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1   **Research Paper**

2   **Title:** Maternal relationships within an Iron Age burial at the High Pasture Cave, Isle of Skye,

3   Scotland

4

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33   Declaration of interest: none.

34 **Highlights**

35 • Rare Iron Age cave burial assemblage of an adult female with foetal remains

36 • First report of complete ancient mitochondrial DNA sequences from foetal-aged bone

37 fragments

38 • Shot-gun sequencing and mitochondrial genome analysis for maternal lineage

39 identification

40 • Female could be excluded as being the mother of one the infants

41

Abstract

Human remains from the Iron Age in Atlantic Scotland are rare, which makes the assemblage of an adult female and numerous foetal bones at High Pasture Cave, on the Isle of Skye, particularly noteworthy. Archaeological evidence suggests that the female had been deposited as an articulated skeleton when the cave entrance was blocked off, marking the end of use of the site. Particularly intriguing is the deposition of disarticulated remains from a foetus and perinate close to the adult female, which opens the possibility that the female might have been the mother of both of the infants. We used shotgun genome sequencing in order to analyse the mitochondrial genomes of all three individuals and investigate their maternal relationship, and we report here, for the first time, complete ancient mitogenomes from foetal-aged bone fragments. While we could not exclude the possibility that the female was the mother of, or maternally related to, the foetus, we could definitely say that she was not the mother of the perinate buried alongside her. This finding is contrary to the standard archaeological interpretation, that women in such burials most likely died in childbirth and were buried together with their foetuses.

Keywords: ancient DNA, foetus, mitochondrial DNA, shot-gun sequencing

Introduction

High Pasture Cave (Uamh an Ard Achadh) is situated in the Parish of Strath on the Isle of Skye in Scotland. The wider environment surrounding the site revealed a diverse archaeological landscape of monuments and sites relating to prehistoric and historic periods, including a number of funerary monuments and hut-circles, combined with well-preserved field systems (Birch and Wildgoose, 2013). Radiocarbon dates of samples from different layers within the cave, and from the complex deposits outside the cave entrance, indicate periodic activity at High Pasture Cave from the 19th century cal. BC to cal. AD 1st century (Birch and Wildgoose, 2013). This, and the diversity of small finds, indicates a complex occupation history with several phases of intense activity during the Iron Age (Birch and Wildgoose, 2013).

Stonework found in the surface trench was associated with a series of entrance arrangements leading down a stone stairwell into the cave, whose system provided direct access to an underground stream. Although the archaeological material found in the cave indicated a fairly typical domestic assemblage (comprising bone, stone and iron tools, ceramics, and residues of metalworking), their depositional context, including the restricted morphology of the cave, raises questions about this interpretation. A well-preserved faunal assemblage was recovered, including animal and fish bones, and shellfish, as well as charred plant remains. Among the

76   animal bones, a high percentage were assigned to domesticated pig, which suggests a
77   feasting deposit (Livarda and Madgwick, 2017), and the bones displayed evidence of unusual
78   butchery practices, such as deliberate division of skeletons into left and right parts, and
79   unusual cut-marks across several ribs (Drew, 2005). The majority of the remains found at this
80   site displayed distinct ritual aspects, such as the burial of animals after butchery, and the
81   presence of both disarticulated human remains and complete human inhumations, including
82   foetal and infant burials (Birch and Wildgoose, 2013; Livarda and Madgwick, 2017).

83   A century or so after the stairwell was back-filled, with the passage being deliberately blocked
84   with boulders and sediments, a final act of closure at High Pasture Cave was carried out (Birch
85   & Wildgoose, 2013). Human remains from three individuals were deposited into the top of the
86   blockage (**Figure 1**), consisting of an adult female, aged 25–40 years and 1.55m tall, together
87   with two infants: one foetus that died between 12 and 26 weeks of gestation, and one perinate
88   that died during the last month of foetal development or during the first two weeks of life. The
89   woman had been laid out in the top of the backfilled stairwell that originally provided access
90   to the natural cave below, and large stones had been placed on top of her. The remains were
91   badly fragmented in the head, chest, abdominal area, and the right upper and lower arm, and
92   left lower arm regions, consistent with blunt force trauma from the stones. This happened
93   either at, or around, the time of deposition, precluding any chance to ascertain if the injuries
94   were the cause of her death.

95

96
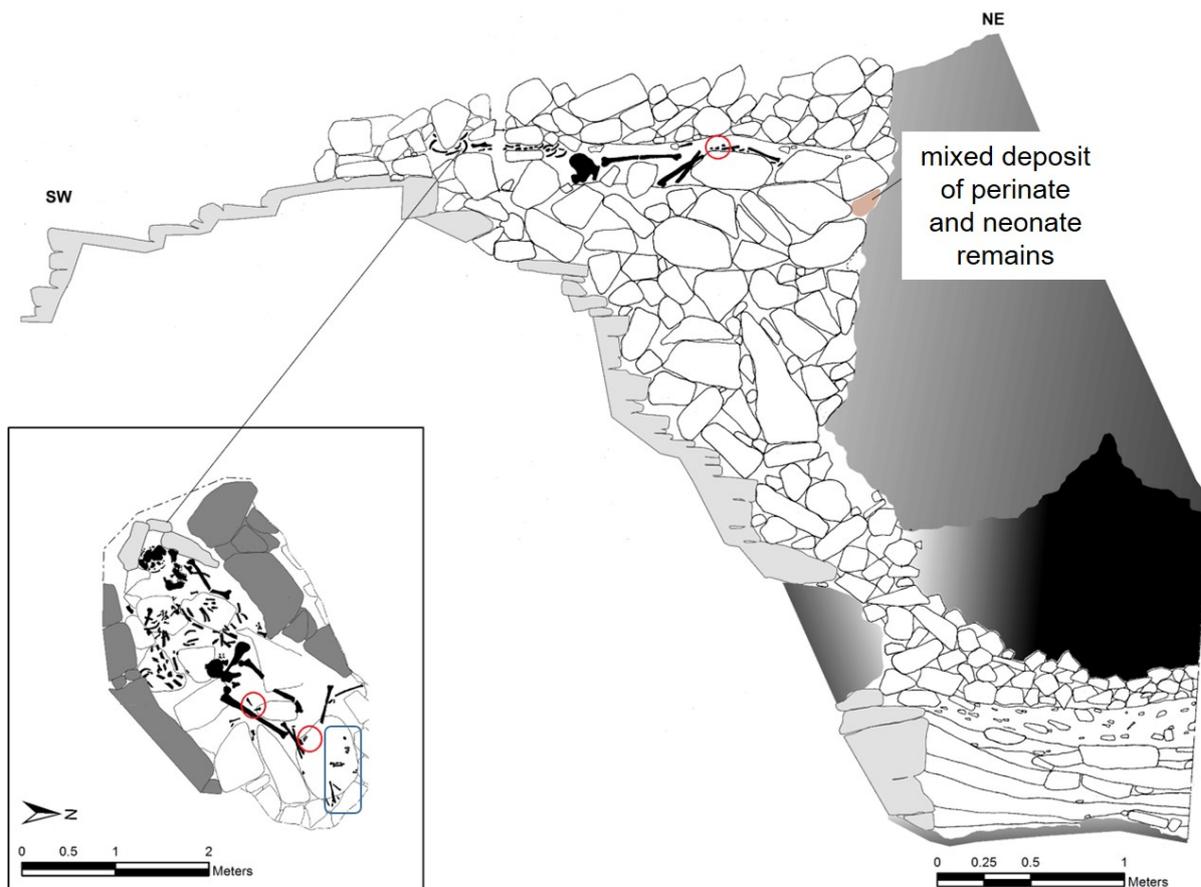
Figure 1. Burial schematic at High Pasture Cave, showing the position of the human remains within the stairwell blockage and, inset, the location of the adult, infant and animal bones. The remains of the foetus and perinate are indicated by red circles, with the foetal bones closer to the female's pelvis than the perinate. The blue rectangle shows the location of the foetal pig and perinatal dog, and also included a mixed deposit of perinate and neonate remains, as shown in the section of the stairwell.

Bones of the foetus were recovered from between the knees of the woman, while two small bundles of bones by her feet contained the skeletal elements from the perinate, and the remains of a foetal pig and a perinatal dog (**Figure 1**). The reasons for the segregation of the bones from the human foetus and perinate, and piglet and puppy are difficult to explain, but this may have formed a major part of the overall internment process. Although the relationship between the infant remains and the adult woman in the burial were uncertain, radiocarbon dates indicate that all three are roughly contemporary. The adult female yielded a radiocarbon date of cal. AD 28–230 (2σ; SUERC-14946), which corresponds to the Iron Age in this region. The infant remains were radiocarbon dated to cal. AD 63–219 (SUERC-66371) for the foetus, and cal. AD 76–228 (SUERC-66372) for the perinate (**Figure 2**). The majority of the other human bones (including infants and foetuses) found at this site (**Figure 1**), belonged to different contexts, and were dated as being older than the here presented analysed individuals

116 (Supplementary Information and **Table S1**). Although one perinate (D) bone, found in the west
117 wall of the stairwell (**Figure 1**), was roughly contemporaneous with the three individuals
118 studied here (**Table S1**), it only consisted of a proximal right humerus, and the entire sample
119 had been used for radiocarbon dating as part of an earlier study (Birch and Wildgoose, 2013;
120 Shapland et al., 2016), and so was unavailable for DNA analysis.

121
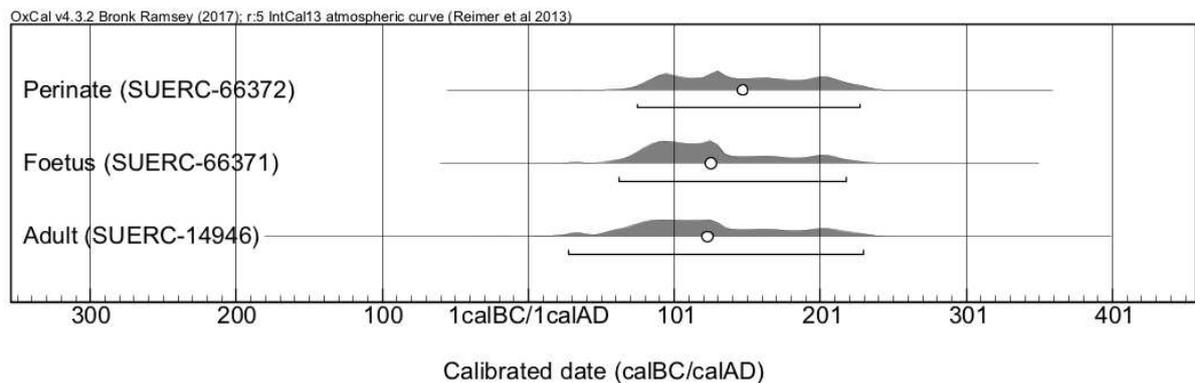
122

123 **Figure 2.** Radiocarbon dates for the human remains analysed from the High Pasture Cave
124 stairwell, calibrated using OxCal v.4.3.2 (Bronk Ramsey, 2017) and the most recent calibration
125 curve IntCal 13 (Reimer et al., 2013).

126

127 Several of the bones from the extremities of the articulated adult woman were missing,
128 suggesting that excarnation was the most likely pre-treatment of the body prior to final
129 deposition (Birch and Wildgoose, 2013). This may also have been the case with the foetus,
130 perinate, foetal piglet and perinatal puppy, as no cut-marks were noted on any of the bone
131 elements, making it unlikely that the bodies were cut up or dissected but rather were left to
132 decompose naturally. This type of funerary tradition would have provided the opportunity for
133 disarticulated human remains to be recovered for curation, use, or deposition, and
134 disarticulated human remains have indeed been recovered from a large number of Iron Age
135 settlement sites (Tucker, 2010). Therefore, it is possible that the remains of either the human
136 foetus or perinate were curated and only interred after the woman had died.

137 There does not appear to have been a tradition of formal burial of the dead throughout the
138 majority of the Iron Age in Atlantic Scotland, and it has frequently been posited that an
139 archaeologically 'invisible' form of disposal of the dead, such as exposure, must have been
140 practiced (Armit and Ginn, 2007). Consequently, human remains from this period are rarely
141 recovered; on Skye, for example, just one other Iron Age site has produced human remains –
142 Fiskavaig, a rock-shelter with evidence of Middle Iron Age occupation, from which a single
143 perforated fragment of adult human cranium was recovered in 2009 (Birch 2009). However,

144   distinctive and specific burials for women, foetuses and infants during the Iron Age in Britain,

145   including Scotland, were rather common (Finlay, 2000; Armit and Ginn, 2007). The general

146   archaeological interpretation has been that the women must have died in childbirth and were

147   thus buried together with their foetuses (Millet and Gowland, 2015). However, to our

148   knowledge, this hypothesis has not, to date, been analysed genetically. In order to test this

149   assumption, we used whole mitochondrial genome (mitogenome) data to analyse the maternal

150   genetic relationship of the adult female to both the foetus and the perinate.

151

Material and Methods

Sampling and DNA extraction from ancient remains

We sampled a petrous bone from the female adult, and two infant bone fragments from the perinate (ischium) and the foetus (scapula) for genetic analysis. We carried out the sampling at the Ancient DNA Facility at the University of Huddersfield under dedicated clean-room conditions. Laboratory researchers wore full body suits, hairnets, gloves and face masks throughout the drilling, extraction and library preparation processes. We constantly cleaned all tools and surfaces with LookOut® DNA Erase (SIGMA Life Sciences), as well as with bleach, ethanol and long exposures to UV light.

We decontaminated all of the bone surfaces by UV radiation for 30 minutes on each side, followed by cleaning with 5µm aluminum oxide powder using a compressed air abrasive system. For the adult sample, we excised the densest part of the petrous bone (Pinhasi et al., 2015) using a circular saw. We obtained bone powder by crushing this excised petrous portion, and the complete bone fragments from the two infants, in a MixerMill (Retsch MM400). We extracted DNA from 150mg of the petrous powder and 50–100mg of each infant bone, following the protocol by Yang et al. (1998), with modifications by MacHugh et al. (2000). We included blank controls throughout the extractions, library preparation and amplification reactions to monitor for possible modern DNA contamination.

Library preparation and sequencing of ancient DNA

We constructed next-generation sequencing libraries from the ancient DNA extracts using the method described in Meyer and Kircher (2010), with modifications outlined in Gamba et al. (2014) and Martiniano et al. (2014). Briefly, the changes were as follows: we used T4 DNA polymerase buffer (Thermo Scientific) instead of Tango buffer in the blunt-end repair step, and we heat inactivated *Bst* polymerase activity by incubating the libraries for 20 minutes at 80°C. We set up indexing amplifications using Accuprime *Pfx* Supermix (Life Technology), primer IS4 (0.2µM), a specific indexing primer (0.2µM) and 3µl of sample library, with a total reaction volume of 25µl. We performed all DNA purification steps using the QIAQuick MinElute purification kit (Qiagen) following the manufacturer's protocol, with the modification of adding 0.05% Tween 20 (Fisher BioReagents) to the elution buffer.

We prepared single-end libraries for both of the infant samples. We ran amplification under the following thermal cycling conditions: 5 minutes at 95°C; 11 cycles of 15 seconds at 95°C,

185   30 seconds at 60°C and 30 seconds at 68°C; with a final extension step of 5 minutes at 68°C.

186   We purified the resulting amplification product using the QIAQuick MinElute purification kit

187   (Qiagen).

188   As the adult was part of an earlier study, four dual-index libraries (Kircher et al., 2012) were

189   made from this individual. We set up amplification using AmpliTaq Gold (5U/µl), Thermopol

190   reaction buffer (10x), dNTPs (10mM each), both indexing primers (10µM each) and 10µl DNA

191   sample library. The following thermal cycling steps were used for amplification: 12 minutes at

192   95°C; 10 cycles of 20 seconds at 95°C, 30 seconds at 60°C, and 40 seconds at 72°C; with a

193   final extension step of 5 minutes at 72°C. We purified the amplification product using the

194   QIAQuick MinElute PCR purification kit. For the second amplification round, we used

195   Accuprime *Pfx* SuperMix (Life Technology), together with primers IS5 (10µM), IS6 (10µM) and

196   2.5µl of sample library. We carried out amplification under the following thermal cycling

197   conditions: 30 seconds at 98°C; 10 cycles of 20 seconds at 98°C, 30 seconds at 60°C, and

198   40 seconds at 72°C; with a final extension of 5 minutes at 72°C. We purified the resulting

199   amplification product as previously described.

200   We quantified all amplification reactions using a Qubit® ds-DNA High Sensitivity assay kit on

201   the Qubit® 3.0 Fluorometer, and additionally we checked the quality of each library using an

202   Agilent 2100 Bioanalyzer High Sensitivity DNA kit prior to pooling equimolarly for next-

203   generation sequencing (NGS) purposes. For the adult, we sent four dual-indexed libraries for

204   100-bp (base-pair) single-end sequencing on a HiSeq2500 (NBAF Liverpool, United

205   Kingdom), whereas we assigned the infant remains one single-indexed library each, and sent

206   them both for 100-bp paired-end sequencing on a HiSeq4000 (Macrogen, South Korea).

207

208   **Analysis of ancient sequence data**

209   We trimmed the NGS reads and aligned to the human mitochondrial genome revised

210   Cambridge Reference Sequence (rCRS, NC_012920; Andrews et al., 1999) and the human

211   reference genome (UCSC hg19). We trimmed the reads using cutadapt (v.1.13; Martin, 2011),

212   allowing a minimum overlap of 1bp between read and adapter (adapter sequence:

213   AGATCGGAAGAGCACACGTCTGAA-CTCCAGTCAC), discarding reads shorter than 30bp.

214   We used BWA aln (v.0.7.12-r1039; Li and Durbin, 2009) to map reads to both the rCRS and

215   hg19, filtering by base quality 15, and disabling seed length, as recommended for ancient DNA

216   data (Schubert et al., 2012), with a maximum edit distance of 0.01 and maximum number of

217   gap opens set to 2. We used SAMtools v.1.3 to sort and filter reads, and to remove PCR

218   duplicates, and assessed DNA damage patterns using mapDamage 2.0 (Jónsson et al., 2013)

219   and bamdamage (Malaspinas et al., 2014). We used picard tools (v.2.18,

220 *http://broadinstitute.github.io/picard*) to add read groups to the sequencing reads aligned to
221 hg19, and assessed the sequencing quality using FastQC (v.0.11.5; Andrews 2010) and
222 qualimap (v.2.2.1; Okonechnikov et al., 2015). We used samtools mpileup and bcftools (v.1.3)
223 to create a consensus FASTQ file of the alignment to the rCRS, and SEQTK (v.1.2-r102-dirty)
224 to convert the FASTQ file into FASTA format. We used schmutzi (Renaud et al., 2015) to
225 detect possible modern mitochondrial contamination, and we assigned haplotypes using
226 Haplogrep 2 (Weissensteiner et al., 2016) and confirmed them manually using the Integrative
227 Genomics Viewer (IGV v.2.4.13; Robinson et al., 2011; Thorvaldsdóttir et al. 2013). The only
228 difference in the treatment of the paired-end reads compared to single-end reads was our use
229 of AdapterRemoval (v.2.1.7; Schubert et al., 2016), which trimmed the adapters from the read
230 pairs and merged the reads.

231

Mitochondrial haplotype calling for ancient samples

233 We created the mitochondrial genome consensus sequences using SAMtools mpileup and
234 BCFtools. As the coverage of the foetus and perinate mitogenomes was very low (see Results
235 section), we also used GATK (v.3.7) to call the variants against the rCRS for the two infant
236 samples. This approach allowed a maximum output of variants in order to determine the
237 mitochondrial haplogroup of the studied individuals, and also allowed us to compare both
238 methods. All other bioinformatic steps were the same for both read types.

239 We called the mitochondrial haplotypes of the newly sequenced modern samples using
240 HaplotypeCaller, with 'Downsampling' set to 250, 'Ploidy' set to 100 and 'Standard Call
241 Confidence' set to 30, and, for SNP filtering, we set the 'Minimum Coverage Filter' to 2. In
242 order to filter the mutations and heteroplasmies, we used bcftools v.1.3 (Li and Durbin, 2009;
243 Li, 2011). We considered mutations with a read frequency ≥0.7 as variants, whilst mutations
244 with an allele frequency >0.3 and <0.7 were considered heteroplasmies. We checked all
245 heteroplasmies manually in Geneious® (version 6.1.8). We excluded variants at positions
246 around 302-315 including 309.1C(C) and 315.1C, AC insertions and deletions at 515-522,
247 16182C, 16183C, and 16193.1C(C) from analyses. We checked the final haplotype
248 classifications with HaploGrep 2 (Weissensteiner et al., 2016) and converted these into
249 FASTA files with HaploFasta.

250

Modern mitochondrial genomic sequences and SNP data

252 We sequenced a total of 13 modern samples belonging to H7a1 (Germany, *n* = 9; Scotland,
253 *n* = 3; Wales, *n* = 1) to accompany the published data (Denmark, 15; England, 2; Finland, 1;

254 Germany, 1; Italy, 3 [including 1 Sardinian]; Russia, 2; Scotland, 1; United Kingdom, 1; USA,

255 2; unknown geographic origin, 9; Bulgaria ancient, 1) (**Table S2**; **Figure S1**). The four newly

256 generated British samples originated from a selection of samples collected by BritainsDNA

257 (now owned by Source BioScience). An overall mitochondrial SNP (single nucleotide

258 polymorphism) dataset of anonymised samples was available to us, also from

259 BritainsDNA/Source, consisting of 5,337 British and Irish individuals. We used these SNP data

260 for mitochondrial haplogroup frequency estimates.

261

Analysis of mitochondrial DNA data

263 We carried out polymerase chain reactions (PCR) for modern samples in the modern genetics

264 lab at the University of Huddersfield. To amplify the complete mitogenome (16,568 bp), we

265 used two fragments for each sample following the method in Brandini et al. (2017). We purified

266 the PCR products using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA)

267 with an elution volume of 50µl. We assessed the DNA concentration of each fragment using

268 1µl of the purified PCR product using a Qubit® 3.0 fluorometer. After quantification, we pooled

269 both fragments equimolarly with a final volume of 40µl and a concentration of 1ng/µl. We then

270 sent the samples for sequencing at the Earlham Institute (Norwich, UK) on a MiSeq250 (PE-

271 Nano x1). We used EAGER (Peltzer et al., 2016) for assessment of quality control, removal

272 of duplicates, mapping and SNP calling, using the following settings: AdapterRemoval for the

273 removal of adapters and read merging; bwa-mem to align sequences to the rCRS (Andrews

274 et al., 1999); and removal of duplicates using DeDup. We aligned published sequences to the

275 rCRS using Sequencher™ version 5.1 (*http://www.genecodes.com*), and converted these to

276 variants using mtDNAGeneSyn (Pereira et al., 2009). We verified haplogroup classifications

277 using Haplogrep 2 (Weissensteiner et al., 2016). We also used HaploGrep2 to call

278 haplogroups from the SNP data of the 5,337 British and Irish individuals.

279

Phylogenetic tree reconstruction

281 We placed all newly generated sequences in a maximum-parsimony phylogenetic tree,

282 constructed by compiling published (*n* = 38) and unpublished (*n* = 15; 13 modern and the two

283 ancient) complete mitogenome data (**Table S2**; **Figure S1**). We also included data generated

284 at Huddersfield as part of a larger British Isles study. We used mtPhyl v.4.015 (Eltsov and

285 Volodko, 2011; *https://sites.google.com/site/mtphyl/home*) to reconstruct the phylogeny

286 following the classification of PhyloTree build 17 (*http://www.phylotree.org/tree/index.htm*),

287 and using the molecular clock developed by Soares et al. (2009) to date nodes. We calculated

288 maximum-likelihood (ML) estimates assuming the HKY85 mutation model with gamma-

289 distributed (32 categories) rates (plus invariant sites) and two partitions: coding region

290 (positions 00577–16023) and control region (positions 16024–00576) with baseml v.4.7 from

291 PAML package (Yang, 1997). We then converted the distances into years using a mutation

292 rate of one substitution in every 3,624 years (Soares et al. 2009), further accounted for

293 purifying selection. We obtained coalescent time estimates using rho (ρ) and sigma (σ) values

294 exported from the in-house developed founder analysis software, applying the same clock. All

295 ancient samples (n = 3) were excluded from the age calculations.

296

Results

We successfully extracted and sequenced DNA from all three ancient samples. The endogenous DNA content of all individuals was quite low, at 11.9%, 8.0% and 7.6% for the adult, perinate and foetus respectively. The nuclear genome of the adult female was sequenced to a coverage of 0.28x, whilst her mitochondrial genome was sequenced to a coverage of 58.07x. For the two infants, the nuclear genome coverage was 0.014x for the perinate and 0.006x for the foetus, with mitochondrial genome coverages of 4.14x and 2.24x, respectively. Due to the low coverage of the nuclear genomes of the foetus and the perinate, and the extremely low number of overlapping SNPs retrieved (<10,000), no meaningful autosomal analysis was possible. The genome-wide data of the adult female is currently being further analysed in a broader Iron Age study (in preparation) and, since the focus of the present study is the maternal relationship of the individuals analysed, and the coverage of the genome-wide data retrieved from the two infant remains was too low to be useful, we do not present any genome-wide analysis here.

Contamination estimation

Contamination estimates ranged from 0.165% to 0.185% for the adult female, and 0 to 0.005% for both infants. We observed no exogenous contamination in extraction or library blanks, supporting the authenticity of the results, and there were no matches between the mitochondrial haplotypes from the three ancient samples and those of the researchers.

Genetic sex identification

We identified the genetic sex using the tool published by Skoglund et al. (2013). The osteological determination that the adult was female was supported by the genetic sex identification. We were unable to assign the genetic sex for the two infants due to their low genomic coverage.

Mitochondrial haplotype determination

To securely determine mitochondrial haplotypes for the infants, we compared the results from two bioinformatic approaches (that is, GATK Variant Call *vs.* calling the consensus sequence, as discussed in the Methods section), and we assigned the same haplotypes to each individual using both methods. Due to the low coverage in both infants' mitochondrial genomes, the

329   haplotypes initially generated presented a number of ambiguous base calls (**Table 1**). In order

330   to reduce the number of ambiguities, and thus simplify any haplotype comparison, we checked

331   each one of the ambiguous positions manually, and made a decision by combining

332   phylogenetic and molecular criteria. We provide a thorough overview of the rationale behind

333   every mutation disregarded in **Table S3**. Although the haplotypes finally reconstructed could

334   still contain some errors (**Table 1**), they show that only one of the infants, the foetus, shares

335   haplogroup (H7a1b) with the adult female and, could, therefore, possibly be related to her. We

336   assigned the perinate to haplogroup J1c3, thus excluding a direct maternal kinship.

337

338 **Table 1.** Comparison of mutations scored for all three ancient mitochondrial genomes using Haplogrep 2.

Sample	Haplogroup	Final Haplotype[a]	Initial Haplotype[b]	Gaps[c]
Adult female	H7a1b	*263G 750G* **1393A** *1438G* **1719A** *4769G* **4793G** *8860G 15326G* **16261T** 16519C	-	-
Foetus	H7a1b	*263G 750G* **1393A 1719A** *4769G* **4793G** (7025G) *8860G* **16261T** 16519C	263G 633M 750G 911K 1393A 1719A 4769G 4793R 5239K 6272R 6359M 6842Y 7022K 7025R 8576K 8860G 10674K 10750R 11629R 12308R 12766Y 12822R 12864K 12873W 13207W 14864K 15601K 16261T 16519C	1438G 15326G
Perinate	J1c3	*73G* **185A 228A** *263G* **295T 462T 489C** *750G 2706G* **3010A 10398G 11251G** *11719A* **12612G 13708A 13934T** *14766T* **14798C** *15326G* **15452A 16069T** 16126C	73G 152Y 185A 228A 263G 295T 462T 489C 539Y 750G 2706G 3010A 3276R 4892M 6243R 9233Y 9477R 9814Y 10398G 10567W 11251R 11719A 12210W 12612G 12835R 12998Y 13708A 13934T 14766T 14798C 15326G 15452A 15854W 16022Y 16069T 16126C	-

[a] *Italics*: changes *vs.* the rCRS up to the H2 root; **bold**: H7 to H7a1b diagnostic mutations in the case of the adult and foetus, and JT to J1c3 diagnostic mutations in the case of the perinate; the dubious possible mutation seen in the foetus is shown in parentheses.
[b] underlined: ambiguous positions
[c] gaps: positions seen in the adult, but not covered in the foetal mitogenome

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Discussion

Here we report for the first time complete ancient mitogenomes from foetal-aged bone fragments. Previous studies have used PCR-based methods to determine the genetic sex of neonates (Faerman et al., 1998; Waldron et al., 1999; Irish et al., 2008), but no mitogenomes or genome-wide data have been published to date. Using next-generation sequencing to generate whole mitogenomes results in a more detailed analysis of the haplotype than a PCR-based approach, where typically only fragments of the non-coding region are amplified. Although we generated whole mitogenomes from the two infants, we were unable to determine the molecular sex of either the perinate or the foetus due to their low nuclear genome coverage.

Mitochondrial genomes are used to trace the female line of descent as they are passed on from mother to child without recombination. The only changes that occur over generations are naturally-occurring mutations, and these can be used for phylogenetic reconstructions over wide geographic areas and long timespans. Both the female and the foetus belong to the very distinctive and uncommon (among present-day populations) haplogroup H7a1b. In contrast, the perinate belongs to haplogroup J1c3, and so can be ruled out as a child of the adult female. In addition, as we saw no other defining mutations, we can say that the mitogenome of this individual is basal to J1c3. For more details about haplogroup J1c, see Supplementary Information.

The main defining mutations of haplogroup H7a1b are the transitions at positions 4793, 1719, 16261 and 1393, which define H7, H7a, H7a1 and H7a1b, respectively. These are all found in both individuals (shown in bold in **Table 1**). After all ambiguities were assessed (**Table S3**), the sequence of the foetus displayed a single plausible difference compared to the adult female, the A7025G transition (**Table 1**). The 7025 transition defines subclade H5a5, but has never been observed in any H7 sequence to date. Further sequencing would be necessary to securely define the base read at position 7025 for the foetal sequence. However, based on the current data, we must recognise that, although the foetus and the adult female share the same rare haplogroup, they might not share the same haplotype (if a mutation at np 7025 is accepted).

In our modern mtDNA SNP database, haplogroup H occurs in 41% of all modern British Isles mitochondrial lineages (2247 out of 5537). However, haplogroup H7 is only a minor European subclade, which is found at 1.6% (85 out of 5337) in our British Isles dataset, while its nested subclade, H7a1b is even rarer, and is found at 0.7% (13 out of 1926) in Scotland, 0.3% (1 out of 374) in Ireland and 0.2% in England (4 out of 2304), while it has not been detected in Wales (0 out of 733). For more details about haplogroup H7, see Supplementary Information. The

16

373 subhaplogroup of H7a1b dates to *c*. 4300 years [2400–6300] and is seen in modern-day Scotland
374 and England, but also in Denmark, Finland and Sardinia (Behar et al., 2012; Li et al., 2014; Raule
375 et al., 2014). The apparently wide geographic distribution and young age of H7a1b suggest that
376 it may have been introduced into Britain more recently than the arrival of the Bell Beaker settlers,
377 *c*. 4450 years ago, when the genome-wide pattern of the British gene pool began to take on its
378 modern form (Olalde et al. 2018) – possibly in the Bronze Age or in the Iron Age itself. The two
379 individuals from High Pasture Cave are, nevertheless, the earliest known H7 sequences from
380 north-west Europe (**Figure S1**). The nodal haplotype represented by the female, and possibly by
381 the foetus, has survived in modern-day Scotland and Denmark.

382 The fact that the two ancient individuals share the same rare haplogroup, and possibly the same
383 haplotype, suggests that, even if they were not directly related, they belonged to a population that,
384 similarly to other Iron Age populations, was most likely small and in-bred. The possibility that the
385 two individuals might not share the same haplotype is potentially even more tantalizing, as it would
386 imply that, although small and geographically isolated, this population carried a level of diversity
387 (represented by the nodal haplotype +7025 mutation) that could have either arisen *in loco*, thus
388 implying some level of prolonged isolation, or been introduced from a more diverse, and possibly
389 larger, and interconnected, metapopulation.

390 Infants and young children were often distinguished in burial customs from the rest of society
391 (Ucko, 1969). In early Iron Age Thrace, young or newborn children were sacrificed for rituals and
392 it was only in the late Iron Age that newborn and young animals were used instead (Nekhrizov
393 and Tzvetkova, 2010). In Roman and Anglo-Saxon periods in England, children who had not yet
394 teethed were, instead of being cremated, buried within building walls or under buildings (Stoodley,
395 2000; Gowland et al., 2014). Also, in Roman Dorset and Buckinghamshire, women who died in
396 pregnancy were only buried after the child had been removed from the womb (embryotomy
397 surgery) (Gowland et al., 2014).

398 The separation of infant burials from the rest of the population appears to have been widespread
399 across the Iron Age and into the Romano-British period (Redfern and Dewitte, 2011). In the British
400 Iron Age, women who died in childbirth, as well as foetuses and young infants, were often given
401 special burials, highlighting the importance of the link between woman and child (Millet and
402 Gowland, 2015). Human remains of infants were often placed into cave systems, and the number
403 and geographic spread of cave burials during this period in Scotland is relatively high – especially
404 given that burials from this period are generally not abundant (Saville and Hallén, 1994), and that

405   the deposition of individual articulated skeletons in Iron Age Scotland is rare (Tucker, 2010).

406   However, there is also evidence of infants being buried in unstable, abandoned buildings or

407   disused settlement sites. For example, an excavation at the site of Howe in Orkney revealed an

408   Iron Age burial of an adult male, aged 35–45 years, along with a perinate and an infant, all of

409   whom had been placed within accumulated rubble in a midden in a disused yard (Armit and Ginn,

410   2007). The bones showed gnaw marks, and their layout has been interpreted as an informal

411   burial; that is, they were deposited without ceremony (Armit and Ginn, 2007). Another example is

412   the Iron Age site at the Knowe of Skea, also in Orkney, where over one hundred, mainly infant,

413   bodies were found deposited in domestic rubble contexts (Armit and Ginn, 2007). Cremations and

414   inhumations of adult women, alongside foetal/neonatal remains, are also known from Ireland,

415   located in reused, separate burial areas (Finlay, 2000).

416   The context of the High Pasture Cave appears to mirror those burials in cave systems, with the

417   adult female being deposited as an articulated skeleton just prior to the closure of the cave.

418   However, the trauma evident on the female (whether inflicted before or after death), and the lack

419   of any wider tradition of inhumation in the region, has led to the suggestion that this burial was a

420   deviant one, or even a sacrifice connected to the closure of the cave stairwell, rather than a

421   ceremonial burial of a mother and her children (Armit, in press). The disarticulated remains of the

422   foetus and perinate appear to have been collected and purposefully deposited with her, raising

423   several questions, especially now that our results show that we can positively exclude a direct

424   maternal kinship for the perinate.

425

Conclusions

The results of our study show that one of the two infants buried alongside the adult female at High Pasture Cave was definitely not her child, whereas the foetus could not be excluded as being maternally related to her. This is an intriguing finding considering the common archaeological understanding of this type of female/child burial, where the infants are assumed to be the children of the women. So far, archaeological interpretations of these types of burial indicate either mother–child relationships, or a midwife buried in the same place as infants. In the case of High Pasture Cave, where the woman had potentially been crushed to death, archaeological questions still remain. Why might she have been chosen for this extremely unusual form of treatment before/after death? Why were the human bones of infants curated, collected, and deposited in the cave with her, and why was she buried with an unrelated child?

Our genetic analysis shows that a general assumption of mother–child relations within such burials can be misleading, and that genetic analysis can aid the analysis and interpretation of such archaeological contexts.

Data availability

Sequencing data deposited in GenBank, accession number xxxx.

454   **References**

455   Achilli, A., Rengo, C., Magri, C., Battaglia, V., Olivieri, A., Scozzari, R., Cruciani, F., Zeviani, M.,

456   Briem, E., Carelli, V. & Moral, P. (2004) The molecular dissection of mtDNA haplogroup H

457   confirms that the Franco-Cantabrian glacial refuge was a major source for the European gene

458   pool. *American Journal of Human Genetics*, **75**, 910-918. doi.org/10.1086/425590

459   Allentoft M.E., Sikora M., Sjögren K.G., Rasmussen S., Rasmussen M., Stenderup J.,

460   Damgaard P.B., Schroeder H., Ahlström T., Vinner L., Malaspinas A.S., Margaryan A., Higham

461   T., Chivall D., Lynnerup N., Harvig L., Baron J., Della Casa P., Dąbrowski P., Duffy P.R., Ebel

462   A.V., Epimakhov A., Frei K., Furmanek M., Gralak T., Gromov A., Gronkiewicz S., Grupe G.,

463   Hajdu T., Jarysz R., Khartanovich V., Khokhlov A., Kiss V., Kolář J., Kriiska A., Lasak I.,

464   Longhi C., McGlynn G., Merkevicius A., Merkyte I., Metspalu M., Mkrtchyan R., Moiseyev V.,

465   Paja L., Pálfi G., Pokutta D., Pospieszny Ł., Price T.D., Saag L., Sablin M., Shishlina N.,

466   Smrčka V., Soenov V.I., Szeverényi V., Tóth G., Trifanova S.V., Varul L., Vicze M.,

467   Yepiskoposyan L., Zhitenev V., Orlando L., Sicheritz-Pontén T., Brunak S., Nielsen R.,

468   Kristiansen K. & Willerslev E. (2015) Population genomics of Bronze Age Eurasia. *Nature* **522**,

469   167. doi.org/10.1038/nature14507

470   Andrews S. (2010) FastQC: a quality control tool for high throughput sequence data. Available

471   at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

472   Andrews R.M., Kubacka I., Chinnery P.F., Lightowlers R.N., Turnbull D.M. & Howell N. (1999)

473   Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA.

474   *Nature Genetics* **23**, 147. doi.org/10.1038/13779

475   Armit I. & Ginn V. (2007) Beyond the grave: human remains from domestic contexts in Iron Age

476   Atlantic Scotland. *Proceedings of the Prehistoric Society* **73**, 113-134.

477   Armit I. (in press) Human remains in Scottish Caves: High Pasture Cave in context. In: *High*

478   *Pasture Cave: Ritual, Memory and Identity in the Iron Age of Skye* (eds. S. Birch, G.

479   Cruickshanks and J. Mckenzie). Oxbow Books, Oxford.

480   Behar D.M., Van Oven M., Rosset S., Metspalu M., Loogväli E.L., Silva N.M., Kivisild T., Torroni

481   A. & Villems R. (2012) A "Copernican" reassessment of the human mitochondrial DNA tree

482   from its root. *American Journal of Human Genetics* **90**, 675-684.

483   doi.org/10.1016/j.ajhg.2012.03.002

484 Birch S. (2009) Latest finds from the Fiskavaig rock shelter site. http://www.high-pasture-
485   cave.org/index.php/latest_finds/comments/178/ (accessed on 22nd January 2019)

486 Birch S. & Wildgoose M. (2013) *Uamh an Ard Achadh (High Pasture Cave) & Environs Project,*
487   *Strath, Isle of Skye 2010/11 (NGR NG 5943 1971). The Preliminary Assessment and Analysis*
488   *of Late Prehistoric Cultural Deposits from a Limestone Cave and Associated Surface*
489   *Features.* Data Structure Report 2013.

490 Brace S., Diekmann Y., Booth T.J., Faltyskova Z., Rohland N., Mallick S., Ferry M., Michel M.,
491   Oppenheimer J., Broomandkhoshbacht N., Stewardson K. Walsh S., Kayser M., Schulting R.,
492   Craig O.E., Sheridan A., Parker Pearson M., Stringer C., Reich D., Thomas M.G. & Barnes I.
493   (2018) Population replacement in early Neolithic Britain. *bioRxiv*. doi.org/10.1101/267443

494 Brandini S., Bergamaschi P., Cerna M.F., Gandini F., Bastaroli F., Bertolini E., Cereda C.,
495   Ferretti L., Gómez-Carballa A., Battaglia V. and Salas A. (2017). The Paleo-Indian entry into
496   South America according to mitogenomes. Molecular Biology and Evolution, **35**, 299-311.
497   doi.org/10.1093/molbev/msx267

498 Bronk Ramsey C. (2017) Methods for summarizing radiocarbon datasets. *Radiocarbon* **59**,
499   1809-1833. doi.org/10.1017/RDC.2017.108

500 Brotherton, P., Haak, W., Templeton, J., Brandt, G., Soubrier, J., Adler, C.J., Richards, S.M.,
501   Der Sarkissian, C., Ganslmeier, R., Friederich, S., Dresely, V., van Oven, M., Kenyon, R., Van
502   der Hoek, M.B., Korlach, J., Luong, K., Ho, S.Y.W., Quintana-Murci, L., Behar, D.M., Meller,
503   H., Alt, K.W., Cooper, A., and The Genographic Consortium (2013) Neolithic mitochondrial
504   haplogroup H genomes and the genetic origins of Europeans. *Nature Communications*,
505   **4**,1764. 10.1038/ncomms2656

506 Cassidy L.M., Martiniano R., Murphy E.M., Teasdale M.D., Mallory J., Hartwell B. & Bradley
507   D.G. (2016) Neolithic and Bronze Age migration to Ireland and establishment of the insular
508   Atlantic genome. *Proceedings of the National Academy of Sciences* **113**, 368-373.
509   doi.org/10.1073/pnas.1518445113

510 Dabney J., Meyer M. & Pääbo S. (2013) Ancient DNA damage. *Cold Spring Harbor*
511   *Perspectives in Biol*ogy, a012567. doi: 10.1101/cshperspect.a012567

512 Drew C. (2005) *Refuse or Ritual? The Mammal Bones from High Pasture Cave, Skye.*
513   Unpublished PhD thesis, Durham University.

514   Eltsov N. & Volodko N. (2011) MtPhyl: Software tool for human mtDNA analysis and phylogeny
515   reconstruction. https://sites.google.com/site/mtphyl/home

516   Faerman M., Bar-Gal G.K., Filon D., Greenblatt C.L., Stager L., Oppenheim A. & Smith P.
517   (1998) Determining the sex of infanticide victims from the late Roman era through ancient
518   DNA analysis. *Journal of Archaeological Science* **25**, 861-865. doi.org/10.1006/jasc.1997.0231

519   Finlay N. (2000) Outside of life: traditions of infant burial in Ireland from cillin to cist. *World*
520   *Archaeology* **31**, 407-422. doi.org/10.1080/00438240009696929

521   Gamba C., Jones E.R., Teasdale M.D., McLaughlin R.L., Gonzalez-Fortes G., Mattiangeli V.,
522   Domboróczki L., Kővári I., Pap I., Anders A. & Whittle A. Dani J., Raczky P., Higham T.F.G.,
523   Hofreiter M., Bradley D.G. & Pinhasi R. (2014) Genome flux and stasis in a five millennium
524   transect of European prehistory. *Nature Communications* **5**, 5257.
525   doi.org/10.1038/ncomms6257

526   Günther T., Valdiosera C., Malmström H., Ureña I., Rodriguez-Varela R., Sverrisdóttir Ó.O.,
527   Daskalaki E.A., Skoglund P., Naidoo T., Svensson E.M., de Castro J.M.B. Carbonell E., Dunn
528   M., Storå J., Iriarte E., Arsuaga J.L., Carretero J.M., Götherström A. & Jakobsson M. (2015)
529   Ancient genomes link early farmers from Atapuerca in Spain to modern-day Basques.
530   *Proceedings of the National Academy of Sciences of the USA 112*, 11917-11922.
531   doi.org/10.1073/pnas.1509851112

532   Haak W., Lazaridis I., Patterson N., Rohland N., Mallick S., Llamas B., Brandt G., Nordenfelt S.,
533   Harney E., Stewardson K., Fu Q., Mittnik A., Bánffy E., Economou C., Francken M., Friederich
534   S., Pena R.G., Hallgren F., Khartanovich V., Khokhlov A., Kunst M., Kuznetsov P., Meller H.,
535   Mochalov O., Moiseyev V., Nicklisch N., Pichler S.L., Risch R., Rojo Guerra M.A., Roth C.,
536   Szécsényi-Nagy A., Wahl J., Meyer M., Krause J., Brown D., Anthony D., Cooper A., Alt K.W.
537   & Reich D. (2015) Massive migration from the steppe was a source for Indo-European
538   languages in Europe. *Nature* **522**, 207. doi.org/10.1038/nature14317

539   Hofmanová Z., Kreutzer S., Hellenthal G., Sell C., Diekmann Y., Díez-del-Molino D., van Dorp
540   L., López S., Kousathanas A., Link V., Kirsanow K., Cassidy L.M., Martiniano R., Strobel M.,
541   Scheu A., Kotsakis K., Halstead P., Triantaphyllou S., Kyparissi-Apostolika N., Urem-Kotsou
542   D., Ziota C., Adaktylou F., Gopalan S., Bobo D.M., Winkelbach L., Blöcher J., Unterländer M.,
543   Leuenberger C., Çilingiroğlu Ç., Horejs B., Gerritsen F., Shennan S.J., Bradley D.G., Currat
544   M., Veeramah K.R., Wegmann D., Thomas M.G., Papageorgopoulou C. & Burger J. (2016)

545 Early farmers from across Europe directly descended from Neolithic Aegeans. *Proceedings of*
546 *the National Academy of Sciences of the USA* **113**, 6886-6891.
547 doi.org/10.1073/pnas.1523951113

548 Irish J.D., Bratlund B., Schild R., Kolstrup E., Królik H., Mańka D. & Boroń T. (2008) A late
549 Magdalenian perinatal human skeleton from Wilczyce, Poland. *Journal of Human Evolution* **55**,
550 736. doi:10.1016/j.jhevol.2008.03.007

551 Jónsson H., Ginolhac A., Schubert M., Johnson P.L. & Orlando L. (2013) mapDamage2.0: fast
552 approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics* **29**,
553 1682-1684. doi.org/10.1093/bioinformatics/btt193

554 Juras A., Krzewińska M., Nikitin A.G., Ehler E., Chyleński M., Łukasik S., Krenz-Niedbała M.,
555 Sinika V., Piontek J., Ivanova S., Dabert M., Götherström A. (2017) Diverse origin of
556 mitochondrial lineages in Iron Age Black Sea Scythians. *Scientific Reports* **7**, 43950.
557 doi.org/10.1038/srep43950

558 King T.E., Fortes G.G., Balaresque P., Thomas M.G., Balding D., Maisano Delser P., Neumann
559 R., Parson W., Knapp M., Walsh S., Tonasso L., Holt J., Kayser M., Appleby J. Forster P.,
560 Ekserdjian D., Hofreiter M. & Schürer K. (2014) Identification of the remains of King Richard III.
561 *Nature Communications* **5**, 5631. doi.org/10.1038/ncomms6631

562 Kircher M., Sawyer S. & Meyer M. (2012) Double indexing overcomes inaccuracies in multiplex
563 sequencing on the Illumina platform. *Nucleic Acids Research* **40**, e3.
564 doi.org/10.1093/nar/gkr771

565 Knipper C., Mittnik A., Massy K., Kociumaka C., Kucukkalipci I., Maus M., Wittenborn F., Metz
566 S.E., Staskiewicz A., Krause J. & Stockhammer P.W. (2017) Female exogamy and gene pool
567 diversification at the transition from the Final Neolithic to the Early Bronze Age in central
568 Europe. *Proceedings of the National Academy of Sciences*, 201706355.
569 doi.org/10.1073/pnas.1706355114

570 Lazaridis I., Nadel D., Rollefson G., Merrett D.C., Rohland N., Mallick S., Fernandes D., Novak,
571 M., Gamarra B., Sirak K., Connell S., Stewardson K., Harney E., Fu Q., Gonzalez-Fortes G.,
572 Jones E.R., Roodenberg S.A., Lengyel G., Bocquentin F., Gasparian B., Monge J.M., Gregg
573 M., Eshed V., Mizrahi A.S., Meiklejohn C., Gerritsen F., Bejenaru L., Blüher M., Campbell A.,
574 Cavalleri G., Comas D., Froguel P., Gilbert E., Kerr S.M., Kovacs P., Krause J., McGettigan
575 D., Merrigan M., Merriwether D.A., O'Reilly S., Richards M.B., Semino O., Shamoon-Pour M.,

576   Stefanescu G., Stumvoll M., Tönjes A., Torroni A., Wilson J.F., Yengo L., Hovhannisyan N.A.,

577   Patterson N., Pinhasi R. & Reich D. (2016) Genomic insights into the origin of farming in the

578   ancient Near East. *Nature* **536**, 419. doi.org/10.1038/nature19310

579   Li H. & Durbin R. (2009) Fast and accurate short read alignment with Burrows–Wheeler

580   transform. *Bioinformatics* **25**, 1754-1760. doi.org/10.1093/bioinformatics/btp324

581   Li, H., 2011. A statistical framework for SNP calling, mutation discovery, association mapping

582   and population genetical parameter estimation from sequencing data. *Bioinformatics*, **27**,

583   2987-2993. doi.org/10.1093/bioinformatics/btr509

584   Li S., Besenbacher S., Li Y., Kristiansen K., Grarup N., Albrechtsen A., Sparsø T., Korneliussen

585   T., Hansen T., Wang J., Nielsen R., Pedersen O., Bolund L. & Schierup M.H. (2014) Variation

586   and association to diabetes in 2000 full mtDNA sequences mined from an exome study in a

587   Danish population. *European Journal of Human Genetics* **22**, 1040.

588   doi.org/10.1038/ejhg.2013.282

589   Lipson M., Szécsényi-Nagy A., Mallick S., Pósa A., Stégmár B., Keerl V., Rohland N.,

590   Stewardson K., Ferry M., Michel M., Oppenheimer J., Broomandkhoshbacht N., Harney E.,

591   Nordenfelt S., Llamas B., Gusztáv Mende B., Köhler K., Oross K., Bondár M., Marton T.,

592   Osztás A., Jakucs J., Paluch T., Horváth F., Csengeri P., Koós J., Sebők K., Anders A.,

593   Raczky P., Regenye J., Barna J.P., Fábián S., Serlegi G., Toldi Z., Gyöngyvér Nagy E., Dani

594   J., Molnár E., Pálfi G., Márk L., Melegh B., Bánfai Z., Domboróczki L., Fernández-Eraso J.,

595   Antonio Mujika-Alustiza J., Alonso Fernández C., Jiménez Echevarría J., Bollongino R.,

596   Orschiedt J., Schierhold K., Meller H., Cooper A., Burger J., Bánffy E., Alt K.W., Lalueza-Fox

597   C., Haak W. & Reich D. (2017) Parallel palaeogenomic transects reveal complex genetic

598   history of early European farmers. *Nature* **551**, 368. doi.org/10.1038/nature24476

599   Livarda A. & Madgwick R. (2017). Ritual and religion: bioarchaeological perspectives. In: *The*

600   *Bioarchaeology of Ritual and Religion* (eds. A. Livarda, R. Madgwick and S. Riera-Mora).

601   Oxbow Books, Oxford; pp. 1-13. ISBN 978-1-178570-828-2

602   MacHugh D.E., Edwards C.J., Bailey J.F., Bancroft D.R. & Bradley D.G. (2000) The extraction

603   and analysis of ancient DNA from bone and teeth: a survey of current methodologies. *Ancient*

604   *Biomolecules* **3**, 81-102.

605   Malaspinas A.S., Tange O., Moreno-Mayar J.V., Rasmussen M., DeGiorgio M., Wang Y.,

606   Valdiosera C.E., Politis G., Willerslev E. & Nielsen R. (2014) bammds: a tool for assessing the

607 ancestry of low-depth whole-genome data using multidimensional scaling (MDS). *Bioinformatics*
608 **30**, 2962-2964. doi.org/10.1093/bioinformatics/btu410

609 Margaryan A., Derenko M., Hovhannisyan H., Malyarchuk B., Heller R., Khachatryan Z.,
610 Avetisyan P., Badalyan R., Bobokhyan A., Melikyan V., Sargsyan G., Piliposyan A., Simonyan
611 H., Mkrtchyan R., Denisova G., Yepiskoposyan L., Willerslev E. & Allentoft M.E. (2017) Eight
612 millennia of matrilineal genetic continuity in the south Caucasus. *Current Biology* **27**, 2023-
613 2028. doi.org/10.1016/j.cub.2017.05.087

614 Martin M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing
615 reads. *EMBnet.Journal* **17**, 10. doi.org/10.14806/ej.17.1.200

616 Martiniano R., Coelho C., Ferreira M.T., Neves M. J., Pinhasi R. & Bradley D.G. (2014) Genetic
617 evidence of African slavery at the beginning of the trans-Atlantic slave trade. *Scientific Reports*
618 **4**, 5994. doi.org/10.1038/srep05994

619 Martiniano R., Caffell A., Holst M., Hunter-Mann K., Montgomery J., Müldner G., McLaughlin
620 R.L., Teasdale M.D., Van Rheenen W., Veldink J.H., van den Berg L.H., Hardiman O., Carroll
621 M., Roskams S., Oxley J., Morgan C., Thomas M.G., Barnes I., McDonnell C., Collins M.J. &
622 Bradley D.G. (2016) Genomic signals of migration and continuity in Britain before the Anglo-
623 Saxons. *Nature Communications* **7**, 10326. doi.org/10.1038/ncomms10326

624 Mathieson I., Lazaridis I., Rohland N., Mallick S., Patterson N., Roodenberg S.A., Harney E.,
625 Stewardson K., Fernandes D., Novak M., Sirak K., Gamba C., Jones E.R., Llamas B.,
626 Dryomov S., Pickrell J., Arsuaga J.L., de Castro J.M., Carbonell E., Gerritsen F., Khokhlov A.,
627 Kuznetsov P., Lozano M., Meller H., Mochalov O., Moiseyev V., Guerra M.A., Roodenberg J.,
628 Vergès J.M., Krause J., Cooper A., Alt K.W., Brown D., Anthony D., Lalueza-Fox C., Haak W.,
629 Pinhasi R. & Reich D. (2015) Genome-wide patterns of selection in 230 ancient Eurasians.
630 *Nature* **528**, 499. doi.org/10.1038/nature16152

631 Mathieson I., Alpaslan-Roodenberg S., Posth C., Szécsényi-Nagy A., Rohland N., Mallick S.,
632 Olalde I., Broomandkhoshbacht N., Candilio F., Cheronet O., Fernandes D., Ferry M.,
633 Gamarra B., Fortes G.G., Haak W., Harney E., Jones E., Keating D., Krause-Kyora B.,
634 Kucukkalipci I., Michel M., Mittnik A., Nägele K., Novak M., Oppenheimer J., Patterson N.,
635 Pfrengle S., Sirak K., Stewardson K., Vai S., Alexandrov S., Alt K.W., Andreescu R., Antonović
636 D., Ash A., Atanassova N., Bacvarov K., Gusztáv M.B., Bocherens H., Bolus M., Boroneanţ A.,
637 Boyadzhiev Y., Budnik A., Burmaz J., Chohadzhiev S., Conard N.J., Cottiaux R., Čuka M.,

638   Cupillard C., Drucker D.G., Elenski N., Francken M., Galabova B., Ganetsovski G., Gély B.,

639   Hajdu T., Handzhyiska V., Harvati K., Higham T., Iliev S., Janković I., Karavanić I., Kennett

640   D.J., Komšo D., Kozak A., Labuda D., Lari M., Lazar C., Leppek M., Leshtakov K., Vetro D.L.,

641   Los D., Lozanov I., Malina M., Martini F., McSweeney K., Meller H., Menđušić M., Mirea P.,

642   Moiseyev V., Petrova V., Price T.D., Simalcsik A., Sineo L., Šlaus M., Slavchev V., Stanev P.,

643   Starović A., Szeniczey T., Talamo S., Teschler-Nicola M., Thevenet C., Valchev I., Valentin F.,

644   Vasilyev S., Veljanovska F., Venelinova S., Veselovskaya E., Viola B., Virag C., Zaninović J.,

645   Zäuner S., Stockhammer P.W., Catalano G., Krauß R., Caramelli D., Zariņa G., Gaydarska B.,

646   Lillie M., Nikitin A.G., Potekhina I., Papathanasiou A., Borić D., Bonsall C., Krause J., Pinhasi

647   R. & Reich D. (2018) The genomic history of southeastern Europe. *Nature* **555**, 197.

648   doi.org/10.1038/nature25778

649   Meyer M. & Kircher M. (2010) Illumina sequencing library preparation for highly multiplexed

650   target capture and sequencing. *Cold Spring Harbor Protocols* **2010**, pdb-prot 5448. doi:

651   10.1101/pdb.prot 5448

652   Millett M. & Gowland R. (2015) Infant and child burial rites in Roman Britain: a study from East

653   Yorkshire. *Britannia* **46**, 171-189. doi.org/10.1017/S0068113X15000100

654   Mittnik A., Wang C.C., Pfrengle S., Daubaras M., Zariņa G., Hallgren F., Allmäe R.,

655   Khartanovich V., Moiseyev V., Tõrv M., Furtwängler A., Andrades Valtueña A., Feldman M.,

656   Economou C., Oinonen M., Vasks A., Balanovska E., Reich D., Jankauskas R, Haak W.,

657   Schiffels S. & Krause J. (2018) The genetic prehistory of the Baltic Sea region. *Nature*

658   *Communications* **9**, 442. doi.org/10.1038/s41467-018-02825-9

659   Neparáczki E., Maróti Z., Kalmár T., Kocsy K., Maár K., Bihari P., Nagy I., Fóthi E., Pap I.,

660   Kustár A., Pálfi G., Raskó I., Zink A. & Török T. (2018) Mitogenomic data indicate admixture

661   components of Central-Inner Asian and Srubnaya origin in the conquering Hungarians. *PLoS*

662   *ONE* **13**, e0205920. doi.org/10.1371/journal.pone.0205920

663   Okonechnikov K., Conesa A. & García-Alcalde F. (2015) Qualimap 2: advanced multi-sample

664   quality control for high-throughput sequencing data. *Bioinformatics* **32**, 292-294.

665   doi.org/10.1093/bioinformatics/btv566

666   Olalde I., Brace S., Allentoft M.E., Armit I., Kristiansen K., Booth T., Rohland N., Mallick S.,

667   Szécsényi-Nagy A., Mittnik A., Altena E., Lipson M., Lazaridis I., Harper T.K., Patterson N.,

668   Broomandkhoshbacht N., Diekmann Y., Faltyskova Z., Fernandes D., Ferry M., Harney E., de

669 Knijff P., Michel M., Oppenheimer J., Stewardson K., Barclay A., Alt K.W., Liesau C., Ríos P.,

670 Blasco C., Miguel J.V., García R.M., Fernández A.A., Bánffy E., Bernabò-Brea M., Billoin D.,

671 Bonsall C., Bonsall L., Allen T., Büster L., Carver S., Navarro L.C., Craig O.E., Cook G.T.,

672 Cunliffe B., Denaire A., Dinwiddy KE., Dodwell N., Ernée M., Evans C., Kuchařík M., Farré JF.,

673 Fowler C., Gazenbeek M., Pena R.G., Haber-Uriarte M., Haduch E., Hey G., Jowett N.,

674 Knowles T., Massy K., Pfrengle S., Lefranc P., Lemercier O., Lefebvre A., Martínez C.H.,

675 Olmo V.G., Ramírez A.B., Maurandi J.L., Majó T., McKinley J.I., McSweeney K., Mende B.G.,

676 Modi A., Kulcsár G., Kiss V., Czene A., Patay R., Endrődi A., Köhler K., Hajdu T., Szeniczey

677 T., Dani J., Bernert Z., Hoole M., Cheronet O., Keating D., Velemínský P., Dobeš M., Candilio

678 F., Brown F., Fernández R.F., Herrero-Corral A.M., Tusa S., Carnieri E., Lentini L., Valenti A.,

679 Zanini A., Waddington C., Delibes G., Guerra-Doce E., Neil B., Brittain M., Luke M., Mortimer

680 R., Desideri J., Besse M., Brücken G., Furmanek M., Hałuszko A., Mackiewicz M., Rapiński A.,

681 Leach S., Soriano I., Lillios K.T., Cardoso J.L., Parker Pearson M., Włodarczak P., Price T.D.,

682 Prieto P., Rey P.J., Risch R., Rojo Guerra M.A., Schmitt A., Serralongue J., Silva A.M.,

683 Smrčka V., Vergnaud L., Zilhão J., Caramelli D., Higham T., Thomas M.G., Kennett D.J.,

684 Fokkens H., Heyd V., Sheridan A., Sjögren K.G., Stockhammer P.W., Krause J., Pinhasi R.,

685 Haak W., Barnes I., Lalueza-Fox C. & Reich D. (2018) The Beaker phenomenon and the

686 genomic transformation of northwest Europe. *Nature* **555**, 190-196.

687 doi.org/10.1038/nature25738

688 Olivieri A., Sidore C., Achilli A., Angius A., Posth C., Furtwängler A., Brandini S., Capodiferro

689 M.R., Gandini F., Zoledziewska M., Pitzalis M., Maschio A., Busonero F., Lai L., Skeates R.,

690 Gradoli M.G., Beckett J., Marongiu M., Mazzarello V., Marongiu P., Rubino S., Rito T.,

691 Macaulay V., Semino O., Pala M., Abecasis G.R., Schlessinger D., Conde-Sousa E., Soares

692 P., Richards M.B., Cucca F. & Torroni A. (2017) Mitogenome diversity in Sardinians: a genetic

693 window onto an island's past. *Molecular Biology and Evolution* **34**, 1230-1239.

694 doi.org/10.1093/molbev/msx082

695 Pala M., Olivieri A., Achilli A., Accetturo M., Metspalu E., Reidla M., Tamm E., Karmin M.,

696 Reisberg T., Kashani B.H., Perego U.A., Carossa V., Gandini F., Pereira J.B., Soares P.,

697 Angerhofer N., Rychkov S., Al-Zahery N. Carelli V., Sanati M.H., Houshmand M., Hatina J.,

698 Macaulay V., Pereira L., Woodward S.R., Davies W., Gamble C., Baird D., Semino O., Villems

699 R., Torroni A. & Richards M.B. (2012) Mitochondrial DNA signals of late glacial recolonization

700 of Europe from Near Eastern refugia. *American Journal of Human Genetics* **90**, 915-924.

701 doi.org/10.1016/j.ajhg.2012.04.003

702 Peltzer, A., Jäger, G., Herbig, A., Seitz, A., Kniep, C., Krause, J., & Nieselt, K. (2016). EAGER:
703   efficient ancient genome reconstruction. *Genome Biology* **17**, 60. doi.org/10.1186/s13059-016-
704   0918-z

705 Pereira, L., Freitas, F., Fernandes, V., Pereira, J.B., Costa, M.D., Costa, S., Máximo, V.,
706   Macaulay, V., Rocha, R. & Samuels, D.C., 2009. The diversity present in 5140 human
707   mitochondrial genomes. *American Journal of Human Genetics*, **84**, 628-640.
708   doi.org/10.1016/j.ajhg.2009.04.013

709 Pereira J.B., Costa M.D., Vieira D., Pala M., Bamford L., Harich N., Cherni L., Alshamali F.,
710   Hatina J., Rychkov S., Stefanescu G., King T., Torroni A., Soares P., Pereira L. & Richards
711   M.B. (2017) Reconciling evidence from ancient and contemporary genomes: a major source
712   for the European Neolithic within Mediterranean Europe. *Proceedings of the Royal Society B:*
713   *Biological Sciences* **284**. doi.org/10.1098/rspb.2016.1976

714 Pinhasi R., Fernandes D., Sirak K., Novak M., Connell S., Alpaslan-Roodenberg S., Gerritsen
715   F., Moiseyev V., Gromov A., Raczky P., Anders A., Pietrusewsky M., Rollefson G., Jovanovic
716   M., Trinhhoang H., Bar-Oz G., Oxenham M., Matsumura H. & Hofreiter M. (2015) Optimal
717   ancient DNA yields from the inner ear part of the human petrous bone. *PLoS ONE* **10**,
718   e0129102. doi.org/10.1371/journal.pone.0129102

719 Raule N., Sevini F., Li S., Barbieri A., Tallaro F., Lomartire L., Vianello D., Montesanto A.,
720   Moilanen J.S., Bezrukov V., Blanché H., Hervonen A., Christensen K., Deiana L., Gonos E.S.,
721   Kirkwood T.B., Kristensen P., Leon A., Pelicci P.G., Poulain M., Rea I.M., Remacle J., Robine
722   J.M., Schreiber S., Sikora E., Eline Slagboom P., Spazzafumo L., Antonietta Stazi M.,
723   Toussaint O., Vaupel J.W., Rose G., Majamaa K., Perola M., Johnson T.E., Bolund L., Yang
724   H., Passarino G. & Franceschi C. (2014) The co-occurrence of mt DNA mutations on different
725   oxidative phosphorylation subunits, not detected by haplogroup analysis, affects human
726   longevity and is population specific. *Aging Cell* **13**, 401-407. doi.org/10.1111/acel.12186

727 Redfern R.C. & DeWitte S.N. (2011) A new approach to the study of Romanization in Britain: a
728   regional perspective of cultural change in late Iron Age and Roman Dorset using the Siler and
729   Gompertz–Makeham models of mortality. *American Journal of Physical Anthropology* **144**,
730   269-285. doi.org/10.1002/ajpa.21400

731 Reimer P.J., Bard E., Bayliss A., Beck J.W., Blackwell P.G., Bronk Ramsey C., Buck C.E.,
732   Cheng H., Edwards R.L., Friedrich M., Grootes P.M., Guilderson T.P., Haflidason H., Hajdas

733 I., Hatté C., Heaton T.J., Hoffmann D.L., Hogg A.G., Hughen K.A., Kaiser K.F., Kromer B.,

734 Manning S.W., Niu M., Reimer R.W., Richards D.A., Scott E.M., Southon J.R., Staff R.A.,

735 Turney C.S.M. & van der Plicht J. (2013) IntCal13 and Marine13 radiocarbon age calibration

736 curves 0-50,000 years cal BP. *Radiocarbon* **55**, 1869-1887.

737 doi.org/10.2458/azu_js_rc.55.16947P

738 Renaud G., Slon V., Duggan A.T. & Kelso J. (2015) Schmutzi: estimation of contamination and

739 endogenous mitochondrial consensus calling for ancient DNA. *Genome Biology* **16**, 224.

740 doi.org/10.1186/s13059-015-0776-0

741 Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., &

742 Mesirov, J. P. (2011). Integrative genomics viewer. *Nature Biotechnology*, **29**, 24. doi:

743 10.1038/nbt.1754

744 Saag L., Varul L., Scheib C.L., Stenderup J., Allentoft M.E., Saag L., Pagani L., Reidla M.,

745 Tambets K., Metspalu E., Kriiska A. Willerslev E., Kivisild T. & Metspalu M. (2017) Extensive

746 farming in Estonia started through a sex-biased migration from the Steppe. *Current Biology* **27**,

747 2185-2193. doi.org/10.1016/j.cub.2017.06.022

748 Saville A. & Hallén Y. (1994) The 'Obanian Iron Age': human remains from the Oban cave sites,

749 Argyll, Scotland. *Antiquity* **68**, 715-723. doi.org/10.1017/S0003598X00047426

750 Schubert M., Ginolhac A., Lindgreen S., Thompson J.F., Al-Rasheid K.A., Willerslev E., Krogh

751 A. & Orlando L. (2012) Improving ancient DNA read mapping against modern reference

752 genomes. *BMC Genomics* **13**, 178. doi.org/10.1186/1471-2164-13-178

753 Schubert M., Lindgreen S. & Orlando L. (2016) AdapterRemoval v2: rapid adapter trimming,

754 identification, and read merging. *BMC Research Notes* **9**, 88. doi.org/10.1186/s13104-016-

755 1900-2

756 Schuenemann V.J., Peltzer A., Welte B., van Pelt W.P., Molak M., Wang C.C., Furtwängler A.,

757 Urban C., Reiter E., Nieselt K., Teßmann, B., Francken M., Harvati K., Haak W., Schiffels S. &

758 Krause J. (2017) Ancient Egyptian mummy genomes suggest an increase of Sub-Saharan

759 African ancestry in post-Roman periods. *Nature Communications* **8**, 15694.

760 doi.org/10.1038/ncomms15694

761 Shapland F., Sinfield L. and McSweeney K. (2016) High Pasture Cave Human Remains. In:

762 Birch, S., Cruickshanks, G. and McKenzie, J. (forthcoming) *High Pasture Cave: Ritual,*

763 *Memory and Identity in the Iron Age of Skye*. Oxbow Books

764 Skoglund P., Storå J., Götherström A. & Jakobsson M. (2013) Accurate sex identification of
765   ancient human remains using DNA shotgun sequencing. *Journal of Archaeological Science*
766   **40**, 4477-4482. doi.org/10.1016/j.jas.2013.07.004

767 Soares, P., Ermini, L., Thomson, N., Mormina, M., Rito, T., Röhl, A., Salas, A., Oppenheimer,
768   S., Macaulay, V. & Richards, M.B., 2009. Correcting for purifying selection: an improved
769   human mitochondrial molecular clock. *American Journal of Human Genetics*, **84**, 740-759.
770   doi.org/10.1016/j.ajhg.2009.05.001

771 Thorvaldsdóttir H., Robinson J.T., Mesirov J.P. (2013) Integrative Genomics Viewer (IGV): high-
772   performance genomics data visualization and exploration. *Briefings in Bioinformatics* **14**, 178-
773   192. doi.org/10.1093/bib/bbs017

774 Tucker F.C. (2010) *Woven into the Stuff of Other Men's Lives: The Treatment of the Dead in*
775   *Iron Age Atlantic Scotland.* PhD thesis, University of Bradford.

776 Waldron T., Taylor G.M. & Rudling D. (1999) Sexing of Romano-British baby burials from the
777   Beddingham and Bignor villas. *Sussex Archaeological Collection* **137**, 71-79.

778 Weissensteiner H., Pacher D., Kloss-Brandstätter A., Forer L., Specht G., Bandelt H.-J.,
779   Kronenberg F., Salas A. & Schönherr S. (2016) HaploGrep 2: mitochondrial haplogroup
780   classification in the era of high-throughput sequencing. *Nucleic Acids Research* **44**, W58-W63.

781 Willerslev E. & Cooper A. (2005) Ancient DNA. *Proceedings of the Royal Society B: Biological*
782   *Sciences* **272**, 3-16. doi.org/10.1098/rspb.2004.2813

783 Yang D.Y., Eng B., Waye J.S., Dudar J.C. & Saunders S.R. (1998) Improved DNA extraction
784   from ancient bones using silica-based spin columns. *American Journal of Physical*
785   *Anthropology* **105**, 539-543. doi.org/10.1002/(SICI)1096-8644(199804)105:4<539::AID-
786   AJPA10>3.0.CO;2-1

787 Yang Z. (1997). PAML: a program package for phylogenetic analysis by maximum likelihood.
788   *Bioinformatics*. **13**. 555–556. 10.1093/bioinformatics/13.5.555