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Ancient DNA gives new insights into a Norman Neolithic monumental cemetery dedicated to male elites

Maïté Rivollat[a,b,c,1], Aline Thomas[d,1], Emmanuel Ghesquière[e,f], Adam Benjamin Rohrlach[b,c,g], Ellen Späth[b], Marie-Hélène Pemonge[a], Wolfgang Haak[b,c], Philippe Chambon[d,2], and Marie-France Deguilloux[a,2]

Edited by Marie Besse, Laboratory of Prehistoric Archaeology and Anthropology, F.-A. Forel Department, Section of Earth and Environmental Sciences, University of Geneva, Geneva, Switzerland; received November 15, 2021; accepted March 1, 2022 by Editorial Board Member Elsa M. Redmond

The Middle Neolithic in western Europe is characterized by monumental funerary structures, known as megaliths, along the Atlantic façade. The first manifestations of this phenomenon occurred in modern-day France with the long mounds of the Cerny culture. Here, we present genome-wide data from the fifth-millennium BCE site of Fleury-sur-Orne in Normandy (France), famous for its impressively long monuments built for selected individuals. The site encompasses 32 monuments of variable sizes, containing the burials of 19 individuals from the Neolithic period. To address who was buried at the site, we generated genome-wide data for 14 individuals, of whom 13 are males, completing previously published data [M. Rivollat et al., *Sci. Adv.* 6, eaaz5344 (2020)]. Population genetic and Y chromosome analyses show that the Fleury-sur-Orne group fits within western European Neolithic genetic diversity and that the arrival of a new group is detected after 4,000 calibrated BCE. The results of analyzing uniparentally inherited markers and an overall low number of long runs of homozygosity suggest a patrilineal group practicing female exogamy. We find two pairs of individuals to be father and son, buried together in the same monument/grave. No other biological relationship can link monuments together, suggesting that each monument was dedicated to a genetically independent lineage. The combined data and documented father–son line of descent suggest a male-mediated transmission of sociopolitical authority. However, a single female buried with an arrowhead, otherwise considered a symbol of power of the male elite of the Cerny culture, questions a strictly biological sex bias in the burial rites of this otherwise "masculine" monumental cemetery.

monumental graves | Middle Neolithic | ancient DNA | Normandy | patrilineality

Significance

By integrating genomic and archaeological data, we provide new insights into the Neolithic French monumental site of Fleury-sur-Orne in Normandy, where a group of selected individuals was buried in impressively long monuments. The earliest individuals buried at Fleury-sur-Orne match the expected western European Neolithic genetic diversity, while three individuals, designated as genetic outliers, were buried after 4,000 calibrated BCE. We hypothesize that different, unrelated families or clans used the site over several centuries. Thirteen of 14 of the analyzed individuals were male, indicating an overarching patrilineal system. However, one exception, a female buried with a symbolically male artifact, suggests that the embodiment of the male gender in death was required to access burial at the monumental structures.

Ancient DNA (aDNA) studies on human populations can shed light on social organization, particularly on rules linked to biological relatedness. Despite varying funerary contexts (monumental vs. simple tombs, collective vs. individual burials) and therefore, contrasting forms of social organization, data from the early to the end of the European Neolithic converge toward patrilineal systems (1–5). Here, prehistoric societies are considered to be organized into groups of filiation/descent (family, clan, or tribe depending on the scale), whose children belong to the group of their biological father, according to the ethnological definition (6). Other scholars have documented patrilineal systems after the advent of metallurgy (7, 8), although these are not always demonstrable with the available data (9). Radiogenic isotope ratios of strontium, either obtained in parallel with DNA data or obtained independently, can help in specifying the pattern (10–12); nonlocal isotope signals in women are often interpreted as evidence of female mobility in patrilocal kinship systems on the basis of female exogamy. Here, the combined evidence suggests postmarital residence in the paternal family of the men, while women come from external groups (7).

The cemetery of Fleury-sur-Orne (Normandy, France) (Fig. 1) belongs to the first monumental funeral manifestations in Europe and predates Atlantic megalithic societies (13). Established during the second quarter of the fifth millennium calibrated (cal.) BCE, Fleury-sur-Orne consisted of earthen long barrows, some measuring up to 300 m in length. These monuments belong to the "Passy" phenomenon, named after the eponymous site (14). They are part of the Cerny culture that originated in the Yonne and upper Seine valley region, hereafter called "the Paris Basin," around 4,700 cal. BCE at the beginning of the local Middle Neolithic (15–18). The Passy-type monuments or STP (structure de type Passy) then spread to Normandy, where Fleury-sur-Orne is located.

Passy-type monuments were probably erected to commemorate high-status individuals. To date, interpretations of the individual's social status are based on bioarchaeological analyses carried out on the Paris Basin corpus. Burial features of the monumental

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[1]To whom correspondence may be addressed. Email: rivollat.maite@gmail.com or aline.thomas@mnhn.fr.

[2]P.C. and M.-F.D. contributed equally to this work.

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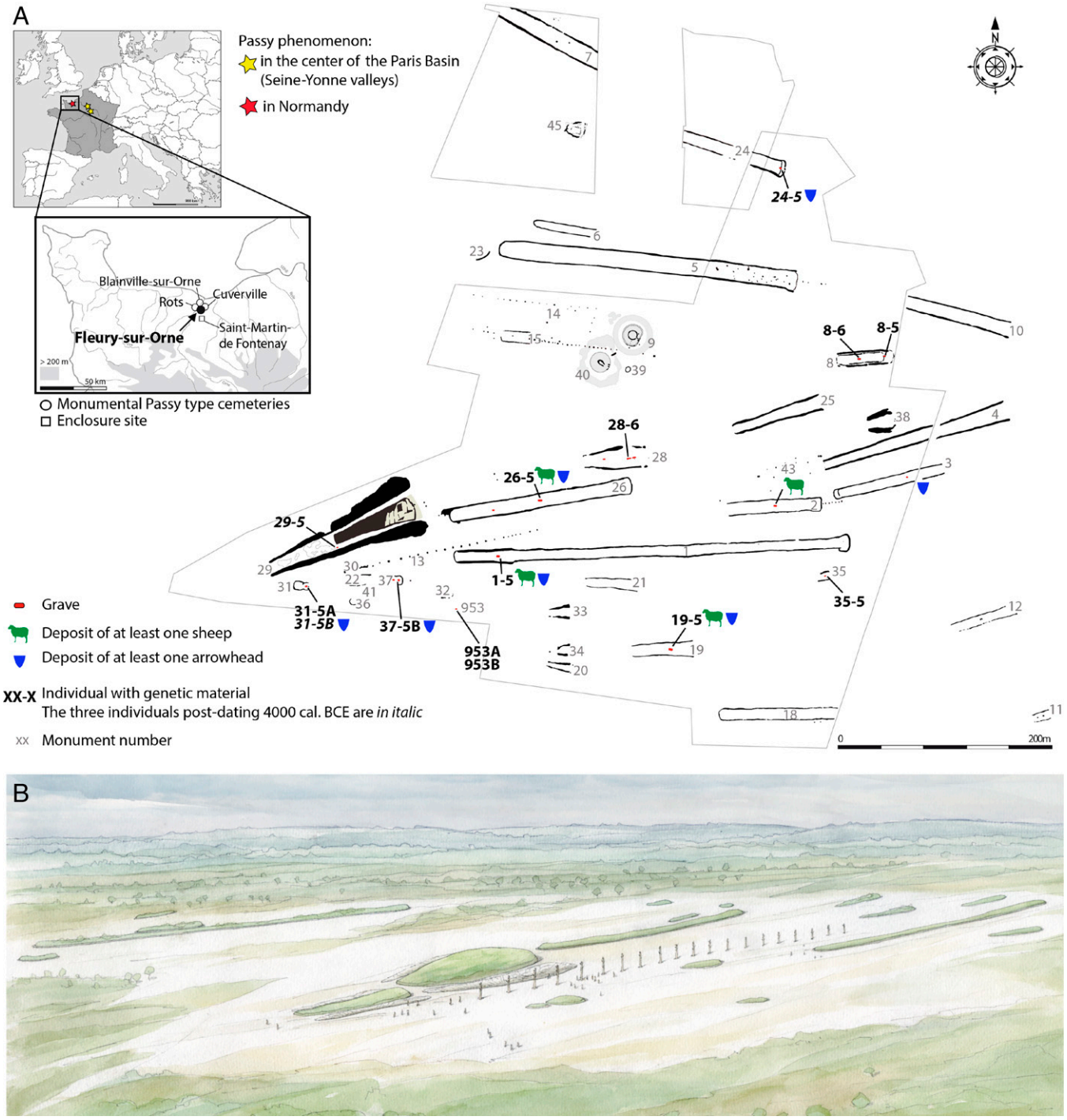


Fig. 1. Site description. (*A*) Location and map of Fleury-sur-Orne in northwestern France. (*B*) Artistic impression of the Fleury-sur-Orne monuments and surrounding landscape. Reproduced with permission from Laurent Juhel.

cemeteries have been studied in detail (19), as well as the spatial organization of the cemeteries according to the biological and social status of the dead (20–23). Both males and females were buried in these cemeteries, as well as subadults. Across Cerny cemeteries, burial practices appear to share a consistent definition of social status and gendered stratification (24). Specifically, one category of individuals of power, buried with arrows, quivers, and possibly, bows, is identified as "hunters" (25). In the adult corpus, these hunters are only men, whose musculoskeletal stress markers are consistent with archery activities (26). Together, the recognition given to the masculine, to archery or to hunting, or even more broadly, to the wild world (27) characterizes the Cerny ideology in the Paris Basin.

In Normandy, the Passy-type monuments also belong to the Cerny culture (28). Besides Fleury-sur-Orne, three other monumental cemeteries are known in the region thanks to aerial survey: Rots, Blainville-sur-Orne, and Cuverville. All four monumental cemeteries form a tight group and are spaced about 10 km apart (Fig. 1*A*). Fleury-sur-Orne is the only cemetery that has been excavated extensively, making it the Norman reference for the Passy phenomenon (13). The cemetery yielded 32 STPs in total, of which one mound (monument [mon.] 29) was still

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preserved. Graves ($n = 17$) were found along the central axis in half of the monuments. Most of the monuments contain one single grave; however, three of the monuments (mon. 8, 31, and 37) contain two graves, and one (mon. 28) contains three. Interestingly, one double burial (grave 953) is not associated with any visible monument (Fig. 1*A*). The data on the monumental architecture, graves, and status of the dead attest to the uniformity of the phenomenon with sites in the Paris Basin. The structure of the Fleury-sur-Orne cemetery adheres to the same rules as those of Cerny cemeteries in the Paris Basin. The graves, the deceased, and certain grave goods (arrows) confirm shared symbolic references. The morphological sex assessment of the skeletons, although only partially preserved, suggests male prevalence. However, several aspects of the burial system and deposition pattern of the dead (e.g., whole skinned sheep offerings in the graves) at Fleury-sur-Orne differ from those of the burials of the Paris Basin, as well as the absence of subadults or burials in the lateral ditches (13). According to the very little information available from other local sites (long barrows, arrowheads, sheep), particularly the partial archaeological survey conducted at Rots (28), we can only assume that the Norman cemeteries belong to the Passy phenomenon without further clarification as to specific affinities to the Paris Basin sites or to Fleury-sur-Orne.

Direct radiocarbon dates from skeletal elements from 15 individuals indicate a main use phase from 4,600 to 4,300 cal. BCE (13). Three Neolithic individuals date to after 4,000 cal. BCE, with all three buried inside an existing monument (mon. 24, 29, and 31). During the first half of the fourth millennium BCE, new mounds of stone were constructed, while occasional burials were added in more recent periods (Bronze Age and Classical Antiquity) inside the existing monuments. These more recent burials are not considered in the present study.

To date, ancient DNA analyses of people buried in Passy-type cemeteries targeted a few individuals from Fleury-sur-Orne and focused on population genetic affinities at a continental level (29). Fleury-sur-Orne's intrasite analysis now offers the possibility to infer the social organization of the group on the basis of biological relatedness and archaeological context. Was the site occupied by an homogenous group? Has the cemetery hosted one or several biological families, and what rules of (biological) descent can be inferred from the genetic structure of the group? Such an investigation addresses indirectly the status of the monuments (related or independent lineages), the status of the cemetery itself (one local elite or several centralized lineages), and more broadly, the occupation of the territory between the end of the fifth and the beginning of the fourth millennium BCE.

Data Overview

Here, we present genome-wide data for the complete Neolithic site of Fleury-sur-Orne, which adds six new individuals to the previously published data (29), resulting in a total of 15 of the 19 Neolithic individuals discovered at the site who yielded ancient DNA results. The four remaining individuals could not be sampled due to very poor skeletal preservation. Additionally, all of the studied individuals have a direct radiocarbon date (Dataset S1). We sampled petrous bones ($n = 8$) when available, teeth ($n = 3$), and long bones ($n = 4$), from which DNA was extracted. From these, DNA libraries were built with a partial uracil-DNA-glycosylase (UDG) treatment, allowing for the assessment of postmortem deamination patterns (6 to 18%) expected for ancient DNA data. An initial screening via shotgun sequencing of 5 million reads was used to select libraries with an amount of endogenous DNA above 0.05%, leading to the exclusion of individual 37-6 (Dataset S2). For the 14 remaining individuals that passed these quality criteria, we subsequently enriched libraries for ∼1.2 million single-nucleotide polymorphisms (SNPs) using targeted in-solution capture ("1,240k" SNP capture) (30), as well as two independent in-house capture arrays for the complete mitogenome (31) modified following ref. 32 and for the Y chromosome (33). The Y-chromosome capture array was also applied to the nine previously published individuals for which Y haplogroups had been assigned from the 1,240k data (29). The sequencing was processed on Illumina platforms to an average depth per site of 0.5× for 1,240k capture, 122× for mitochondrial capture, and 0.6× for Y-chromosome capture (Datasets S3–S5). We estimated potential contamination on the nuclear genome by testing for heterozygosity of polymorphic sites on the X chromosome in males (34) and set the threshold to 5%, which none exceeded (Dataset S1). For the 1,240k data, we randomly called one allele per position considering the human genome as a pseudohaploid genome. We called the SNPs according to the Affymetrix Human Origins panel (HO; ∼600,000 SNPs) (35, 36) and the 1,240k panel (Dataset S1) (30). Thirteen individuals with more than 20,000 SNPs on the 1,240k panel were used for the downstream genome-wide analyses, excluding individual 31-5A (10,297 SNPs) for whom only genetic sex and uniparentally inherited mitochondrial DNA (mtDNA) and Y haplogroups are reported (Dataset S1). The poor macroscopic preservation of human remains from Fleury-sur-Orne allowed only limited osteological observations. Morphological age classes remain broad, but all individuals can be assigned to the adult category. Osteological male sex could be estimated for four individuals, two using the pelvis bone (37, 38) and two following a secondary diagnosis method (39) based on comparative skeletal data from Cerny individuals in the Paris Basin (Dataset S1). Genetic (chromosomal) sex could be assigned to 14 individuals, of which 13 were male and 1 was female. Despite the overall poor macroscopic preservation, we document a high success rate of 87% for DNA retrieval, especially for individuals 8-6, 28-6, 31-5B, and 953B for whom only unidentified bone splinters were sampled (Dataset S1). This high success rate was made possible by a sampling strategy that anticipated ancient DNA analyses during the excavation and by the targeted capture of SNPs (1,240k array).

We determined uniparentally inherited haplogroups using the data obtained from mitochondrial and Y-chromosome captures (Fig. 2*A*). We reconstructed complete mitogenomes for all individuals and assigned 11 different haplotypes (Datasets S1 and S5). Fleury-sur-Orne individuals mostly carry subhaplogroups of J, K, T, and H, which are characteristic of Neolithic farmers (40, 41). Three individuals (1-5, 8-6, and 953A) carry haplogroups on the derived branch of U5b (U5b1c and U5b2b3a), and the individual 31-5A carries the haplogroup U8a1, which is likely to have been inherited from European Mesolithic hunter-gatherers (HGs). The proportion of U5 and U8 haplogroups is consistent with the proportion of HG-like ancestry on both the nuclear and mitochondrial genomes observed in western Europe for the Middle Neolithic period (1, 29, 32, 42–44).

Y-chromosome haplogroups were assigned for all Fleury-sur-Orne males (Dataset S4) who carry at least three different haplogroups (Fig. 2*A*). Some individuals (1-5, 8-5, 8-6, 24-5, 26-5, and 35-5) were analyzed in a previous study (33). Haplogroup H2 (P96) is carried by six individuals and has also been found at another French Neolithic site in the Paris Basin

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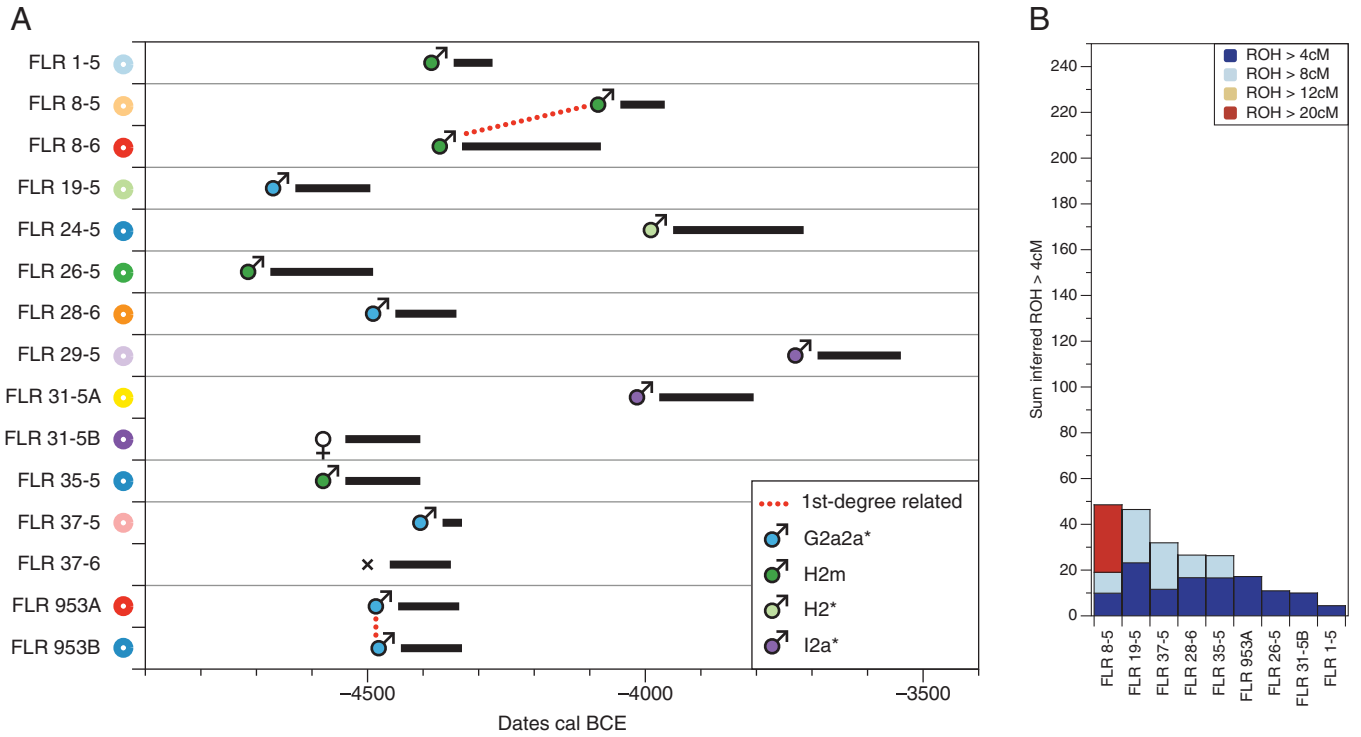


Fig. 2. (*A*) Genetic sex, Y chromosome (colored symbols), and mitochondrial haplogroups (colored circles on the *y* axis) distribution and biological relatives along the chronology of the necropolis (two-sigma ranges of calibrated radiocarbon dates are given in cal. BCE). Datasets S1 and S5 have details. (*B*) ROH for individuals with more than 300,000 SNPs on the 1,240k panel (*SI Appendix*, Fig. S6 shows the scale).

(29). G2a2a1a (PF3177) is carried by three individuals, and two more carry G2a2a (PF3147) with no more diagnostic SNPs available for further resolution. Both haplogroups H2 and G2a were common during the Early and Middle Neolithic in regions of modern-day Germany (29, 30, 32, 42) and France (29, 43) and represent the predominant Y haplogroups carried by the majority of Anatolian and related early European farmers (45, 46). Moreover, we found in Fleury-sur-Orne the specific haplogroup H2m (P96), which was recently described in ancient Neolithic individuals associated with the Mediterranean Neolithic route of diffusion (33) and also found in Ireland (1), thus linking the French Neolithic groups with the Mediterranean Neolithic expansion. Due to a very low number of Y-chromosome SNPs, individual 24-5 is assigned to haplogroup H2*, without further resolution possible. Finally, individuals 29-5 and 31-5A carry Y-haplogroups I2a1a and I2a1a2, respectively, which belong to haplogroup I2a, a lineage common in Upper Paleolithic and Mesolithic HGs that is also found in western Europe during the Early to Late Neolithic, persisting until today.

Genetic Identity, Demographic History

We used principal component analysis (PCA) on a dataset of modern-day western Eurasians and projected the ancient individuals onto the genetic variation to explore qualitatively the extended dataset (Fig. 3*A*). The group of Fleury-sur-Orne (FLR) falls within the variability of contemporaneous Middle Neolithic groups from France (29, 43), as well as generally western European Neolithic individuals, except two outlier individuals, 24-5 and 29-5, who postdate 4,000 cal. BCE and are described separately. To test the group diversity formally, we applied an f_3-outgroup test of the form f_3(individual 1, individual 2; Mbuti) on all individuals from Fleury-sur-Orne added to three individuals originating from contemporaneous sites as controls (*SI Appendix*, Figs. S1 and S2). A cluster of five individuals (19-5, 26-5, 28-6, 35-5, and 37-5), who are among the oldest of the site, stands out (Fig. 2*A* and *SI Appendix*, Fig. S1), which suggests a small community during the early stages of the cemetery. However, these five individuals carry both paternal lineages H2* and G2a*, suggesting that at least two paternal lines were using the site from its very beginning. All of the individuals from Fleury-sur-Orne have a higher within-group affinity than between distinct regional groups (*SI Appendix*, Fig. S2). This finding supports the hypothesis of the early use of the site by communities that were regionally confined to Normandy and less likely from comparative, contemporaneous individuals from the Paris Basin, east or west of France.

Echoing the Y-chromosome H2m haplogroup findings, the Fleury-sur-Orne group predating 4,000 cal. BCE (FLR pre-4,000) shows slightly higher affinities to Early Neolithic Iberian groups during the first phase of the Neolithic expansion than to groups from central Europe or eastern France according to the *f* statistic of the form f_3(FLR, test; Mbuti) (*SI Appendix*, Fig. S3). From 5,000 cal. BCE onward, we observe highly similar f_3 values, first in France and later in Britain and Ireland, compared with more eastern regions or Italian groups. We then tested for cladality between Fleury-sur-Orne and other French groups using f_4 statistics of the form f_4(Mbuti, test A; FLR pre-4,000 cal. BCE, test B), where test A represents groups from Neolithic Europe and test B represents five groups from different Neolithic time periods and regions in France. We find that FLR pre-4,000 is cladal with Middle Neolithic French groups, such as Gurgy (Paris Basin) (29), Bergheim (Alsace, Michelsberg) (43), and Clos de Roque (southern France) (47), regardless of test A (*SI Appendix*, Fig. S4), suggesting large-scale genetic homogeneity at this time. However, FLR pre-4,000 is

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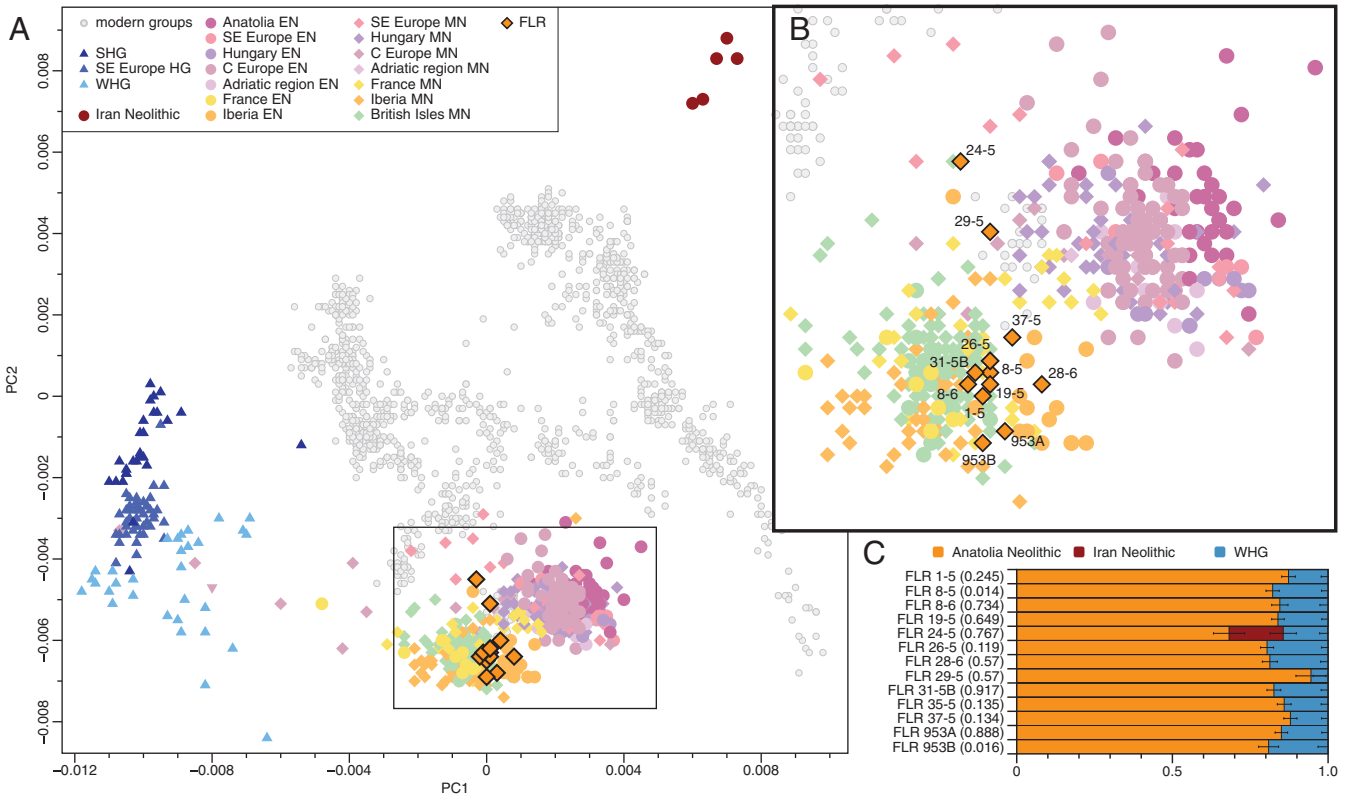


Fig. 3. Population genetics analyses. (*A*) PCA of published ancient (symbols with no outline) and Fleury-sur-Orne individuals (new and published; black outlined symbols) projected onto 777 present-day west Eurasians (gray circles). SGE, Scandinavian Hunter-Gatherers; SE, Southeastern; WHG, Western Hunter-Gatherers; EN, Early Neolithic; C, Central; MN, Middle Neolithic. (*B*) Zoomed in view of Neolithic European groups with grave numbers of Fleury-sur-Orne individuals. (*C*) Modeled ancestry components of Fleury-sur-Orne individuals using qpAdm and Anatolian Neolithic, WHG, and Iranian Neolithic groups as sources. *P* values are given in parentheses.

not cladal with the preceding Early Neolithic French Linear Pottery Culture (LBK) group but instead, has higher affinities with all western European groups from Italy, Iberia, and Germany from the fifth millennium BCE, as well as British and Irish groups postdating 4,000 cal. BCE. This confirms the genetic affinity of LBK French groups with Early Neolithic groups from southeastern and central Europe observed in the PCA and a clear break with the western Middle Neolithic European cluster to which FLR pre-4,000 belongs. Interestingly, however, when we test for cladality between FLR pre-4,000 and Pendimoun, an Early Neolithic site from southern France, FLR pre-4,000 shares more ancestry with Neolithic groups from the early phase of the Danubian wave of expansion as well as with Early Neolithic groups from Italy and Iberia. The excess HG ancestry present in Pendimoun (29) explains this pattern and highlights the distinctiveness of this region during the Early Neolithic. The higher HG proportion in southern France decreases in the later Chasséen period, and the French and western European gene pool becomes more homogeneous during the Middle Neolithic. Overall, the early phase of Fleury-sur-Orne is characterized by a genetic affinity to Iberian groups during the Early Neolithic and then more broadly to the whole of western Europe (i.e., the Iberian Peninsula and the Atlantic facade including Britain and Ireland), echoing the connections previously demonstrated for the Neolithic (1, 29, 44, 47, 48) and in line with the Y-chromosome lineage H2m reported above.

Individuals 24-5 and 29-5, who are both shifted upward from the main FLR group on the PCA, are dated to after 4,000 cal. BCE (Fig. 2*A*). Individual 24-5, although higher up on Principal Component 2 (PC2), does not appear different from the rest of the group in the f_3-outgroup analysis (*SI Appendix*, Figs. S1 and S2). However, individual 29-5 indeed shows less affinity with the FLR group (*SI Appendix*, Fig. S1), consistent with its position on PC2, but also with the regional variability known during the Middle Neolithic. In addition, this individual also carries the lowest amount of HG ancestry (Fig. 3*C*).

As shown before, Neolithic Europeans carry different HG components from genetically differentiated local groups (29, 42, 49, 50). We explored the presence of diverse HG components in both outliers 24-5 and 29-5 using an f_4 test of the form f_4(outgroup, FLR_outlier; HG_test, Loschbour) but did not detect any specific HG attraction beyond the local HG individual Loschbour we conditioned on (Dataset S6). We then applied f_3 statistics of the form f_3(FLR, test; Mbuti) to test for specific affinities to European Neolithic groups, but this resulted in inconsistent observations, which is likely to be linked to coverage issues and the limit of resolution this method offers to discern very subtle differences (*SI Appendix*, Fig. S3). Lastly, we also explored distal sources of ancestry in individual 24-5 via qpAdm and detected about 17% ancestry of a component maximized in Neolithic Iranians (*P* value = 0.76) (Fig. 3*C*). This represents another Neolithic source than the source mainly involved in the diffusion across Europe, traces of which have also been found in a few Neolithic groups all over Europe and contemporaneous Chalcolithic groups from southeastern Europe (Dataset S7 and *SI Appendix*, Fig. S5) (51, 52). This component might have been sporadically carried along with the western Anatolian component by expanding early farmers during the Neolithic diffusion and

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explains the clear differentiation of our individual 24-5 compared with the Fleury-sur-Orne group before 4,000 cal. BCE. In contrast, qpAdm models fail to distinguish between any specific additional components for individual 29-5, probably due to the relatively low coverage, where the lack of power to reject models (53) results in imprecise genetic characterization.

The outlier individuals 24-5, 29-5, and 31-5A belong to the second phase of the site postdating 4,000 cal. BCE (Fig. 2*A*), but only grave 29-5 shows deviant burial features. This grave lies along the southern ditch of monument 29, outside the mound (Fig. 1*A*), and the presence of a polished axe head among the grave goods, which appears in the archaeological records after 4,000 cal. BCE, suggests that it was placed there long after the monument was built. On the contrary, nothing in the burial treatment of the individuals 24-5 and 31-5A distinguishes them from the individuals of the older phase (both lay along the central axis of the monuments, while grave 24-5 contains arrowheads). Individual 31-5A was buried 10 cm above individual 31-5B, whose date falls within the main phase of the cemetery. The temporal gap of a half millennium in the radiocarbon dates from these two individuals is surprising given the stratigraphic position. However, the chronological break of the most recent individuals matches with the genetic outlier status and suggests newly arriving groups to the region between 4,000 and 3,500 cal. BCE, unrelated to the preceding community. The nature of the link between the two outliers 31-5B and 24-5 and the individuals of the main phase remains unclear in light of the contradiction between burial continuity and biological discontinuity. The funerary link is so close that it makes it unlikely that we are dealing with random, opportunistic reuse of old funerary monuments at later Neolithic periods, as it will be the case with the graves from the Bronze Age and Classical Antiquity. We speculate that individuals of the later phase were buried intentionally to establish a symbolic social and/or genealogical link to the forebears at the site. It is noteworthy that human remains dating to after 4,000 cal. BCE in western France are mainly found in passage tombs (i.e., burials in which the dead were undoubtedly selected) (54). Thus, these three individuals represent a particularly rare burial context for the period.

Genetic Relatedness, Spatial Organization, and Social Inferences

As commonly observed during the Neolithic period (40, 55–57), the mitochondrial diversity at Fleury-sur-Orne is also relatively high. In fact, 11 of 14 individuals carry different mitochondrial haplogroups, while K1a+195 and U5b1c are shared by three and two individuals, respectively (Dataset S1). In contrast, the level of Y-chromosomal diversity is low, while the two Y-chromosomal haplogroups identified (G2a2a and H2m) during the first phase of the site before 4,000 cal. BCE, were common at this time. We applied the software HapROH (58) to identify long runs of homozygosity (ROH) in our individuals (Fig. 2*B* and *SI Appendix*, Fig. S6). Of the nine high-coverage individuals suitable for this analysis, only individual 8-5 shows evidence for long ROH, suggesting some degree of inbreeding among his parents who could have shared a relatively close biological relationship, such as second cousins. This overall absence of long ROH (except 8-5) indicates that the group using the necropolis was sufficiently large or with controlled union rules to avoid inbreeding.

To shed further light on the social organization, we explored the genetic relatedness between individuals coanalyzed with the funerary organization. Two pairs of individuals (8-5/8-6 and 953A/953B) are first-degree related, indicating they are either parent–offspring or full siblings. Given that in both cases, the related pair of individuals does not share the same mitochondrial haplogroup, we can infer a father–son relationship. The father–son pair 8-5/8-6 shares the same monument (mon. 8) that was extended eastward in a posterior phase of use for the individual 8-5 (Fig. 1*A*). The status of the individual buried in the elongated part (8-5) appears to perpetuate the status of the first individual (8-6). The inheritance of high social status from father to son is suggested here by the position of the grave. The fact that both father and son are buried in a single monument is in line with the interpretation of a familial monument. Unfortunately, the two other monuments enclosing two graves (mon. 31 and 37) provided genomic data for only one individual of each pair and thus, no further support for our scenario. We, therefore, caution that it cannot be ruled out that the filiation seen in monument 8 is an exception within Fleury-sur-Orne. However, there is a second father–son pair in the double burial 953, although not associated with any visible monument (Fig. 1*A*). This burial is either isolated or its monument has been completely plowed flat. According to field observations and the analysis of the arrangement of the two skeletons, a simultaneous deposition of the two bodies is the most plausible interpretation. Given that a simultaneous death can explain the common grave, the status of this pair is likely to be different from the pair in monument 8.

Genetic relationships beyond the second degree cannot be reliably detected with Relationship Estimation from Ancient DNA (READ) (59). However, pairwise outgroup f_3 statistics of the form f_3(Mbuti; individual 1, individual 2) between all individuals from Fleury also did not reveal any other closer affinities between unrelated individuals (*SI Appendix*, Fig. S1). As it stands, we detect no close biological connections between the monuments. Considering that each monument at the Fleury-sur-Orne cemetery represents a single lineage, the number of unrelated individuals corresponds broadly to the number of groups in a biological sense. Therefore, our data suggest that the site was a central place where independent male elite figures were gathered in different monumental individual graves according to paternal lineages. If the pattern observed in the monuments that yield genetic material is applied to/transposed onto the other 32 monuments built at the site, we can hypothesize that as many lineages must have used the cemetery. However, considering the 200 y of the site's first occupation given by the absolute radiocarbon dates (that might be overestimated), the number of individuals recovered at the site does not meet the expected number of individuals under a strict patrilineal transmission model. It can be assumed that many of the missing individuals were related to those who were excavated. The link between the number of lineages and the size of the clans, in a social sense, remains an open question, even if the results yielded from HapROH suggest that the overall group was sufficiently large. Unfortunately, the occupation of the area is poorly understood for the period due to the fact that the contemporaneous residential sites of the monumental cemeteries have not been identified (60). The enclosure site of Diguet at Saint-Martin-de Fontenay, located 3 km from Fleury-sur-Orne (Fig. 1*A*), may be an exception, but if the palisades suggest a defensive role, the residential function of the site is not determined (61). As long as the number and the size of the settlements surrounding Fleury-sur-Orne are unknown, the size of the area over which the important individuals buried in the cemetery were drawn is impossible to assess.

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The interindividual genomic relatedness and the burial organization of the cemetery suggest a patrilineal system of biological kin. The monumentality of the burials suggests that this transmission by father to son accords with a male transmission of sociopolitical authority. We speculate that this must have been a highly codified context, which makes the presence of one female (31-5B) noteworthy. Her grave is positioned in the central axis of a monument (mon. 31), during the first phase of use, as were all males. In addition, she was buried with four arrowheads, a type of artifact that is considered to be exclusively male in its associations in the Cerny culture (26). Here, the attribution of the male-gendered artifact goes beyond the biological sexual identity. This implies a sine qua non condition for this woman and thus, a gender presented as masculine, which has granted her access, through the funerary rites, to this monumental cemetery.

Concluding Remarks

The specific architecture of the Passy phenomenon contrasts strongly with other monumental contexts known for later periods on the Atlantic shore, mainly in Britain and Ireland, from where genomic data are available (1, 2, 5). In Ireland, passage tombs contain rooms that can accommodate the bodies of several individuals (several dozen sometimes). Adult males, adult females, and subadults are buried together, and genetic relatives suggest that the funerary organization was built around full family systems, in the sense of biological and social kin, at the island level (1). In Britain, the Neolithic cairn of Hazleton North contains a five-generation family of 27 individuals, with a complex pattern of unions, showing a funerary system strongly built on close biological relatedness (5). The treatment of the deceased in the passage tombs contrasts strikingly with the few individual burials in the massive long barrows of the Passy type. Although both being substantial in size, these two types of burial monument refer to different selective rules and probably to different forms of social organizations and status systems, implying that not all monumental structures are equivalent in meaning.

Ancient DNA, now available for the entire group of Fleury-sur-Orne, significantly increases our understanding of the site. The specific selection of individuals buried at the site cannot be considered a representative snapshot of the greater community of the time. The male elite burials in Fleury-sur-Orne share many similarities with the system observed in the Paris Basin, the heart of the Passy-type monumental burial phenomenon. It should be noted, however, that even if Normandy and the Paris Basin were part of the same burial phenomenon, it is expressed differently in each region. We caution that the observations from Fleury-sur-Orne cannot be applied to the entire Passy phenomenon. For example, the absence of subadults in the necropolis of Fleury-sur-Orne constitutes an important point of contrast. In the Paris Basin, a few children hold a social rank or function that is similar to that of the male adults (62). The apparently exclusive patrilineal system from father to adult son suggested by the genetic signature at Fleury-sur-Orne contrasts with the situation in the Paris Basin and questions the strategy of inheritance during the life of the privileged figures and the status of the children. Either the children were not found due to bone preservation issues or to the shallowness of their graves causing them to disappear over time, or else, acquisition of high status was only granted at a more advanced age. Lastly, the integration of only one woman within the burial pattern of Fleury-sur-Orne is different from the selection of the dead in monumental cemeteries in the Paris Basin, where women are equally numerous as men. However, the discrimination between male and female burials at Fleury-sur-Orne leads to an increased valuing of men and therefore, to a low visibility of women (24). In Fleury-sur-Orne, the presence of only one woman, endowed with a male symbol, underlines the importance of the male identity in the regional expression of the Cerny culture.

Materials and Methods

Fieldwork Strategy. The genetic study of Fleury-sur-Orne was part of a broad scientific archaeological program. As early as the excavation phase, the sampling was made with all recommended precautions, which maximized the probability of successful DNA sequencing given the poor preservation of the bones.

Laboratory Processing. All samples were processed at the Max Planck Institute for the Science of Human History in Jena, Germany. We sampled one petrous bone, three teeth, and two unspecified bones, adapting to available material (Dataset S1). Samples were first irradiated with ultraviolet light for 30 min on all sides. A layer of the surface was mechanically removed from the petrous bone, which was then drilled from the outside (63). All teeth were cleaned with a low concentration bleach solution (3%) and cut along the cementum/enamel junction, with the powder collected by drilling into the pulp chamber (64). A layer of the surface was mechanically removed from the unspecified bones, and the bones were powdered with a mill.

DNA was extracted following the protocol described in ref. 65. Nine double-stranded libraries were built with unique index pairs (66, 67). We applied the partial UDG (half) protocol to remove most of the ancient DNA damage while preserving the characteristic damage pattern in the terminal nucleotides (68). We first screened all indexed libraries via shotgun sequencing of 5 million reads on an Illumina HiSeq4000 using the double-end (2- × 50-bp reads) kit. We used EAGER (69) to process the raw data and to select libraries with >0.05% endogenous human DNA and those showing characteristic damage aDNA patterns for downstream SNP capture. The sample 37-6 did not pass these quality controls and hence, was excluded from further analyses. Selected libraries were hybridized in solution to different oligonucleotide probe sets synthetized by Agilent Technologies to enrich for 1,233,013 informative nuclear SNP markers (30) and an in-house capture for the complete mitogenome following Maricic et al. (31) and modified after Haak et al. (32). We also applied the Y-mappable capture assay (YMCA) to target Y-chromosome variants for all male individuals (33).

Data Processing. After demultiplexing, raw sequence data were processed using EAGER. This included clipping adaptors with Adaptor Removal (70), mapping with BWA v0.7.12 (71) against the Human Reference Genome hs37d5, and removing duplicate reads with the same orientation and start and end positions. After using MapDamage v.2.0.6 to observe characteristic aDNA damage patterns, we used BamUtil (https://genome.sph.umich.edu/wiki/BamUtil:_trimBam) to clip two bases at the ends of each read for each sample to remove residual deaminations (Dataset S3). We merged together the libraries belonging to the same individuals using samtools v1.3.1 (72).

Analyses. Following Mittnik et al. (73), we determined the genetic sex by calculating the relative coverage for each of the sex chromosomes with respect to the autosomes. We set a threshold of Y ratio < 0.05 for a female and Y ratio > 0.4 for a male (Dataset S1).

We used the ANGSD package to test for the heterozygosity of polymorphic sites on the X chromosome in male individuals, applying a contamination threshold of 5% (34) (Dataset S1).

We genotyped our bam files with pileupCaller (https://github.com/stschiff/sequenceTools/tree/master/src/SequenceTools) by randomly calling one allele per position, effectively considering the human genome to be a pseudohaploid genome. We called the SNPs according to the HO (~600,000 SNPs) (31, 32) and the 1,240k panel (30). Numbers of SNPs covered at least once are given in Dataset S1.

To process mitochondrial DNA data, we extracted reads from mitocapture data using samtools v1.3.1 (72) and mapped these to the revised Cambridge

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Reference Sequence (74). We merged data from mitochondrial and 1,240k captures as well as shotgun data, called consensus sequences using Geneious R8.1.974 (75), and used Haplogrep 2 to determine mitochondrial haplotypes (76) (Datasets S1 and S5).

Merging reads obtained from the shotgun data, the 1,240k capture, and the Y-chromosome capture, we applied the method described in Rohrlach et al. (33) to manually assign Y-chromosomal haplogroups (Dataset S4).

We estimated the degree of genetic relatedness between our individuals by applying READ (59). We calculated the length of ROH using the software HapROH on individuals carrying more than 300,000 SNPs (Fig. 2 and *SI Appendix*, Fig. S6) (58).

For population genetics analysis, we merged our dataset with published ancient ($n = 598$) and modern ($n = 2583$) data to the HO (35, 36) as well as to the 1,240k SNP panel (30). We performed PCA using the program "smartpca" v10210 (EIGENSOFT) on the HO dataset (77). We computed principal components from 777 present-day west Eurasians on which ancient individuals were then projected (lsqproject: YES; shrinkmode: YES) (Fig. 3 *A* and *B*). We calculated outgroup f_3 statistics using qp3Pop and f_4 statistics using qpDstat with the f_4 mode from ADMIXTOOLS on the 1,240k SNP panel (Dataset S6) (35). We used qpAdm and the 1,240k SNP panel to estimate ancestry proportions (ADMIXTOOLS) (32). We used as sources Anatolia_Neolithic, Western Hunter-Gatherers (WHG), and Iran Ganj_Dareh_Neolithic_published and as outgroups Mbuti.DG Papuan.DG Onge.DG Han.DG Karitiana.DG Ethiopia_4500BP.SG Ust_Ishim_HG_published.DG Russia_MA1_HG.SG Czech_Vestonice Italy_Villabruna Israel_Natufian Caucasian Hunter-Gatherers (CHG) and Anatolian Hunter-Gatherers (AHG) (Fig. 3*C* and Dataset S7).

Data Availability. Wide-genome data have been deposited in the European Nucleotide Archive (ENA, accession no. PRJEB51061).

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Author affiliations: [a]De la Préhistoire à l'Actuel, Culture, Environnement, Anthropologie, Université de Bordeaux, CNRS, Pessac 33615, France; [b]Department of Archaeogenetics, Max Planck Institute for Science of Human History, Jena 07745, Germany; [c]Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology, Leipzig 04103, Germany; [d]Eco-Anthropologie, Muséum national d'Histoire naturelle, CNRS, Université de Paris Musée de l'Homme, Paris 75116, France; [e]Inrap Grand Ouest, Bourguébus 14540, France; [f]Centre de Recherche en Archéologie, Archéosciences, Histoire, Université de Rennes 1, CNRS, Rennes 35042, France; and [g]Australian Research Council Centre of Excellence for Mathematical and Statistical Frontiers, School of Mathematical Sciences, University of Adelaide, Adelaide, SA, 5005, Australia

1. L. M. Cassidy *et al.*, A dynastic elite in monumental Neolithic society. *Nature* **582**, 384–388 (2020).
2. F. Sánchez-Quinto *et al.*, Megalithic tombs in western and northern Neolithic Europe were linked to a kindred society. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 9469–9474 (2019).
3. H. Schroeder *et al.*, Unraveling ancestry, kinship, and violence in a Late Neolithic mass grave. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 10705–10710 (2019).
4. R. Yaka *et al.*, Variable kinship patterns in Neolithic Anatolia revealed by ancient genomes. *Curr. Biol.* **31**, 2455–2468.e18 (2021).
5. C. Fowler *et al.*, A high-resolution picture of kinship practices in an Early Neolithic tomb. *Nature* **601**, 584–587 (2021).
6. M. Godelier, *Métamorphoses de la parenté* (Fayard, 2004).
7. A. Mittnik *et al.*, Kinship-based social inequality in Bronze Age Europe. *Science* **366**, 731–734 (2019).
8. K.-G. Sjögren *et al.*, Kinship and social organization in Copper Age Europe. A cross-disciplinary analysis of archaeology, DNA, isotopes, and anthropology from two Bell Beaker cemeteries. *PLoS One* **15**, e0241278 (2020).
9. A. Žegarac *et al.*, Ancient genomes provide insights into family structure and the heredity of social status in the early Bronze Age of southeastern Europe. *Sci. Rep.* **11**, 10072 (2021).
10. R. A. Bentley *et al.*, Community differentiation and kinship among Europe's first farmers. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 9326–9330 (2012).
11. G. Grupe *et al.*, Mobility of Bell Beaker people revealed by strontium isotope ratios of tooth and bone: A study of southern Bavarian skeletal remains. *Appl. Geochem.* **12**, 517–525 (1997).
12. C. Knipper *et al.*, Female exogamy and gene pool diversification at the transition from the Final Neolithic to the Early Bronze Age in central Europe. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 10083–10088 (2017).
13. E. Ghesquière *et al.*, "Monumental cemeteries of the 5th millenium BC: The Fleury-sur-Orne contribution" in *Megaliths–Societies–Landscapes, Early Monumentality and Social Differentiation in Neolithic Europe*, J. Müller, M. Hinz, M. Wunderlich, Eds. (Verlag Dr. Rudolf Habelt GmbH, Bonn, Germany, 2019), pp. 177–191.
14. P. Duhamel, M. Fonton, H. Carré, "La nécropole monumentale Cerny de Passy (Yonne): Description d'ensemble et problèmes d'interprétation" in *La culture de Cerny, nouvelle économie, nouvelle société au Néolithique. Actes du Colloques International de Nemours, 9-10-11 mai 1994*, C. Constantin, D. Mordant, D. Simonin, Eds. (Mémoires du Musée de préhistoire d'Ile-de-France, 1997), vol. 6, pp. 397–448.
15. C. Constantin, D. Mordant, D. Simonin, *La culture de Cerny. Nouvelle économie, nouvelle société au Néolithique. Actes du Colloque International de Nemours, 9-10-11 mai 1994* (Mémoires du Musée de Préhistoire d'Île-de-France, 1997), **vol. 6**.
16. P. Duhamel, D. Mordant, "*Les nécropoles monumentales Cerny du bassin Seine-Yonne" in* La culture de Cerny, nouvelle économie, nouvelle société au Néolithique. Actes du Colloques International de Nemours, 9-10-11 mai 1994, C. Constantin, D. Mordant, D. Simonin, Eds. (Mémoires du Musée de Préhistoire d'Île-de-France, 1997), vol. 6, pp. 481–488.
17. P. Duhamel, M. Midgley, "Espaces, monumentalisme et pratiques funéraires des sociétés néolithiques en voie de hiérarchisation - Les nécropoles monumentales Cerny du bassin Seine-Yonne" in *Archéologie des Pratiques Funéraires. Approches Critiques*, L. Barry, Ed. (Bibracte 9, Glux-en-Glenne, France, 2004), pp. 211–248.
18. P. Chambon, A. Thomas, The first monumental cemeteries of western Europe: The "Passy type" necropolis in the Paris basin around 4500 BC. *J. Neolithic Archaeol.*, 10.12766/jna.2010.37 (2010).
19. P. Chambon, D. Mordant, J.-G. Pariat, "Sépultures du Néolithique moyen en Bassin parisien: Le cas des architectures sépulcrales" in *Relations interrégionales au Néolithique entre Bassin parisien et Bassin rhénan. Actes du 26e Colloque interrégional sur le Néolithique (Luxembourg, 8–9 November 2003)*, F. Le Brun-Ricalens, F. Valotteau, A. Hauzeur, Eds. (Archeologia Mosellana, 2007), pp. 445–467.
20. P. Chambon, Revoir Passy à la lumière de Balloy: Les nécropoles monumentales Cerny du bassin Seine-Yonne. *Bull. Soc. Préhist. Fr.* **100**, 505–515 (2003).
21. P. Chambon, "La nécropole de Balloy Les Réaudins: Approche archéo-anthropologique" in *La culture de Cerny, nouvelle économie, nouvelle société au Néolithique. Actes du Colloque International de Nemours, 9-10-11 mai 1994*, C. Constantin, D. Mordant, D. Simonin, Eds. (Mémoires du Musée de préhistoire d'Ile-de-France, 1997), vol. 6, pp. 489–498.
22. P. Chambon, A. Thomas, "Approche structurale des nécropoles monumentales du Ve millénaire dans le bassin Seine-Yonne. *Bull. Soc. Préhist. Fr.* **111**, (2014), pp. 275–290.
23. A. Thomas, "Identités funéraires, variants biologiques et facteurs chronologiques: Une nouvelle perception du contexte culturel et social du Cerny (Bassin parisien, 4700-4300 avant J.-C.)," PhD thesis, University Bordeaux 1, Bordeaux, France (2011).
24. A. Thomas, Social discriminations through the grave: Identity and status of the dead in the Cerny culture (Middle Neolithic, Paris Basin, France). *Anthropologie* **52**, 171–182 (2014).
25. P. Chambon, J.-M. Pétillon, Des chasseurs Cerny? *Bull. Soc. Préhist. Fr.* **106**, 761–783 (2009).
26. A. Thomas, Bioarchaeology of the middle Neolithic: Evidence for archery among early European farmers. *Am. J. Phys. Anthropol.* **154**, 279–290 (2014).
27. I. Sidéra, "Le mobilier en matières dures animales en milieu funéraire Cerny: Symbolisme et socio-économie" in *La culture de Cerny, nouvelle économie, nouvelle société au Néolithique. Actes du Colloque International de Nemours 9-10-11 mai 1994*, C. Constantin, D. Mordant, D. Simonin, Eds. (Mémoires du Musée de Préhistoire d'Île-de-France, 1997), vol. 6, pp. 499–513.
28. J. Desloges, "Les premières architectures funéraires de Basse-Normandie" in *La culture de Cerny, nouvelle économie, nouvelle société au Néolithique. Actes du Colloque International de Nemours, 9-10-11 mai 1994*, C. Constantin, D. Mordant, D. Simonin, Eds. (Mémoires du Musée de Préhistoire d'Île-de-France, 1997), vol. 6, pp. 515–539.
29. 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).
30. I. Mathieson *et al.*, Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* **528**, 499–503 (2015).
31. T. Maricic, M. Whitten, S. Pääbo, Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. *PLoS One* **5**, e14004 (2010).
32. W. Haak *et al.*, Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* **522**, 207–211 (2015).
33. A. B. Rohrlach *et al.*, Using Y-chromosome capture enrichment to resolve haplogroup H2 shows new evidence for a two-path Neolithic expansion to Western Europe. *Sci. Rep.* **11**, 15005 (2021).
34. T. S. Korneliussen, A. Albrechtsen, R. Nielsen, ANGSD: Analysis of next generation sequencing data. *BMC Bioinformatics* **15**, 356 (2014).
35. N. Patterson *et al.*, Ancient admixture in human history. *Genetics* **192**, 1065–1093 (2012).
36. I. Lazaridis *et al.*, Genomic insights into the origin of farming in the ancient Near East. *Nature* **536**, 419–424 (2016).
37. J. Bůžek, A method for visual determination of sex, using the human hip bone. *Am. J. Phys. Anthropol.* **117**, 157–168 (2002).
38. J. Bůžek, F. Santos, B. Dutailly, P. Murail, E. Cunha, Validation and reliability of the sex estimation of the human os coxae using freely available DSP2 software for bioarchaeology and forensic anthropology. *Am. J. Phys. Anthropol.* **164**, 440–449 (2017).
39. P. Murail, J. Bruzek, J. Braga, A new approach to sexual diagnosis in past populations. Practical adjustments from Van Vark's procedure. *Int. J. Osteoarchaeol.* **9**, 39–53 (1999).
40. G. Brandt *et al.*; Genographic Consortium, Ancient DNA reveals key stages in the formation of central European mitochondrial genetic diversity. *Science* **342**, 257–261 (2013).
41. B. Bramanti *et al.*, Genetic discontinuity between local hunter-gatherers and central Europe's first farmers. *Science* **326**, 137–140 (2009).
42. M. Lipson *et al.*, Parallel palaeogenomic transects reveal complex genetic history of early European farmers. *Nature* **551**, 368–372 (2017).

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43. 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).
44. S. Brace *et al.*, Ancient genomes indicate population replacement in Early Neolithic Britain. *Nat. Ecol. Evol.* **3**, 765–771 (2019).
45. I. Mathieson *et al.*, The genomic history of southeastern Europe. *Nature* **555**, 197–203 (2018).
46. M. Feldman *et al.*, Late Pleistocene human genome suggests a local origin for the first farmers of central Anatolia. *Nat. Commun.* **10**, 1218 (2019).
47. I. Olalde *et al.*, The Beaker phenomenon and the genomic transformation of northwest Europe. *Nature* **555**, 190–196 (2018).
48. L. M. Cassidy *et al.*, Neolithic and Bronze Age migration to Ireland and establishment of the insular Atlantic genome. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 368–373 (2016).
49. Q. Fu *et al.*, The genetic history of Ice Age Europe. *Nature* **534**, 200–205 (2016).
50. V. Villalba-Mouco *et al.*, Survival of Late Pleistocene hunter-gatherer ancestry in the Iberian Peninsula. *Curr. Biol.* **29**, 1169–1177.e7 (2019).
51. D. M. Fernandes *et al.*, The spread of steppe and Iranian-related ancestry in the islands of the western Mediterranean. *Nat. Ecol. Evol.* **4**, 334–345 (2020).
52. V. Villalba-Mouco *et al.*, Genomic transformation and social organization during the Copper Age-Bronze Age transition in southern Iberia. *Sci. Adv.* **7**, eabi7038 (2021).
53. É. Harney, N. Patterson, D. Reich, J. Wakeley, Assessing the performance of qpAdm: A statistical tool for studying population admixture. *Genetics* **217**, iyaa045 (2021).
54. G. Bailloud, "Le Néolithique et Chalcolithique en France" in *La Protohistoire de l'Europe*, J. Lichardus, M. Lichardus, Eds. (PUF, Paris, France, 1985), pp. 516–568.
55. W. Haak *et al.*; Members of the Genographic Consortium, Ancient DNA from European early neolithic farmers reveals their near eastern affinities. *PLoS Biol.* **8**, e1000536 (2010).
56. A. Szécsényi-Nagy *et al.*, The maternal genetic make-up of the Iberian Peninsula between the Neolithic and the Early Bronze Age. *Sci. Rep.* **7**, 15644 (2017).
57. M. Lacan *et al.*, Ancient DNA reveals male diffusion through the Neolithic Mediterranean route. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 9788–9791 (2011).
58. H. Ringbauer, J. Novembre, M. Steinrücken, Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nat. Commun.* **12**, 5425 (2021).
59. J. M. Monroy Kuhn, M. Jakobsson, T. Günther, Estimating genetic kin relationships in prehistoric populations. *PLoS One* **13**, e0195491 (2018).
60. A. Chancerel, C. Marcigny, E. Ghesquière, "Le plateau de mondeville (calvados): Du néolithique à l'âge du bronze" (Documents d'archéologie française 99, Edition de la Maison des Sciences de l'Homme, Paris, France, 2006).
61. C.-C. Besnard-Vauterin, E. Ghesquière, M. Besnard, C. Marcigny, Une enceinte Néolithique moyen à Saint-Martin-de-Fontenay (Calvados). *Bull. Soc. Préhist. Fr.* **101**, 141–146 (2004).
62. A. Thomas, P. Chambon, P. Murail, Unpacking burial and rank: The role of children in the first monumental cemeteries of Western Europe (4600-4300 BC). *Antiquity* **85**, 772–786 (2011).
63. E. Orfanou, M. Himmel, F. Aron, W. Haak, Minimally-invasive sampling of pars petrosa (os temporale) for ancient DNA extraction V.2. (2020). dx.doi.org/10.17504/protocols.io.bqd8ms9w. Accessed 11 December 2020.
64. G. U. Neumann, A. Andrades Valtueña, J. A. Fellows Yates, R. Stahl, G. Brandt, Tooth sampling from the inner pulp chamber for ancient DNA extraction V.2. (2020). https://dx.doi.org/10.17504/protocols.io.bqebmtan. Accessed 11 December 2020.
65. I. Velsko, E. Skourtianoti, G. Brandt, Ancient DNA extraction from skeletal material. (2020). https://dx.doi.org/10.17504/protocols.io.baksicwe. Accessed 11 December 2020.
66. F. Aron, G. U. Neumann, G. Brandt, Half-UDG treated double-stranded ancient DNA library preparation for Illumina sequencing. (2020). dx.doi.org/10.17504/protocols.io.bmh6k39e. Accessed 11 December 2020.
67. R. Stahl *et al.*, Illumina double-stranded DNA dual indexing for ancient DNA. (2020). https://dx.doi.org/10.17504/protocols.io.bakticwn. Accessed 15 June 2021.
68. N. Rohland, E. Harney, S. Mallick, S. Nordenfelt, D. Reich, Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20130624 (2015).
69. A. Peltzer *et al.*, EAGER: Efficient ancient genome reconstruction. *Genome Biol.* **17**, 60 (2016).
70. M. Schubert, S. Lindgreen, L. Orlando, AdapterRemoval v2: Rapid adapter trimming, identification, and read merging. *BMC Res. Notes* **9**, 88 (2016).
71. H. Li, R. Durbin, Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* **26**, 589–595 (2010).
72. H. Li *et al.*; 1000 Genome Project Data Processing Subgroup, The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
73. A. Mittnik, C.-C. Wang, J. Svoboda, J. Krause, A molecular approach to the sexing of the triple burial at the upper paleolithic site of Dolní Věstonice. *PLoS One* **11**, e0163019 (2016).
74. R. M. Andrews *et al.*, Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat. Genet.* **23**, 147 (1999).
75. M. Kearse *et al.*, Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649 (2012).
76. H. Weissensteiner *et al.*, HaploGrep 2: Mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Res.* **44**, W58–W63 (2016).
77. N. Patterson, A. L. Price, D. Reich, Population structure and eigenanalysis. *PLoS Genet.* **2**, e190 (2006).

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