. *Antiquity* **91**, 1501–1514 (2017).
47. A. Arzelier *et al.*, Neolithic genomic data from southern France showcase intensified interactions with hunter-gatherer communities. *iScience* **25**, 105387 (2022).
48. C. B. Ramsey, Bayesian analysis of radiocarbon dates. *Radiocarbon* **51**, 337–360 (2009).
49. R. Fernandes, C. Rinne, M.-J. Nadeau, P. Grootes, Towards the use of radiocarbon as a dietary proxy: Establishing a first wide-ranging radiocarbon reservoir effects baseline for Germany. *Environ. Archaeol.* **21**, 285–294 (2016).
50. R. Peyroteo-Stjerna *et al.*, Multidisciplinary investigation reveals an individual of West African origin buried in a Portuguese Mesolithic shell midden four centuries ago. *J. Archaeol. Sci. Rep.* **42**, 103370 (2022).
51. D. Y. Yang, B. Eng, J. S. Wayne, J. C. Dudar, S. R. Saunders, Improved DNA extraction from ancient bones using silica-based spin columns. *Am. J. Phys. Anthropol. Off. Am. Assoc. Phys. Anthropol.* **105**, 539–543 (1998).
52. J. Dabney *et al.*, Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 15758–15763 (2013).
53. E. Svensson *et al.*, Genome of Peștera Muierii skull shows high diversity and low mutational load in pre-glacial Europe. *Curr. Biol.* **31**, 2973–2983 (2021).
54. R. Pinhasi *et al.*, Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PLoS One* **10**, e0129102 (2015).
55. H. B. Hansen *et al.*, Comparing ancient DNA preservation in petrous bone and tooth cementum. *PLoS One* **12**, e0170940 (2017).
56. S. Sawyer, J. Krause, K. Guschanski, V. Savolainen, S. Pääbo, Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. *PLoS One* **7**, e34131 (2012).
57. M. Meyer, M. Kircher, Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* 2010, pdb-prot5448 (2010).



58. H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
59. P. Skoglund, J. Storå, A. Götherström, M. Jakobsson, Accurate sex identification of ancient human remains using DNA shotgun sequencing. *J. Archaeol. Sci.* **40**, 4477–4482 (2013).
60. Q. Fu *et al.*, A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* **23**, 553–559 (2013).
61. M. Rasmussen *et al.*, An Aboriginal Australian genome reveals separate human dispersals into Asia. *Science* **334**, 94–98 (2011).
62. H. Weissensteiner *et al.*, HaploGrep 2: Mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Res.* **44**, W58–W63 (2016).
63. A. L. Price *et al.*, Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genetics* **38**, 904–909 (2006).
64. P. Skoglund *et al.*, Genetic evidence for two founding populations of the Americas. *Nature* **525**, 104–108 (2015).
65. S. Purcell *et al.*, PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genetics* **81**, 559–575 (2007).
66. L. Chaitanya *et al.*, The HirisPlex-S system for eye, hair and skin colour prediction from DNA: Introduction and forensic developmental validation. *Forensic Sci. Int. Genet.* **35**, 123–135 (2018).
67. L. G. Simões *et al.*, PRJEB71770. European Nucleotide Archive. <https://www.ebi.ac.uk/ena/browser/view/PRJEB71770>. Deposited 15 January 2024.