5. ENVIRONMENTAL ANALYSIS

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Environmental analysis was focussed on the human and animal remains retrieved during the excavation works. Radiocarbon dates were retrieved from three separate samples of human remains (Individuals 'A', 'B', and 'E'), with the dates derived from samples of their ribs. A further radiocarbon date was retrieved from a sheep radius found in midden deposit C026. Stable isotope analysis (to assess diet) was also completed for samples from Individuals 'A', 'B', and 'E', with analysis of a tooth, rib bone, and femur of Individuals 'A' and 'B', and tooth and humerus for Individual 'E'. During excavation of Individuals 'A' and 'B' soil samples were taken from the anterior aspect of the sacrum for palaeoparasitological analysis, and a bone and tooth sample from Individuals 'A' and 'B' were submitted for aDNA analysis at the Francis Crick Institute to form part of the 1,000 Ancient British Genomes project.

5.1 Radiocarbon dates

Samples were retrieved from rib bones of Individuals 'A', 'B', and 'E' and submitted for radiocarbon dating. A further sample (sheep radius) was retrieved from midden material C026 and submitted for radiocarbon dating. The samples were processed by SUERC and were calibrated to the calendar timescale using the Oxford Radiocarbon Accelerator Unit calibration program OxCal 4 (Bronk Ramsey 2009). The provided date ranges were calibrated using the IntCal20 atmospheric calibration curve (Reimer et al 2020). The radiocarbon dating results are provided in Table 2.

Radiocarbon dates for Individuals 'A' and 'B' (C028 & C029) display a very similar date range, which supports the theory that the two individuals were buried at the same time, and evinced in the archaeological results. These two individuals were probably interred around the mid-15th century, and earlier than Individual 'E' (C054) located in Section 6 to the north. The date range for Individual 'E' is less precise than Individuals 'A' and 'B', with a possible interment ranging from the early-16th to the mid-17th century. This may indicate a progression of burial plots expanding to the north from the 15th century onwards, however, it must be borne in mind that the remains of Individual 'E' were heavily disturbed by the construction of the 'rampart', which may have impacted the date ranges retrieved. Dating for the sheep radius from C026 provides a date range in the first half of the 15th century, and likely prior to the interment of Individuals 'A' and 'B'. This also suggests that the midden material and wall C023 pre-dated these burials.

Table 2 Radiocarbon dates from osteological samples retrieved in Section 1 and Section 6

Sample Number	Context Number	Individual	Laboratory Code	Uncalibrated Date BP	Calibrated Date (AD) at 95.4% Probability	Percentage Likelihood (95.4% probability)
3	028	A	SUERC–100093 (GU58635)	416 +/-29	1429–1513	85.5%
					1591–1620	10.0%
5	029	B	SUERC100094 (GU58637)	428 +/-29	1425–1500	91.6%
					1600–1615	3.9%
7	054	E	SUERC100095 (GU58639)	278 +/-29	1508–1594	52.5%
					1617–1666	40.0%
					1784–1795	3.0%
21	026	N/A	SUERC–100906 (GU59012)	513 +/-24	1400–1443	95.4%

5.2 Stable isotope analysis

Stable isotope analysis was conducted on samples retrieved from Individuals 'A', 'B', and 'E'. The analysis included $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. A sample of femur or humerus fragments (to assess long term diet) and rib fragments (to assess later life diet) were submitted for Individuals 'A', 'B', and 'E'. Tooth samples (to assess early life diet) were submitted for Individuals 'A' and 'B'. The analysis was completed by SUERC, and the results are provided in Table 3.

Stable isotope analyses have been used extensively to infer ancient diet, with often simplistic analysis of the data used to suggest marine based or terrestrial diets (Makerewicz & Sealy 2015); a higher $\delta^{15}N$ ratio coupled with a less negative $\delta^{13}C$ ratio suggesting a marine based diet, and a lower $\delta^{15}N$ ratio coupled with a more negative $\delta^{13}C$ ratio suggesting a terrestrial, plant-based diet with varying diets plotted in between these extremes. On this basis alone, the data for Individuals 'A', 'B', and 'E' would suggest a terrestrial based diet, with Individuals 'A' and 'B' displaying a more meat rich diet than Individual 'E'. More detailed factors should be considered in isotope analyses, as local environmental factors and cultivation practices (such as manuring) can affect levels of $\delta^{15}N$, resulting in a higher ratio than 'baseline' levels would suggest (ibid: 148–9). In comparison to the isotope levels returned for the sheep radius from C026 however, it appears that at a (presumed) local level an herbivorous diet would return a significantly lower $\delta^{15}N$ ratio, and a more negative $\delta^{13}C$ ratio than displayed in Individuals 'A', 'B', and 'E'. Analyses from 24 contemporary burials in Portmahomack (dating from the 12th to 16th centuries) displayed a higher average $\delta^{15}N$ ratio ranging from 12.7‰ to 16.6‰, and less negative $\delta^{13}C$ ratio ranging from -20.4‰ to -17.1‰ indicating a mixed terrestrial and marine diet (Curtis-Summers 2016: D31).

Whilst the isotope analysis of the samples from Jedburgh Abbey Rampart seems to indicate a terrestrial diet, it is far too small a sample size to make broader generalisations regarding diet in the area in the 15th and 16th centuries from these analyses alone. Excavations in the 1980s at Jedburgh Abbey uncovered 41 burials (both lay people and monastic burials), however, stable isotope analyses have not been carried out on those remains to provide a more nuanced understanding of late medieval diet in Jedburgh (Lewis & Ewart 1995). Direct comparisons between the individuals indicate that both Individuals 'A' and 'B' likely had a very similar diet, whilst Individual 'E' probably had a less

Table 3 Stable isotope analysis results

Sample Number	Context Number	Sample Type	d13C ‰	d15N ‰	C/N Molar ‰	d34S ‰	C/S Molar	N/S Molar
1	028	Human right maxillary second incisor	-20.7	10.5	3.4	17.6	494	147
2	028	Human right femur fragment	-20.5	10.5	3.4	16.4	488	145
3	028	Human right rib fragment	-20.5	10.8	3.3	15.2	484	146
4	029	Human left maxillary canine	-20.4	10.5	3.4	16.6	447	131
5	029	Human right rib fragment	-20.3	11.1	3.5	15.6	527	150
6	029	Human left femur fragment	-20.6	10.7	3.4	15.7	489	144
7	054	Human rib fragment	-20.4	10.5	3.4	14.6	521	154
8	054	Human left humerus fragment	-20.6	9.5	3.4	14.3	490	145
21	026	Sheep radius	-21.8	5.8	3.2	–	–	–

meat-rich diet, although still well within the ranges of an omnivorous one. The $\delta^{13}C$ ratios remained consistent for all three individuals into later life, with very marginal fluctuation; slight increases in later life are noted in the $\delta^{15}N$ ratios, particularly Individuals 'B' and 'E', which may indicate an increase in meat consumption in later life.

The $\delta^{34}S$ (sulphur) analysis compares the ratio of 34S:32S in a sample against the equivalent ratio in a known reference standard known as the Vienna-Canyon Diablo Troilite (VCDT). We can then compare this ratio to a map of the available Sulphur δ34S‰ (VCDT) from plants in the UK biosphere. The small sample size also restricts the analysis of the $\delta^{34}S$ results, with all three individuals displaying ratios that fall within the parameters of any UK coastal zone on the British Geological Society Biosphere Isotope Domains. What these results do show however, is that the $\delta^{34}S$ ratios for all three individuals decreased in later life. This may suggest a move inland, but it must be noted that there is no inland sulphur data for the Scottish Borders, or indeed inland Scotland, (BGS 2022) so it is possible that their ratio could also be similar to the local area as well.

5.3 Palaeoparasitology

Analysis of soil samples from the pelvic regions of Individuals 'A' and 'B' was completed by the Ancient Parasites Laboratory of the Department of Archaeology, University of Cambridge to determine if intestinal parasites could be identified in these two individuals. No intestinal parasite eggs were identified within the samples, however, this does not mean that the individuals were not affected by parasites, merely that none were identified within the retrieved samples. Given the small sample size, intestinal parasites cannot be ruled out from the wider community.

5.4 aDNA analysis

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A tooth and an auditory ossicle from both Individuals 'A' and 'B' were sent to the Ancient Genomics Institute at the Francis Crick Institute for DNA analysis. The auditory ossicles were sampled because they preserve human DNA exceptionally well and can be sampled directly without any additional impact to the skeleton (Sirak et al 2020). A tooth from each individual was sampled additionally to assess for the presence of DNA from ancient pathogens. Teeth preserve DNA reasonably well and have a direct blood supply via the pulp cavity which is likely to act as a reservoir of infectious pathogens (Margaryan et al 2018).

The bones and teeth were sampled and processed in the clean room facility of the ancient genomics laboratory at the Francis Crick Institute. The teeth were sampled by drilling 50–100 milligrams of powder from the tooth root with an EV410-230 EMAX Evolution Dentistry drill. The tooth powders and whole ossicles were then lysed with 300ul (<10mg of powder) or 1000ul (>10mg of powder) of lysis buffer (0.5 EDTA pH 8.0, 0.05% Tween-20, 0.25mg/ml Proteinase K) and incubated overnight at 37°C. Lysates were centrifuged for two minutes at maximum speed (13,200 rpm) in a table centrifuge and 140ul of the lysate was transferred into FluidX tubes for automated extraction on an Agilent Bravo Workstation (Dabney et al 2013; Rohland et al 2018). Extracts were turned into single-stranded double-indexed Next Generation Sequencing DNA libraries automatically on an Agilent Bravo Workstation with no treatment to remove uracils (Gansauge et al 2020). The two DNA libraries derived from the auditory ossicles were subject to shallow Next Generation shotgun sequencing on an Illumina HiSeq instrument to assess DNA preservation. Both showed excellent DNA preservation as defined by the endogenous content (proportion of reads aligning with the human genome – see Table 4). DNA sequences in both libraries showed patterns of damage consistent with authentic ancient DNA (genuinely ancient DNA would be expected to show damage rates of around 10% or above). These two libraries were subject to deeper whole genome shotgun sequencing on an Illumina NovaSeq Instrument to produce higher coverage (higher quality) whole genomes.

Both the shallow and deeper sequencing data indicate that Individual 'A' was genetically female (two X-chromosomes) and Individual 'B' was genetically male (one Y-chromosome), in agreement with the osteological assessments of biological sex (Skoglund et al 2013). We were able to use the higher

coverage whole genome data to call the paternal (Y-chromosome haplogroup) and maternal lineage (mitochondrial haplogroup) of Individual 'B' and the maternal lineage of Individual 'A' using Y-Leaf and HaploGrep (Weissensteiner et al 2016; Ralf et al 2018). The two burials had different maternal lineages, suggesting they were not directly related on their maternal line of descent. Two methods of estimating genetic relatedness were applied to the shallow screening data: the pairwise mismatch rate (PMR) (Kennett et al 2017) and TKGWV2 (Fernandes et al 2021). The results from both analyses suggested that the two Jedburgh individuals were unlikely to have been close relatives (third degree or closer - PMR = 0.0332±0.006 with a baseline of 0.04; TGWV2, Halved Relatedness Coefficient (HRC) = -0.0566 when Unrelated < 0.0625, 2nd Degree between 0.0625 and 0.1875, 1st Degree > 0.1875). The number of Single Nucleotide Polymorphisms (SNPs) that could be included in both analyses was low (783 SNPs for PMR, 1697 for TKGWV2), however, and so this result is tentative until further analyses can be performed on the whole genome data. It is not possible to assess whether they were non-biological kin, for example spouses.

Individual 'B"s paternal lineage is within the sub-clade R1b-L21. R1b-L21 lineages were introduced to Britain by migrations from continental Europe around 2500 BC associated with the development of the Beaker phenomenon and are almost ubiquitous amongst men who lived in Britain through the Bronze Age (Olalde et al 2018; Patterson et al 2022). While frequencies of this subclade have decreased as a result of post-Bronze Age migrations, R1b-L21 is still in high frequency amongst present-day populations from Britain, particularly western Britain and Scotland, as well as in Ireland and Brittany (Patterson et al 2022). Maternal lineage sub-clades represented in both Individual 'B' (J1c1) and Individual 'A' (X2b) have been in Britain since the Neolithic and are still relatively common in present-day north-western Europe (Olalde et al 2018; Brace et al 2019). Uniparental markers are single loci which represent a small proportion of an individual's genetic ancestry and may not be representative. Therefore, any conclusions about an individual's ancestry based only on these loci will be tentative. However, with this in mind, the paternal and maternal lineages of

Table 4 Preliminary results from shallow screening and deep sequencing of DNA libraries from Jedburgh Individuals A and B

Individual	Element	Lab No.	Run	% Endogenous Human DNA	C-T DNA Damage	Nuclear Coverage	Genetic (Karyotypic) Sex	Y-Chromosome Haplogroup	Mitochondrial Haplogroup
A	R. Malleus	C11178	Screening	86	22.77	0.01x	XX	–	–
A	R. Malleus	C11178	Deep Sequencing	41	19.26	2.4x	XX	–	X2b
B	R. Malleus	C11180	Screening	58	19.63	0.008x	XY	–	–
B	R. Malleus	C11180	Deep Sequencing	27	15.88	1.3x	XY	R1b1a1b1a1a2c1a5b1a1a	J1c1

both Individuals 'A' and 'B' are consistent with what we would typically expect from the local ancestry of medieval people from Scotland.

We plan to undertake further analyses on the whole genome data from Individuals 'A' and 'B' to provide a firmer account of their ancestry and genetic relatedness. The DNA libraries produced from powder taken from their teeth have not yet been sequenced and analysed but may give indications as to whether either individual was suffering from a specific systemic infection at the time of their death.