Article

# Lasting Lower Rhine–Meuse forager ancestry shaped Bell Beaker expansion

https://doi.org/10.1038/s41586-026-10111-8

Received: 17 March 2025

Accepted: 8 January 2026

Published online: 11 February 2026

Check for updates

Iñigo Olalde[1,2,3,28]✉, Eveline Altena[4,28]✉, Quentin Bourgeois[5,28]✉, Harry Fokkens[5,28]✉, Luc Amkreutz[5,6], Steffen Baetsen[7], Marie-France Deguilloux[8], Alessandro Fichera[9], Damien Flas[10,11], Francesca Gandini[9], Jan F. Kegler[12], Lisette M. Kootker[13], Judith van der Leije[7], Kirsten Leijnse[14], Constance van der Linde[15], Leendert Louwe Kooijmans[5], Roel Lauwerier[16], Rebecca Miller[10], Helle Molthof[17], Pierre Noiret[10], Daan C. M. Raemaekers[18], Maïté Rivollat[8,19,20], Liesbeth Smits[21], John R. Stewart[22], Theo ten Anscher[17], Michel Toussaint[20], Kim Callan[23], Olivia Cheronet[24,25], Trudi Frost[23], Lora Iliev[23], Matthew Mah[23,26,27], Adam Micco[23,27], Jonas Oppenheimer[23], Iris Patterson[23], Lijun Qiu[23], Gregory Soos[23], J. Noah Workman[23], Ceiridwen J. Edwards[9], Iosif Lazaridis[2,23], Swapan Mallick[23,26], Nick Patterson[27], Nadin Rohland[23], Martin B. Richards[9], Ron Pinhasi[24,25], Wolfgang Haak[19], Maria Pala[9] & David Reich[2,23,26,27]✉

Ancient DNA studies revealed that, in Europe from 6500 to 4000 BCE, descendants of western Anatolian farmers mixed with local hunter-gatherers resulting in 70–100% ancestry turnover[1], then steppe ancestry spread with the Corded Ware complex 3000–2500 BCE[2]. Here we document an exception in the wetland, riverine and coastal areas of the Netherlands, Belgium and western Germany, using genome-wide data from 112 people 8500–1700 BCE. A distinctive population with high (approximately 50%) hunter-gatherer ancestry persisted 3,000 years later than in most European regions, reflecting incorporation of female individuals of Early European Farmer ancestry into local communities. In the western Netherlands, the arrival of the Corded Ware complex was also exceptional: lowland individuals from settlements adopting Corded Ware pottery had hardly any steppe ancestry, despite a Y-chromosome characteristic of people associated with the early Corded Ware complex. These distinctive patterns may reflect the specific ecology that they inhabited, which was not amenable to full adoption of the early Neolithic type of farming introduced with Linearbandkeramik[3], and resulted in distinct communities where transfer of ideas was accompanied by little gene flow. This changed with the formation of Lower Rhine–Meuse Bell Beaker users by fusion of local people (13–18%) and Corded Ware associated migrants of both sexes. Their subsequent expansion then had a disruptive impact across a much wider part of northwestern Europe, especially in Great Britain where they were the main source of a 90–100% replacement of local Neolithic ancestry.

Whole-genome ancient DNA (aDNA) analysis has illuminated long-standing debates about cultural and demographic transformations in Holocene Europe. Two major prehistoric events have been characterized: the spread of genetic ancestry originating from western Anatolian farmers into Europe associated with the introduction of farming in the Early Neolithic[4], and the spread of ancestry characteristic of Yamnaya steppe pastoralists during the third millennium BCE[2,5–7], mediated by the dispersal of the Corded Ware (CW) and Bell Beaker (BB) complexes. However, the demographic processes at the regional level are still not clearly understood and have been shown to follow variable patterns. For example, while the spread of Anatolian ancestry in central Europe was primarily propelled by the expansion of Linearbandkeramik (LBK) farmers[1,4,5], in the Baltic region and Scandinavia adoption of the farming lifestyle took place much later and, in some cases, there was even a return to hunting, gathering and fishing[8–10].

Here we focus on the unique trajectory of communities from water-rich environments in the wider Lower Rhine–Meuse area in western and central Netherlands, Belgium, and northern and northwestern Germany. Around 5500 BCE, the southern part of this region witnessed the arrival of LBK-associated farmers, who settled across the fertile loess soils in the south of the Netherlands and parts of Belgium, Germany and France. Within these communities, there is evidence of contact with hunter-gatherer groups, as documented by Limburg and La Hoguette pottery[11], although the origin of these ceramics and the importance[12] of these contacts are debated[3]. Once established, these LBK communities developed into regional variants such as the Blicquy, Rössen, Villeneuve-Saint-Germain, Bischheim and the later southern Michelsberg groups.

North of the loess, large rivers such as the Scheldt, Meuse and Rhine created a dynamic landscape that included fertile soils favoured by

A list of affiliations appears at the end of the paper.

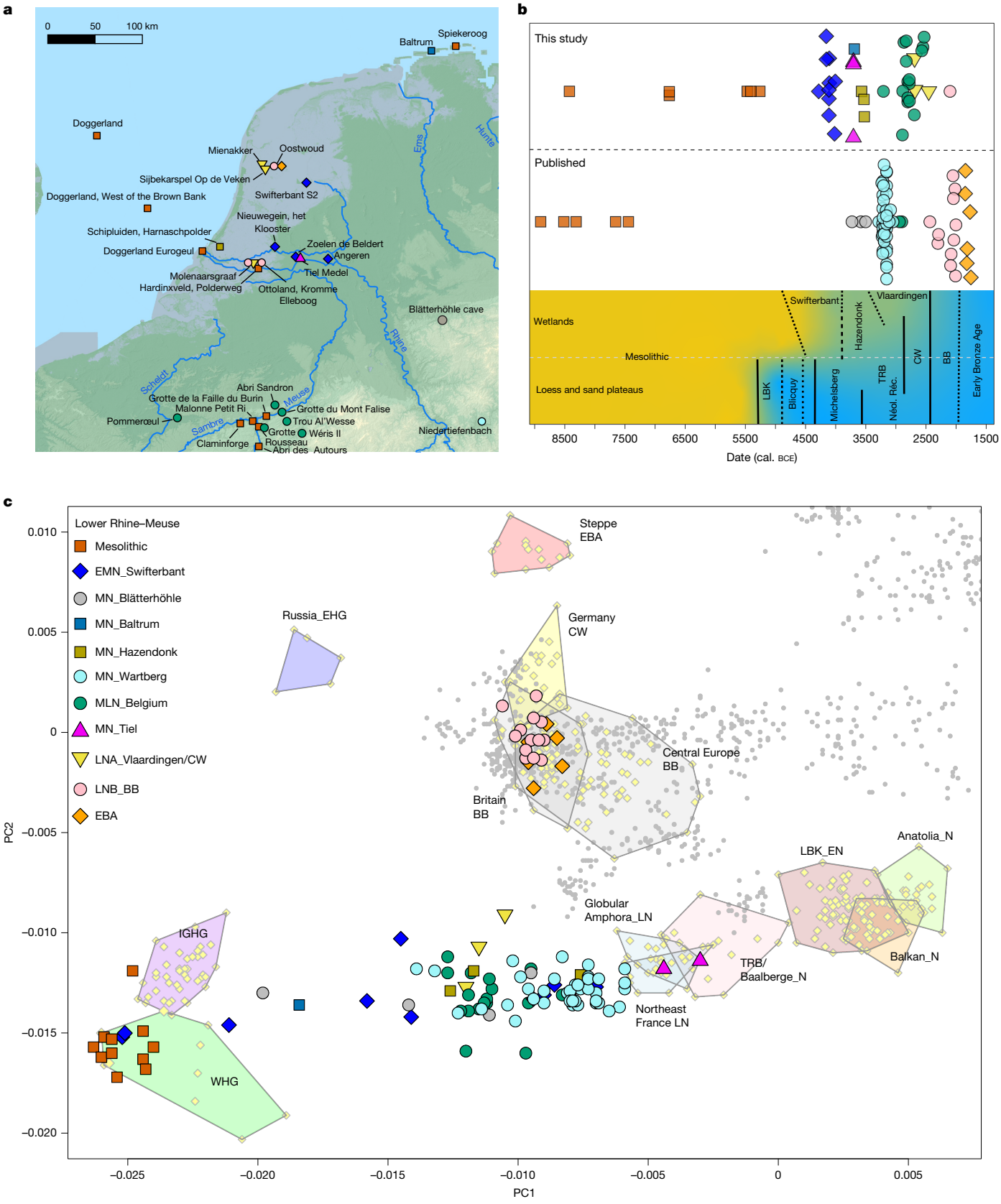



**Fig. 1** | See next page for caption.

farmers, alongside coastlines, beach barriers, river delta wetlands and forested river dunes that continued to support hunting, gathering and fishing practices after the full adoption of farming around 4200 BCE[3,13–16]. This contrasts with other areas of Europe (with the exception of northern Scandinavia, the Baltic region and the eastern European taiga), where farming practices quickly became dominant[8]. In the Lower Rhine–Meuse area, the wetland communities of the Swifterbant (fifth millennium BCE) and Hazendonk cultures (4000–3500 BCE)

**Fig. 1 | Overview of ancient individuals analysed in this study. a**, Map showing archaeological sites with genome-wide data in the Lower Rhine–Meuse area and adjacent regions. The elevation map was downloaded from https://www.mapsforeurope.org/datasets/euro-dem. **b**, Chronological placement of the individuals from the Lower Rhine–Meuse region included in this study. Bottom, the local chronology of archaeological cultures. The black lines indicate the degree of changes; the dashed lines represent a gradual change in material culture; and the solid lines indicate a more abrupt change in material culture. The colour gradient indicates the general reliance on hunting and gathering (yellow) to farming (blue). Néol. Réc. refers to Néolithique Récent. **c**, PCA with the ancient individuals projected onto the principal components computed on present-day individuals from West Eurasia. EMN, Early-Middle Neolithic; MLN, Middle–Late Neolithic; EN, Early Neolithic; MN, Middle Neolithic; LN, Late Neolithic; LNA, Late Neolithic A; LNB, Late Neolithic B; N, Neolithic; EBA, Early Bronze Age; IGHG, Iron Gates hunter-gatherers; EHG, Eastern hunter-gatherers.

settled on elevated areas (river and coastal dunes, crevasse splays and river levees) in a region dominated by water courses and peat bogs. They relied mostly on hunting, gathering and fishing, but also practiced farming. Around 3500 BCE, the Vlaardingen culture succeeded the Swifterbant/Hazendonk tradition, while remaining settled in approximately the same region[17]. Simultaneously, farmers associated with the Funnelbeaker culture (*Trechterbekercultuur* (TRB) in Dutch) settled on the Frisian–Drenthian plateau in the northeast and its surrounding sandy uplands, in regions where no evidence of earlier habitation, neither burials or settlements, has been found. The Swifterbant, Hazendonk and Vlaardingen settlements were all located near water streams, while TRB farmers settled mostly on forested sandy plateaus and their fringes, as did the Michelsberg communities to the south.

A mixed subsistence strategy of hunting, gathering and farming persisted in the western/central Netherlands until the third millennium BCE, when a more intense farming-based economy emerged in association with the Late Vlaardingen complex and the introduction of the ard plough around 3000 BCE[18]. The spread of CW influence to the wider Lower Rhine–Meuse area was more complex than in many other areas of central and eastern Europe. In the uplands, where skeletal material tends to be poorly preserved and no aDNA data are available, the complete CW package emerged as marked by the construction of CW burial mounds, the general absence of settlements and sparse pottery finds[19]. By contrast, in wetland areas along the coast, the Rhine–Meuse delta[20] and other low-lying regions[21], CW-associated pottery was incorporated into Vlaardingen settlement contexts, but the characteristic CW-style burials were not[20,22,23].

The arrival of the BB complex around 2500 BCE marked another major cultural transition, as settlements spread across the wetlands and coastal areas, replacing Vlaardingen and CW settlements, although generally not using the same sites[21]. The BB economy was similar to the previous CW one and consisted of predominantly farming mixed with low-intensity hunting and gathering. In the sandy uplands, there was a continuation of the barrow ritual, but with distinct BB characteristics and material culture replacing the CW repertoire[19,24]. BB groups were also well attested south of the Rhine, as evident in BB burial mounds on the sandy soils of the southern Netherlands and Belgium[25–27]. BB settlement sites remain just as elusive in this area as CW settlements. However, the presence of ploughland dated to the Late Neolithic suggests that the lack of settlement evidence is not the result of nomadism but rather of settlements in lower lying places where there is little chance for detection by archaeologists[21].

Archaeogenetic data have the potential to deepen our understanding of the nature of the dynamic changes in the Lower Rhine–Meuse region. We generated genome-wide data using in-solution enrichment for more than a million single-nucleotide polymorphisms (SNPs) from 44 individuals dated between 8500 and 1700 BCE, sampling cultural contexts that fill gaps in the aDNA record of this region (Fig. 1a,b and Supplementary Tables 1 and 2). The mean number of SNPs covered from a core set of 1.15 million autosomal targets is 492,551, with a mean coverage of 1.09. Together with 69 published individuals[1,6,28–31], the time-transect includes 112 individuals. We also report 14 new direct radiocarbon dates on newly analysed individuals (Supplementary Table 16).

## Late persistence of forager ancestry

We report six new Mesolithic individuals who traced all of their ancestry from Mesolithic western hunter-gatherers (WHGs) (Supplementary Table 5), matching previous genetic results from Mesolithic hunter-gatherers from the region[28]. Based on principal component analysis (PCA) (Fig. 1c), Neolithic individuals from the Lower Rhine–Meuse area fall along the central/western European Neolithic cline, but much closer to WHG than most European Neolithic farmers. This suggests elevated WHG-related ancestry, which we confirmed through modelling using qpAdm (Supplementary Table 6). We found that the earliest Neolithic individuals (4400–3800 BCE), associated with the Swifterbant culture, are genetically highly heterogeneous, with a mother and her daughter (I12093–I12094; Nieuwegein het Klooster) entirely descending from hunter-gatherer populations, one individual (I38442 from Angeren Kampsepad) with 84% of such ancestry; three individuals (I12091–I17968 from Nieuwegein het Klooster and I33739 from Zoelen de Beldert) with 60–63%; and four individuals (SWA001, SWA002 and SWA004 from Swifterbant-S2 and I33738 from Zoelen de Beldert) with 37–45% (Extended Data Fig. 2 and Supplementary Table 6). These results differ from the overall patterns of hunter-gatherer and farmer admixture elsewhere in central and western Europe, where the arrival of a farming economy generally reduced local WHG ancestry to less than 30%. However, the results perhaps make more sense in light of the equally limited economic transformation, which combined farming with continued core reliance on the rich wild resources from the Lower Rhine–Meuse wetlands and river valleys. Genetic mixing of local groups with high WHG ancestry continued for the next approximately 1,500 years, with stable proportions of around 40–50% WHG and 50–60% Early European Farmer (EEF) ancestry. Rare exceptions include one Middle Neolithic individual from the island of Baltrum (BLR001) and one individual from the Blätterhöhle cave (I15651), both with over 75% hunter-gatherer ancestry. The fact that this relatively high WHG ancestry extended not only to the Lower Rhine–Meuse wetlands, but also to further along the Rhine and Meuse rivers and the northern coast is consistent with archaeological evidence of continued cultural engagement of people across this region[32]. Three individuals from Tiel Medel de Roeskamp who can be indirectly dated to around 3700 BCE (Supplementary Information 2 and Supplementary Table 14) deviate from this pattern of high WHG ancestry, with only around 20% (Extended Data Fig. 2), possibly representing new arrivals from neighbouring parts of northwest Europe with lower WHG-associated ancestry. Their distinct genetic profile, in combination with parallels in pottery and lithic technology[32], suggests an origin to the southeast among contemporaneous fully Neolithic communities in that region such as Bischheim groups. As such, the Tiel Medel de Roeskamp settlement represents a regional outlier, both in ancestry and material culture, and highlights that the Lower Rhine–Meuse area was not isolated, but part of a dynamic frontier characterized by mobility, encounter and interaction across cultural boundaries.

Compared with other regions of central, southern and western Europe where farming was practiced, the Lower Rhine–Meuse area stands out for its long survival of high proportions of WHG-related ancestry on a population scale (as opposed to isolated cases[33–35]) until the BB transition, halfway through the third millennium BCE (Fig. 2).

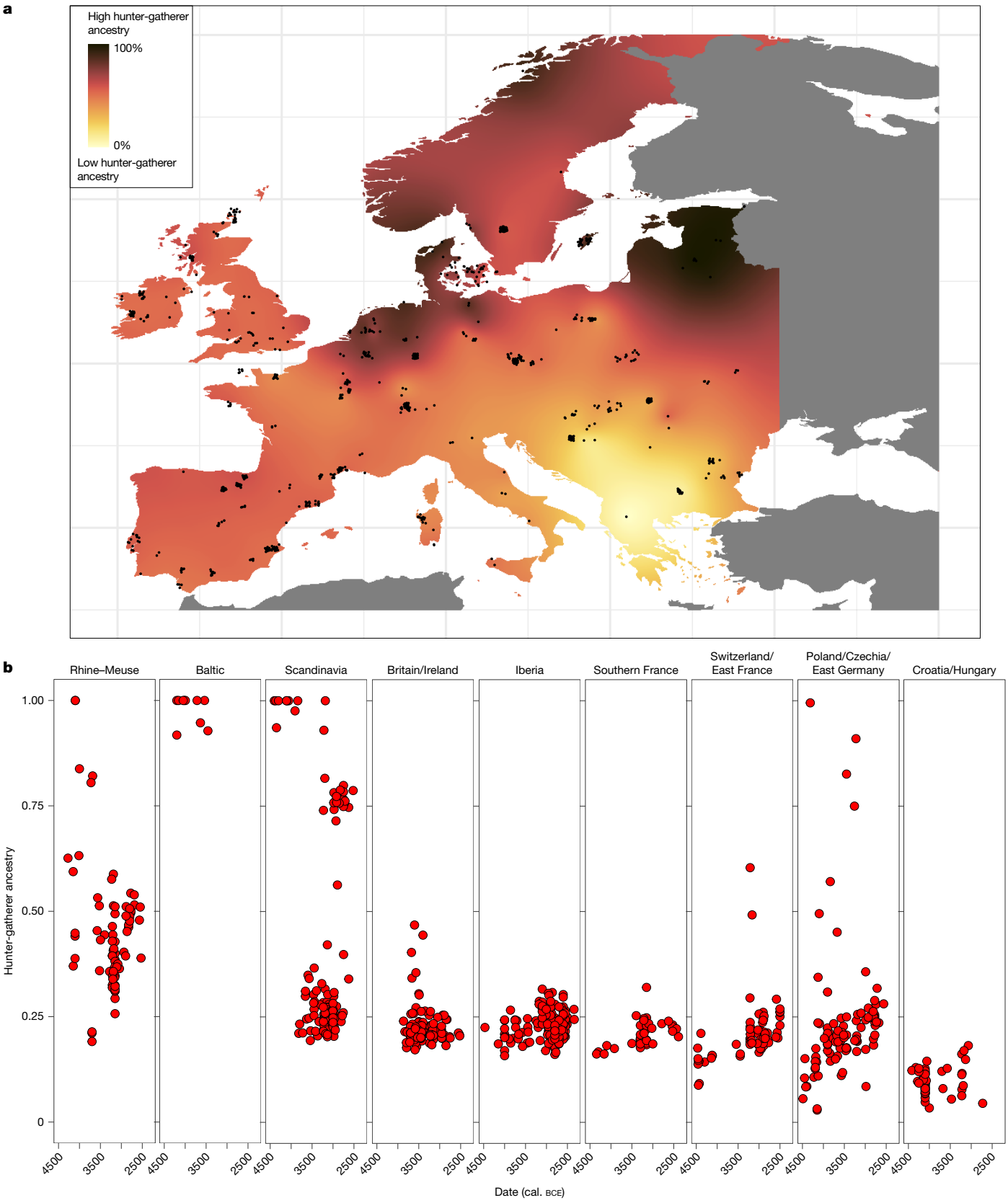



**Fig. 2 | Hunter-gatherer ancestry proportions across Europe between 4500 and 2500 BCE. a**, Spatial kriging of hunter-gather ancestry. The colours represent the predicted ancestry proportion at each point in the grid. Map data are from the R package maps. **b**, Hunter-gatherer ancestry levels in individuals from different European regions. Ancestry proportions were estimated using qpAdm (Supplementary Tables 6 and 7).

To identify other instances in which WHG ancestry on a population scale endured in such high proportions to the dawn of the Bronze Age, it is necessary to go to parts of the Baltic coast where populations with high EEF ancestry never made a substantial impact[9], and to Scandinavia where hunter-gatherers with full WHG ancestry persisted until the early third millennium BCE alongside EEF-ancestry-rich farmers[10] (Fig. 2 and Supplementary Table 7).

The unique ancestry makeup of Lower Rhine–Meuse Neolithic groups is also evident from their EEF–WHG admixture time estimates (Extended Data Fig. 4), which point to ongoing admixture well into the fourth millennium BCE, in contrast to other European regions (Supplementary Table 15). The Tiel Medel de Roeskamp individuals represent a deviation from this pattern with older admixture dates, again highlighting their likely recent origin outside the Lower Rhine–Meuse area.

## Female-mediated early farming ancestry

We find that the EEF ancestry proportions in Lower Rhine–Meuse area Neolithic people were significantly higher on chromosome X than the autosomes (normally distributed $Z$ score = 5; Supplementary Table 8), indicating a higher ancestral contribution from women with EEF ancestry. Independent confirmation is provided by analysis of the two uniparentally inherited parts of the genome (Supplementary Table 13). Among the Early and Middle Neolithic men ($n$ = 43 excluding close relatives), we observed only Y-chromosome lineages common in Mesolithic hunter-gatherers (haplogroups I2a, R1b-V88 and C1a2). By contrast, the maternally transmitted mitochondrial lineages are predominantly of Neolithic farmer origin (50 out of 71), based on their absence in sampled European Mesolithic individuals[4,6,9,10,28,35–37]. For example, the earliest individual with EEF ancestry, a female individual associated with the Swifterbant culture and dated to around 4342–4171 calibrated years BCE (cal. BCE) (I17968, Nieuwegein het Klooster) at the start of the transition to farming in the region[14,16], has only 37% EEF ancestry in her autosomes but farmer-associated mitochondrial haplogroup H+152. A previous study[36] reported similar sex-biased admixture in Neolithic farmers of Iberia and in Funnel Beaker farmers of northern Europe. A plausible scenario is that in all three regions, hunter-gatherer communities incorporated farmer women, who plausibly mediated the exchange of ideas and technologies related to farming. This scenario of sex-biased admixture of Neolithic ancestry contrasts with one of almost complete displacement of local ancestry by incoming farmers and migration of entire groups, a process that occurred in other parts of Early Neolithic Europe[38].

The Middle Neolithic populations of the Lower Rhine–Meuse region were highly genetically interconnected, as reflected in large segments (>12 cM) of the genome being identical by descent (IBD), which is expected to be observed only for individuals who share common ancestors in the last dozens of generations[39] (Supplementary Table 14). We also find several cases of IBD segments over 20 cM, suggesting even closer relationships between sites such as Blätterhöhle, Niedertiefenbach and Abri Sandron, as well as between sites in the Lower Rhine–Meuse area and nearby areas of central Europe and Northern France (Fig. 4a). A notable case is a relationship (~50 cM in IBD) between an individual from Blätterhöhle, modern western Germany, and a father–daughter pair from Mont-Aimé[34] in modern northern France, who are also clear ancestry outliers exhibiting more hunter-gatherer ancestry than other individuals from Mont-Aimé.

## CW using groups with minimal steppe DNA

In many areas of Europe, the emergence of the CW complex is associated with large-scale demographic change due to the arrival of groups carrying steppe ancestry. The ancestry change in three sampled individuals from Vlaardingen/CW contexts in the Western Netherlands is far smaller. These individuals were buried within settlements with CW

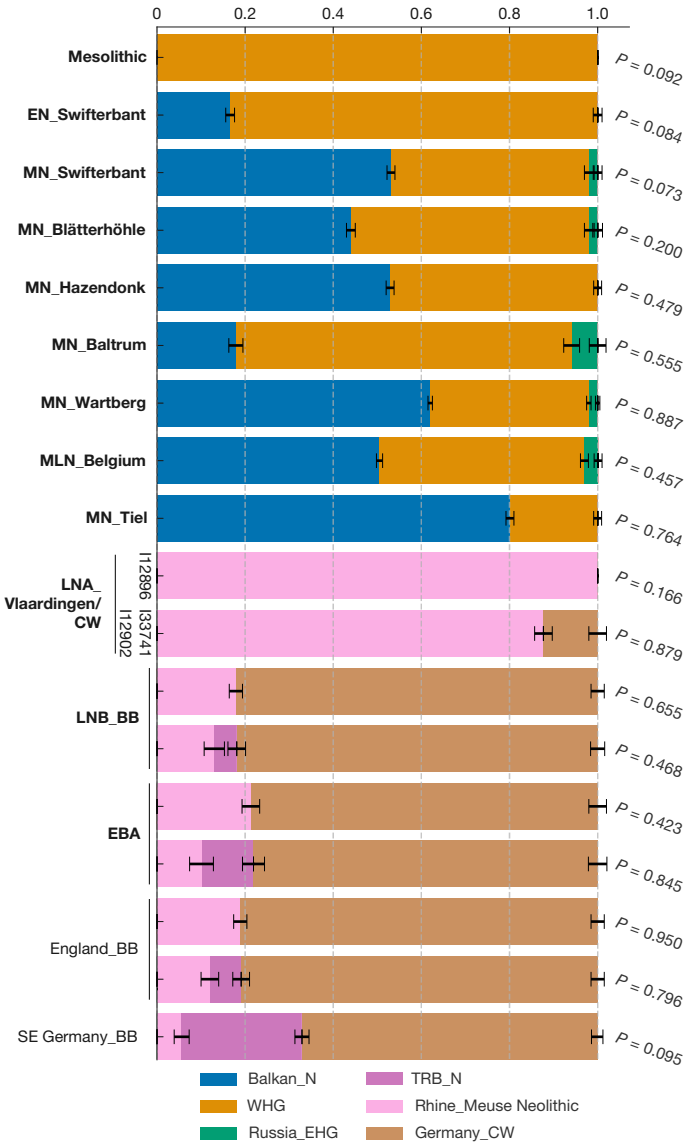



**Fig. 3 | Admixture proportions for Lower Rhine–Meuse populations and other relevant groups.** Admixture proportions were estimated using qpAdm (Supplementary Tables 5, 6 and 10). Groups from the Rhine–Meuse region are shown in bold. The error bars indicate the s.e. of estimates from 5-cM-block jackknife analysis. $P$ values for the fit of each qpAdm model to the genetic data are provided. SE, southeast.

complex material goods, but not the typical CW single grave burials, which are overall absent from the Vlaardingen culture region. One female (I12896 from Molenaarsgraaf-24A) has no steppe-related ancestry at all and instead shares ancestry with local late Neolithic farmers of the region (Fig. 3 and Supplementary Table 9). However, the other two individuals (I12902 and I33741) from Opmeer Mienakker and Sijbekarspel op de Veken, north of the Rhine River delta, can be modelled as a mixture of 12–16% ancestry associated with the main cluster of CW groups, and 84–88% derived from Lower Rhine–Meuse area Neolithic populations with high hunter-gatherer ancestry, similar to Late Neolithic Belgium or Hazendonk (Fig. 3 and Supplementary Table 10). Despite a low steppe ancestry proportion in the autosomes, the male I12902 from Opmeer Mienakker carries Y haplogroup R1b-U106, also known in one early CW-associated individual from Bohemia[7]. Furthermore, one of his bones yields a date of 2852–2574 cal. BCE; one of the earliest CW complex associated dates in Europe outside of Bohemia and the Baltic region[40]. These results suggest that the male ancestor

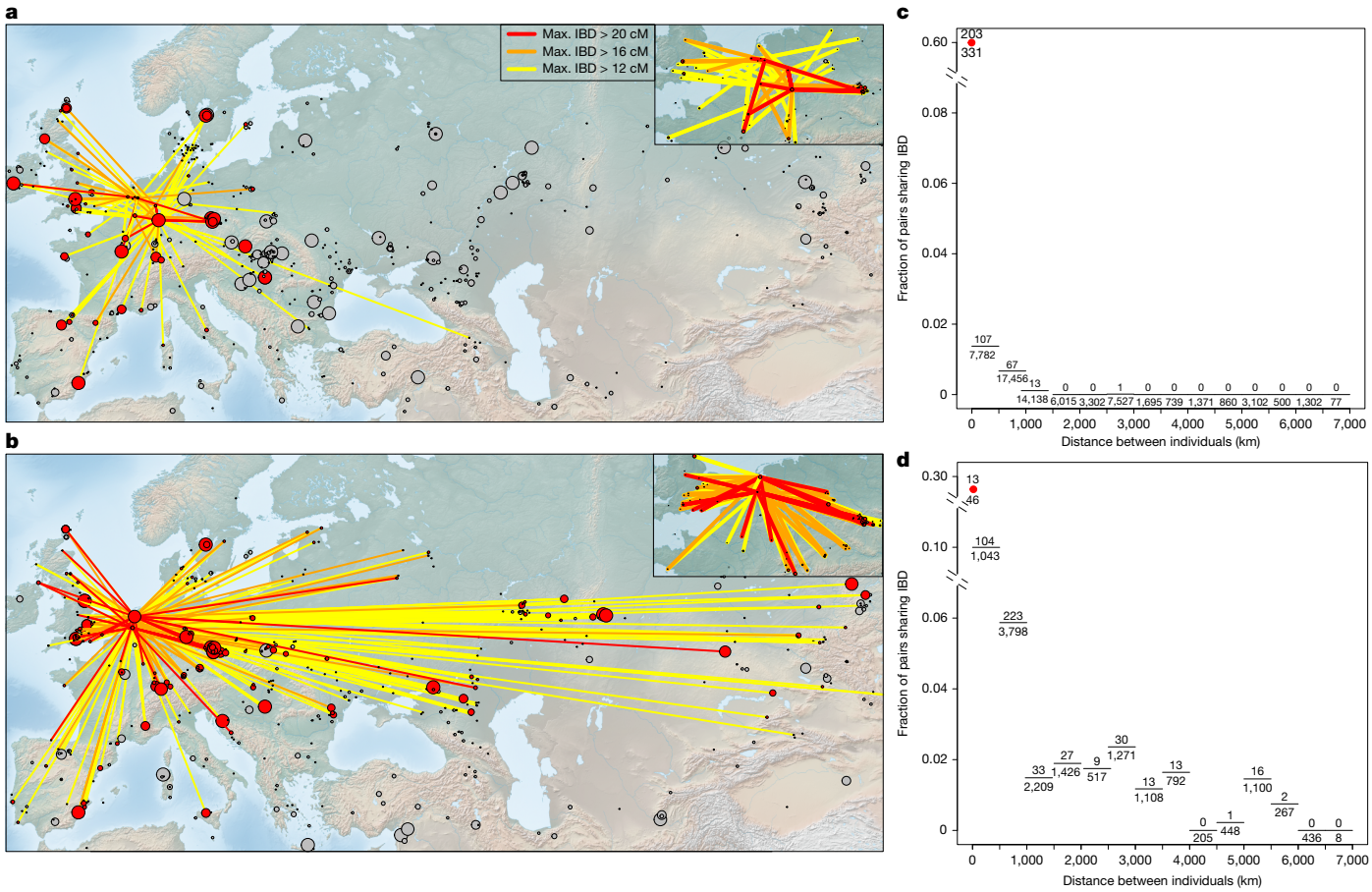



**Fig. 4 | Genetic connections of Lower Rhine–Meuse groups. a**,**b**, IBD connections of Lower Rhine–Meuse Early, Middle and Middle–Late Neolithic individuals ($n = 35$) (**a**) and Lower Rhine–Meuse BB-associated individuals ($n = 11$) (**b**). Sites are represented by circles; the size is proportional to the number of individuals amenable to IBD calling. The grey circles indicate archaeological sites between 5500 and 2500 BCE (top) and between 3000 and 1500 BCE (bottom) with no IBD connections to Lower Rhine–Meuse individuals. **c**,**d**, Decay of IBD sharing, with the geographical distance for Lower Rhine–Meuse Early, Middle and Middle–Late Neolithic individuals and Lower Rhine–Meuse BB-associated individuals (**d**). Pairs were considered to share IBD if they share at least one segment of >12 cM. Maps were drawn using public-domain Natural Earth data using the rnaturalearth package in R.

who brought this Y haplogroup to the Lower Rhine–Meuse region was part of the early CW expansion.

While limited to three people, the IBD analysis for the Vlaardingen/CW individuals revealed two additional notable signals. First, the two individuals with around 11% CW ancestry, excavated at nearby sites, have an IBD match that represents approximately a seventh-degree relationship (Supplementary Table 14), hinting at a small community size. Second, the geographical range of their IBD links extends much further east than previous groups (Extended Data Fig. 3). Among their closest connections, we found a Yamnaya-associated individual from Samara in far eastern Europe[41] (ID: I6733) and CW-associated individuals from present-day Poland[42] (ID: pcw362) and Estonia[43] (ID: NEO306), with whom Vlaardingen/CW individuals share one IBD segment of around 19 cM long that was very unlikely to survive for more than a handful of generations. We also detected connections to other Lower Rhine–Meuse area Late Neolithic individuals (Supplementary Table 14), providing direct evidence of a major local ancestry component in Vlaardingen/CW individuals. These patterns reflect their dual sources of ancestry: a minor component (potentially completely along the male line) from central/eastern CW groups (and through them to Yamnaya steppe pastoralists) and a major component from the local Neolithic population. Although our individuals do not represent the initial generation of admixture between CW-related and local groups, patterns from nearby northeastern France could serve as a good model for this interaction. There, around 2500 BCE, a man with ancestry similar to CW-associated individuals (including nearby CBV95[44] buried with an All-Over Ornamented CW type Beaker) fathered a child with a local woman[45]. His son did the same, rapidly reducing steppe ancestry to levels comparable to those seen in the Vlaardingen/CW individuals I12902 and I33741.

## BB users had local admixture

With the advent of the BB complex in the Lower Rhine–Meuse delta after 2500 BCE, we see a strong difference in genetic ancestry compared with the previous CW/Vlaardingen individuals. All 13 available BB-associated individuals appear genetically close to eastern European CW-associated groups, but not to the preceding Vlaardingen/CW individuals in the PCA (Fig. 1c). They can be modelled with around 82% ancestry from the main cluster of eastern European CW-associated individuals (Fig. 3 and Supplementary Table 10), and their remaining ancestry from a Wartberg Neolithic-related group (Supplementary Table 11), representing the Neolithic population from the Lower Rhine–Meuse area with the lowest level of hunter-gatherer ancestry, or as a mixture between Middle and Late Neolithic groups from outside the Lower Rhine–Meuse area (for example, Poland Globular Amphora, Czechia TRB, Germany Baalberge, Iberia Neolithic–Chalcolithic) and Late Neolithic populations from Belgium. All of these scenarios point to an approximately 82–87% (but not 100%) ancestry change associated with the arrival of the BB complex in the Lower Rhine–Meuse region (Supplementary Table 10).

A contribution of local farmers, with their distinctive signature of high hunter-gatherer ancestry, is essential to model the formation of Lower Rhine–Meuse delta BB-associated individuals. Even at 13–18%, we can be confident that this local admixture occurred: models lacking this unique Lower Rhine–Meuse farmer genetic contribution are rejected with strong statistical significance (Supplementary Table 10). This suggests that the observed mixture between CW culture associated groups and European farmers that formed the genetic profile of Lower Rhine–Meuse BB must have occurred, at least in part, in the region itself. In the context of these genetic results, it is notable that radiocarbon dates suggest that the Lower Rhine–Meuse area was one of the earliest places where the BB cultural phenomenon arose[46]. Although the earliest appearance of BB cultural material has been located in Iberia[47], our results show that the earliest formation of BB-associated groups that were influenced not just culturally but also genetically by CW users may have occurred in western Europe including the Lower Rhine–Meuse area.

As there are possibly several centuries between the analysed CW/Vlaardingen and BB populations, we need to consider the option that the considerable change in genetic ancestry could have been a more gradual process, rather than a sudden change. However, what is different compared with the previous ancestry changes in our time transect, is that it involved both sexes this time. Among BB men, all yielded R1b-L151 haplogroups, which were absent in earlier Neolithic European populations, except for early CW-associated individuals from Czechia in central Europe[7]. All nine available BB-associated men from Oostwoud Tuithoorn and Ottoland Kromme Elleboog belonged to the derived R1b-L151-P312 lineage (Supplementary Table 1), the major lineage among BB groups across Europe[6]. Three P312 individuals could be further subtyped to DF19, a minor P312 subtype today mostly present in central/northern European populations (https://www.yfull.com/tree/R-DF19/). At Molenaarsgraaf, the only man with enough resolution to determine an L151 subtype belonged to R1b-L151-U106 (I13025), matching the Vlaardingen/CW-associated male individual from Opmeer Mienakker, referred to above, and suggesting a similar CW-related source for the patrilineal ancestry in both Vlaardingen/CW and BB men in the Lower Rhine–Meuse area. This would be consistent with the hypothesis that, even if there was limited local continuity within the lowlands, the same male lineages that were associated with the arrival of CW pottery to the region (in the Vlaardingen/CW context) were at least partially associated with subsequent BB emergence. This suggests that the BB-associated population in the Lower Rhine–Meuse area could have emerged from sustained influx of 'classic' CW-related groups to the region (such as those documented in the uplands to the east, where skeletal preservation is absent but classic CW burial features are present), who mixed with the local Vlaardingen or other Lower Rhine–Meuse Neolithic populations who had high hunter-gatherer ancestry.

Further evidence for a major influx from outside regions during the BB period comes from inspection of IBD networks, which, after this point, expand thousands of kilometres further to the east and northeast compared to the CW/Vlaardingen period (Fig. 4b). These show strong links to CW- and BB-associated individuals from Bohemia, as well as evidence of distant Early Bronze Age relatives from England and Scotland (Supplementary Table 14), corroborating findings of a shared origin between these groups located on opposite shores of the North Sea[6]. The BB horizon is the first period in our time transect when people of the Lower Rhine–Meuse region became intensively integrated within a much wider European IBD network, in contrast to the previous more regional patterns—yet another indication for the high level of mobility that has become evident from isotope studies[48].

After this period of considerable demographic change, Early Bronze Age individuals from the Lower Rhine–Meuse area had similar ancestry to BB predecessors (Figs. 1c and 3), with around 3% additional Neolithic-related ancestry (Supplementary Table 10), potentially reflecting small-scale admixture with neighbouring populations. Local continuity from the BB period to the Middle Bronze Age is also reflected in the abundant familial links connecting Early Bronze Age individuals with both earlier BB individuals and later Middle Bronze Age ones (Supplementary Table 14), with pairs sharing up to 100 cM IBD as expected for approximately sixth-degree relationships.

**Table 1 | Modelling ancestry**

| | Lower Rhine–Meuse BB | British BB |
|---|---|---|
| *P* value for models with northern Iberian farmers | 0.00079 | 0.0072 |
| *P* value for models with northeast French farmers | 0.00015 | 0.00077 |
| *P* value for models with German Baalberge farmers | 0.000015 | 0.000085 |
| *P* value for models with Globular Amphora farmers | 0.0012 | 0.0071 |
| *P* value for models with TRB farmers | 0.00000018 | 0.00000042 |
| *P* value for models with Middle–Late Neolithic Belgium (Lower Rhine–Meuse) | 0.0605 | 0.0057 |
| *P* value for models with Middle Neolithic Wartberg (Lower Rhine–Meuse) | 0.65 | 0.95 |
| Proportion of ancestry from Late Neolithic Wartberg (Lower Rhine–Meuse) (%) | 17.9 | 18.9 |
| Minimum proportion of ancestry from Middle–Late Neolithic Belgium, allowing three-way qpAdm models with CW and other farmer sources outside the Lower Rhine Meuse area (%) | 10.9 | 9.3 |

We modelled the ancestry of Lower Rhine–Meuse BB-associated individuals and the main group of British BB-associated individuals using qpAdm models involving a CW group from present-day Germany and different Neolithic populations from within and outside the Lower Rhine–Meuse area. Further details are provided in Supplementary Table 10.

## Lower Rhine–Meuse expansion to Britain

The study identifies the Lower Rhine–Meuse region as the likely origin of a secondary expansion of BB users who had a greater demographic impact than the postulated initial Iberian expansion.

To document this, we used our statistical modelling framework to re-examine the genetic evidence of the arrival of BB users in Britain. Previous work proposed an origin of British BB-associated groups in the Lower Rhine–Meuse based on Y-chromosome data[7], and showed a minimum of around 90% ancestry change[6], but had not been able to distinguish models in which the EEF ancestry came from Britain itself or elsewhere. We analysed 28 BB-associated individuals from Great Britain from the main homogeneous cluster (Supplementary Tables 3 and 4) and obtained the same result as for Rhine–Meuse delta BB individuals (Table 1 and Supplementary Table 10). Both require around 12–19% ancestry from Lower Rhine–Meuse Neolithic populations with high levels of hunter-gatherer ancestry, plus ancestry from the main CW genetic cluster (Fig. 3). This is consistent with no contribution at all from British Neolithic farmers. However, in an outlier subset of four BB-associated individuals from Britain (including the high-status 'Amesbury Archer') with lower proportions of steppe ancestry than the main cluster (Extended Data Fig. 1 and Supplementary Table 3), models featuring CW-complex-associated groups and Lower Rhine–Meuse farmers provide a poor fit (Supplementary Table 10). We cannot rule out the possibility that some of the ancestry of these outliers could derive from local British Neolithic populations, but it could also plausibly come from separate migratory streams into Britain, such as the one suggested for the Amesbury Archer, whose isotopic genetic signatures indicate an origin in the Alps[49]. By the Early Bronze Age, when ancestry proportions in Britain stabilized, we estimate around 92% Lower Rhine–Meuse BB ancestry and at most 8% local British Neolithic ancestry, but

possibly as little as 0% (Supplementary Table 12), providing new information about the magnitude of the demographic transition associated with the BB transition in Britain.

## Discussion

A notable finding in our study is that the long-term persistence of high hunter-gatherer genetic ancestry proportions was not limited to the previously reported Blatterhöhle cave[1] and Wartberg culture[29] in the Rhine region, but also occurred in the core wetlands of the Netherlands and the inland regions of the Meuse. In the context of archaeological evidence, this suggests the co-existence of two distinct but interdigitated Neolithic spheres in the entire Lower Rhine–Meuse region, persisting well into the third millennium BCE. One of these consisted of communities centred around water, not only those in the wetland core but also connected to it through waterways, and practicing a semi-agrarian lifestyle[3]. Water links were central for these communities, and connections to people living along the waterways were often more important than connections to physically closer neighbours. Surrounding the waterways, the other sphere consisted of early full-blown farming communities that preferred the fertile loess soils and kept to their culturally specific traditions of settlement, housing, material culture and burial. This supports the frontier mobility model proposed previously[50], albeit in a more geographically restricted context. These communities exchanged ideas, with women introducing EEF genetic ancestry and probably also new technological knowledge in the more hunting–gathering-practicing communities but their distinct cultures persisted for millennia.

The very high proportion of WHG ancestry was observed from the earliest Neolithic Swifterbant communities to the period of the introduction of the CW complex. CW pottery is present in the Rhine–Meuse delta, but other aspects of this culture are lacking, in particular the characteristic burial rituals[19,20,24]. This is matched by limited population influx and high retention of the genetic ancestry of previously established groups.

The BB complex related communities harbour evidence of a break with the previously established pattern, as they show a profound change in genetic ancestry. This was introduced by new incoming people of both sexes with CW related ancestry. Admixture with local Lower Rhine–Meuse delta populations did occur, but was limited to 13–18%. Owing to the uncertainty of the radiocarbon-dated individuals in our transect, we need to consider a potentially slightly larger time-gap between the three analysed Vlaardingen/CW-associated individuals and the 13 BB-associated individuals. Thus, the time course and exact location in the region where people with a CW-associated genetic profile and a Lower Rhine–Meuse Neolithic genetic profile came together remains unclear. However, the homogeneity of ancestry within the three BB-associated sites that we sampled shows that the mixture had largely taken place by that time (around 2400–1900 BCE).

The evidence for a large-scale demographic change in the Lower Rhine–Meuse region by the time of the spread of Beaker users is important in light of the evidence from Britain, where Beaker users spread at around the same time. As the British Neolithic populations encountered by Beaker users practiced cremation and therefore did not yield samples amenable for aDNA analysis, it has been unclear whether there was a sharp population break or a period of extended co-existence[51]. In the Lower Rhine–Meuse region, we do not have this issue, as cremation was rarely practised by previous groups, yet the genetic turnover seems to have been similarly profound as in Britain, with the great majority of local BB burials being consistent with no local British Neolithic ancestry and the contribution of the local British Neolithic population by the Early Bronze Age estimated at ≤9%. Although we do not know what triggered this large-scale mobility, it is clear that the genetic legacy of local populations both in the Lower Rhine–Meuse area and Britain collapsed relatively rapidly.

Despite the evidence of a cultural break, in both the Lower Rhine–Meuse area and Britain, local cultural traditions and knowledge remained intact for some time between 2450 and 2200 BCE[49,52,53]. British archaeologists stress that continuity is witnessed in the building and use of Late Neolithic monuments like Stonehenge, Avebury, Woodhenge and Silbury Hill, and that culturally substantial changes only occur in the twenty-third century BCE[52,54]. Similarly, in the Lower Rhine–Meuse area, BB groups used the same areas, although not the exact same settlement sites as their Vlaardingen/CW predecessors. They continued to settle in river valleys, on crevasse splays and along river dunes in a way that was oriented explicitly towards a hunting–farming mixed economy. This indicates that the newcomers with their distinct genetic ancestry came from a similar landscape elsewhere or were in close contact with the local communities to learn how to handle this specific type of landscape.

## Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-026-10111-8.


1. Lipson, M. et al. Parallel palaeogenomic transects reveal complex genetic history of early European farmers. *Nature* **551**, 368–372 (2017).
2. Allentoft, M. E. et al. Population genomics of Bronze Age Eurasia. *Nature* **522**, 167–172 (2015).
3. Amkreutz, L. W. S. W. *Persistent traditions. A Long-Term Perspective on Communities in the Process of Neolithisation in the Lower Rhine Area (5500–2500 Cal BC)* (Sidestone Press, 2013).
4. Lazaridis, I. et al. Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* **513**, 409–413 (2014).
5. Haak, W. et al. Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* **522**, 207–211 (2015).
6. Olalde, I. et al. The Beaker phenomenon and the genomic transformation of northwest Europe. *Nature* **555**, 190–196 (2018).
7. Papac, L. et al. Dynamic changes in genomic and social structures in third millennium BCE central Europe. *Sci. Adv.* **7**, eabi6941 (2021).
8. Robb, J. Material culture, landscapes of action, and emergent causation: a new model for the origins of the European Neolithic. *Curr. Anthropol.* **54**, 657–683 (2013).
9. Mittnik, A. et al. The genetic prehistory of the Baltic Sea region. *Nat. Commun.* **9**, 442 (2018).
10. Skoglund, P. et al. Genomic diversity and admixture differs for Stone-Age Scandinavian foragers and farmers. *Science* **344**, 747–750 (2014).
11. Hofmann, D. in *Something Out of the Ordinary? Interpreting Diversity in the Early Neolithic Linearbandkeramik and Beyond* (eds Amkreutz, L. W. S. W. et al.) 191–226 (Cambridge Scholars Press, 2016).
12. Kirschneck, E. The phenomena la Hoguette and Limburg—technological aspects. *Open Archaeol.* **7**, 1295–1344 (2021).
13. Crombé, P. et al. New evidence on the earliest domesticated animals and possible small-scale husbandry in Atlantic NW Europe. *Sci. Rep.* **10**, 20083 (2020).
14. Brusgaard, N. Ø et al. Early animal management in northern Europe: multi-proxy evidence from Swifterbant, the Netherlands. *Antiquity* **98**, 654–671 (2024).
15. Kooijmans, L. P. L. & Jongste, P. F. B. *A Neolithic Settlement on the Dutch North Sea Coast c. 3500 CAL BC* (Analecta Praehistorica Leidensia, 2006).
16. Dreshaj, M., Dee, M., Brusgaard, N., Raemaekers, D. & Peeters, H. High-resolution Bayesian chronology of the earliest evidence of domesticated animals in the Dutch wetlands (Hardinxveld-Giessendam archaeological sites). *PLoS ONE* **18**, e0280619 (2023).
17. Menne, J. & Brunner, M. Transition from Swifterbant to Funnelbeaker: a Bayesian chronological model. *Open Archaeol.* **7**, 1235–1243 (2021).
18. Goossens, T. A. *Opgraving Hellevoetsluis-Ossenhoek. Een Nederzetting van de Vlaardingen-Groep op een Kwelderrug in de Gemeente Hellevoetsluis* (ARCHOL, 2009).
19. Bourgeois, Q. *Monuments on the Horizon: The Formation of the Barrow Landscape throughout the 3rd and 2nd Millennium BC* (Sidestone Press, 2013).
20. Beckerman, S. M. *Corded Ware Coastal Communities: Using Ceramic Analysis to Reconstruct Third Millennium BC Societies in the Netherlands* (Sidestone Press, 2015).
21. Fokkens, H., Steffens, B. J. W. & van As, S. F. M. Farmers, fishers, fowlers, hunters: knowledge generated by development-led archaeology about the Late Neolithic, the Early Bronze Age and the start of the Middle Bronze Age (2850–1500 cal BC) in the Netherlands. *Ned. Archeol. Rapp.* **53**, 978–990 (2016).
22. Furholt, M. Re-integrating archaeology: a contribution to aDNA studies and the migration discourse on the 3rd millennium BC in Europe. *Proc. Prehist. Soc.* **85**, 115–129 (2019).
23. Kroon, E. J. *Serial Learners. Interactions between Funnel Beaker West and Corded Ware Communities in the Netherlands during the Third Millennium BCE from the Perspective of Ceramic Technology* (Sidestone Press, 2024).
24. Wentink, K. *Stereotype. The Role of Grave Sets in Corded Ware and Bell Beaker Funerary Practices* (Sidestone Press, 2020).

25. Dyselinck, T. et al. *Gent-Hogeweg, Vlakdekkende Opgraving. Mariakerke* BAAC-Rapport A-11.0045 (BAAC, 2013).
26. Vander Linden, M. What linked the Bell Beakers in third millennium BC Europe? *Antiquity* **81**, 343–352 (2007).
27. Lanting, J. N. De NO-Nederlandse/NW-Duitse Klokbekergroep: culturele achtergrond, typologie van het aardewerk, datering, verspreiding en grafritueel. *Palaeohistoria* **49–50**, 11–326 (2008).
28. Posth, C. et al. Palaeogenomics of Upper Palaeolithic to Neolithic European hunter-gatherers. *Nature* **615**, 117–126 (2023).
29. Immel, A. et al. Genome-wide study of a Neolithic Wartberg grave community reveals distinct HLA variation and hunter-gatherer ancestry. *Commun. Biol.* **4**, 113 (2021).
30. Veselka, B. et al. Assembling ancestors: the manipulation of Neolithic and Gallo-Roman skeletal remains at Pommercœul, Belgium. *Antiquity* **98**, 1576–1591 (2024).
31. Patterson, N. et al. Large-scale migration into Britain during the Middle to Late Bronze Age. *Nature* **601**, 588–594 (2021).
32. ten Anscher, T. J., Knippenberg, S., van der Linde, C. M., Roessingh, W. & Willemse, N. W. *Doorbraken aan de Rijn. Een Swifterbant-Gehucht, een Hazendonk-Nederzetting en Erven en Graven uit de Bronstijd in Medel-De Roeskamp* RAAP-Rapport 6519, Archol Rapport 742, ADC Rapport A-16.0207 (BAAC, 2023).
33. Arzelier, A. et al. Neolithic genomic data from southern France showcase intensified interactions with hunter-gatherer communities. *iScience* **25**, 105387 (2022).
34. Seguin-Orlando, A. et al. Heterogeneous hunter-gatherer and steppe-related ancestries in Late Neolithic and Bell Beaker genomes from present-day France. *Curr. Biol.* **31**, 1072–1083 (2021).
35. Rivollat, M. et al. Ancient genome-wide DNA from France highlights the complexity of interactions between Mesolithic hunter-gatherers and Neolithic farmers. *Sci. Adv.* **6**, aaz5344 (2020).
36. Mathieson, I. et al. The genomic history of southeastern Europe. *Nature* **555**, 197–203 (2018).
37. Olalde, I. et al. Derived immune and ancestral pigmentation alleles in a 7,000-year-old Mesolithic European. *Nature* **507**, 225–228 (2014).
38. Gelabert, P. et al. Social and genetic diversity in first farmers of central Europe. *Nat. Hum. Behav.* https://doi.org/10.1038/s41562-024-02034-z (2024).
39. Ringbauer, H. et al. Accurate detection of identity-by-descent segments in human ancient DNA. *Nat. Genet.* **56**, 143–151 (2023).
40. Bourgeois, Q. P. J. et al. Spatiotemporal reconstruction of Corded Ware and Bell Beaker burial rituals reveals complex dynamics divergent from steppe ancestry. *Sci. Adv.* **11**, eadx22622262 (2025).
41. Lazaridis, I. et al. The genetic origin of the Indo-Europeans. *Nature* https://doi.org/10.1038/s41586-024-08531-5 (2025).
42. Linderholm, A. et al. Corded Ware cultural complexity uncovered using genomic and isotopic analysis from south-eastern Poland. *Sci. Rep.* **10**, 6885 (2020).
43. Allentoft, M. E. et al. Population genomics of post-glacial western Eurasia. *Nature* **625**, 301–311 (2024).
44. Brunel, S. et al. Ancient genomes from present-day France unveil 7, 000 years of its demographic history. *Proc. Natl Acad. Sci USA* https://doi.org/10.1073/pnas.1918034117 (2020).
45. Parasayan, O. et al. Late Neolithic collective burial reveals admixture dynamics during the third millennium BCE and the shaping of the European genome. *Sci. Adv.* **10**, eadl2468 (2024).
46. Heyd, V. in *Yamnaya Interactions. Proceedings of the International Workshop held in Helsinki, 25–26 April 2019. The Yamnaya Impact on Prehistoric Europe* (eds Heyd, V. et al.) 383–414 (Archaeolingua Alapítvány, (2021).
47. Jeunesse, C. The dogma of the Iberian origin of the Bell Beaker: attempting its deconstruction. *J. Neolit. Archaeol.* **16**, 158–166 (2015).
48. Price, T. D., Knipper, C., Grupe, G. & Smrcka, V. Strontium isotopes and prehistoric human migration: The Bell Beaker period in Central Europe. *Eur. J. Archaeol.* **7**, 9–40 (2004).
49. Parker Pearson, M. et al. *The Beaker People: Isotopes, Mobility and Diet in Prehistoric Britain* Vol. 7 (Oxbow Books, 2019).
50. Zvelebil, M. in *Archaeogenetics: DNA and the Population Prehistory of Europe* (eds Renfrew, C. & Boyle, K.) 57–79 (McDonald Institute Monographs, 2000).
51. Armit, I. & Reich, D. The return of the Beaker folk? Rethinking migration and population change in British prehistory. *Antiquity* **95**, 1464–1477 (2021).
52. Cleal, R. M. J. & Pollard, J. in *Is There a British Chalcolithic? People, Place and Polity in the Later 3rd Millennium* (eds Allen, M. J. et al.) Vol. 4, 317–332 (Oxbow Books, 2012).
53. Booth, T. J., Brück, J., Brace, S. & Barnes, I. Tales from the supplementary information: ancestry change in Chalcolithic–Early Bronze Age Britain was gradual with varied kinship organization. *Camb. Archaeol. J.* https://doi.org/10.1017/s0959774321000019 (2021).
54. Gibson, A. Beakers in Britain. The Beaker package reviewed. *Préhist. Méd.* **8**, 32 (2020).





[1]Department of Zoology and Animal Cell Biology, University of the Basque Country EHU, Vitoria-Gasteiz, Spain. [2]Department of Human Evolutionary Biology, Harvard University, Cambridge, MA, USA. [3]Ikerbasque–Basque Foundation of Science, Bilbao, Spain. [4]Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands. [5]Faculty of Archaeology, Leiden University, Leiden, The Netherlands. [6]National Museum of Antiquities, Leiden, The Netherlands. [7]Archeologisch Onderzoek Leiden, Leiden, The Netherlands. [8]PACEA UMR, University of Bordeaux, Pessac, France. [9]School of Applied Sciences, University of Huddersfield, Huddersfield, UK. [10]Service de Préhistoire, Université de Liège, Liege, Belgium. [11]LAMPEA, Aix-Marseille Université, Aix-en-Provence, France. [12]Department of Archaeology, Ostfriesische Landschaft, Aurich, Germany. [13]Faculty of Science, Department of Earth Sciences—Isotope Archaeology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands. [14]BAAC Archaeology, Hertogenbosch, The Netherlands. [15]Tot op het Bot, Amsterdam, The Netherlands. [16]Formerly Cultural Heritage Agency of the Netherlands, Ministry of Education, Culture and Science, Amersfoort, The Netherlands. [17]RAAP, Weesp, The Netherlands. [18]Groningen Institute of Archaeology (GIA), University of Groningen, Groningen, The Netherlands. [19]Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany. [20]ArcheOs, Research Laboratory for Biological Anthropology, Department of Archaeology, University of Ghent, Ghent, Belgium. [21]Faculty of Humanities, University of Amsterdam, Amsterdam, The Netherlands. [22]Faculty of Science and Technology, Bournemouth University, Poole, UK. [23]Department of Genetics, Harvard Medical School, Boston, MA, USA. [24]Department of Evolutionary Anthropology, University of Vienna, Vienna, Austria. [25]Human Evolution and Archaeological Sciences, University of Vienna, Vienna, Austria. [26]Howard Hughes Medical Institute, Harvard Medical School, Boston, MA, USA. [27]Broad Institute of MIT and Harvard, Cambridge, MA, USA. [28]These authors contributed equally: Iñigo Olalde, Eveline Altena, Quentin Bourgeois, Harry Fokkens. ✉e-mail: inigo.olalde@ehu.eus; e.altena@lumc.nl; Q.P.J.Bourgeois@arch.leidenuniv.nl; h.fokkens@arch.leidenuniv.nl; reich@genetics.med.harvard.edu

# Methods

### Sampling, extraction, library preparation, capture and sequencing

Our initial selection for aDNA analysis comprised 116 ancient individuals from the Lower Rhine–Meuse area for aDNA analysis (Supplementary Table 2), including five previously reported individuals[1,6,31] for whom we generated additional data. We also selected ten previously reported[6,36] individuals from relevant contexts outside the Lower Rhine–Meuse area and generated additional data (Supplementary Tables 2–4). We performed laboratory work in dedicated clean rooms. We removed the outer layer of teeth and long bones and collected powder from beneath the cleaned surface. This process minimized the risk of exogenous DNA contamination, and low-speed drilling was used to prevent heat-induced DNA damage[55]. In the case of temporal bones, we removed cochleae through sandblasting[56] and then milled them. We incubated the resulting powder in lysis buffer and cleaned and concentrated the DNA from one-fifth of the lysate. We did this either manually or using an automated protocol with silica magnetic beads[57] and Dabney binding buffer[58,59] for manual extraction. The samples from Trou Al'Wesse, Abri Sandron and Grotte du Mont Falise were prepared and extracted following the method outlined previously[60].

We built 254 libraries (Supplementary Table 2) using two different protocols. Double-stranded barcoded libraries were prepared with truncated adapters from the extract and subjected to partial (half) uracil–DNA–glycosylase (UDG) treatment before blunt-end repair to substantially reduce the characteristic damage pattern of aDNA[61,62]. Single-stranded libraries were prepared using automated protocols described previously[63]. A fraction was subjected to USER treatment[63].

DNA libraries were enriched for human DNA using probes that target 1,233,013 (1240k capture)[64] or 1,352,535 (Twist BioScience)[65] nuclear SNPs and the mitochondrial genome. We performed two rounds of capture for the 1240k reagent and one for the Twist BioScience reagent. Captured libraries were sequenced on the Illumina HiSeq X10 instrument with 2 × 101 cycles and 2 × 7 cycles to read out the two indices[66] or on the Illumina NextSeq 500 instrument with 2 × 76 cycles and 2 × 7 cycles to read out the two indices.

For three previously reported individuals (I4075, I0103 and I0104), we generated shotgun data (Supplementary Table 2) from the same libraries that were 1240k captured in the original publications[6,36].

### Bioinformatics: demultiplexing, adapter removal, mapping and PCR duplicate removal

Reads for each sample were extracted from the raw sequencing data based on sample-specific indices introduced during wet-laboratory processing, permitting up to one mismatch. Adapters were removed and paired-end sequences were merged into single-ended sequences with a required 15-base-pair overlap (allowing one mismatch with high-quality bases or three mismatches with low-quality bases), using a modified version of SeqPrep v.1.1 (https://github.com/jstjohn/SeqPrep). This process was applied by selecting the highest-quality base in the overlapping region. Reads that could not be merged were discarded before aligning to the human reference genome (hg19) and the RSRS version of the mitochondrial genome using the samse command in bwa (v.0.7.15)[67]. We removed duplicates based on the alignment coordinates and orientation of the aligned reads. Aligned sequences from different libraries of the same sample were merged accordingly into a single BAM file. The computational pipelines are available at GitHub (https://github.com/dReichLab/ADNA-Tools, https://github.com/dReichLab/adna-workflow).

### Evaluation of authenticity

We established aDNA authenticity using several criteria. Libraries with a deamination rate below 3% at the terminal nucleotide were excluded from further analysis. We computed the ratio of Y-chromosome to X- and Y-chromosome reads. Libraries with ratios above 0.03 and below 0.32 were excluded from further analysis. We estimated mismatch rates to the consensus mitochondrial sequence using contamMix (v.1.0.1051)[68], and performed X-chromosome contamination estimates using ANGSD[69] in male individuals with sufficient coverage. Libraries with evidence of contamination were excluded from further analysis. Finally, individuals without a minimum of 20,000 targeted 1240k SNPs with at least one overlapping sequence were discarded from population genetic analysis. After applying these filters, 122 libraries from 59 individuals remained (Supplementary Table 2); we merged data from the libraries to increase sequencing coverage. Of these 59 individuals, 44 were newly reported individuals from the Lower Rhine–Meuse area, five previously reported from the same area and 10 previously reported from other areas.

### Analysis datasets

In addition to the 49 individuals with newly generated data from the Lower Rhine–Meuse area, we also included data from 63 previously published individuals[1,6,28–31] from the region, for a total of 112 individuals from the Lower Rhine–Meuse region and adjacent areas between 8500–1700 BCE (Fig. 1 and Supplementary Table 1). The time–transect dataset includes three new Mesolithic individuals from Belgium, two from the Netherlands and one from northwest Germany, as well as published data from three individuals from now submerged areas of Doggerland[28]; ten Early-Middle Neolithic individuals from semi-agrarian Swifterbant contexts (4600–4000 BCE) (Netherlands)—the first data from this unique culture; the first three Middle Neolithic individuals from Late Swifterbant or Hazendonk archaeological contexts (Netherlands); three likely Middle Neolithic individuals from Tiel (Netherlands) and one Middle Neolithic individual from Baltrum island (northwest Germany); four published Middle Neolithic individuals from Blätterhöhle cave (3500–3000 BCE)[1] (northwest Germany); 40 published Middle Neolithic individuals from a Wartberg context (3500–2800 BCE)[29] (Niedertiefenbach, northwest Germany); 18 Late Neolithic individuals buried in caves from the Ardennes region (3300–2500 BCE) (Belgium); three Late Neolithic individuals from Vlaardingen/CW contexts, including the first data from this culture from the Lower Rhine–Meuse area (3000–2500 BCE) (Netherlands); 13 Late Neolithic individuals from BB contexts (2500–2000 BCE) (Netherlands); and six individuals from an Early Bronze Age context (2000–1700 BCE) (Netherlands).

To aid the analysis of the Lower Rhine–Meuse area individuals, the analysis dataset was further complemented by previously published data from ancient individuals from other regions (Extended Data Fig. 1 and Supplementary Tables 3 and 4). For genome-wide analyses, we assembled two datasets. The HO dataset included the ancient individuals, and 1,036 present-day West Eurasian individuals genotyped on the Affymetrix Human Origins Array[4,70,71]. We retained 591,642 SNPs shared between the 1240k capture and the Human Origins Array. The HOIll dataset included only the ancient individuals and 1,233,013 SNPs in common between 1240k and Twist reagents. In both datasets, we randomly sampled one allele at each SNP position for each individual, discarding the first and the last two nucleotides of each sequence.

### Haplogroup assignment of uniparentally inherited markers

We created consensus mitochondrial haplotypes with samtools and bcftools. We restricted to sequences with a mapping quality of more than 30 and a base quality of more than 30. We then called haplogroups with Haplogrep3[72] (Supplementary Table 1). We called Y-chromosome haplogroups (Supplementary Table 1) following the methodology described previously[73], based on the YFull v.8.09 phylogeny (https://www.yfull.com/). Haplogroups found in Neolithic individuals were classified as either 'hunter-gatherer related' if they were already present among Mesolithic hunter-gatherers (mitochondrial haplogroups U5, U4′9, U2, U* and K1e; Y-chromosome haplogroups I2a, C1a and R1b-V88) or 'Neolithic related' if they were most likely introduced by

incoming EEF populations (all mitochondrial and Y-chromosome haplogroups except for those mentioned above) (Supplementary Table 13). Using this approach, we understand that we might be underestimating the number of lineages contributed by Neolithic farmers, both in the mtDNA and Y chromosome, as some lineages considered to be Mesolithic hunter-gatherer related, based on their presence during the Mesolithic period, might have been incorporated by farmer populations during their path from Anatolia to the Lower Rhine–Meuse area.

### Molecular sex determination

Genetic sex was determined by calculating the ratio of reads mapped to Y-chromosome SNP positions to the total reads mapped to sex-chromosome SNP positions. Individuals with a ratio of <0.03 were classified as female, while those with a ratio >0.32 were classified as male (Supplementary Table 1).

### Biological relatedness

To estimate close biological relatedness up to the third degree, mismatch rates were computed between all possible pairs of Lower Rhine–Meuse area individuals, randomly sampling one read for each individual at each of the 1.15 million autosomal SNPs. Mismatch rates were converted to relatedness coefficients as described previously[74] using three different baseline mismatch rate values to account for the different ancestral backgrounds found in the dataset. If both individuals in the pair had fully Mesolithic hunter-gatherer ancestry, we use a baseline mismatch rate of 0.225. If at least one individual has EEF ancestry and both lack steppe-associated ancestry, we use a baseline mismatch rate of 0.252. If at least one individual has steppe-associated ancestry, we use a baseline mismatch rate of 0.258. Close kinship relationships are annotated in Supplementary Table 1.

### IBD

We called IBD segments between the Lower Rhine–Meuse delta individuals with high-quality data ($n$ = 59) and all the previously published ancient individuals from Eurasia with high-quality data ($n$ = 7,034). We followed the same procedure described previously[39], which involves imputing and phasing the aligned sequenced data with GLIMPSE[75] using haplotypes in the 1000 Genome Project as the reference panel[76], and detecting IBD segments with ancIBD (https://github.com/hringbauer/ancIBD). Pairs of individuals connected by IBD are displayed in Supplementary Table 14. Three individuals from the site of Tiel Medel de Roeskamp have uncertain chronology. The site has a Middle Neolithic Swifterbant occupation but also a Bronze Age occupation phase and the sampled individuals were not amenable to radiocarbon dating. We therefore used their IBD connections to estimate an approximate chronology. Their largest IBD sharing is with I33738, a Middle Neolithic Swifterbant individual from Zoelen de Beldert (Netherlands) dated to 4200–3800 BCE, with whom one of Tiel Medel-de Roeskampindividuals shares four IBD segments longer than 8 cM (the longest being 20.5 cM), for a total share of 53 cM (Supplementary Table 14). The second and third largest IBD sharing are with a Neolithic individual from Hazleton North (England)[74] who lived 3750–3500 BCE (3 IBD segments for a total of 39 cM) and with a Neolithic individual from Gurgy les Noisats[77] dated to 4836–4606 cal. BCE (5855 ± 40 BP, Lyon-4446, SacA-8629) (2 IBD segments for a total of 33 cM). On the basis of these IBD results, a Bronze Age chronological attribution is extremely implausible for these individuals, and we therefore approximate their date to the range 3800–3600 BCE, therefore within the Middle Neolithic. This chronology fits well with their lack of steppe-associated ancestry in the autosomal genome, which already suggested a pre-2500 BCE date.

### Runs of homozygosity

We called runs of homozygosity using hapROH[78] for individuals with more than 300,000 available SNPs from the 1240k capture panel (Supplementary Table 1).

### PCA

We ran PCA on the HO dataset using the smartpca software from the EIGENSOFT package[79]. We computed PCs on 1,036 present-day West Eurasians genotyped on the Affymetrix Human Origins Array. Ancient individuals were projected onto those PCs using lsqproject:YES and shrinkmode:YES.

### qpAdm

We used qpAdm[5] to estimate ancestry proportions. We set the parameters allsnps: YES and inbreed: YES to account for the use of pseudo-haploid data (Supplementary Information 4).

We ran each qpAdm model with four different setups:

(1) Using all 1240k autosomal SNPs.
(2) Using 469k autosomal SNPs reported to greatly reduced the bias when co-analysing 1240k data with Twist and shotgun data[65].
(3) Using 711k autosomal SNPs with reduced bias identified using an approach (https://github.com/rmnfournier/compatibility-panel) to filter out biased SNPs[80].
(4) Using all 1240k autosomal SNPs, but featuring only Twist or shotgun data in the target, source and outgroup populations (Supplementary Table 4).

Throughout this Article, we use terms such as WHG, eastern hunter-gatherers and EEF as genetic shorthand for ancestry components maximized in western European hunter-gatherers, eastern European hunter-gatherers and Neolithic farmers of Anatolian origin, respectively. These labels refer solely to patterns of shared genetic ancestry and do not imply any specific subsistence strategy, cultural affiliation or social identity for individuals in whom these components are detected.

### Admixture date estimates

We used DATES[81] to estimate admixture dates leveraging linkage disequilibrium decay (Supplementary Table 15 and Extended Data Fig. 4). We used the same ancestral population as in qpADM modelling. For Neolithic groups/individuals without steppe ancestry, we used Balkan_N + WHG to study EEF–WHG admixture. To study CW–European Neolithic admixture, we used MN_Wartberg + Germany_CordedWare for Lower Rhine–Meuse BB, Lower Rhine–Meuse EBA and England BB; MLN_Belgium + Germany_CordedWare for Vlaardingen/CordedWare; and GlobularAmphora_LN + Germany_CordedWare for SEGermany_BB. To convert the number of generations since admixture given by DATES into years, we assumed 28 years per generation[82]. We used the following parameters: binsize: 0.001; seed: 77; maxdis: 1; qbin: 10; jackknife: YES; afffit: YES; runfit: YES; lovalfit: 0.45 and minparentcount: 1. We considered the earliest, the most recent and the mean possible chronological dates for each group when estimating admixture dates (Supplementary Table 15). The estimates obtained for Vlaardingen/CordedWare group were considered to be invalid, as the s.e. was higher than the estimated number of generations.

### Radiocarbon chronology

The chronology for each site is described in Supplementary Information 2. All dates were obtained from the original excavation reports, follow-up publications or from direct consultation with the excavators. These have been supplemented by 14 new measurements. Full laboratory codes, pretreatment steps and quantitative quality indicators are reported in Supplementary Table 16. All reported ages were calibrated with the IntCal20 calibration curve using OxCal (v.4.4.4)[83].

Several of the dates are potentially susceptible to marine or freshwater reservoir effect (FRE). However, owing to the dynamic hydrological regime of the Rhine Meuse lowlands, it is often impossible to accurately estimate the source of the FRE, and there is currently no consensus on the correction factor to use for the region[16,84–86]. Thus, all reported radiocarbon dates have not included an FRE correction factor and we

address a potential bias qualitatively in the site descriptions (Supplementary Information 2).

### Ethics

The individuals studied here were all analysed with the goal of minimizing damage to their skeletal remains, with permission from local authorities in each location from which they came. Every sample is represented by stewards, such as archaeologists or museum curators, who are either authors or thanked in the Acknowledgments. Open science principles require making all data used to support the conclusions of a study maximally available, and we support these principles here by making fully publicly available not only the digital copies of molecules (the uploaded sequences) but also the molecular copies (the aDNA libraries themselves, which constitute molecular data storage). Those researchers who wish to carry out deeper sequencing of libraries published in this study should send a request to the corresponding author D.R. We commit to granting reasonable requests as long as the libraries remain preserved in our laboratories, with no requirement that we be included as collaborators or co-authors on any resulting publications.

### Reporting summary

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

## Data availability

Genotype data for individuals included in this study can be obtained from the Harvard Dataverse repository (https://reich.hms.harvard.edu/datasets). The DNA sequences reported in this paper have been deposited in the European Nucleotide Archive under accession number PRJEB105335. Other newly reported data, such as radiocarbon dates and archaeological context information, are included in the Article and its Supplementary Information.


55. Adler, C. J., Haak, W., Donlon, D. & Cooper, A. Survival and recovery of DNA from ancient teeth and bones. *J. Archaeol. Sci.* **38**, 956–964 (2011).
56. Pinhasi, R., Fernandes, D. M., Sirak, K. & Cheronet, O. Isolating the human cochlea to generate bone powder for ancient DNA analysis. *Nat. Protoc.* **14**, 1194–1205 (2019).
57. Rohland, N., Glocke, I., Aximu-Petri, A. & Meyer, M. Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nat. Protoc.* **13**, 2447–2461 (2018).
58. Dabney, J. et al. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl Acad. Sci. USA* **110**, 15758–15763 (2013).
59. Korlević, P. et al. Reducing microbial and human contamination in DNA extractions from ancient bones and teeth. *BioTechniques* **59**, 87–93 (2015).
60. Dulias, K. et al. Ancient DNA at the edge of the world: continental immigration and the persistence of Neolithic male lineages in Bronze Age Orkney. *Proc. Natl Acad. Sci. USA* **119**, e2108001119 (2022).
61. Briggs, A. W. et al. Removal of deaminated cytosines and detection of in vivo methylation in ancient DNA. *Nucleic Acids Res.* **38**, e87 (2009).
62. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil–DNA–glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B* **370**, 20130624 (2015).
63. Gansauge, M. T., Aximu-Petri, A., Nagel, S. & Meyer, M. Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nat. Protoc.* **15**, 2279–2300 (2020).
64. Fu, Q. et al. An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216–219 (2015).
65. Rohland, N. et al. Three assays for in-solution enrichment of ancient human DNA at more than a million SNPs. *Genome Res.* **32**, 2068–2078 (2022).
66. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).
67. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
68. Fu, Q. et al. A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* **23**, 553–559 (2013).
69. Korneliussen, T. S., Albrechtsen, A. & Nielsen, R. ANGSD: analysis of next generation sequencing data. *BMC Bioinform.* **15**, 356 (2014).
70. Patterson, N. et al. Ancient admixture in human history. *Genetics* **192**, 1065–1093 (2012).
71. Biagini, S. A. et al. People from Ibiza: an unexpected isolate in the Western Mediterranean. *Eur. J. Hum. Genetics* **27**, 941–951 (2019).
72. Schönherr, S., Weissensteiner, H., Kronenberg, F. & Forer, L. Haplogrep 3—an interactive haplogroup classification and analysis platform. *Nucleic Acids Res.* **51**, W263–W268 (2023).
73. Lazaridis, I. et al. The genetic history of the Southern Arc: a bridge between West Asia and Europe. *Science* **377**, eabdm4247 (2022).
74. Fowler, C. et al. A high-resolution picture of kinship practices in an Early Neolithic tomb. *Nature* **601**, 584–587 (2021).
75. Rubinacci, S., Ribeiro, D. M., Hofmeister, R. J. & Delaneau, O. Efficient phasing and imputation of low-coverage sequencing data using large reference panels. *Nat. Genet.* **53**, 120–126 (2021).
76. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65 (2012).
77. Rivollat, M. et al. Extensive pedigrees reveal the social organization of a Neolithic community. *Nature* https://doi.org/10.1038/s41586-023-06350- (2023).
78. Ringbauer, H., Novembre, J. & Steinrücken, M. Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nat. Commun.* **12**, 5425 (2021).
79. Patterson, N., Price, A. L. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* **2**, e190 (2006).
80. Fournier, R., Fulton, A. P. & Reich, D. A SNP panel for co-analysis of capture and shotgun ancient DNA data. Preprint at *bioRxiv* https://doi.org/10.1101/2025.07.30.667733 (2025).
81. Narasimhan, V. M. et al. The formation of human populations in South and Central Asia. *Science* **365**, eaat7487 (2019).
82. Fenner, J. N. Cross-cultural estimation of the human generation interval for use in genetics-based population divergence studies. *Am. J. Phys. Anthropol.* **128**, 415–423 (2005).
83. Reimer, P. J. et al. The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* **62**, 725–757 (2020).
84. Philippsen, B. The freshwater reservoir effect in radiocarbon dating. *Herit. Sci.* **1**, 24 (2013).
85. van der Plicht, J. & Streurman, H. J. A new model for radiocarbon dating of marine shells from the Netherlands. *Radiocarbon* **67**, 378–411 (2025).
86. Kamjan, S., Gillis, R. E., Cakirlar, C. & Raemaekers, D. C. M. Specialized cattle farming in the Neolithic Rhine-Meuse Delta: results from zooarchaeological and stable isotope ($\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$) analyses. *PLoS ONE* **15**, e0240464 (2020).
87. Massicotte, P. & South, A. *rnaturalearth: World Map Data from Natural Earth* (2024); docs.ropensci.org/rnaturalearth.



**Acknowledgements** We thank N. Adamski, N. Broomandkhoshbacht, E. Curtis, I. Greenslade, K. Stewardson and F. Zalzala for laboratory work; the staff at the Provinciaal Depot voor Archeologie van Noord-Holland (M. Veen and R. van Eerden), Provinciaal Archeologisch depot Zuid-Holland (I. Riemersma and M. Phlippeau), Archeologisch Depot Gelderland (S. Weiss-König) and National Museum of Antiquities Leiden (L. Amkreutz) for granting permission to sample ancient remains and assistance in sampling; T. Grange and E.-M. Geigl for facilitating access to Bréviandes genomic data. The aDNA data generation and analysis was supported by the National Institutes of Health (R01-HG012287); the John Templeton Foundation (grant 61220); a private gift from J.-F. Clin; the Allen Discovery Center program, a Paul G. Allen Frontiers Group advised program of the Paul G. Allen Family Foundation; the Howard Hughes Medical Institute (D.R.); grant RYC2019-027909-I and project PID2022-140886NA-I00 funded by MCIN/AEI/10.13039/501100011033, "ESF Investing in your future" and FEDER, UE (I.O.); the Basque Government under "Grupos Consolidados" grant no. IT1633-22 (I.O.); NWO-VIDI grant "The Talking Dead" VI.VIDI.191.149 (Q.B.); the Max Planck Society, by the French Research Foundation and German Research Foundation (to M.R., W.H. and M.-F.D., project INTERACT, ANR-17-FRAL-0010 and DFG-HA-5407/4-1, 2018-21); and the Leverhulme Trust (A.F., F.G., C.J.E., M.B.R., M.P.). The accepted version of this article (before the editing, proofreading and formatting changes following the paper being accepted) is subject to the Howard Hughes Medical Institute (HHMI) Open Access to Publications policy; HHMI laboratory heads have previously granted a non-exclusive CC BY 4.0 licence to the public and a sublicensable licence to HHMI in their research articles. Pursuant to those licences, the accepted manuscript can be made freely available under a CC BY 4.0 licence immediately after publication.


**Author contributions** I.O., E.A., Q.B., H.F. and D.R. wrote the manuscript with the input of all of the other authors. M.B.R., R.P., W.H., M.P. and D.R. supervised parts of the study. I.O., I.L., N.P. and M.R. analysed genetic data. E.A., Q.B. and H.F. edited archaeological information. E.A, L.A., S.B., M.-F.D., A.F., D.F., F.G., J.F.K., L.M.K., C.v.d.L., J.v.d.L., K.L., L.L.K., R.L., R.M., H.M., P.N., D.C.M.R., M.R., L.S., J.R.S., T.t.A., M.T. and C.J.E. sampled anthropological remains and/or contributed to the creation of the archaeological supplement. G.S., M.M., A.M. and S.M. processed bioinformatic data. K.C., O.C., T.F., L.I., J.O., I.P., L.Q., J.N.W., C.J.E. and N.R. carried out wet laboratory work.

**Competing interests** The authors declare no competing interests.

**Additional information**

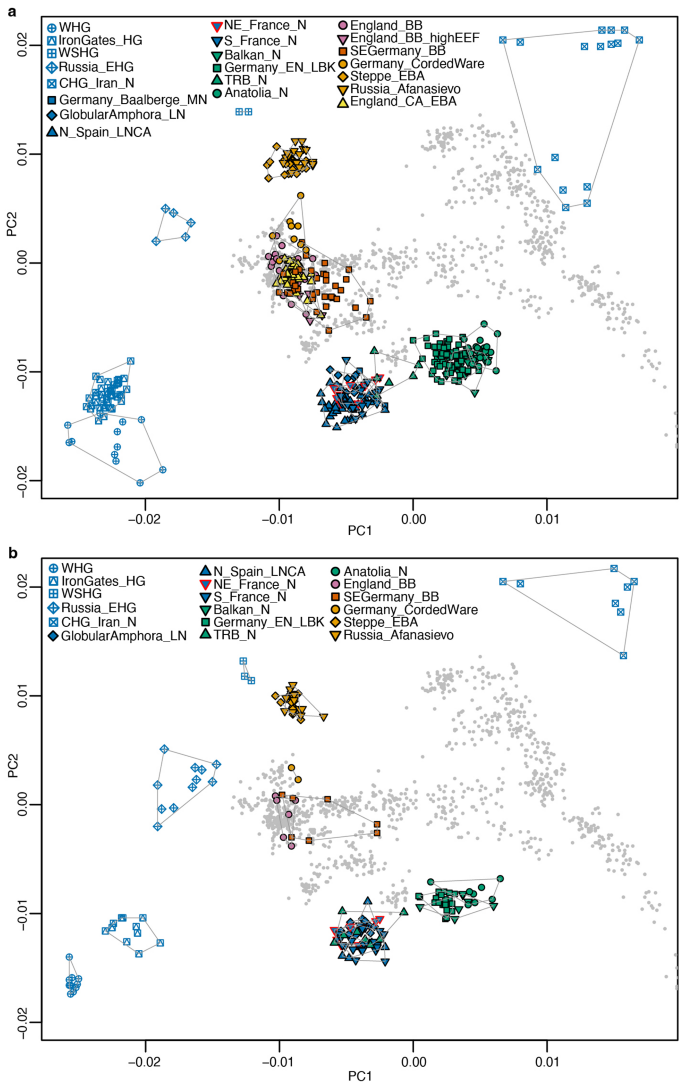

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s41586-026-10111-8.



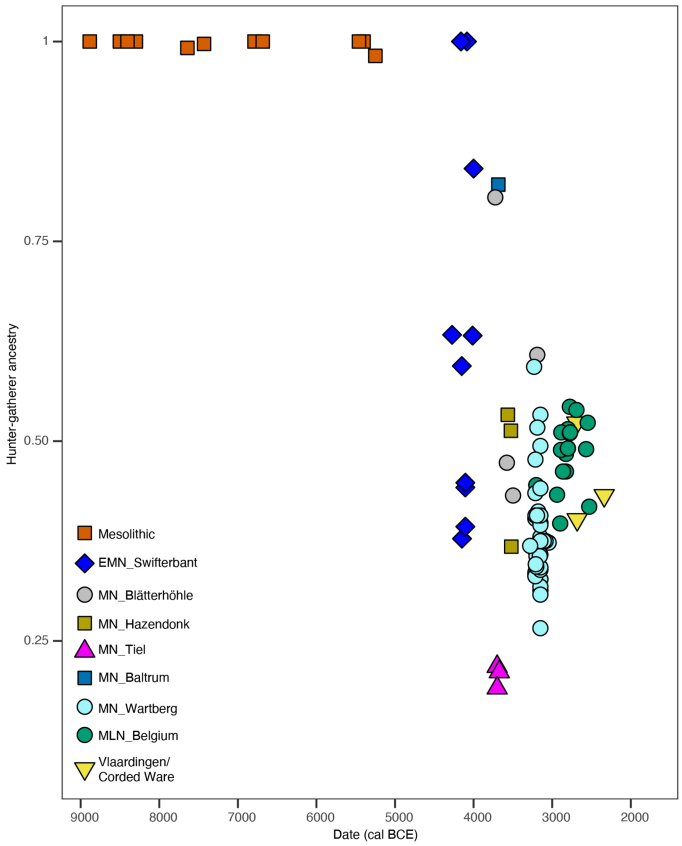
**Correspondence and requests for materials** should be addressed to Iñigo Olalde, Eveline Altena, Quentin Bourgeois, Harry Fokkens or David Reich.



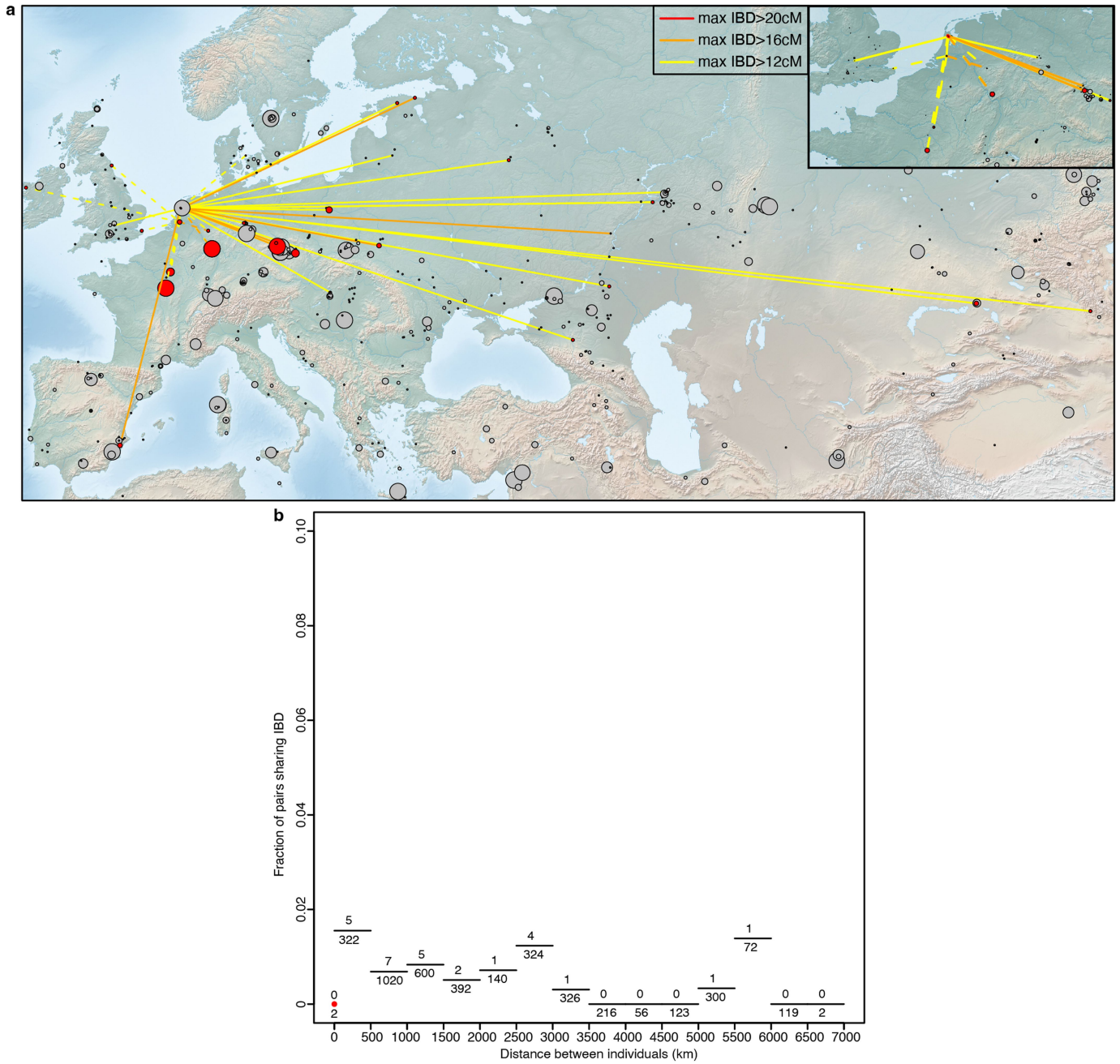
**Peer review information** *Nature* thanks Catherine Frieman, Eva-Maria Geigl, Choongwon Jeong, Oguzhan Parasayan and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.



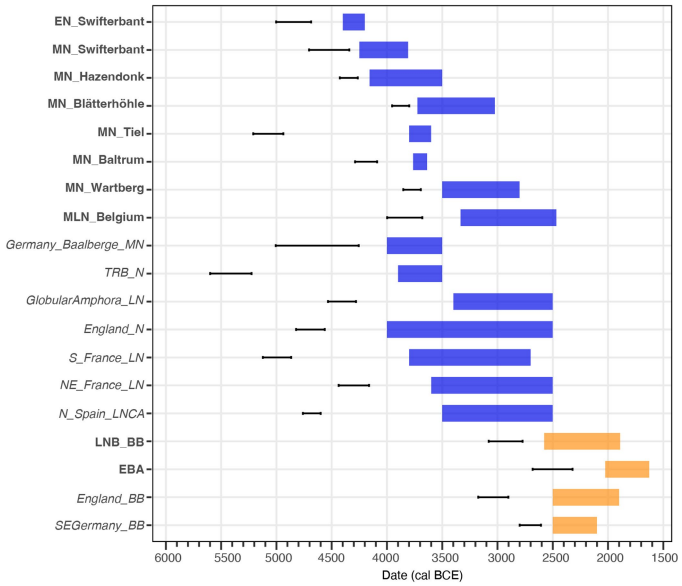

**Extended Data Fig. 1 | Genetic structure of relevant ancient groups used in *qpAdm* analysis.** PCA of sources and outgroups used under *qpAdm*: **a**, setup 1-3; and **b**, setup 4 (Supplementary Tables 3, 4).




**Extended Data Fig. 2 | Hunter-gatherer ancestry proportions across time in the Lower Rhine-Meuse area.** Ancestry proportions were estimated using *qpAdm* (Supplementary Table 6).




**Extended Data Fig. 3 | Genetic connections of Lower Rhine-Meuse CW/Vlaardingen individuals (n = 3). a**, IBD sharing of Lower Rhine-Meuse CW/Vlaardingen individuals. Sites are represented by circles with size proportional to the number of individuals amenable to IBD calling. Grey circles indicate archaeological sites between 3000-2000 BCE with no IBD connections to CW/Vlaardingen individuals. The map was drawn using public-domain Natural Earth data with the rnaturalearth package in R[87]. **b**, Decay of IBD sharing with geographic distance for Lower Rhine-Meuse CW/Vlaardingen individuals. Pairs were considered to share IBD if they share at least one segment of >12 cM. Dotted lines represent IBD connections involving at least one individual without steppe-related ancestry.




**Extended Data Fig. 4 | Admixture time estimates using DATES[81].** Boxes represent the chronological range for each population. Confidence intervals represent admixture date ranges, using 28 years per generation and the average date of the chronological range. We tested Balkan_N + WHG admixture for groups in blue, and MN_Wartberg+Germany_CordedWare admixture for groups in orange. In bold, groups from the Lower Rhine-Meuse region.

nature portfolio

Corresponding author(s): Iñigo Olalde, Eveline Altena, Quentin Bourgeois, Harry Fokkens, David Reich

Last updated by author(s): November 7, 2025

# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☐ | ☒ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | BWA version 0.7.15 and other bioinformatics tools and data workflows (https://github.com/DReichLab/ADNA-Tools and https://github.com/DReichLab/adna-workflow). A detailled description of the data preprocessing steps is given in the Methods section of the manuscript. |
| Data analysis | Relevant published software we used (including version):<br>ancIBD version 0.5, hapROH version 0.63, SeqPrep 1.1, smartpca version 18150, qpAdm version 1201, Yfull version 8.09, HaploGrep3 , contamMix version 1.0-12, ANGSD version 0.923, OxCal version 4.4.4, SAMtools 1.3.1, EIGENSOFT package version 8.0.0, DATES version 1520 |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Genotype data for individuals included in this study can be obtained from the Harvard Dataverse repository through the following link (https://

reich.hms.harvard.edu/datasets). The DNA sequences reported in this paper are deposited in the European Nucleotide Archive under accession number PRJEB105335. Other newly reported data, such as radiocarbon dates and archaeological context information, are included in the manuscript and supplementary files.

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

| | |
|---|---|
| Reporting on sex and gender | N/A |
| Reporting on race, ethnicity, or other socially relevant groupings | N/A |
| Population characteristics | N/A |
| Recruitment | N/A |
| Ethics oversight | N/A |

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

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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

# Life sciences study design

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

| | |
|---|---|
| Sample size | The sample size and geographic distribution is determined by accessibility of relevant osteological remains with sufficient aDNA preservation; it is not something we can choose a priori. We report new ancient DNA data from contexts where ancient DNA has not previously been reported, analyzing all samples with sufficient aDNA preservation. Although even more powerful inferences could have been made if sample sizes were larger, we can make many meaningful new inferences with the available samples. |
| Data exclusions | We excluded samples that did not fall within the geographic scope of the study. After collecting genetic data, we excluded individuals from the analysis dataset as described in the Methods section entitled "Evaluation of authenticity". Specifically: "We established aDNA authenticity using several criteria. Libraries with a deamination rate below 3% at the terminal nucleotide were excluded from further analysis. We computed the ratio of Y-chromosome to X- and Y-chromosome reads. Libraries with ratios above 0.03 and below 0.32 were excluded from further analysis. We estimated mismatch rates to the consensus mitochondrial sequence using contamMix-1.0.1051, and X-chromosome contamination estimates using ANGSD in males with sufficient coverage. Libraries with evidence of contamination were excluded from further analysis. Finally, individuals without a minimum of 20,000 targeted 1240k SNPs with at least one overlapping sequence were discarded from population genetic analysis. After applying these filters, 86 libraries from 42 individuals remained, and we merged data from the libraries to increase sequencing coverage." |
| Replication | Only a single library can be made from each extract aliquot so no replication from the same extract is possible. For the individuals with more than one library, we confirmed in all cases that the libraries were from the same individuals. Another measure of replication also derives from the fact that the ancestry distributions in individuals from the same periods tended to be very similar. As a result of this, key findings in this study are not dependent on single samples. |
| Randomization | Historical studies are retrospective rather than prospective -- and the actual trajectory of human history has occurred only once -- so randomization of the data into independent processes is not possible. The mansucript discusses a caveat about possible biases due to non-random sampling. |
| Blinding | Co-analysis of the genetic and archaeological data was central to the study, so we could not be blind to the sample identity. |

# Reporting for specific materials, systems and methods

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

## Materials & experimental systems

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

## Methods

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

# Palaeontology and Archaeology

| | |
|---|---|
| Specimen provenance | We describe the provenance of all archaeological specimens in the Supplementary Information Section 2 "Archaeological context information about the newly reported and published individuals from the Rhine-Meuse area." |
| Specimen deposition | The bone and tooth parts that remain after analysis for ancient DNA are under the stewardship of the archaeologists and cultural institutions from which they were sampled. At present, they are either already returned to the sample stewards or they are stored on long-term loan at the ancient DNA laboratories where they were analysed. They can be re-examined upon request to the sample stewards. Researchers who wish to replicate analyses from this study or gather new data on the libraries generated for this study are welcome to make a request for aliquots of those libraries to corresponding author David Reich who will fulfill all reasonable requests |
| Dating methods | We obtained 12 new accelerator mass spectrometry-based dates on bone, using the standardized methods described at the service laboratories. |

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

| | |
|---|---|
| Ethics oversight | All human skeletons analysed in this study were sampled with written permission of the stewards of the skeletons and every individual is represented by at least one co-author. Researchers who wish to obtain further information about specific individuals should write to the corresponding authors and/or the authors. The individuals studied here were all analyzed with permission from local authorities in each location from which they came. Every sample is represented by stewards, such as archaeologists or museum curators, who are either authors or thanked in the Acknowledgments. |

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

# Plants

| | |
|---|---|
| Seed stocks | N/A |
| Novel plant genotypes | N/A |
| Authentication | N/A |