Supplementary Materials for

Ancient genome-wide DNA from France highlights the complexity of interactions between Mesolithic hunter-gatherers and Neolithic farmers


*Corresponding author. Email: maite.rivollat@u-bordeaux.fr (M.R.); haak@shh.mpg.de (W.H.)

Published 29 May 2020, Sci. Adv. 6, eaaz5344 (2020)
DOI: 10.1126/sciadv.aaz5344

The PDF file includes:

Legends for tables S1 to S17
Table S18
Figs. S1 to S18
Supplementary Text SI1 to SI10
References

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/22/eaaz5344/DC1)

Tables S1 to S17
Supplementary Tables

Tables S1-S5. Descriptive results.
Combined as tabs in Microsoft Excel spreadsheet:

Table S1. “Sum data”
Summary of archaeological, temporal and geographic context and main genetic results.

Table S2. “EAGER output”
Summary of output statistics of the sequence processing pipeline EAGER for shotgun and 1240k SNP capture sequencing.

Table S3. “Mt contam est”
Overview of the mitochondrial contamination estimates for all libraries in this study.

Table S4. “Mt hg assignment”
Overview of the mitochondrial haplogroup assignment for all individuals in this study.

Table S5. “Y chr hg assignment”
Overview of the Y chromosome haplogroup assignment for all individuals in this study.

Tables S6-S17. Supplementary analyses of genome-wide data.
Combined as tabs in Microsoft Excel spreadsheet:

Table S6. “Group labels”
Overview of group labels for newly generated data and the complete comparative dataset of published ancient individuals used in this study.

Table S7. “f4 subgroups sites”
Summary of f4-tests exploring the genetic differentiation of sub-groups at the Neolithic sites PEN, LBR, and OBN.

Table S8. “f4 ImpressaCardial”
Summary of f4-tests exploring the genetic affinities to Impressa-Cardial groups in Neolithic groups from southern France.

Table S9. “f4 affinity Neo-HG”
Summary of f4-tests exploring the genetic affinities of Neolithic groups to a combined set of European HG individuals consisting of Loschbour, KO1 and La Braña.

Table S10. “qpAdm MODEL A”
Summary of results of qpAdm ancestry modelling of Neolithic groups using MODEL A, which tests a two-way mixture model with European_HG- and Anatolia_Neolithic as sources.

Table S11. “qpAdm LBK OBN”
Summary of results of qpAdm ancestry modelling of Neolithic individuals from the site OBN and the LBK group using MODEL A, which tests a two-way mixture model with European_HG- and Anatolia_Neolithic as sources.

Table S12. “qpAdm MODEL B”
Summary of results of qpAdm ancestry modelling of European HG individuals using MODEL B, which tests a four-way mixture model with Villabruna, EHG, Goyet Q2 and Anatolia_Neolithic as sources.
Table S13. “qpAdm MODEL C”
Summary of results of qpAdm ancestry modelling of Neolithic groups using MODEL C, which tests a three-way mixture model with Anatolia_Neolithic, Loschbour and KO1 as sources.

Table S14. “qpAdm results Fig 4”
Summary of qpAdm results that were used to create Figure 4 and Figure S9, including a detailed description of the group constellations.

Table S15. “qpAdm MODEL D”
Summary of results of qpAdm ancestry modelling of Neolithic groups using MODEL D, which tests a three-way mixture model with Anatolia_Neolithic, Villabruna and Goyet Q2 as sources.

Table S16. “TGM009”
Summary of f4-statistics and qpAdm ancestry modelling results of individuals TGM009. Detailed exploration of the genetic ancestry profile of outlier individual TGM009.

Table S17. “DATES”
Summary of results of admixture dates estimates for all Neolithic groups using the software package DATES.

Table S18. Second degree related individuals from the Neolithic site Gurgy.
Supplementary Figures

Figure S1. Heat map showing pairwise outgroup f3-statistics between European HG individuals. Lighter colours indicate higher shared genetic affinity between individuals.
**Figure S2.** Summary of f4-tests that explore the shared genetic affinities of European HG individuals with Villabruna and EHG (A) and with Loschbour and KO1 (B).
Figure S3. Summary of f4-tests that explore the excess affinity to Anatolian Neolithic farmers in all published Iron Gates HG individuals.
Figure S4. Summary of results of qpAdm ancestry modelling of European HG individuals as a four-way mixture with Villabruna, EHG, Goyet Q2 and Anatolia_Neolithic as sources (MODEL B).
Figure S5. Summary of f4-tests that explore the differential affinities of Neolithic groups to various combinations of HG individuals, such as WHG-like (Villabruna, Loschbour, BDB) and EHG-like (EHG and KO1) ancestries. In panels A, B and C we use Mbuti as outgroup, while in panels D, E and F we use Anatolia_Neolithic.
Figure S6. Summary of results of qpAdm ancestry modelling of Neolithic groups as a three-way mixture with Anatolia Neolithic, Loschbour, and KO1 as sources.
Figure S7. Two-dimensional plot of $f_3$-statistics comparing the differential attraction to two HG sources (Loschbour and KO1) in published and newly reported Neolithic groups sorted by geography (east and west of the Rhine river).
Figure S8. Summary of qpAdm ancestry modelling results of Neolithic groups as a three-way mixture with Anatolia Neolithic, Villabruna and Goyet Q2-like ancestry.
Figure S9. Temporal series of maps summarizing the variable sources of HG and Anatolian Neolithic ancestries in prehistoric Europeans using qpAdm ancestry modelling with MODEL B, C and D (see Material & Methods and Table S14 for model details).
Figure S10. Summary of f4-tests that explore the genetic affinities of Neolithic groups from continental Europe to those of the British Isles.
SI 1. Archaeological context

*European Mesolithic*

The Mesolithic period in Europe begins around 9700 calibrated Before Common Era (cal BCE) with the global climatic change, marking the transition from the Pleistocene to the Holocene, and gradually lasts until the arrival of the first Neolithic farmers, or as late as 5000 cal BCE on the Atlantic coast or 4000 cal BCE in the British Isles and in Scandinavia. A general tendency toward smaller microliths including a wide typology variability and subsequently many cultural entities is observed in the archaeological record all over Europe except in the northern lowlands. The Early Mesolithic cultural diversity reproduces the very wide Final Upper Palaeolithic diversity. Diverse environments, such as sea shores, forests, high-altitude lawns or humid continental areas, are exploited using a combination of gathering, hunting, and fishing, and various plants are ground and stored. Residential mobility seems to be relatively high with no indications of long-term occupations (61, 62).

In western and central Europe, a discontinuity of technologies and a relative homogenization is visible between Early and Late Mesolithic in the middle of the 7th millennium cal BCE. Then, new operative schemes replace previous industries with massive lithic assemblages of blades and trapezes during the Late Mesolithic (Blade and Trapeze Complex, BTC). Several cultural entities are defined geographically, for example the Castelnovian in Italy and south of France. The projectile shapes spread from the Mediterranean area towards central and northern Europe but the operative scheme of using blade pressure techniques remains localized in south-western Europe. The origin of the Mesolithic BTC around 6500 cal BCE is also unclear, and links in the lithic industry appear to be consistent with the Upper Capsian from the Maghreb (63-65), but similarities have also been suggested eastwards within the Pontic area (66). The cultural background is diverse and complex enough to be observed at the time of the Neolithic farmers’ arrival, for instance in the Italian Peninsula where a few technical differences can be seen in assemblages of the Castelnovian and the first farmers (63).

*Origin of European Neolithic*

The first sparse evidence of farmer settlements outside the near-eastern and central Anatolian core areas are dated c. 6700 cal BCE around the Aegean, in western Anatolia, Crete and the Argolis (1, 67, 68). Some of these sites lack pottery entirely (such as Knossos X or Uluçak VI), while in others pottery has been found, albeit in small quantities. The question of whether such assemblages indeed represent a pre-ceramic phase, maybe even a belated Pre-Pottery Neolithic (PPN) tradition, or whether they are a-ceramic, i.e. contemporary to other early ceramic sites but lacking ceramics due to either cultural preferences or samples size, is still debated (69). The exact origin of this “Pioneer Neolithic” around 6700 cal BCE is still unclear, but several authors (1, 70) suggest that the Levant currently appears more likely than central Anatolia, despite the lack of Neolithic ‘stepping stones’ close to the southern coast of Turkey.

More frequent Pottery Neolithic (PN) sites occur from c. 6500-6400 cal BCE onwards with several styles of monochrome, slipped, painted and even impressed wares in the Lakes region of Turkey (71), north-western Turkey (72) and Thessaly (70) without a clear connection to either the PPN or to the earliest PN sequences.

The Impresso-Cardial Complex (ICC), which covered large parts of the Adriatic coast, Italy, southern France and part of the Iberian Peninsula, represents the Mediterranean route of the Neolithic expansion, and the earliest dispersal of farming towards Europe during the first half of the 6th millennium cal BCE (for an historiographical review, see 3).

The earliest archaeological cultural complex associated with the beginning of farming and animal husbandry in central Europe during the second half of the 6th millennium cal BCE is the Linear Pottery culture (or LBK after German *Linearbandkeramik*, or Rubané in
French), which, from its origin in Transdanubia in the Carpathian Basin, reached across Austria, the Czech Republic, Germany, Benelux, and northern France in the West, and Poland and Ukraine in the East. Extending from a spread of the Neolithic along the Danube, it thus represents the continental route (2, 73).

Specific pottery styles, such as La Hoguette and Limburg generally associated to LBK contexts in eastern France and the Benelux, raise the question of potential links with Mediterranean ICC pottery traditions (16, 74).

Figure S11. Simplified map of the Pottery Neolithic expansion (dates are given in years cal BCE).

The Mediterranean stream

In the western Mediterranean region, the earliest Neolithic settlements date to the beginning of the 6th millennium cal BCE in southern Italy. These offer a broad set of cultural aspects that cannot be easily distinguished by their pottery styles and are therefore considered in their entirety as the Impresso-Cardial Complex (ICC) (3, 22, 75).

The origin of the ICC and its links with the Balkans are still unclear, since the earliest farming contexts in continental Greece (ca. 6500 cal BCE) are not well understood (68, 76).

The spread of the ICC out of SE Italy to the West has been very fast since it reached the Ligurian and French coastlines around 5800 cal BCE following a mainly maritime route (22, 77). Recent work on pottery technology (41) shows that the groups at the origin of the Neolithic dispersal in the Tyrrenian Sea and westwards differed from those which were localised south-east of the Apennine mountain chain. This raises the question of a very early cultural differentiation among the south-Italian ICC groups.

The relationship of the ICC and the rare Late Mesolithic Mediterranean BTC is still challenging with only a few clear cases of continuity of the lithic industries (e.g. in Sicily, 78). The evidence of a persistence of Castelnovian in the Tusco-Emilian Apennine, the Po Plain and the Alps until c. 5500 cal BCE highlights the diversity of interaction processes between farmers and hunter-gatherers (63, 64, 75).
Most of the evidence for expansion of the ICC tradition in southern France is observed after 5500 cal BCE, and even more extensively after 5300 cal BCE. From 5300 to 4800 cal BCE, the Neolithic expansion in southern France, as well as in the Iberian Peninsula, was mainly carried by groups with Cardial and/or Epicardial pottery styles (79). A Cardial (or Epicardial) expansion towards the Atlantic coasts of France could also have occurred during this period, despite geographical gaps in southern France and chronological uncertainties around controversial typological connections as well as older and contested radiocarbon dates (80-82).

The continental stream

The LBK emerges in Transdanubia around 5500 cal BCE resulting from a first pulse of expansion of the Starčevo-Körös-Criş cultural complex around 6000 cal BCE between the Black Sea, the Carpathian Basin and the Dinaric Alps (83). The LBK develops quickly in western Transdanubia, while the Alföld Linear Pottery (AVK) represents a related counterpart east of the Tisza river in the Carpathian Basin. The early LBK shows a dense spatial occupation of arable lands, and is characterised by an improved control of the environment, intensification of agriculture, and cultural contact with local Mesolithic groups (14). These farmer groups then spread north-westwards following the natural Danube river route to reach the south of Germany, leaving the AVK behind. They quickly and broadly cross the loess lands in a dispersed but nonetheless connected way (84), eventually reaching the Paris Basin in the West and the Ukraine in the East. Microlithic projectiles show continuous contacts between expanding farmers and local hunter-gatherers across the entire region.

At the turn of the 5th millennium, the LBK diversifies into various derived local cultural groups such as Stichbandkeramik, Großgartach or Rössen, which cover parts of Germany, Poland and the Czech Republic from 4700 to 4200 cal BCE (85). During the 5th millennium cal BCE, the northeastern part of today’s Germany is considered to be an interface between Neolithic societies and Late Mesolithic groups such as the Ertebølle culture in Scandinavia and northern Germany, with mutual signs of exchanges. It is only with emerging Funnel Beaker culture (or Trichterbecherkultur, TRB) around 4100 cal BCE that the traditional hunting, fishing and gathering subsistence economy of these groups disappears in favour of farming (37). The TRB culture includes diverse groups, especially along the southern fringe such as the Altentiefstich, Walternienburg or Elb-Havel contexts (86).

Reaching western Germany around 5300 cal BCE, the LBK spread to the west of the Rhine. In the subsequent periods, a diversification of the cultural complexes becomes visible in the archaeological record. In Alsace and the Upper and Middle Rhine Valley, the Middle Neolithic period was represented by different successive cultures deriving from the Rubané: after 5000 cal BCE the Hinkelstein culture appeared, which evolved into the Großgartach culture after 4950 cal BCE, followed by Rössen (ca. 4750 cal BCE) and then Bischheim after 4600 cal BCE. Around 5100 cal BCE, the terminal manifestation of the local LBK reached the Paris Basin where it is called Rubané Récent du Bassin Parisien (RRBP; 87). The end of the Early Neolithic period in the Paris Basin around 4900 cal BCE is characterised by the emergence/development of a new culture called Villeneuve-Saint-Germain (VSG), the funerary aspects of which clearly stand in Early Neolithic tradition (88). While the VSG culture also shares numerous other characteristics with the Rubané, some elements in the material culture indicate a southern influence, identifying the VSG as a mixed culture combining eastern and southern Neolithic features, as well as HG components (42). In Normandy, where the Mesolithic substratum is poorly defined, the arriving RRBP and VSG communities appear clearly connected to the Paris Basin (89). The emergence of the Cerny culture (ca. 4700 cal BCE) marks the transition to the Middle Neolithic. This culture is distributed from the Paris Basin to Normandy and Brittany (90) and monumental burial structures can be found in different regions. Some archaeological elements of the Cerny context, such as the abandonment of blade production and the increase of funerary items...
related to hunting may indicate a legacy of Mesolithic traditions (91). The complexity of cultural contexts was described by Augereau (92) as the result of various degrees of assimilations between local Mesolithic HG and incoming farmers.

Development and interactions during the 5th millennium BCE

Evidence of contact between LBK and ICC during the Late Cardial and Epicardial phases stems from a small set of LBK pottery fragments within ICC contexts from the Massif central and the Vaucluse department (5200-5100 cal BCE; 93, 94). Symmetrical influence from the South is demonstrated by the intensification of the trade of Mediterranean shells (i.e. perforated Columbella rustica, Cardium sp. disks; 95) and bracelets made of limestone from the Vaucluse plateau (96), in which the Rhone valley must have played a crucial role.

In northern Italy (Po Plain, Tosco-Emilian Apennine, Pre-Alps and Alps) during the second half of the 6th millennium, specific aspects of Pottery Neolithic (i.e. Vho, Fiorano) are probably the result of complex interactions between Castelnovian Mesolithic, Mediterranean ICC, Balkans and central Europe (75, 97). During the transition from the 6th to the 5th millennium, this leads to a massive renewal of the material culture and the emergence of the Square Mouth Pottery complex (SMP; 98), which shows connections to the Balkans and central Europe (97). The ‘Middle Neolithic’ SMP culture is understood to have been involved in the formation of the southern Chassey culture (from the site of Chassey), as highlighted by SMP pottery and lithics in the Pré-Chasséen and Proto-Chasséen (4550-4350 cal BCE) of south-eastern France. Intricately linked with Jade axe production, the role of the SMP culture in connecting European cultures at large scale has to be strongly considered (99).

Lastly, western France represents another important region where the two Neolithic expansion routes converged at around 5000 cal BCE. Although details of the chronology are still debated, the archaeological record indicates influences from both Neolithic streams leading to a specific local profile called NACA (Néolithique Ancien Centre-Atlantique) which seems to integrate both stylistic traditions (25, 100). The first Mediterranean signs seem as early as the late 6th millennium in the pottery records, while elements from the VSG culture, deriving from the LBK tradition, are visible at the early 5th millennium in the continental part of the middle Loire region. These first farmer groups also show interactions with local late hunter-gatherers, carrying a material culture partly based on Mesolithic traditions (25, 62). The development of Megalithic constructions, starting in the 5th millennium on the Atlantic coast, also shows a complex legacy, combining diverse elements (monumental graveyards, round funerary huts, box-like stone cists, graves under boulders, etc.) which could have their origin in the late developments of the first continental and Mediterranean Neolithic, as well as from local hunter-gatherer background (101).
SI 2. Archaeological sites

Bad Dürenberg (Saale district, Saxony-Anhalt, Germany)

Contact: Harald Meller, Susanne Friederich

The site of Bad Dürenberg (BDB) is a Mesolithic open-air double burial of an adult female and an infant. BDB is located 20 km south-east of Halle in the Saale district, a region rich in mineral salts, that was excavated during emergency drainage works in 1934 with only minimal documentation. Three direct radiocarbon dates from the adult female provide a late Boreal age for the individual. The burial is not connected with any known camp site, although it was proposed that a camp of hunter-gatherer-fishers must have been eroded by the waters of the nearby river Saale.

The skeleton of the adult woman is well-preserved and almost complete (101). The remains of an infant between four and twelve months are associated with the adult but are less complete. Both individuals were buried in a small rectangular pit in an upright seated position. Heated ochre was found in the sediment, as well as an elaborate amount of grave goods. These consist of a complete unshed roe deer antler, numerous animal tooth pendants, bone and stone tools, remains from mammals, birds, reptiles and molluscs as well as a polished stone adze head. This amount of goods ascribed to the burial as well as anatomical variations in the craniovertebral junction causing neurological distortions have led to the interpretation of the discovery as that of a possible shaman grave (102).

A previous mitochondrial DNA (mtDNA) analysis on both individuals was performed in the ancient DNA lab of the Institute of Anthropology at the University of Mainz. The female individual was reported to carry mitochondrial haplogroup U4, characteristic of Palaeolithic and Mesolithic hunter-gatherers, but no evidence of kinship was provided (4).

For this study we sampled one femur fragment as well as the petrous bone of the adult individual. Calibrated date intervals are given at 2-sigma level.

- BDB001 / HK34:823a; 7060-6611 cal BCE (7930±90BP; OxA-3136), 6593-6253 cal BCE (7580±80BP; Bln-2221)

Bottendorf (Roßleben, Kyffhäuser district, Thuringia, Germany)

Contact: Harald Meller, Susanne Friederich, Kurt W. Alt

The open-air site of Bottendorf (BOT) is located 45 km south-west of Halle. It was excavated by the local community in 1939 after the discovery by workmen during sand exploitation. A detailed documentation was made by experts called to the site. The site is composed of three Mesolithic burials, two single and one double, about 15 meters apart from each other, as well as an Early Bronze Age funeral space (103).

The first single burial, Bottendorf I (HK39:47a), contains an adult male, in an upright seated position. Ochre and two relatively large and obliquely retouched flint blades were associated with the individual. The double burial of an adult female and a five to seven years old child is called Bottendorf II (HK39:147a, b). Partly disturbed by the sand excavation, the documentation remains incomplete. The adult is seated upright facing the north-west, with the left arm placed on the left leg. No grave goods were found, but the bones were colored with red ochre. Recently, a third single burial has been identified as Mesolithic. It contains the skeletal remains of an immature individual of around seven to ten years old, without grave goods or ochre (104).

Dietary isotope analysis was conducted on these individuals which showed an expected opportunistic subsistence strategy, homogenous between individuals (104). PCR-
based ancient DNA analysis were carried out in the Institute of Anthropology at the University of Mainz, but yielded no results (4).

We re-sampled both individuals from Bottendorf BII/1 (femur) and BII/2 (tooth) for this study. Calibrated date intervals are given at 2-sigma level.

- BOT004 / HK39:147a-b BII/1; 5555-5497 cal BCE (6570±36; OxA-27246), 5599-5517 cal BCE (6604±35; OxA-27247)
- BOT005 / HK39:147b BII/2; 5636-5572 cal BCE (6685±38; OxA-27248)

Pendimoun (Castellar, Alpes-Maritimes, Provence-Alpes-Côte-d'Azur, France)

Contact: Didier Binder, Henri Duday

Pendimoun (PEN) is a rock-shelter discovered in 1955, which is 4 km north of Menton, very close to the French-Italian border. In 1956, preliminary field work provided a male burial attributed to the Cardial period, but this individual was not considered in this study due to contradictory dates from chemical preservatives (105). More extensive excavations performed between 1985 and 2006 (106, 107) revealed a detailed archaeological sequence from the Early Mesolithic (Sauveterrian) to modern times, with a specific important sequence attributed to the Impresso-Cardial complex, dated to between 5750 and 5200/5150 cal BCE. The latter includes three inhumations - two females and one male (107) - dated to the Early Cardial stage, which postdates the Impressa stage. Direct radiocarbon dates and stable isotopes on human remains provide a narrow range between 5480 and 5330 cal BCE for this phase, placing them amongst the oldest Neolithic individuals analysed in this part of the Mediterranean coast. The burials are primarily deposited in individual pits, with distinctive stone architectures for the females and the male. All three individuals show signs of pathologies or trauma, raising questions about a selective recruitment.

Unpublished DNA analyses from the ancient DNA labs in Bordeaux and Madrid by M.-F. Deguilloux, E. Fernandes, C. Gamba, E. Arroyo-Pardo and M.-H. Pemonge have established that the mitochondrial DNA (mtDNA) was preserved.

We sampled for this study one petrous bone for each of the three individuals. Calibrated date intervals are given at 2-sigma level.

- PEN001_real1 / Pendimoun F1; 5480-5370 cal BCE (6445±40BP; GrA-26893)
- PEN001_real2 / Pendimoun F2; 5480-5360 cal BCE (6440±40BP; GrA-26894)
- PEN003 / Pendimoun H2; 5480-5337 cal BCE (6450±40BP; GrA-32061)

Les Bréguières (Mougins, Alpes-Maritimes, Provence-Alpes-Côte-d'Azur, France)

Contact: Didier Binder, Henri Duday

Located on the Mediterranean coast 5 km north from Cannes, Les Bréguières (LBR) is a deep rocky fault line exposed by a quarry in the 1960's with a length of 7 to 8 meters, and a width of one meter. Access must have been located in the missing portion of the cavity. An archaeological rescue operation under basic conditions in 1966 and 1967 allowed researchers to excavate about a thousand faunal bones and over 6000 human remains, primarily bones and teeth. The relative stratigraphic position of the skulls acquired from the excavator allowed us to obtain a set of 11 direct dates on human and faunal remains (108), and a supplementary date for this study (LBR001/BR 8). A Bayesian modelling approach
indicates that the human remains were more probably deposited between 5250 cal BCE and 4600 cal BCE, i.e. between the late Cardial stage at the earliest, and by the Square Mouth Pottery culture for the most recent. Therefore, the oldest individuals of Les Bréguières postdate the pioneer Impressa settlements at the Tyrrhenian area (ca 5850-5500 cal BCE) (22).

An intensive anthropological study was performed on the scattered remains (108), allowing the identification of 61 individuals, amongst them 35 adults and 26 subadults. Some anatomical connections indicated primary deposits, as well as secondary or deferred burial practices, illustrated by several traces of artificial modifications and by the marked deficit of extremity bones. Both sexes are equally represented, on the basis of what could be identified, but a lack of younger individuals has led to the supposition of restricted access to the cavity.

Preliminary aDNA analyses on 30 petrous bones has been performed in PACEA, University of Bordeaux, corresponding to 30 different individuals. Mitochondrial haplogroups have been obtained for 20 samples, and will be provided in a forthcoming study.

For this study we resampled the petrous bones of 5 individuals out of the available 20, using the associated mitochondrial results as proxy for good DNA preservation. Calibrated date intervals are given at 2-sigma level.

- LBR001 / BR 8; 4896-4713 cal BCE (5920±35BP; LTL-18523A); phase C2-C4; Cf. Early SMP / PreChasse
- LBR002 / BR 19; 5120-4820 cal BCE (6104±45BP; LTL-8482A); phase C5; Cf. Cardial / SMP transition
- LBR003 / BR 20bis; 5032-4747 cal BCE (5864±45BP; LTL-12315A); phase C5; Cf. Cardial / SMP transition
- LBR004 / BR 28; 5032-4778 cal BCE (5964±45BP; LTL-12316A); phase C5; Cf. Cardial / SMP transition
- LBR005 / BR 32; 5369-4979 cal BCE (6151±45BP; LTL-13784A); phase C8-C6, Cf. Late Cardial

Viesenhäuser Hof (Stuttgart-Mühlhausen, Baden-Württemberg, Germany)

Contact: Eva Rosenstock, Joachim Wahl, Detlef Gronenborn

Located 20 km north of Stuttgart, the site Viesenhäuser Hof (SMH) is an important Linearbandkeramik (LBK) necropolis with 177 graves out of 247 graves present on the site associated with this culture. Excavations took place in three phases, one phase in 1931, one phase in 1977 and then in 1982, and finally one phase between 1993 and 1998. The site is thought to be amongst the oldest LBK funerary areas in south-western Germany. Divided into two areas by a road, the site is separated in Stuttgart-Mühlhausen I in the south-east and Stuttgart-Mühlhausen II in the north-west. Area II is thought to be older, and strontium isotopes obtained from these individuals indicate a high percentage of non-local profiles (109, 110). On the contrary, area I is thought to be later and strontium data do not indicate significantly non-local individuals. These differences suggest a variance in subsistence and/or mobility during the LBK period. In general, carbon and nitrogen stable isotopes point to a mixed terrestrial diet (111).

Burials are mainly simple, flat and homogenous in size. Some double burials can be found in area II. A sex ratio of 1.32 in favour of males is observed, and grave goods are more numerous for males, and increasing with time, while for females they decrease in time, although it has to be noted that organic grave goods might have been deposited near the head or feet. In general, the female area I burials are also poorer in grave goods than those from area II. For males, grave goods are more numerous and various, including red chalk.
and pottery, food items, polished stone tools, arrowheads, bone tools as well as fire-making equipment and *spondylus* shell ornaments. According to the artefact assignment, the hypothesis of social differences among the buried has been proposed. Different groups include several rather poor burials grouped around a preferably furnished grave in the area II.

Eighteen new radiocarbon dates have been obtained from the *Klaus-Tschira-Archäometrie-Zentrum*, Mannheim, Germany, for graves 9, 13, 20, 22, 24, 32, 34, 37, 38, 44, 45, 56, 59, 61, 67, 71, 107, and 114 (see below). Two additional radiocarbon dates have been obtained from the Laboratory for C-14 Dating, Heidelberg, Germany, for graves 26 and 79.

Previous ancient DNA analysis has been performed on the site. One deep-sequenced individual was published in Lazaridis *et al.* 2014 (34), with new libraries published then in Lipson *et al.* 2017 (6). Eight individuals were also published by Haak *et al.* 2015 (5), performed with the 390K SNPs probe set, and three of them were captured with the 1240K SNPs probe set and published in Mathieson *et al.* 2018 (7).

We report genome-wide data from 26 new individuals, for which one tooth per individual was sampled (see Table S1). Calibrated date intervals are given at 2-sigma level.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Grave</th>
<th>Site</th>
<th>Calibrated Date BCE</th>
<th>MAMS Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMH004</td>
<td>61, P44</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5296-5070</td>
<td>MAMS-36741</td>
</tr>
<tr>
<td>XN164</td>
<td>22/205</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5294-5068</td>
<td>MAMS-36728</td>
</tr>
<tr>
<td>XN165</td>
<td>9/3</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5291-5063</td>
<td>MAMS-36725</td>
</tr>
<tr>
<td>XN166</td>
<td>129/2119</td>
<td>Stuttgart-Mühlhausen II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XN167</td>
<td>67/339</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5310-5079</td>
<td>MAMS-36743</td>
</tr>
<tr>
<td>XN168</td>
<td>38/518</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5288-5060</td>
<td>MAMS-36734</td>
</tr>
<tr>
<td>XN169</td>
<td>13/507</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5222-5054</td>
<td>MAMS-36726</td>
</tr>
<tr>
<td>XN170</td>
<td>32/209</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5207-4985</td>
<td>MAMS-36730</td>
</tr>
<tr>
<td>XN171</td>
<td>24/29</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5293-5066</td>
<td>MAMS-36729</td>
</tr>
<tr>
<td>XN172</td>
<td>34/517</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5292-5064</td>
<td>MAMS-36732</td>
</tr>
<tr>
<td>XN173</td>
<td>114/1993</td>
<td>Stuttgart-Mühlhausen II</td>
<td>5211-5013</td>
<td>MAMS-36733</td>
</tr>
<tr>
<td>XN174</td>
<td>26/195</td>
<td>Stuttgart-Mühlhausen I</td>
<td>4987-4711</td>
<td>MAMS-25933</td>
</tr>
<tr>
<td>XN175</td>
<td>59/530</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5287-5059</td>
<td>MAMS-36740</td>
</tr>
<tr>
<td>XN176</td>
<td>105/1975</td>
<td>Stuttgart-Mühlhausen II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XN180</td>
<td>71/535</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5216-5022</td>
<td>MAMS-36745</td>
</tr>
<tr>
<td>XN182</td>
<td>79/381</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5301-5049</td>
<td>MAMS-36736</td>
</tr>
<tr>
<td>XN183</td>
<td>44/262</td>
<td>Stuttgart-Mühlhausen I</td>
<td>5299-5073</td>
<td>MAMS-36735</td>
</tr>
</tbody>
</table>
• XN191 / gr. 107/1977, Stuttgart-Mühlhausen II; 5317-5209 cal BCE (6267±30BP; MAMS-25931)
• XN205 / gr. 130/2120, Stuttgart-Mühlhausen II
• XN206 / gr. 56/528, Stuttgart-Mühlhausen I; 5285-5054 cal BCE (6202±25BP; MAMS-36738)
• XN207 / gr. 120/2150, Stuttgart-Mühlhausen II
• XN211 / gr. 37/256, Stuttgart-Mühlhausen I; 5213-5040 cal BCE (6165±25BP; MAMS-36733)
• XN215 / gr. 20/208, Stuttgart-Mühlhausen I; 5206-4951 cal BCE (6115±25BP; MAMS-36727)
• XN224 / gr. 68/1428, Stuttgart-Mühlhausen II
• XN225 / gr. 111/1979, Stuttgart-Mühlhausen II

Schwetzingen (Karlsruhe district, Baden-Württemberg, Germany)

Contact: Eva Rosenstock, Joachim Wahl, Detlef Gronenborn

Located 20 km south-east of Mannheim in the Rhine valley, the site of Schwetzingen (SCH) is another large LBK necropolis with 203 identified graves. Discovered in 1988 during construction works, the site was excavated in the following months, followed by archaeological and anthropological studies (112). Direct radiocarbon dates on some individuals, as well as pottery decoration ascribe the site to the younger period of LBK culture, between 5200 and 5000 cal BCE (unpublished data). In total, 194 graves including 202 individuals and 15 cremations were excavated. Of these, 74 were identified as females and 56 as males and a high proportion of sub-adult individuals was observed, especially for the 0-4-year-olds and 5-9-year-olds. The bodies were mainly flexed in a left-sided position, though with multiple exceptions. The necropolis has no uniform skeletal orientation but north-east to south-west is the most common form. Half of the deceased received grave goods, showing a poorer equipment compared to other LBK graveyards. Among these, pottery, stone artefacts, flint, bone, antler, shells, snails, millstones, minerals and traces of the use of powdered minerals and pigments are well represented. Flint blades seem to be typical for older male individuals, whereas the younger individuals had more fragmented blades. Large ensembles of arrowheads also characterize the northern section, while only one or two can be found in the southern section. Of note are four specific stone tools, among these two perforated adzes, one of which was found in a child’s grave. A large number of triangular bone tips are male-specific, and contrary to the arrowheads, concentrated in the southern area. The site was interpreted as burials of a hierarchical/stratified society, with a difference in time and/or furnishing traditions. Strontium isotope analyses showed that non-local individuals are mostly orientated toward north or north-east (113). Schwetzingen along with other sites also provided data for a higher mobility of females in LBK societies, consistent with a patrilocal system (114). Moreover, nitrogen and carbon stable isotope analyses confirmed a mixed terrestrial diet (111).

Ten samples (mainly teeth, and one phalange) were selected for this study (Schwetzingen 122; Table S1). Calibrated date intervals are given at 2-sigma level (115).

• SCH001 / Schwetzingen 9
• SCH004 / Schwetzingen 33
• SCH007 / Schwetzingen 71
• SCH009 / Schwetzingen 99
• SCH010 / Schwetzingen 100
• SCH011 / Schwetzingen 107
• SCH014 / Schwetzingen 122
Halberstadt, mass grave (Harz district, Saxony-Anhalt, Germany)

Contact: Harald Meller, Susanne Friederich, Kurt W. Alt

Located 55 km south-west of Magdeburg, Halberstadt-Sonntagsfeld (HBS) is a large LBK site, grouping several dozen typical longhouses and at least 38 regular LBK inhumations associated with the houses, representing so-called settlement burials (116). An expected representation of both sexes and across all ages appears in these regular graves. Contrasting with this domestic settlement, a mass grave lies in the south-eastern area of the settlement, also dated with six direct radiocarbon dates to the late LBK, yielding an overall date range of 5289-4856 cal BCE, which can be constrained to probably 5080-4997 cal BCE (117). Several mass graves have been excavated in Germany and Austria from the younger phase of the LBK period. There are clear signs of perimortem lethal violence on the remains, often localized on the skulls, and representative population samples lead to surprise attacks on villages, possibly by neighbouring communities.

The mass grave from Halberstadt includes nine individuals in different positions, with few fragmentary grave goods (117). Seven individuals are identified anthropologically as males, one as a possible male and one as a possible female. This pattern differs from the other known LBK mass graves, which consist of young adults or adults (<40 years-old). The absence of children also differs from what is usually observed. Numerous perimortem trauma on the skulls, as well as on some parts of the body are described. Isotope analysis (strontium and carbon/nitrogen) revealed a non-local signature for the HBS mass grave individuals. The association of all these elements leads to the hypothesis of an attacking rather than an assaulted group, which was then killed by the resident community (117).

We sampled and report seven individuals from the mass grave, mainly teeth, as well as a femur for HBS002. Calibrated date intervals are given at 2-sigma level.

- HBS002. / Fnr. 38 und 67; 5207-4855 cal BCE (6093±42BP; MAMS-23988), 5207-4857 cal BCE (6096±42BP; MAMS-23989)
- HBS004 / Fnr. 5
- HBS005 / Fnr. 104
- HBS006 / Fnr. 51
- HBS007 / Fnr. 52; 5212-4961 cal BCE (6134±43BP; MAMS-23992), 5209-4950 cal BCE (6122±43BP; MAMS-23993)
- HBS008 / Fnr. 63
- HBS009 / Fnr. 87

Gurgy “Les Noisats” (Yonne, Bourgogne – Franche-Comté, France)

Contact: Stéphane Rottier

Gurgy "Les Noisats" (GRG) is a necropolis located in the south of the Paris Basin, in the Yonne department. The complete excavation of the necropolis took place between 2004 and 2007. Direct radiocarbon dates on human remains range between 5000 and 4000 cal BCE, but the most intensive occupation period ranges from 4900 to 4500 cal BCE. Around
4700 cal BCE monumental funerary structures appeared in the Paris Basin along with the Cerny culture, characterized by the Structures de Type Passy (STP). However, the Gurgy necropolis does not correspond to this type as the site lacks any monuments or visible structuring of the burial ground. Some perishable casing discovered at the site may echo the Chamblandes cists in western Switzerland and in the Massif central. It is also worth mentioning that the cultural influences from the Mediterranean at the regional level indicate the existence of an already established network at the time.

A total of 134 pits were excavated in the Gurgy necropolis, uncovering 128 individuals. An anthropological investigation (118) determined adults of both sexes (30 males, 20 females and 23 with an undetermined sex) as well as 62 subadult individuals, representing no apparent selection in the buried individuals. Burials are simple and double, with a diversity of grave types. The pit sizes vary from 0.71m to 2.83m according to the grave type. The bodies decomposed in an empty space, proving the presence of a rigid container/coffin. Bodies were placed in a left-side flexed position, and mainly orientated north-south. Grave goods are scarce and randomly distributed across the necropolis.

Previous aDNA analyses have been performed at PACEA, University of Bordeaux, on 102 individuals, providing 55 mitochondrial DNA profiles (26). Mitochondrial haplotypes (based on HVR-1 data) showed an expected Neolithic variability albeit with a higher hunter-gatherer proportion of U5 haplotypes compared to published data from central Europe. A connection with Mediterranean sphere has also been suggested (26, 119).

For this study we sampled one petrous bone from 22 out of the 55 individuals that were successfully typed for mtDNA, using this as a proxy for good DNA preservation. Calibrated date intervals are given at 2-sigma level.

- GRG003 / GLN 204
- GRG008 / GLN 210
- GRG015 / GLN 215B
- GRG016 / GLN 220; 4539-4367 cal BCE (5635±35BP; Lyon-4680(SacA-9832))
- GRG018 / GLN 223; 4765-4540 cal BCE (5795±45BP; Lyon-5875(SacA-14823))
- GRG019 / GLN 225
- GRG021 / GLN 227
- GRG022 / GLN 229
- GRG023 / GLN 231A
- GRG025 / GLN 232C
- GRG027 / GLN 243A; 4829-4623 cal BCE (5870±40BP; Lyon-4444(SacA-8627))
- GRG028 / GLN 243B; 4795-4616 cal BCE (5855±40BP; Lyon-4446(SacA-8629))
- GRG032 / GLN 248; 4316-3984 cal BCE (5303±40BP; Ly-12933)
- GRG035 / GLN 256
- GRG041 / GLN 276
- GRG043 / GLN 280
- GRG047 / GLN 287
- GRG049 / GLN 289B; 4772-4662 cal BCE (5773±58BP; Ly-12934)
- GRG050 / GLN 294
- GRG052 / GLN 299
- GRG056 / GLN 313
- GRG057 / GLN 317; 4784-4546 cal BCE (5815±45BP; Lyon-4449(SacA-8632))

Fleury-sur-Orne "Les Hauts de l'Orne" (Calvados, Normandie, France)

Contact: Emmanuel Ghesquière
The cemetery Fleury-sur-Orme (FLR) is located in Normandy, south of Caen. Covering 21 hectares, the site has not been completely investigated. A rescue excavation in 2014 and 2016 was led by the Institut National de recherches archéologiques préventives (Inrap). The earliest occupation dates to around 4700 cal BCE, but extends beyond 4000 cal BCE. Unique to this site are the STP monuments, in which the deceased were buried. Thirty-two of these monuments have been identified, ranging from 8 to 400 meters in length (120). Usually, but sometimes two burials, are found in such monuments in an axial position. Their shallow depth indicates monuments with a semi-underground structure. Of the 17 individuals that were buried in the necropolis, only two come from a double burial. The macroscopic preservation of the bones is poor, but some hypotheses about orientation of the body and the funerary rites have been proposed, such as positioning on the side and empty space during decomposition, with additional elements suggesting structured chambers/coffins made of organic material.

Almost no grave goods are available for the entire cemetery, which makes an attribution to the Cerny culture difficult, even though it would be consistent with the overall chronology and the STP, similar to what can be found in the Paris Basin. The few grave goods are mainly arrow heads, linked to an archer status, and possibly hunters. Some burials also provide faunal remains, e.g. complete sheep, from which the skulls and hides (because of missing hooves) had been removed. One of the prevalent hypotheses is that the cemetery is a burial place for leaders of different regional groups.

Previous mitochondrial analyses have been performed in the frame of the PhD of M. Rivollat (unpublished data). Fourteen individuals have provided mitochondrial profiles, in line with the characteristic Neolithic diversity and showing no maternal relationship between individuals.

For this study we sampled either a petrous bone or a tooth for nine out of the 14 individuals who provided mitochondrial results, using this as a proxy for good DNA preservation (see Table S1). Date intervals are given at 2sigmas.

- FLR001 / FLR 1-5: 4345-4275 cal BCE (5640±30BP; Beta-421634)
- FLR002 / FLR 8-5: 4045-3965 cal BCE (5210±30BP; Beta-416654)
- FLR003 / FLR 19-5: 4630-4495 cal BCE (5610±30BP; Beta-416656)
- FLR004 / FLR 26-5: 4675-4490 cal BCE (5720±30BP; Beta-413158)
- FLR005 / FLR 37-5: 4365-4330 cal BCE (5500±30BP; Beta-416661)
- FLR007 / FLR 953A: 4445-4335 cal BCE (5520±30BP; Beta-416662)
- FLR010 / FLR 28-6: 4450-4340 cal BCE (5550±30BP; Beta-416659)
- FLR013 / FLR 31-5B: 4540-4405 cal BCE (5580±30BP; Beta-413162)
- FLR014 / FLR 35-5: 4540-4405 cal BCE (5650±30BP; Beta-413164)

Obernai (Bas-Rhin, Alsace, France)

Contact: Philippe Lefranc, Hélène Réveillas

The Obernai necropolis (OBN) is located in Alsace, about 30 km south-west of Strasbourg. The site was excavated in 2013 led by Inrap as part of a rescue excavation and revealed a large temporal sequence from the Palaeolithic to the early Middle Ages. Given the time pressure, the excavation of the Neolithic necropolis was not exhaustively detailed. Moreover, the natural slope of the site may have led to a strong erosion of the archaeological elements in the southern part of the site. Both direct radiocarbon dates on Neolithic human remains and typological dates based on pottery and ornament items gave a consistent chronological range from 5000 to 4400 cal BCE (121). Based on archaeological data (specifically the pottery styles), these inhumations were associated with the different successive post-LBK groups known in the region: Großgartach, Planig-Friedberg, and
Rössen. The site is organised in three different areas which are chronologically successive. The southern-most area, with three Großgartach attributed graves, is the most ancient. The central area contains two Planig-Friedberg culturally-related burials, while the northern part is the densest area, containing Planig-Friedberg and Rössen attributed burials. Given that a clear succession of Großgartach, Planig-Friedberg, and Rössen cultures could be established at the regional scale (122), it has been proposed that the Obernai necropolis was successively used by culturally differentiated farmer communities. However, it is worth noting that radiocarbon dates overlap and are not consistent with the culturally successive division of the areas, echoing the general problems encountered in the establishment of cultural chronological limits (23).

A total of 27 individuals were excavated. Burials were all single and characterized by the presence of a wooden structure (121). Anthropological investigations identified 12 adults and 14 immature individuals, and one individual was too badly preserved to be assigned to any category. The majority of bodies were deposited along the west-east axis, with the head pointing west. The deceased bodies were mostly lying on their backs and presented upper limbs either in extension or with flexed arms near the abdomen, and their lower limbs were systematically in extension. These positions are in line with the post-LBK of the Rhine Region and with the funerary tradition from LBK (123), showing a clear cultural continuity between these successive aspects at Obernai.

Seven new radiocarbon dates have been obtained from the Centro di Fisica applicate, Datazione e Diagnostica (CEDAD) in Lecce, Italy, for OB 4088-1, OB 4090-1, OB 4730-1, OB 4001-1, OB 4035-1 and OB 4664-1.

Previous aDNA analyses have been performed at PACEA, University of Bordeaux, on 23 individuals, providing 17 mitochondrial profiles (124). Mitochondrial haplotypes (based on HVR-1 data) showed the characteristic central European Neolithic variability and a connection to LBK individuals.

We sampled one petrous bone each from nine out of the 17 individuals that provided mitochondrial results as a proxy for good DNA preservation. Two additional individuals were sampled (OB 4035-1 and OB 4664-1), which had not been analysed before. Calibrated date intervals are given at 2-sigma level.

- OBN001 / OB 4008-1; 4766-4550 cal BCE (5809±33BP; OxA-30277)
- OBN002 / OB 4017-1; dating failed
- OBN003 / OB 4022-1; 4689-4504 cal BCE (5744±33BP; OxA-30278)
- OBN004 / OB 4088-1; 4681-4499 cal BCE (5732±25BP; LTL-18525A)
- OBN005 / OB 4090-1; 5007-4847 cal BCE (6044±25BP; LTL-18526A)
- OBN006 / OB 4730-1; 5208-4986 cal BCE (6124±25BP; LTL-18527A)
- OBN007 / OB 4001-1; 4518-4350 cal BCE (5601±45BP; LTL-18528A)
- OBN008 / OB 4015-1; 4688-4505 cal BCE (5743±30BP; SUERC-52378)
- OBN009 / OB 4035-1; 4521-4369 cal BCE (5628±25BP; LTL-18529A)
- OBN010 / OB 4106-1; dating failed
- OBN011 / OB 4664-1; 4886-4533 cal BCE (5749±25BP; LTL-18531A)

**Tumulus C du Péré (Prissé-la-Charrière, Deux-Sèvres, Nouvelle-Aquitaine, France)**

Contact: Luc Laporte, Chris Scarre, Ludovic Soler

The Péré tumulus C at Prissé-la-Charrière (PRI) is located in central-western France. The site was excavated over more than 20 fieldwork campaigns since 1995 (and still ongoing), and consisted of a 100-meter-long mound. The sequence of building phases and use has been directly dated, with the help of human remains between 4300 and 4000 cal BCE. The complex architectural setting is the result of specific stages of construction for
each distinct, and at least partly successive, architectural project. The mound includes three separate burial chambers, each associated with a distinct stage of construction. One of the chambers (III) had not been opened since the Neolithic time and had survived totally intact, free from human or animal disturbance. Remains of at least eight individuals, corresponding to four adults and four children, had been deposited in the chamber. Spatial analysis of the skeletal material provided arguments (persistence of specific articulations after initial decomposition, consistency of the distribution of disarticulated elements) in favour of bodies that were deposited in sitting position along the walls that subsequently collapsed into the empty chamber space (125, 126).

Previous aDNA analyses have been performed at PACEA, University of Bordeaux, on six individuals, which yielded three mitochondrial DNA profiles, including haplogroup N1a, which is common in Neolithic central Europe (127).

Teeth from three individuals from the previous study were re-sampled for this study. Calibrated date interval is given at 2-sigma level.

- PRI001 / Prissé 1; 4340-4171 cal BCE (5406±32BP; OxA-15063)
- PRI005 / Prissé 5
- PRI006 / Prissé 6

**Tangermünde (Stendal district, Saxony-Anhalt, Germany)**

*Contact: Harald Meller, Susanne Friederich*

Located 10 km south-east of Stendal, the site of Tangermünde (TGM) is a flat grave necropolis. Discovered in 1883 during sand exploitation, the site was excavated in different campaigns from 1947 to 1950, during which 36 graves were unearthed, of which 21 were located on the final archaeological map (128). Typological features and direct radiocarbon dates associate the site with the Elb-Havel context of the Funnelbeaker culture (or TRBK; Trichterbecherkultur); a Neolithic context that is mainly found in the north of central Europe during the 4th millennium cal BCE.

All the individuals were deposited laying on their back, except one in a flexed position from a double burial. It was suggested that the orientation of the bodies was gender- and age-specific, with males and children more in an ESE-WNW orientation, and females in an E-W orientation. Grave goods were also distributed according to sex-specific rules. Arrows, quivers and bracelets were considered male-specific, with awls and tooth jewellery being found around the males and children’s heads and necks. Archaeological documentation describes a kind of ‘goods bag’, including bones, punches and stones as well as crossed knives or arrow holders and a tail axe, and was interpreted as “hunter” or “warrior” equipment specific to male individuals. Ornaments and pottery elements indicate differential spatial distribution, while other elements do not.

We sampled petrous bones from two individuals for this study. Two new radiocarbon dates have been obtained from Curt-Engelhorn-Zentrum Archäometrie in Manheim, for both individuals. Calibrated date intervals are given at 2-sigma level.

- TGM008 / Grave 17, HK50:707; 3330-2936 cal BCE (4442±29BP; MAMS-41244)
- TGM009 / Grave 21, HK51:121; 3369-3109 cal BCE (4556±26BP; MAMS-41243)
SI 3. Sex determination

We determined the genetic sex by calculating the number of reads mapping to each of the sex chromosomes relative to the autosomes (52). X-ratio (mapped X-Chromosome reads / mapped autosomal reads) and Y-ratio (mapped Y-Chromosome reads / mapped autosomal reads) are plotted in Figure S12.

Applying a threshold of < 0.05 for the Y-ratio for females and a Y-ratio > 0.4 for males (see Table S2), we determined the genetic sex of a total of 101 individuals (42 females and 59 males). One individual (PRI005) yielded a Y-ratio of 0.063, slightly outside of our established threshold. Two individuals, SCH018 and XN206, yielded a Y-ratio of 0.38 and 0.35, respectively, which is outside the threshold for males. All three individuals also had a relatively small number of reads, which could explain this deviation, but we cautiously flagged them with a question mark in tables S1 and S2.

Individual HBS006 shows an X-ratio of 0.68, compatible with females, and a Y-ratio of 0.48, compatible with males, at the exclusion of contamination from the opposite sex. This deviation cannot be explained by a low number of reads, as this individual has more than 260,000 covered SNPs on the 1240K panel. This finding is consistent with karyotype XXY, which has been described as Klinefelter’s syndrome, or 47,XXY, and represents an aneuploidy specific to sexual chromosomes. The prevalence is about 0.1-0.2% in the general population (129). With over 2000 prehistoric individuals genotyped so far and following the first reported case in a historic Icelandic individual (130), this case is consistent with the prevalence reported.

Figure S12. Summary plot of X and Y ratios for each individual to determine the genetic sex.
SI 4. Mitochondrial data

For the majority of individuals, we generated complete mitogenomes using an in-house mitochondrial capture probe set following the method published in Maricic et al. 2010 (27) and modified according to Haak et al. 2015 (5; see Table S4). For the LBK sites HBS and SCH, as well as the Mesolithic sites and TGM, we did not perform a separate mitochondrial capture, and instead harvested the mitochondrial DNA (mtDNA) reads from the by-catch of the 1240K data, which in most cases resulted in low-coverage haplotype calls (between 0.1X and 18.1X).

Amongst the Mesolithic individuals, BDB001, as published in Bramanti et al. 2009 (4), carries the HG-characteristic haplotype U4, which is confirmed by our findings. Haplogroup U4 is found in many HG individuals from Scandinavia, Russia and south-eastern Europe, corresponding geographically to the KO1-like or EHG component (7, 8, 131). In contrast, none of the WHG cluster individuals is carrying U4, but U5 or U8 instead (4, 34, 132). No mitochondrial haplogroup could be determined for both BOT individuals.

The 43 newly typed LBK individuals were assigned to haplogroups H, T, K, N1a, W, I, J and U5. These are all considered characteristic for the early farmer population (4-8, 133), with the exception of haplogroup U5, typically found among WHG individuals (4, 34, 132). Haplogroup N1a, originally described as a characteristic signal for early central European farmers (134), but later also found in Spain (5), is confirmed to be present in high frequency in LBK groups. We observed a frequency of 20.9% in our new groups, closely matching the previously reported frequency in German LBK individuals (22.2%, N=27), and resulting in an overall estimate of 20% for all German LBK (N=70). Here, the site of SMH stands out with an N1a frequency of 32%, while no first- or second-degree relationship have been observed (see part SI 6), suggesting this high percentage could have arisen from closely related family groups. Interestingly, among the 43 new LBK individuals, only one individual carries the HG-associated haplogroup U5 (XN173), in line with the low HG nuclear component found in other LBK groups.

ICC and derived groups in southern France also present the expected diversity for Neolithic groups, with individuals assigned to haplogroups H, HV, K, J. However, two individuals from LBR carry haplogroup U5 (LBR002) and U8 (LBR004), which are both characteristic for HG, as described above. This is consistent with the higher proportion of HG component in the nuclear data.

The mtDNA data from the French Neolithic dated to the 5 th millennium cal BCE also exhibits the characteristic diversity of Neolithic groups, carrying haplogroups J, K, T, H, N1a, V, X2, as well as U5 and U8. Previous mitochondrial PCR-based results from OBN, GRG and PRI individuals (26, 124, 127) could be confirmed. Out of 35 individuals, 29 had been correctly assigned, 2 are newly reported (OBN009 and OBN011) and three haplogroups could be refined and reassigned based on complete mitochondrial genome data (Table S4). OBN003 haplogroup changes from K1a4a1e to K1a1b1, GRG003 from H to K1a+195, and GRG032 from U5b to K1a+195. As described in the previous studies, we observe a much higher proportion of HG-associated haplogroups (15.5%) compared to Neolithic groups further east (1.4% in all LBK groups combined). However, haplogroup N1a, a characteristic feature of LBK groups (134), has also been found in all northern French groups, from Alsace to the Atlantic coast, and confirms the link with the LBK sphere. These findings are consistent with our nuclear data, and shows that mitochondrial DNA – when studied at the population level – can provide sufficiently robust observations with respect to population history.

The latest individual, TGM009, did not yield sufficient coverage to precisely determine a haplogroup, which remains a basal JT. Nevertheless, J or T are both characteristic for the early farmer groups, and in line with the 23.4% of farmer component seen in the nuclear DNA of this individual.
SI 5. Y chromosome data

Y chromosome haplotypes have been assigned manually using pileups of Y-SNPs included in the 1240K SNP panel that overlap with SNPs included on the ISOGG SNP index v.14.07, last downloaded 07/01/2020 (57).

The only male amongst our Mesolithic individuals, BOT004, carries the haplogroup I. Due to the low coverage of this individual, we cannot obtain a better resolution on the branch of the haplogroup I (see Table S5). However, haplogroup I is very common among post-Late Glacial Maximum (LGM) hunter-gatherer individuals from Europe who carry the haplogroup I in high proportion during the post-LGM (59.2%, N=49). For instance, Goyet Q2 (15), Loschbour (34) and Bichon (135) are all members of subgroups of I2a. The other main Y-haplogroups contributing to the HG Y-chromosome diversity in Europe are C, J, and R (33).

Among our three LBK sites, male individuals from Schwetzingen (SCH) yielded a very low number of Y-SNPs allowing only few detailed assignments. Male individuals from SMH in south-western Germany carry mainly haplogroup H2 (10 out of 13). A few individuals with low coverage data resulted in limited-resolution typing, such as F (XN206, XN215) and CT (XN191, XN207). The male LBK individuals from eastern Germany carry exclusively subgroups of haplogroup G2, such as G2a2a and G2a2b. The individuals from the mass grave in Halberstadt that includes almost exclusively males, who were non-local according to strontium data and with signs of pre- and perimortem trauma, are believed to be of a group of attackers that was killed or executed in situ. However, the male individuals buried in the regular graves and attributed to the settlement nearby also carry G2a2 (6), not allowing any group differentiation at the Y chromosome level. Overall, H2 (SMH) and G2a2 (HBS) are the predominant Y-haplogroups carried by the majority of Anatolian and related early European (8, 136), which seem to replace local Y chromosome haplogroup profiles (I, C, and R), an observation that is consistent with results of the autosomal data.

Among male individuals from the new ICC and post-ICC groups from south of France, four males carry the HG-associated haplogroup I. The male from PEN carries haplogroup I2a1a2b (M423), and the three individuals from LBR haplogroup I2a1b (M436) or I2a1b1a1b (Z2057). Later during the Neolithic, haplogroup I can be found mainly in Spain (39, 137) and in the British Isles (39, 40). Interestingly, haplogroup I can also be found in post-ICC individuals from south of France (39), suggesting local/regional continuity of male lineages that are associated with HG ancestry. As shown previously, haplogroup I had been very common in HG individuals in Europe. Unlike LBK groups, French ICC individuals do not carry the common male-signal from Anatolia. Again, in line with nuclear data, ICC and derived groups from southern France show a stronger signal of HG contribution, which became established in the subsequent Middle Neolithic period.

During the post-LBK Neolithic in France, the pattern of Y chromosome diversity seems to be structured according to sites. Males from OBN carry both haplogroups I2a1a2 (M423), and C1a2 (Z38888), which is known from other Mesolithic HG individuals such as La Braña (8, 12) but also from Anatolian and other first farmer groups (7, 8). Male individuals from GRG in the Paris Basin carry mostly G2a2b2a, as well as H2 (P96) in two individuals (GRG022 and GRG041). Both haplogroups are characteristic for incoming farming groups and are commonly found among male LBK individuals (see above; 6, 8, 136). Males from FLR in Normandy also show both haplogroups G2a2 and H2, but here the G2a2 sub-branch (i.e. G2a2a1) is different from GRG and also found in German LBK groups (HBS). The only male individual from PRI on the Atlantic coast shows the haplogroup I2a1a2a (L161.1). When plotted on a map, we observe that Y haplogroups during the Middle Neolithic in France are not distributed geographically, but rather according to site. The general, limited within-site Y chromosome variability observed suggests patrilinear societies during the Neolithic.

Another noteworthy observation is that males from the British Isles carry exclusively haplogroup I2 (12, 40). While we could show with nuclear data that the early farmers from the British Isles are connected through Normandy and the Paris Basin to the Mediterranean
region, the specific Neolithic Y-haplogroups from northern France do not seem to have been carried across the Channel.

Finally, the male individual TGM009 from the TRBK context in Germany, showing 76.6% of HG component, is also carrying a HG-associated haplogroup I2, albeit of branch I2a2a1b (S6596), which have not been described in prehistoric individuals yet.

Overall, a Y-chromosome specific HG signal is still present in male lineages in the newly typed Neolithic groups from southern France, e.g. OBN in Alsace and PRI on the Atlantic coast, as well as in TGM009. Individuals from all three sites also show an affinity with HGs at the autosomal level, albeit for different reasons that are linked to specific local histories (see main text).

\[ \text{Figure S13. Times series and distribution of Y chromosome haplogroups in prehistoric European individuals from 14,000 to 3,000 years cal BCE.} \]
SI 6. Kinship analysis

We used the software READ (Relationship Estimation from Ancient DNA) (30) to estimate the degree of genetic relatedness between our newly typed individuals. This method has been shown to reliably identify first and second-degree relatedness between pairs of individuals. To take the different genetic ancestry profiles of our main meta-populations into consideration, we separated our dataset into a Mesolithic and a Neolithic subgroup.

To test for relatedness between both individuals from BOT, we included all published Mesolithic individuals from western Europe, except the deeply covered and diploid Loschbour from Luxembourg who would have biased the analysis. We then calculated the proportion of non-matching alleles (P0) between individuals BOT004 and BOT005. The resulting normalised value for the pair BOT004/BOT005 is 0.78539, corresponding to first-degree related individuals. Given the low coverage of both BOT individuals, we merged them and used them together in the subsequent analyses.

We applied the same rationale to our Neolithic dataset, using only the newly generated dataset as the cohort size is sufficiently large with known unrelated individuals. Only the site of Gurgy showed several second-degree related individuals (values ranging between 0.90625 and 0.8125). We present the table of related individuals as well as the plot of all combinations for Gurgy individuals below (Table S18).

Table S18. Second degree related individuals from the Neolithic site Gurgy.

<table>
<thead>
<tr>
<th>Individual 1</th>
<th>Individual 2</th>
<th>P0</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRG008</td>
<td>GRG057</td>
<td>0.8823</td>
</tr>
<tr>
<td>GRG015</td>
<td>GRG041</td>
<td>0.87562</td>
</tr>
<tr>
<td>GRG018</td>
<td>GRG057</td>
<td>0.88734</td>
</tr>
<tr>
<td>GRG021</td>
<td>GRG027</td>
<td>0.88381</td>
</tr>
</tbody>
</table>

Figure S14. READ analysis results of pairwise comparisons of 22 individuals from the site Gurgy (GRG). The first four pairs are the 2nd degree related individuals shown in Table S18.
SI 7. Phenotypic and functional SNPs

We investigated individual genotypes of 13 SNPs associated with phenotypes of interest (8, 138). We calculated the genotype likelihood (using the UnifiedGenotyper module of the Genome Analysis Toolkit (GATK) v.3.5.) based on the number of reads from our bam files (phred-scale mapping quality score (MAPQ)>30 and base quality score (BASEQ)>30) for each specific position to determine the presence of the ancestral (non-effect) or derived (effect) alleles. We restricted the analysis to individuals with >200,000 SNPs mapping to the Human Origins panel (N=52). Descriptive results are shown in Figure S15. We also provide the allele frequencies calculated for each group (SMH, LBR-PEN, OBN, GRG, FLR), bearing in mind the limited number of individuals in each group (Figure S16).

A total of five allele variants in different genes associated with predicting light-skin color have been investigated. We observed the derived states in rs16891982 (SLC45A2), rs7119749 (GRM5), rs10831496 and rs1042602 (TYR) following Mathieson et al. 2015 (8), with an additional variant in rs1426654 (SLC24A5) (139). Derived alleles are absent in our Mesolithic BDB001 individual, in accordance with previous findings that showed that light pigmentation alleles appear fixed in Neolithic populations and this is likely linked to migration (8). Neolithic individuals show a mixed pattern with either homozygosity for the derived allele or high heterozygosity. The derived allele in rs1426654 (SLC24A5) seems fixed in most of our groups (Figure S16) except for in LBR-PEN with a lower frequency, but this position has a low-coverage overall. Individuals with higher HG component (TGM009 (76.6%), PEN001_real2 (55.3%), OBN003 (43.3%)) also show various genotypes for all loci considered.

An allele variant associated with light eye color, rs12913832 in OCA2, has also been included. Here, BDB001 carries the derived allele, as do TGM009, OBN003, and the three individuals from PEN with higher amounts of HG ancestry, whereas the remainder of the Neolithic individuals show a mixed pattern of dark and light eye color.

One of the strongest signals of selection detected in past populations in Europe predicts lactose tolerance in rs4988235 in LCT (8). Previous studies showed that the selection for lactase persistence in Europe must have occurred during the Bronze Age or even later (8, 140, 141). In agreement with this statement, our Mesolithic and Neolithic individuals do carry the ancestral allele for this locus, with the exception of a single read carrying the derived allele for PEN001_real1 and PEN001_real2, which make these individuals heterozygous for this SNP. According to the current state of knowledge, this seems highly unlikely, and given the low coverage we cannot rule out a sequencing error or residual DNA damage, especially for PEN001_real1 who shows 23 reads with the ancestral allele.

A signal likely linked to adaptation to different diets is located in rs174546 in the FADS1 gene, involved in fatty acid metabolism by decreasing triglyceride levels (142). The overall frequency of this SNP is high, as early as at the Mesolithic period, which is not in line with a pattern of selection linked to changes of diet at the Mesolithic/Neolithic transition.

Genes SLC22A4 (rs272872) and ATXN2/SH2B3 (rs653178) have been shown to be potentially linked to coeliac diseases, following a selection to protect against ergothioneine deficiency in agricultural diets, although the high frequencies observed in modern-day populations have been reached recently (8, 143). Our Mesolithic individual BDB001 has the ancestral allele for both positions, while the derived state of the allele can be found in a substantial number of Neolithic individuals, at least with a heterozygous profile, especially for rs272872 in SLC22A4.

The position rs4833103 of the gene cluster TLR1-TLR6-TLR10, possibly linked to resistance to leprosy, tuberculosis and other mycobacteria, has been investigated, as well as rs2269424 of the Major Histocompatibility Complex (MHC) suggested to be associated with mycobacteria resistance, as they showed the strongest signal of selection for these genes (8, 144). In line with previous results showing a low frequency in Neolithic Europeans for rs4833103 (TLR1-TLR6-TLR10) (8), only four of our Neolithic individuals are homozygous derived, and two are heterozygous. The frequencies observed in our groups for...
the MHC rs2269424 position are higher, with 27 individuals out of 52 having a homozygous derived genotype, including the Mesolithic individual BDB001, and 16 being heterozygous at this locus.

Finally, the derived allele at position rs1229984 in ADH1B gene is well-known to significantly reduce the clearance rate of alcohol from the liver, leading to a reduction of alcohol consumption (145). Our Mesolithic BDB001 is the only one to show a heterozygosity at this position, while all the Neolithic individuals are homozygous ancestral.

**Figure S15.** Summary of select phenotypic and functional variants in high coverage individuals. Colours indicate the most likely genotype, shading indicates genotype likelihood and numbers in cells indicate the number of reads for each state (# Ancestral / # Derived).
Figure S16. Allele frequencies for 13 targeted loci in five Neolithic groups. Dots show maximum likelihood frequency estimates and ±1SE for the derived allele frequency in each ancient group (number of individuals considered between bracket for each position).
Using the program ‘smartpca’ v10210 (EIGENSOFT), we performed a PCA on the HO dataset (59) calculated with 777 present-day west Eurasians on which ancient individuals were projected using the options lsqproject:YES and shrinkmode:YES. Individuals with less than 10,000 SNPs covered were excluded.

In the main text, we present a zoomed-in PCA plot, focusing on our new samples (Figure 1C). We present below the complete PCA with all modern-day west-Eurasian individuals (Figure S17).

![PCA plot](image)

**Figure S17.** PCA plot based on 777 modern-day west-Eurasian individuals (grey circles) and relevant ancient individuals (coloured symbols).
SI 9. qpAdm analysis

We used qpAdm on the 1240K panel/dataset to investigate the different sources of ancestry in our Mesolithic and Neolithic target groups. This method was first described in Haak et al. 2015 (5) and then implemented in the software compilation ADMIXTOOLS (https://github.com/DReichLab/AdmixTools). Following Olalde et al. 2018 (39), we used a core set of outgroups from worldwide modern-day populations (Mbuti, Papuan, Onge, Han, Karitiana, Mota, Ust_Ishim and MA1), to which we added ancient Czech_Vestonice, Caucasian HG (CHG) and Israel_Natufian. We used these as our basic set of eleven outgroups. Depending on which sources were used and which specific hypotheses were being addressed, we augmented the setup by adding additional outgroups, as shown below. We ran all models with the option ALLSNPS=NO and we opted for the nested fitting model in all cases.

Quantifying universal hunter-gatherer ancestry in European farmer groups

**MODEL A – 2-source model**
Target: all individuals between 7000 and 3000 BC with a direct radiocarbon date
Sources: Anatolia_Neolithic, European_HG (Luxembourg_Loschbour, Hungary_KO1, Iberia_LaBrana)
Supp. outgroups: Italy_Villabruna

We first estimated the proportion of HG ancestry in our new Neolithic individuals along with published individuals (Table S10). We used Anatolia_Neolithic and a set of hunter-gatherers labelled European_HG, including Luxembourg_Loschbour, Hungary_KO1 and Iberia_LaBrana, to have a representative and broader coverage of HG in Europe. Here, we added Italy_Villabruna to the basic set of 11 outgroups. We tested all newly reported individuals and all published individuals with a direct radiocarbon date (Table S10). In Figure 2, we plotted the resulting estimates for the HG ancestry and the mean calibrated radiocarbon date from the 2-sigma interval.

Genetic structure in European hunter-gatherers

**MODEL B – 4-source model**
Target: all HG individuals/groups of Europe
Sources = Italy_Villabruna, EHG, Belgium_GoyetQ2, Anatolia_Neolithic
Supp. outgroups: Belgium_GoyetQ116-1

We used this model to characterise the genetic composition of all published and newly generated European HG by modelling ancestry from up to four distal sources and added Belgium_GoyetQ116-1 to the basic set of outgroups (Figure S4, Table S12). We first tested Luxembourg_Loschbour and EHG as they represent the two main HG components in Europe along a genetic cline (7; see Figure 3, Figures S1 and S2). We added Belgium_GoyetQ2 representing the residual post-LGM Magdalenian-associated ancestry in Iberia (15) and Anatolia_Neolithic to account for trace ancestry in some HG groups with specific recent history of admixture.

Tracing different hunter-gatherer ancestries in farmer groups

**MODEL C – 3-source model**
Target: all Neolithic individuals/groups of Europe
Sources = Anatolia_Neolithic, Luxembourg_Loschbour, Hungary_KO1
We initially applied the 4-source MODEL B also to Neolithic groups (Table S12). However, the model fails in most cases as it is unable to identify the EHG component reliably. While the EHG component is present in HG from south-eastern, central Europe and Scandinavia, as shown in Figure 3 and Figure S2, it becomes negligibly small given the already minor overall proportion of HG ancestry in Neolithic individuals. In fact, MODEL B fails to detect the minor HG component itself in many groups from south-eastern or central Europe (Table S12). We therefore applied the MODEL C restricting to three sources (Anatolia_Neolithic, Luxembourg_Loschbour and Hungary_KO1) and selecting geographically and temporally more proximal groups as sources in the right populations to increase the resolution of our analyses (Figure S6 and Table S13). In turn, we added Italy_Villabruna, EHG, Germany_Meso_BDB001 and Iron Gates HG to the basic set of outgroups. Of note, individuals with a significant affinity to Anatolian Neolithic (Z<-3; 3<Z; Figure S3) were excluded from the Iron Gates HG group.

In some cases, both nested models Anatolia_Neolithic / Luxembourg_Loschbour and Anatolia_Neolithic / Hungary_KO1 explain the admixture in the Neolithic population with statistical support (p-value >0.05) equally well. We therefore chose the best fitting model according to qpAdm program, retaining both values for these specific groups in Table S13.

Neolithic groups with such a "floating" signal are mostly located in central Europe, which is consistent with the genetic profile of the modelled source Hungary_KO1. This signal is different compared to all groups located west of the Rhine, where a strong and unambiguous Luxembourg_Loschbour component is observed, which argues for different processes of admixture with local HG in the respective regions.

**Tracing Goyet Q2 ancestry in France**

**MODEL D – 3-source model**

Target: all new Neolithic individuals/groups

Sources = Anatolia_Neolithic, Italy_Villabruna, Belgium_GoyetQ2

Supp. outgroups: Hungary_KO1, Belgium_GoyetQ116-1

To test for the presence of Goyet_Q2 ancestry in our new European Neolithic populations, we also explored a model analogous to the one described in Villalba-Mouco et al. 2019 (15). Here, we followed the published model by adding Hungary_KO1 and Belgium_GoyetQ116-1 to the basic set of outgroups (see Figure S8 and Table S15).

We combined the results of qpAdm MODEL B and MODEL C (Table S14) in Figure 4 in order to represent a synthetic summary for hunter-gatherers and Neolithic farmers. Each model and the respective parameters are described in Table S14 and above. In attempting to organize the different maps chronologically and geographically, we had to rearrange and regroup some individuals and/or groups, by way of which some were split according to chronological layers, while others were grouped together. We chose to mark the individuals/groups that are not supported (p-value < 0.05) with transparent colours. All details concerning the structure of the synthetic maps are given in Table S14.

We also integrated the results of qpAdm MODEL B, C and D (Table S14) in Figure S9 to highlight the presence of the GoyetQ2 component in Neolithic groups from south-western Europe. Of note, MODEL D results in a much better fit for the MN group GRG, which carries extra-GoyetQ2 ancestry, and which otherwise is not supported by MODEL C (p-value < 0.05). All details are provided in Table S14.
SI 10. DATES analysis

We estimated the admixture date with the weighted covariance statistic according to the software DATES v.753 recently developed by modifying the algorithm presented by Narasimhan et al. (146) (https://github.com/priyamoorjani/DATES). The method will soon be published by Chintalapati and colleagues. The method assumes a simple two-way admixture and requires a genotype vector (G) of the admixed target individual across markers, as well as allele frequency estimates for the markers in the two source populations \( f_{\text{source1}} \) and \( f_{\text{source2}} \), as input. It estimates the global ancestry proportions of the two sources by solving the following linear equation for \( \alpha \), the ancestry proportion from the first source:

\[
\mathbf{G} = \alpha \times \mathbf{f}_{\text{source1}} + (1 - \alpha) \times \mathbf{f}_{\text{source2}}
\]

Then, by taking the regression residual and weighting this by the difference between the frequency in two sources, it provides an estimate of local ancestry for each marker \( L \):

\[
\mathbf{L} = (\mathbf{f}_{\text{source1}} - \mathbf{f}_{\text{source2}})(\mathbf{G} - [\alpha \times \mathbf{f}_{\text{source1}} + (1 - \alpha) \times \mathbf{f}_{\text{source2}}])
\]

By taking a correlation of this measure between markers across genetic distance which breaks down as recombination, it estimates admixture date, assuming a single pulse model. This method can be applied on a single sample from the admixed population.

We applied this method to estimate the admixture date between the European_HG (see Text S19, MODEL A) ancestry and the Anatolian_Neolithic ancestry to all the European Neolithic groups. As we work on recent admixture, we set the maximum distance (maxdist) option at 1.

Table S17 presents results for all groups showing reliable values. We excluded failed runs for groups that have too small a proportion of HG ancestry, as well as unrealistically high values, probably linked to the low coverage of the target group.

Figure S18 shows the ancestry covariance plots for our new groups.

In specific case, such as XX and YY, we were interested in whether a one or a two-pulse model best fits our data. This is analogous to whether or not fitting two lines, and attributing the points to the closest line in Figure S18 significantly reduces the residual sum of squares.

To test this we applied an optimal Box-Cox transformation to the data (147), as implemented in the boxcox() function from the MASS package (148). We fit transformed linear regressions with one or two curves, and then compared the two model fits using the Integrated Completed Likelihood criterion, an information criterion that accounts for clustering, as implemented in the stepFlexmix() function from the flexmix package (149). In no case did we find statistical support for a two-pulse model.
Figure S18. Ancestry covariance for each group of our Neolithic dataset based on DATES.
REFERENCES AND NOTES


24. C. Jeunesse, S. van Willigen, Westmediterranes Frühneolithikum und westliche Linearbandkeramik: Impulse, Interaktionen, Mischkulturen, in *Die Neolithisierung*


57. M. Morgan, H. Pagès, V. Obenchain, N. Hayden, Rsamtools: Binary alignment (BAM), FASTA, variant call (BCF), and tabix file import, R package version 1.34.1 (2019).


98. P. Mazzieri, *I siti di Via Spezia (Beneficio e Via Guidorossi) et Pontetaro a Parma nel quadro della cultura diei Vasi a Bocca Quadrata in Emilia Occidentale* (Università di Pisa, 2010).


103. J. Grünberg, The Mesolithic burials of the Middle Elbe-Saale region, in *Mesolithic Burials—Rites, Symbols and Social Organisation of Early Postglacial Communities, International Conference Halle (Saale), Germany, 18 to 21 September 2013.*
104. M. Stecher, J. M. Grünberg, K. W. Alt, Bioarchaeology of the Mesolithic individuals from Bottendorf (Thuringia, Germany), in Mesolithic Burials—Rites, Symbols and Social Organisation of Early Postglacial Communities, International Conference Halle (Saale), Germany, 18 to 21 September 2013.


110. H. C. Strien, Untersuchungen zur Bandkeramik in Württemberg (Habelt, 2000).


149. F. Leisch, Flexmix: A general framework for finite mixture models and latent class regression In R (2004).