

# Population Replacement in Early Neolithic Britain

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**The roles of migration, admixture and acculturation in the European transition to farming have been debated for over 100 years. Genome-wide ancient DNA studies indicate predominantly Aegean ancestry for continental Neolithic farmers, but also variable admixture with local Mesolithic hunter-gatherers<sup>1-11</sup>. Neolithic cultures first appear in Britain ca. 4000 BCE, a millennium after they appear in adjacent areas of continental Europe. The pattern and process of this delayed British Neolithic transition remains unclear<sup>12-14</sup>. We assembled genome-wide data from six Mesolithic and 67 Neolithic individuals found in Britain, dating from 8500-2500 BCE, a dataset that includes 22 newly-reported individuals and the first genomic data from British Mesolithic hunter-gatherers. Our analyses reveal persistent genetic affinities between Mesolithic British and Western European hunter-gatherers. We find overwhelming support for agriculture being introduced to Britain by incoming continental farmers, with small, geographically-structured levels of hunter-gatherer ancestry. Unlike other European Neolithic populations, we detect no resurgence of hunter-gatherer ancestry at any time during the Neolithic in Britain. Genetic affinities between British and Iberian Neolithic populations suggest that British Neolithic people were mostly descended from Aegean farmers who followed the Mediterranean route of dispersal and likely entered Britain from northwestern mainland Europe. We also demonstrate variation in pigmentation levels coexisted in Europe by ca. 6000 BCE.**

The transition to farming marks one of the most important ecological shifts in human evolution. The processes by which this transition occurred have been a matter of intense debate for over a century<sup>12-14</sup>, although across continental Europe ancient DNA studies indicate a predominant role for expanding Neolithic farmer populations of mostly Aegean ancestry (Aegean Neolithic Farmers; ANF)<sup>1-11</sup>. Aegean farmer-derived populations dispersed throughout Europe via two major routes; one along the Mediterranean and the other through Central and into Northern Europe<sup>8</sup>. Both dispersing populations introgressed repeatedly with local Mesolithic foragers, which gradually increased their proportion of European Mesolithic ancestry<sup>4, 10-12</sup>.

The nature of the Neolithic transition in Britain remains unclear because of the millennium-long delay in its appearance after the establishment of farming in adjacent regions of continental Europe<sup>13-15</sup>, and the lack of genome-wide data from British Mesolithic hunter-gatherers. Whilst there is universal agreement amongst archaeologists that there was a dramatic change in material culture in Britain around 4000 BCE, there are divergent views regarding the extent to which this change was influenced by cultural or demographic processes<sup>13-15</sup>. The British Isles lie farthest from the Aegean origin<sup>1-11</sup> of the migrating farmers that influenced the development of the Neolithic across Europe, were geographically isolated from continental Europe by large bodies of water, and had maritime climates which differ from the majority of mainland Europe; all factors which may alter the nature of the adoption of farming. The relationship between British and continental European Mesolithic populations is also of interest, as Britain geographically abuts two genetically-distinct but contemporaneous populations: Western European and Scandinavian (WHGs & SHGs) Mesolithic hunter-gatherers, and could have potentially harboured ancestry from earlier (~19,000 to 15,000 BCE) Magdalenian Palaeolithic hunter-gatherer populations<sup>16-18</sup>.

Here, we report the first whole genome data from six Mesolithic (including ‘Cheddar Man’ from Gough’s Cave, Somerset) and 16 Neolithic British individuals, and combine it with new and reported data from 51 previously published Neolithic British individuals<sup>9</sup> to characterise the Mesolithic to Neolithic transition in Britain (Figure 1, Supplementary Figure S16). The dates of our Mesolithic samples range from 8750-8459 cal. BCE (Early Mesolithic Aveline’s Hole, Somerset, England) to 4256-3803 cal. BCE (Late Mesolithic Cnoc Coig, Oronsay, western Scotland). Our Neolithic samples range from 3951-3780 cal. BCE (Early Neolithic McArthur Cave, western Scotland) to 2570-2347 cal. BCE (Late Neolithic Isbister, Orkney, Scotland). We combined data generated in two different ways. For 35 individuals, we generated new whole genome shotgun sequencing data, including the first full genomes from the British Mesolithic (at 2.3x) and Neolithic (at 10.7x). For all individuals, we enriched next generation sequencing libraries for approximately 1.24 million single nucleotide polymorphisms (SNPs) (median coverage 0.88x). When available we merged data obtained from both methods and chose the most likely base to represent the allele at each locus (see Material and Methods). These were combined with ancient genomic data from 67 previously reported individuals<sup>1-4, 6-9, 11, 16-22</sup> (see Supplementary Table S1) and modern genomic data from diverse global populations<sup>23</sup>.

All British Mesolithic individuals cluster with Western and Scandinavian hunter-gatherers in a principal components analysis (Figure 2). By contrast, all directly-dated individuals who post-date 4000 BCE, and undated individuals associated with Neolithic monuments, cluster tightly near Iberian and Central European Middle Neolithic individuals. By examining the degree of allele sharing of British Mesolithic individuals to various European hunter-gatherer individuals/groups (SHG, EHG and El Mirón, see Supplementary Figures S1-S4), we were able to attribute them confidently to the WHG group. Comparison of British Mesolithic individuals to different Mesolithic WHGs (Berry au Bac - France, Ranchot88 - France, Loschbour - Luxembourg, La Braña - Spain, KO1 - Hungary; Supplementary Figures S5-S6, S11-S14) indicates that all most closely resemble Loschbour. When we compared the remaining British Mesolithic genomes to Loschbour and Cheddar Man (our highest-coverage British Mesolithic sample; ~2.3X), we found no significant excess of shared drift for either individual, indicating that Loschbour, Ranchot and the British Mesolithic samples do not form separate clusters (Supplementary Figure S7).

To investigate the proportions of Aegean farmer-related ancestry in the British samples we modelled them as mixtures of ANFs and European WHGs using the *qpAdm* method, which studies ensembles of  $f_4$  statistics (Figure 3, Supplementary Figure S8)<sup>24</sup>. The genomes of all British Mesolithic individuals can be explained almost entirely by WHG ancestry, the remainder (<7.3%) likely stemming from a poorly matching portions of the genome, that – at least for Cheddar Man – can be seen also in our Chromopainter analysis (see Figure 5). Most of the ancestry in all British Neolithic individuals could be attributed to ANFs (>56%, ~74% on average), indicating a substantial demographic shift with the transition to farming. To investigate the proximate source of ANF ancestry in British Neolithic individuals, we examined affinities with Early Neolithic individuals from Iberia and Central Europe. We compare Early over Middle Neolithic individuals as the latter are contemporary with the British Early Neolithic, making them an unlikely direct source. For all British Neolithic individuals considered we inferred more shared drift with Early Neolithic Iberians (Figure 4A,

Supplementary Figure S9). However, these  $f_4$  statistic-based inferences may be sensitive to levels of WHG admixture, such that the similarity in WHG admixture proportions in Early Neolithic Iberian and British samples, but lower estimates in Central European Early Neolithic individuals, is driving the inference of an Iberian rather than Central European source for Early British farmers. To examine this possibility in more detail we performed a more powerful haplotype-based analysis.

Using a chromosome painting approach<sup>25</sup> we obtained patterns of haplotype matching between our high coverage British Neolithic sample and a global modern reference panel (Supplementary Materials Section 7). We observed similar patterns of donor haplotype matching in the British Neolithic genome to those inferred for other high coverage Neolithic genomes from Ireland and Iberia. These were more similar than the same profiles obtained for high coverage Neolithic genomes from Central Europe (Figure 5). Inferred ancestry coefficients (see methods) further support this connection between the British, Irish and Iberian Neolithic<sup>3</sup> and are consistent with the same ancestral populations bringing the Neolithic to Britain and Ireland (Figure 5). Additional modelling using global modern populations<sup>26</sup> suggests this population is best represented today by ancestry components found in French and Spanish peoples (Figure 5c).

In order to test for a potential second ANF ancestry stream from Central Europe, we explicitly modeled WHG and Early Neolithic populations in *qpGraph* (see Supplementary Fig. S23 and Supplementary Table S10). The results suggest that the limited Central European Neolithic admixture we find in British Neolithic populations is regionally structured, with populations from England showing the highest levels of admixture, followed by populations from Scotland. Neolithic farmers from Wales show no Central European admixture. However, we caution that the model fits are poor and the results therefore potentially inconclusive.

We inferred some significant geographic structure in WHG admixture proportions among the British Early Neolithic individuals (see Supplementary Table S4 for statistical comparison of inferred WHG admixture proportions); those from Wales retain the lowest levels of WHG admixture, followed by those from South-West and Central England. Neolithic individuals from South East England and Scotland show significantly higher WHG admixture proportions. These proportions remain stable from the Early into the Middle/Late Neolithic. To infer levels of WHG introgression occurring between Iberian Early Neolithic populations and early British farmers, we estimated admixture proportions using *qpAdm*<sup>24</sup>. We detected little excess (~10%) WHG ancestry beyond that already present in Iberian Early Neolithic individuals, supporting the notion of little or no additional admixture with British hunter-gatherers, particularly in Wales, South West and Central England (Figures 3 and 4B; Supplementary Table S4). This result appears to be slightly at odds with the  $f_4$  results presented in Supplementary Figure S7, which indicate that some British Neolithic samples share genetic affinities with Cheddar Man over Loschbour, although it is difficult to say in these cases whether this is due to genuine substantial admixture with British WHGs or others in northern Europe. We regressed individual WHG ancestry proportions in British Neolithic farmers (shown in Supplementary Figure S8) against latitude and longitude and found a significant positive southwest to northeast cline (Supplementary Figure S15).

We used ALDER<sup>27</sup> on pairs of Early Neolithic regional samples to estimate the timing of WHG/ANF admixture events and to further explore WHG introgression in Britain (Supplementary Table S3). Only Early Neolithic farmers from western Scotland show evidence for WHG introgression within 10 generations; two individuals from Raschoille Cave had estimated introgression events occurring  $4.0 \pm 3.4$  generations before they lived, which is sufficiently recent in their past that it likely occurred in Britain. The elevated levels of WHG ancestry we see in Neolithic samples from South East England are older, and therefore probably a result of farmer-forager interactions in mainland Europe. Chronological modelling (using OxCal 4.3<sup>28</sup>) of available direct Early Neolithic radiocarbon data from individuals showing ANF ancestry suggests that continental farmers arrive in Britain by 3963-3720 cal. BCE (95% confidence), 482 years after to 16 years before (95% confidence) the death of our latest Mesolithic individual showing no ANF ancestry (Supplementary Materials Section 6). Our model suggests that continental farmers arrive marginally earlier in the west (although see the discussion in the Supplementary Materials), and rapidly disperse into other regions of Britain (including the Orkney Islands). The latest regional appearance of ANF ancestry is in Central England and occurs 58 to 380 years (95% confidence) after it first appears in Britain.

To explore the variation in pigmentation of European populations, we predicted pigmentation in higher-coverage Mesolithic and Neolithic Europeans using Hlrisplex-S<sup>29</sup>. Cheddar Man mostly likely had blue/green eyes, dark brown possibly black hair, and dark or dark to black skin, while our highest coverage early Neolithic individual had brown eyes, black possibly dark brown hair, and intermediate to dark skin (see Supplementary Materials Section 3). Together with the pigmentation prediction outcomes we obtained for Loschbour and La Braña, these results imply that different pigmentation levels coexisted in Europe by around ca.6000 BCE.

The six British Mesolithic genomes examined here are typical of WHGs, indicating that this population spread to the furthest northwestern point of early Holocene Europe after moving from southeastern Europe, or further east, from approximately 12,000 BCE<sup>17</sup>. This genetic similarity among British and European Mesolithic individuals spans a period in Britain (ca. 8,500-4,000 BCE) that includes the cultural transition to the Late Mesolithic and the separation of Britain from continental Europe. Our analyses indicate that the appearance of Neolithic practices and domesticates in Britain ca. 4,000 BCE was mediated overwhelmingly by immigration of farmers from continental Europe<sup>13-14</sup>, and strongly reject the hypothesised adoption of farming by indigenous hunter-gatherers as the main process<sup>15</sup>. British farmers were substantially descended from Iberian Neolithic-related populations whose ancestors had expanded along a Mediterranean route<sup>3,8</sup>, although with a minority portion of their ancestry from populations who took the Danubian route<sup>9</sup>. The affinities we find between Neolithic individuals from the British Isles and modern individuals from France are consistent with populations sharing ancestry with Neolithic groups in Iberia moving into northern France via the Atlantic seaboard and/or southern France, mixing to a limited degree with Neolithic populations from Central Europe before travelling across the Channel<sup>14, 15, 30</sup>.

One explanation for the British Neolithic cline in WHG ancestry is that a single population moved across Britain from a western entrypoint, and progressively admixed with local hunter-gatherers. This scenario is consistent with the western distribution of megalithic cultures along the Atlantic seaboard<sup>31</sup>, and is supported by the radiocarbon evidence suggesting a marginally earlier date for the arrival of ANF ancestry in the west of Britain. However, the lack of evidence for substantive WHG introgression into British Neolithic populations – outside of western Scotland – favours this cline reflecting multiple source populations with variable proportions of WHG admixture having entered different parts of Britain. This interpretation is consistent with archaeological evidence for regional British Neolithic cultures showing links to varied parts of mainland Europe<sup>14</sup> and our qpGraph analysis showing geographically-structured Neolithic Central European admixture. Overall, the regional variation in ancestry of British Neolithic populations likely reflects both differing degrees of admixture between farmers and local foragers (e.g. western Scotland), and multiple continental source populations carrying variable WHG and Neolithic Central European ancestry.

Evidence for only low levels of WHG introgression among British Early Neolithic people is striking given the extensive and complex admixture processes inferred for continental Neolithic populations<sup>4, 10-12, 32-33</sup>. Low levels of admixture between these two groups on the wave front of farming advance in continental Europe have been attributed to the maintenance of cultural and reproductive boundaries for several centuries after initial contact, before more extensively mixing<sup>32</sup>. Similarly, isotopic and genetic data from the west coast of Scotland suggest the potential coexistence of genetically distinct hunter-fisher-gatherers and farmers, albeit for a maximum of a few centuries<sup>34</sup>. However, there is no evidence for a resurgence of WHG ancestry in the British Neolithic, consistent with limited evidence for Mesolithic cultural artefacts in Britain beyond 4000 BC<sup>14</sup>, and with a major dietary shift from marine to terrestrial resources at this time (see Supplementary Materials Section 5)<sup>35</sup>.

In contrast to other European regions, the transition to farming in Britain occurred with little introgression from resident foragers – either during initial colonization, or throughout the Neolithic. This may reflect low Late Mesolithic population density in Britain and/or an introduction of farming by populations who had mastered the technologies needed to thrive in northern and western Europe during the previous two millennia<sup>13</sup>.

## *Materials and Methods*

*Ancient DNA Extraction and Sequencing*—DNA extractions and library preparations for all samples with newly reported data were conducted in a dedicated ancient DNA laboratory (NHM, London). We used approximately 25mg of finely drilled bone powder and followed the DNA extraction protocol described in Dabney *et al.*<sup>36</sup> but replaced the Zymo-Spin V column binding apparatus with a high pure extender assembly from the High Pure Viral Nucleic Acid Large Volume Kit (Roche). Library preparations followed the partial uracil–DNA–glycosylase treatment described in Rohland *et al.*<sup>37</sup> and a modified version of the Meyer & Kircher<sup>38</sup> protocol. Library modifications: the initial DNA fragmentation step was not required; all clean-up steps used MinElute PCR purification kits (Qiagen). The index PCR step included

double indexing<sup>39</sup>, the polymerase AmpliTaq Gold and the addition of 0.4mg/mL BSA. The index PCR was set for 20 cycles with three PCR reactions conducted per library. Libraries were screened for DNA preservation on an Illumina NextSeq platform, with paired-ends reads. Promising libraries were further enriched in two ways, one at the NHM using in-solution hybridisation capture enrichment kits (Mybait-3) from MYcroarray. The baits were designed to cover ca. 20K SNP's (5,139 functional and 15,002 neutral SNP's) at 4x tiling. The capture protocol followed the manufacturer's instructions in the Mybait manual v3. Captured libraries were sequenced on an Illumina NextSeq platform (NHM) using paired-ends reads. Newly reported data from 36 of these libraries was also obtained at the dedicated ancient DNA lab in Harvard Medical School by enriching in solution for approximately 1.24 million targeted SNPs. We sequenced these libraries on an Illumina NextSeq500 instrument, iteratively sequencing more until we estimated that the additional number of targeted SNPs hit per newly generated sequence was less than 1 per 100.

**Bioinformatics**—All sequence reads underwent adapter and low-quality base trimming, and overlapping reads pairs were collapsed with AdapterRemoval<sup>40</sup>. Non-collapsed reads and those below 30bp were discarded, and the remaining aligned against the hs37d5 human reference genome with BWA<sup>41</sup>. Mapped reads with MAPQ at least 30 were merged per individual and re-aligned around InDels with GATK<sup>42</sup>. Resulting BAM files were split by flowcell and lane, and empirical ATLAS<sup>43</sup> post mortem damage patterns estimated per individual per lane for lanes with at least 5.5 million reads, otherwise per individual per flowcell. ATLAS BQSR base quality score recalibration tables were generated per lane for lanes with at least 5.5 million reads, otherwise per flowcell. We generated recalibrated BAM files per individual with ATLAS recalBAM, and used those to estimate mitochondrial contamination and determine Mitochondrial and Y-chromosome haplogroups with ContamMix<sup>44</sup>, Yleaf<sup>45</sup> and Phy-Mer<sup>46</sup>. We considered Mitochondrial contamination to be tolerable if 0.98 was included in the confidence intervals. Haploid genotypes were called with ATLAS allelePresence with theta fixed at 0.001, determining the most likely base at a position. Heterozygosity estimates shown in Supplementary Fig. S10 were computed with ATLAS estimate Theta and default window size of 1Mbp, excluding windows that overlap with telo- or centromeres.

**PCA**—Principal component analysis was performed with LASER<sup>47</sup> following the approach described previously<sup>6</sup>. After generating a reference space of modern Western Eurasian individuals<sup>7</sup>, we projected the BAM files of ancient reference individuals (see Supplementary Table S1 for references) and the British individuals presented here into the reference space via Procrustes analysis implemented in LASER.

***f*-statistics**—The various flavours of *f*-statistics presented here, *i.e.* outgroup  $f_3$ ,  $f_4$ ,  $qpAdm$ , and  $qpGraph$  were computed with  $qpPop$ ,  $qpDstat$  in  $f_4$  mode,  $qpAdm$ , and  $qpGraph$  from the ADMIXTOOLS<sup>23</sup> package with default parameters on the positions defined by the *HOIII* set of SNPs<sup>7</sup>. Ancient individuals analysed here are listed in Supplementary Table S1, including the explanation of all population labels used (WHG, SHG, etc.). Modern reference individuals were first published in Mallick *et al.*<sup>22</sup>. All  $qpAdm$  runs used the set of outgroups Han, Karitiana, Mbuti, Onge, Papuan, Mota, Ust'-Ishim, MA1, El Mirón, GoyetQ116-1.

**ALDER**—We used ALDER<sup>27</sup> to estimate admixture event dates between WHG and ANF. All experiments we ran are listed in Supplementary Table S3, which consisted of the pairs or groups of individuals specified in the first column and WHG and ANF (individuals constituting WHG and ANF are given in Supplementary Table S1).

**Chronological Modelling** - We used OxCal 4.3<sup>28</sup> to produce chronological models of the arrival and spread of ANF ancestry into Britain. We only used Early Neolithic (4000-3500 BC) radiocarbon dates that had been obtained from material or individuals where there was palaeogenetic data indicating ANF ancestry. We divided these samples into five regional populations: Western Britain, Central England, Eastern England, Western Scotland and the Orkney Isles. Dates associated with each region were grouped as Phases (Supplementary Figure S19). We used the Boundary function to produce probability distributions for the arrival of ANF ancestry in Britain as a whole, and for each region. We used the Difference function to produce probability distributions for the time between the death of the latest individual with wholly WHG ancestry and the arrival of populations with ANF ancestry, as well as between the arrival of ANF ancestry in Britain as a whole and the different regions of Britain. Further information is provided in Supplementary Materials Section 6.

**Haplotype-based analyses** - We used CHROMOPAINTER<sup>25</sup> to summarize DNA patterns in our ancient individuals, including other high coverage publicly available ancient genomes from relevant cultures and time periods, to infer the proportion of DNA for which ancient individuals most closely matched to individuals from a global panel of modern groups<sup>48-50</sup>. This panel included many population samples from across West Eurasia, as well as 35 labelled groups from within the British Isles. We generated matching profiles when considering SNPs independently (allele sharing) and also when considering the correlations between neighbouring SNPs (haplotype sharing). To do so we first merged high quality diploid calls for our selected high coverage ancient genomes and jointly phased the resultant dataset of 159,287 SNPs using SHAPEITv2<sup>51</sup>. We performed additional mixture modelling using our allele and haplotype sharing profiles implemented in SOURCEFIND<sup>26</sup> to form target groups as mixtures of the DNA sharing profiles of other included groups. We performed two sets of analyses: i) using all modern groups (or a subset of) to model the ancestry of ancient individuals, and ii) using different sets of ancient individuals, plus the modern Yoruba and Han, to model the ancestry of modern world-wide groups. Further details are provided in Supplementary Materials Section 7.

## Figure legends

**Figure 1:** *Map of sample locations.* Geographical locations of British samples analysed here. Numbers indicate the number of samples from a given location.

**Figure 2:** *PCA of modern and ancient West-Eurasians.* British and additional ancient samples are projected onto the reference space computed on present-day West-Eurasian



populations. See Material and Methods for computational details and Supplementary Table 1 for information on the samples.

*Abbreviations:* European (Eur.), Pleistocene (Plei.), hunter-gatherer (H.-G.), British Isle (Brit.-I.), Middle Neolithic (M.-Neo.)

**Figure 3:** *WHG and ANF ancestry components of British and Central European Neolithic populations.* The relative WHG and ANF ancestry in Early and Middle Neolithic British and continental European populations quantified by *qpAdm*. Percentages indicate error estimates. See Material and Methods for computational details and Supplementary Table 1 for the lists of samples grouped into WHG and the different Neolithic populations.

*Abbreviations:* Neolithic (Neo.), Early-, Middle-, and Late Neolithic (EN, MN, LN), South East (SouthE), South West (SouthW)

**Figure 4:** **(A)** *Affinities of British and continental Neolithic populations.* We compute  $f_4$  symmetry testing statistics for different British EN, MN, and LN and continental MN populations comparing shared drift with Central European EN and Iberian EN populations. A positive Z-score above 2 corresponds to a significant affinity to the Iberian EN over Central European EN population. **(B)** *Quantifying excess WHG ancestry in British EN compared to the Iberian EN population.* We compute *qpAdm* estimates of WHG and Anatolian and Iberian ANF populations in EN samples from Wales, England, and Scotland. See Supplementary Table 1 for the lists of samples grouped into WHG and the different Neolithic populations.

**Figure 5:** **(A)** Hierarchical clustering of total-variation-distance (TVD) between CHROMOPAINTER inferred haplotype sharing profiles of seven high coverage Neolithic individuals when compared to a global modern reference panel. **(B)** Inferred ancestry proportions (SOURCEFIND inferred mixing coefficients) of high coverage ancient genomes, coloured as per the legend and outer pie ring colour, relative to a panel of ancient genomes, plus the modern day Yoruba and Han (as given in the legend at top). **(C)** Inferred ancestry proportions of five high coverage Neolithic individuals (triangles coloured as in (B)) relative to a global modern reference panel. The size of the blue circle provides the size of the inferred contribution, with all possible modern contributors shown with a grey dot.

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## Methods

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