

Genetic Analysis of Eynsham Burial 325

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A left temporal bone from the Middle Neolithic Eynsham Burial 325 was sent to the Francis Crick Institute's Ancient Genomics Laboratory for DNA analysis to investigate genetic sex and ancestry. The temporal bone was sampled and analysed as part of the Wellcome Trust-funded 'aGB: A Thousand Ancient Genomes from Great Britain' project.

Methods

Highly sensitive ancient DNA techniques were applied for the preparation of sequencing libraries from bone powder in the specialised ancient DNA cleanroom facility at The Francis Crick Institute. The Eynsham left temporal bone was drilled in the inferior aspect through the jugular fossa to reach the cochlea and remove 50-100 milligrams of bone powder using a modified version of Sirak et al. (2017). The left malleus was discovered in the auditory meatus during drilling. The auditory ossicles are comparable and sometimes superior sources of DNA than the cochlea and so laboratory methods were applied initially to the left malleus Sirak et al. (2020).

DNA extraction and single stranded library preparation was performed on automated Agilent Bravo Workstations following the protocols by Rohland et al. (2018) and Gansauge et al. (2020). The libraries were amplified with unique double index combinations and screened for endogenous DNA on the HiSeq4000 platform. Downstream analysis was carried out through the nf-core/eager v.2.3 pipeline (Yates et al. 2020). Mainly, a 35bp cutoff was set before alignment against the hs37d5 assembly in BWA (Li and Durbin 2009). Duplicates were removed via Dedup and DNA damage was estimated thanks to DamageProfiler.

Preservation metrics for the Eynsham malleus were excellent, with 62% of sequences retrieved aligning to the human genome (Table 1). The human sequences showed characteristic patterns of DNA damage which authenticated them as genuinely ancient. Several contamination metrics found little evidence for recent human contamination (Renaud et al. 2015, Peyrégne and Peter 2020, Green et al. 2009). The DNA library from the Eynsham malleus was submitted for deeper production shotgun sequencing on an Illumina NovaSeq instrument, following a gel-excision protocol to increase the proportion of sequences above 35 bp (Gansauge et al. 2020). Sequences were mapped with vg (Garrison et al. 2018) against GRCh38. This deeper sequencing produced genome-wide data at 0.58x coverage (Table 1).

Results and Discussion

Estimates of genetic (karyotypic) sex in both the screening and production data using (Skoglund et al. 2013) indicated the skeleton was that of a female (two X chromosomes). This result is in agreement with the osteological assessment of the remains.

The development of Neolithic across Europe was usually accompanied by the arrival of groups of people carrying ancestry from populations of Neolithic Anatolia, known as 'Early European Farmer (EEF)' ancestry (Skoglund et al. 2014, Lazaridis et al. 2016, Skoglund et

al. 2012, Skoglund and Mathieson 2018). Dispersals of people carrying EEF ancestry over two millennia largely drove the development of the Neolithic farming practices across Europe. Groups carrying EEF ancestry mixed with local populations who predominantly carried genetic ancestry commonly known as 'Western-European Hunter-Gatherer (WHG)'. Admixture between incoming and local groups and their descendants was regionally variable, with some groups mixing gradually and others in episodic pulses, sometimes hundreds-to-thousands of years after groups carrying EEF ancestry had arrived.

Groups carrying EEF ancestry first appear in Britain c.4000 BC, which is broadly coincident with the development of Neolithic practices (Brace et al. 2019, Olalde et al. 2018). It is likely groups of people carrying EEF ancestry moved into Britain from adjacent regions of continental Europe, present-day northern France and Belgium. While there is direct evidence for admixture between local groups descended from the Mesolithic population of Britain and incoming Neolithic farmers in western Scotland, the Mesolithic population of Britain have only a small genetic legacy in Neolithic Britain over the long term. Unlike other areas of Neolithic Europe, there is no evidence for a later resurgence or pulse of local WHG ancestry in Neolithic Britain (Patterson et al. 2022, Brace et al. 2019). Analysis of shared genetic drift between different populations of Neolithic Europe suggest that Neolithic populations of Britain and Ireland remained relatively similar to one another, but became relatively differentiated from Neolithic groups in continental Europe through time (Ariano et al. 2022). This suggests that after an intense period of migration into Britain and Ireland in the first half of the 5th Millennium BC, cross-Channel movements of people into Britain and Ireland substantially attenuated and their populations became fairly insular. After 2450 BC, a novel genetic ancestry known as 'Western Steppe Herder (WSH)' ancestry, which ultimately derived from people who lived on the Pontic-Caspian steppe from c.3000 BC, predominates amongst people in Britain. Migrations of people carrying WSH ancestry appear to coincide with the development of Bell Beaker cultures, although there is some argument as to whether people carrying WSH ancestry could have arrived earlier (Armit and Reich 2021, Carlin 2020).

We projected the genetic data from the Eynsham burial onto a Principal Component Analysis (PCA) defined by genetic ancestries typical of present-day Western Eurasia alongside relevant ancient populations ((Lazaridis et al. 2016); Figure 1). The PCA distributed samples in space based on the extent to which they share genetic variants. The Eynsham burial clustered with the Neolithic populations of Britain and Ireland. We used qpAdm ((Haak et al. 2015)) to investigate what distal ancestries could explain the Eynsham burial ancestry. We initially attempted a three-way model including WHG, EEF and WSH ancestries, which indicated that the Eynsham burial did not harbour any WSH ancestry (Table 2). A two-way model using just EEF and WHG ancestry produced a good fit with 77% of ancestry of the Eynsham burial explained by EEF ancestry (Table 2; Figure 2). These ancestry proportions are typical of people who inhabited Britain during the Early Neolithic.

These results suggest that the Eynsham burial was wholly descended from Early Neolithic populations carrying EEF ancestry who arrived in Britain from c.4000 BC. The similarity in ancestry proportions between the Eynsham burial and the inhabitants of Early Neolithic Britain suggests that the Eynsham burial derived little, if any, ancestry from the Mesolithic population of Britain and therefore provides no evidence for any resurgence of local Mesolithic-derived ancestry in the Middle Neolithic. This is all consistent with the broader

population history of Neolithic Britain involving initial migrations and minimal early admixture followed by relative isolation from continental Europe and no later resurgence of ancestry derived from local Mesolithic groups. The absence of WSH ancestry from the Eynsham burial is unsurprising given their radiocarbon date predates the earliest evidence for groups carrying WSH ancestry moving off the Pontic-Caspian steppe (Allentoft et al. 2022).

Lab No	Element	Site	Sk No.	Sequencing Run	No. Sequences Generated	No. Human Sequences	% Endogenous	% 5pCtoT (Damage)	X Contam.	% Contam.	Sex Assignment	MtDNA Coverage	Nuclear Coverage	MtDNA Haplogroup
C10738	L. Malleus	Eynsham	Sk 325	Screening	977134	608047	62.23	34.04	0	-2.15	XX	0.92	0.001	-
				Production	104679200	37822368	36.13	33.33	-0.14	1.66	XX	51.53	0.58	H,H+152,H+195

Table 1: Summary metrics for screening and production sequencing of the *L. Malleus* from Eynsham Sk 325. The Mitochondrial haplogroup was called using HaploGrep2 (Weissensteiner et al. 2016).

Sources	Outgroups	Balkan_N (EEF)	SE	WHG	SE	Steppe (WSH)	SE	p-value
WHG Balkan_N Steppe	subSaharan_Africa IronGates_Mesolithic Anatolia_Neolithic Afanasiovo	0.78	0.02	0.24	0.02	-0.01	0.02	0.39
WHG Balkan_N	subSaharan_Africa IronGates_Mesolithic Anatolia_Neolithic Afanasiovo	0.77	0.02	0.23	0.02	-	-	0.74

Table 2: Results of qpAdm modelling of distal ancestries for Eynsham burial 325 with standard errors (SE; Haak et al. 2015 using 303,203 autosomal SNPs overlapping with '1240k panel', called with pileupCaller "--singleStrandMode" (<https://github.com/stschiff/sequenceTools>)).

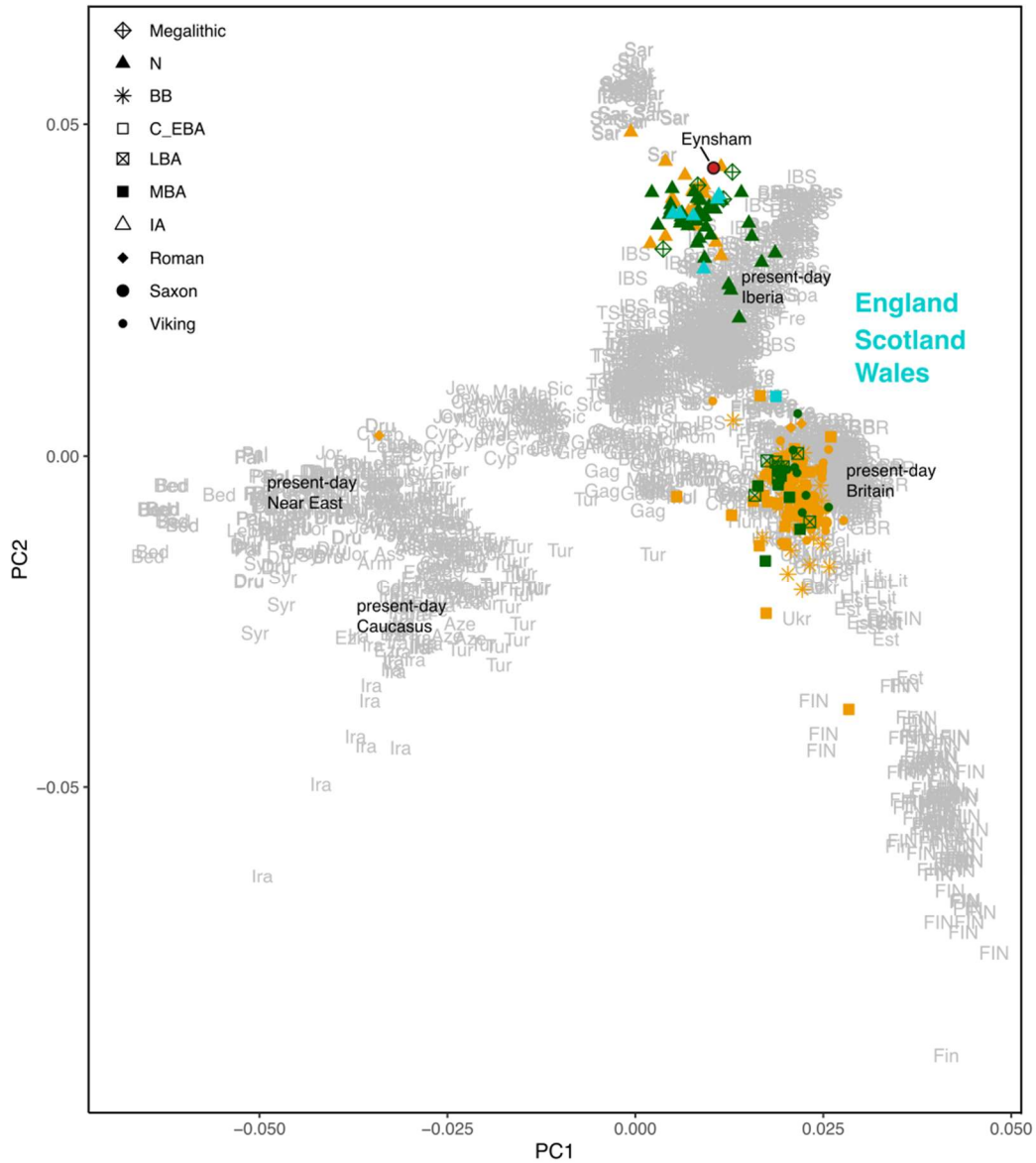


Figure 1: Principal Component Analysis (PCA) defined by present-day populations with ancestries from western Eurasia from the Human Origins reference panel with projected genetic data from Eynsham burial 325 and other relevant ancient individuals from Britain (Lazaridis et al. 2016, Brace et al. 2019, Olalde et al. 2018, Patterson et al. 2022, Martiniano et al. 2016, Schiffels et al. 2016, Margaryan et al. 2020). 250,564 autosomal SNPs from the Eynsham burial overlapped with the Human Origins reference panel. Ancient genomes from Britain are coloured, with yellow for present-day England, green for Scotland and blue for Wales. The Eynsham burial plots with other Neolithic burials from present-day England.

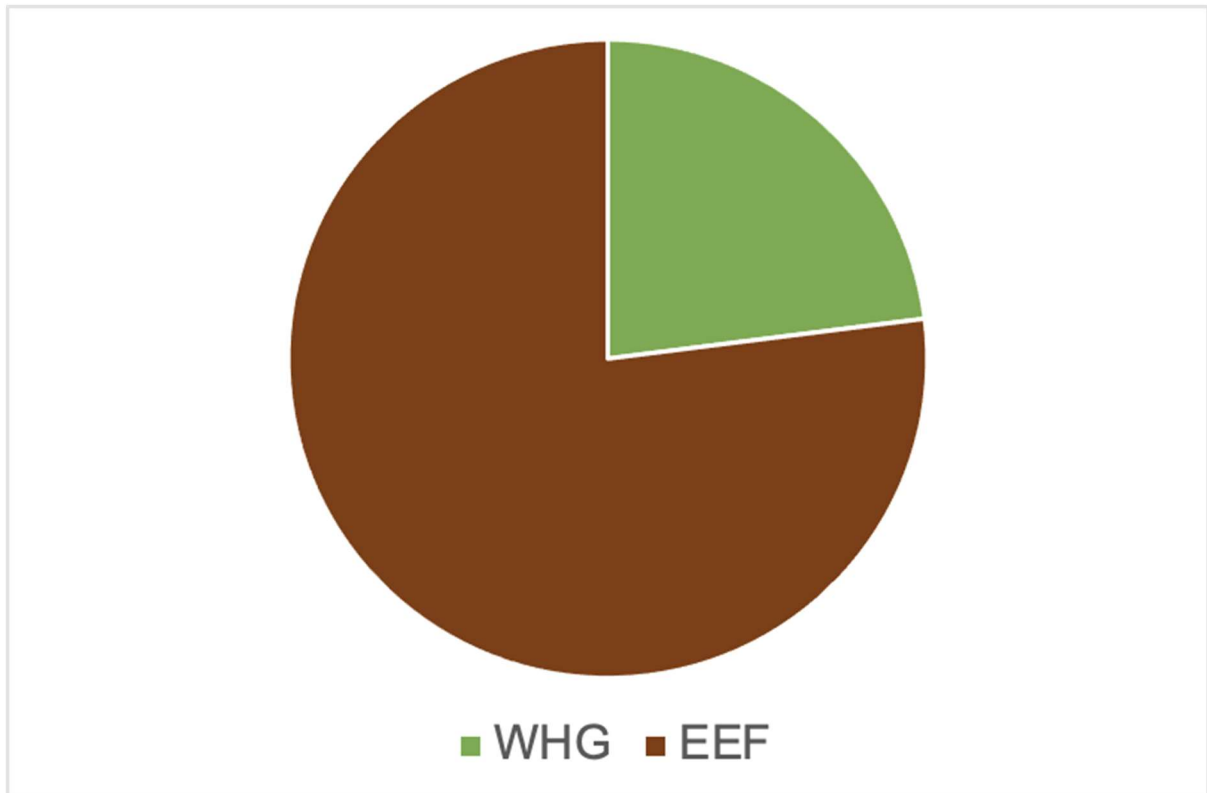


Figure 2: Proportions of the genetic ancestry of the Eynsham burial 325 derived from Western European Hunter-Gatherers (WHG) and Early European Farmers (EEF), as defined by qpAdm modelling (Haak et al. 2015). 303,203 autosomal SNPs overlapping with the ‘1240k panel’ were called with pileupCaller “--singleStrandMode” (<https://github.com/stschiff/sequenceTools>).

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