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# Supplementary Information

## A high-resolution picture of kinship practices in an Early Neolithic tomb

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### **Genotype dataset provided as Supplementary Data file**

## 26 **SI Section 1: Osteological summary of human remains from Hazleton North**

27 The human skeletal remains from Hazleton North (Extended Data Figure 1) were first reported  
28 on as part of the excavation report in 1990<sup>1</sup>, and subsequently re-examined in 2016 as part of a  
29 PhD thesis on the burials within long barrows in southern Britain<sup>2</sup>. This summary updates the  
30 published osteological report with the findings of the 2016 thesis. Supplementary Table 1 details  
31 the genetic and osteological information together with the location of the excavated human  
32 remains found within the monument for those individuals that have been sampled for aDNA,  
33 while Supplementary Table 2 details information on all 19 individuals that are distinguishable  
34 from one another on an osteological basis, whether or not they provided aDNA for the current  
35 publication. Extended Data Table 1 supplies a summary of the key osteological data and  
36 biological relationships for each of the 35 individuals successfully sampled. As some ancient  
37 DNA samples were taken from loose teeth there is little osteological information for them, while  
38 in other cases the skeletal element sampled derived from a largely-complete skeleton (e.g.  
39 Skeleton 1). It should be noted that this is not the minimum number of individuals (MNI)  
40 identified from the site: a minimum of 41 individuals can be identified from within the tomb, of  
41 which 22 were adults, two were juveniles, ten were children between 3 and 12 years of age and  
42 seven were infants under three years of age. Eighteen individuals were excavated from the  
43 northern side of the tomb (nine adults, three children, and six infants) and 23 from the southern  
44 side (13 adults, two juveniles, seven children, and one infant). One of the adults, one child and  
45 one infant in the northern entrance were cremated. An additional eight fragments of human  
46 remains from a minimum of two individuals were located in the south quarry (from which stone  
47 was sourced to build the cairn). The individuals from the quarry would bring the total MNI from  
48 the excavations at the site as a whole to 43.

49 The MNI assessment for the adults was based on the presence of the right calcaneus, plus one  
50 cremated adult. The MNI for children was based on the dentition from six mandibles (two from  
51 the northern side of the tomb and four from the southern side) and one maxilla (from the  
52 southern side). The MNI of two for juveniles located in the southern side of the monument was  
53 based on the presence of two axis bones. The MNI for the infants was based on different  
54 elements of varying ages: the left ischium of a neonate (from the northern side); the mandible of  
55 a nine month old infant (from the northern side); two unfused *pars basilaris* bones from an  
56 individual aged 18 months and one aged two years and three months at death (both from the  
57 northern side); two radii from individuals aged three months old and one aged between two and a  
58 half and three years of age at death (one from the northern side and one from the southern).

59 The sex of the individuals for whom we successfully obtained ancient DNA was inferred from  
60 the genetic data itself. The osteological determination of sex from the assemblage was based on  
61 the right ox coxae (as the most reliable indicator of sex in the skeleton), which was only able to  
62 be identified in five males and one probable male, and three females. The aDNA identification of  
63 sex adds considerably to the number of individuals for whom biological sex can be determined,  
64 as follows:

65  
66 Osteology North Side: Adults (9), juvenile (--), children (3), infants (6), male (5), female (2),  
67 **total (18).**

68 Genetics North Side: Adults (n/a), juvenile (n/a), children (n/a), infants (n/a), male (11), female  
69 (3), **total (14).**

70 Osteology South Side: Adults (13), juvenile (2), children (7), infants (1), male (8), female (1),  
71 **total (23).**

72 Genetics South Side: Adults (n/a), juvenile (n/a), children (n/a), infants (n/a), male (15), female  
73 (6), **total (21).**

74 The genetic results include seven males and three females from the north chamber, four males  
75 from the north entrance, eight males and five females from the south chamber, four males from  
76 the south passage, and three males and one female from the south entrance.

77 There is no reliable way to assess the original number of individuals whose remains were  
78 interred in a tomb on the basis of an osteological assessment of MNI alone. Following Monte  
79 Carlo simulations of taphonomy, Robb concluded that ‘a tomb assemblage containing 50 people  
80 could equally well represent 50, 100 or 1000 original depositions’<sup>3</sup>. Identifying individuals using  
81 ancient DNA offers new possibilities for assessing whether the tomb population was originally  
82 similar to the minimum number of individuals or much higher. In the case of the current study,  
83 the number of individuals identified as genetically distinct is not far below the osteological MNI  
84 (see above), even though we have not been able to obtain results for all individuals identified as  
85 distinct on the basis of osteological assessment. This requires careful interpretation given the  
86 different methods used to reach these numbers. The genetic evidence comes from 17 petrous  
87 portions of the temporal bone, 45 teeth (some of which were ‘loose’ in the chambered areas  
88 when found; maxillary canines were preferentially selected from among these to reduce the  
89 chance of duplicate genetic results on the same individual), and four other bone elements,  
90 whereas MNI estimates drew on a wider range of skeletal elements. The genetic sample does not  
91 necessarily cover all of the individuals identified as distinct on the basis of osteology *and*  
92 includes individuals whose remains might not be present among those used to calculate the  
93 osteological MNI (particularly where ‘loose’ teeth were sampled). Nonetheless, the comparison  
94 suggests the genetic sample provides good coverage of the number of individuals whose remains  
95 survived within the tomb: in particular, there are genetic results for 21 individuals from the south  
96 side for which osteological analysis suggests an MNI of 23. The real number of individuals is  
97 likely higher than 23, but since the 21 distinct genetic individuals were identified from 39  
98 samples yielding successful results from the south side, including loose teeth, it is possible that  
99 the actual number of individuals whose remains are present in the tomb overall may be not  
100 significantly higher than the MNI: that is, tens rather than hundreds. Analysis of a later tomb in  
101 Iberia suggests that only teeth survived from an early phase of use while the surviving bones  
102 derived from later individuals<sup>4</sup>. In such a case a calculation of MNI based on bone would

103 overlook earlier individuals, but in that particular tomb there was a significant span of time  
104 between the dates obtained from teeth and those from bone. Although the three aDNA samples  
105 from first generation individuals at Hazleton North were all from teeth, skeletal remains do exist  
106 for two of them, and while we have not carried out an equivalent analysis to Aranda Jiménez et  
107 al., there is no apparent gap between the dates from teeth and those from bone (SI section 4).  
108 There is no clear indication that the chambered areas were used prior to the introduction of the  
109 individuals assessed in this study, and, indeed, the combination of the excavator's assessment of  
110 the sequence of site construction and use, the chronological models, and the patterning we have  
111 identified in the placement of individuals from the two branches of the lineage on either side of  
112 the monument, suggests that a previous use of the monument for remains that have decayed  
113 completely or have been removed is unlikely.

114

#### 115 *Mortuary transformation and taphonomy*

116 Mortuary practices vary slightly on either side of the tomb, but discrete deposits of bones from  
117 specific individuals are evident in both cases. These do not seem to have been significantly  
118 disturbed following deposition: although the excavators' analysis of this was limited, conjoining  
119 bones from the same individual were all kept within the same compartments in the north  
120 chambered area, and a similar picture is evident in the southern chambered area where only a  
121 few bones from the south chamber conjoined or were paired with bones in the south passage or  
122 entrance<sup>1</sup>. Among the sampled remains, skeletal elements belonging to SC3m, SC6m and SC8m  
123 were found both in the south chamber and in the south passage, while the predominance of bones  
124 from SE4m were found at the rear of the entrance with one in the passage, suggesting some  
125 further displacement of remains within the south chambered area. There are no recorded  
126 instances of bones from the south chambered area combining with those from the north  
127 chambered area, and we detected no genetic duplicates between the south and north chambered  
128 areas, suggesting that remains were not moved between the two burial zones.

129 Fifty-one bone elements from more than five individuals in the north chambered area had been  
130 gnawed by canids when the bone was fresh, attested by helical fractures, longitudinal fractures,  
131 gnaw marks on the diaphyses, some crenulated edges (especially on the ribs), and tooth furrows<sup>5-  
132 7</sup>. This suggests that some individuals were left exposed to the elements where the bodies were  
133 scavenged (excarnation). Most of the affected bone elements could not be assigned to specific  
134 individuals, but osteologically defined individuals A, C, G and H (i.e. NE2m(A), NC2f(C),  
135 NC5m(G) and NC6m(H)), all from the northern chambered area, bear clear signs of canid  
136 gnawing (e.g. Extended Data Figure 2). Scavenging by canids was not identified in the original  
137 osteological report<sup>8</sup>.

138 Individual G (NC5m), a child of between three and four years of age at death, exhibited not only  
139 gnawing by canids on the distal end of the right humerus but also signs of weathering on the  
140 bone, suggesting a period of exposure to heat, cold, wet or dry environments<sup>9</sup>. Individual C  
141 (NCf2), a young female aged less than 24 years at death, exhibited extensive gnawing to all of

142 the long bones present and to four of the left tarsals. Furthermore, the right humerus had been  
143 extensively chewed at both ends and on the shaft, and exhibited a helical fracture on the  
144 proximal end. The right tibia exhibited extensive damage to the proximal end in the form of  
145 crenulated edges, and clear signs of chewing and puncture marks were present on the right  
146 femoral head together with gnawing of the greater and lesser trochanters. Significantly, all of the  
147 long bones were weathered and match the description of those detailed in Stage 1 of  
148 Behrensmeyer's scheme<sup>9</sup>, equating to a period of exposure of between a few days, and up to  
149 three years. While all of the limbs are present, the proximal and distal ends of some long bones  
150 were chewed including the right femoral head which must therefore have been disarticulated  
151 from the pelvis. Research<sup>10</sup> on the effects of human remains subjected to scavenging by dogs  
152 suggests that if this stage of disarticulation has been reached, a period of not more than a year  
153 (between two and eleven months) has passed since the body was exposed to faunal activity.  
154 Thus, we can infer a period in which canids had access to the remains before they were collected  
155 and deposited in the tomb (access to the tomb was blocked by stone slabs which were  
156 presumable replaced each time a set of remains was added<sup>1</sup>). Only three bones showing signs of  
157 canid gnawing, representing at least two individuals (an adult and a child), were excavated from  
158 the southern chambered area (adult rib 4805 and clavicle 10499 in the entrance, and child's tibia  
159 11438 from the chamber).

160 Remains deposited in the northern chambered area were more likely to have been exposed to the  
161 elements than in the south: 51 bone fragments in the north versus 3 in the south. Cremated  
162 remains from at least three individuals — one adult of unknown sex, one child of unknown age  
163 and one infant of unknown age — were concentrated at the north entrance, where over 187  
164 fragments were recovered. By contrast, only 26 fragments of cremated bone were found in the  
165 south chambered area<sup>8</sup>. Some differences are therefore detectable in the treatment of human  
166 remains prior to deposition in the north compared with the south chambered area.

167 The range of treatments of human remains at Hazleton North is not particularly unusual in the  
168 context of other Early Neolithic sites in southern Britain, although these display a notable  
169 diversity in mortuary practices<sup>11</sup>. The introduction of intact or almost intact corpses into  
170 chambers is now suspected for many Cotswolds tombs, with varying degrees of disturbance or  
171 deliberate movement of the remains during successive activity within chambers, including the  
172 placement of further human remains. Evidence of canid gnawing is known from a few sites, most  
173 notably Adlestrop in the Cotswolds, where they are interpreted as evidence of the exposure of  
174 remains prior to selective inclusion in the chamber<sup>12</sup>. Cremated remains are attested in small  
175 numbers at several long barrows<sup>2,11</sup>. Tool marks from cutting flesh from bones or deliberately  
176 disarticulating body parts was not detected at Hazleton North but is known elsewhere, including  
177 Adlestrop<sup>13</sup>. Finally, the remains of the Early Neolithic dead were not only placed in stone  
178 chambered tombs in southern Britain. They are also found in wooden chambers covered by  
179 earthen mounds, in caves, in pit graves, in the ditches of causewayed enclosures, and  
180 occasionally in features associated with occupation<sup>11</sup>.

181

182 *Health, pathology and trauma*

183 Six individuals suffered from conditions suggesting periods of poor nutrition. Four children  
184 suffered from scurvy, including SC6m and SC9f, both born into the core lineage in the third and  
185 fourth generation, and two not yet sampled for aDNA (an infant from the north entrance and a  
186 nine-month old from the north chamber). Scurvy is caused by a lack of vitamin C in the diet,  
187 which is crucial to combat infections, allow the normal development of all bodily tissues, in  
188 particular collagen, and to facilitate iron absorption<sup>14</sup>. SC6m and SC9f did not survive past six  
189 and nine years of age respectively. Two adults suffered from both cribra orbitalia (CO) and  
190 porotic hyperostosis (PH), which are conditions considered to be non-specific indicators of  
191 physiological stress. One was SC10f (Individual viii), who was not born into the lineage and for  
192 which we have no evidence of bearing lineage children; another was NC2f (Individual C), whose  
193 first-generation union with NC1m was key to the foundation of a maternal sub-lineage within the  
194 patriline. These conditions are usually found in children, and where evidence of the lesions  
195 caused by CO or PH are found in adults it is a relic of childhood<sup>15-17</sup>. Lack of vital nutrients,  
196 such as vitamin B12, vitamin B9, and Vitamin C (in the case of CO), together with a lack of  
197 animal protein in the mother's diet, are all passed on in breastmilk causing megaloblastic  
198 anaemia in small children. Furthermore, poor sanitary living conditions and weaning foods  
199 lacking in sufficient dietary value contribute to the development of these conditions<sup>16</sup>. The  
200 presence of scurvy in four individuals is significant, as this condition is rarely reported in  
201 assemblages of this period. Cuthbert's study of human remains from 42 Neolithic long barrows  
202 in southern Britain found that three sites had evidence of scurvy, and that 4 out of 6 (67%) of the  
203 cases were from Hazleton North<sup>2</sup>; an additional possible case of scurvy was noted at West Tump  
204 long barrow, Gloucestershire by Smith and Brickley<sup>13</sup>. The prevalence of CO and PH at Hazleton  
205 North was low compared to other sites of the same period<sup>2</sup>.

206 The cases of poor nutrition need to be set alongside Carbon and Nitrogen stable isotope analyses  
207 on 22 human femurs from the tomb (16 from adults, 6 from subadults) which indicate that all  
208 sampled individuals consumed a very high level of animal protein consistent with a meat-rich  
209 diet<sup>18</sup>, and a proteomic analysis of dental calculus on four individuals from the tomb which  
210 suggests the ingestion of processed bovine milk products<sup>19</sup>. This suggests that diets in this  
211 community were generally similar to those in the wider region, where animal protein formed a  
212 substantial dietary contribution and there is good evidence for the use of dairy products<sup>20</sup>.

213 The assemblage as a whole exhibited a high prevalence of dental disease. Three adults detailed in  
214 Supplementary Table 1 exhibited evidence of periodontal disease (PD), a condition caused by the  
215 inflammation of the soft tissues surrounding the tooth, forming a pocket where bacteria  
216 proliferate<sup>21</sup>. Without treatment, the alveolar bone will become infected and reabsorption occurs,  
217 culminating in the loss of the affected tooth<sup>22</sup>. Two further individuals also had PD but were not  
218 tested for aDNA. PD is the most common cause of ante-mortem tooth loss (AMTL)<sup>23</sup>, a  
219 condition that was exhibited by six individuals sampled for aDNA analysis (Supplementary

220 Table 1). A further six individuals in the assemblage suffered from ATML. Attrition (wearing) of  
221 the dentition was evident in eight individuals and is generally associated with advancing old age,  
222 a coarse diet, or technological activities<sup>24</sup>. Nine other individuals who are not featured in  
223 Supplementary Table 1 also suffered from attrition. Dental abscesses were exhibited by four  
224 individuals and are caused by bacteria entering the pulp cavity of a tooth, causing inflammation  
225 and a build-up of pus. When the pressure in the jaw becomes excessive, a hole forms, allowing  
226 the pus to escape<sup>25</sup>. A further seven individuals from the tomb also suffered from dental  
227 abscesses. Calculus, dental plaque that is not removed and becomes mineralized<sup>26</sup>, was present  
228 on eight individuals for whom we definitely obtained genetic data (listed in Supplementary Table  
229 1) and on a further five individuals and reflects lack of dental hygiene. Dental caries were  
230 present on three loose teeth excavated from the monument, but could not be assigned to a  
231 particular individual. The incidence of these particular dental pathologies is greater at Hazleton  
232 North than other long barrows from southern Britain<sup>2</sup>.

233 At least six individuals exhibited evidence of osteoarthritis (OA). Four mature adult male  
234 individuals from Supplementary Table 1 exhibited signs of the condition. OA only affects the  
235 synovial joints of the skeleton, where the degeneration of the articular cartilage results in  
236 subchondral changes which affect the efficacy of the joint<sup>8</sup>. Two individuals in particular had  
237 widespread osteoarthritis. One had OA of the left and right sternoclavicular joints, the spine, left  
238 and right shoulder and left wrist, and the other had OA of the spine, right hip, right knee and  
239 right foot. The other two individuals did not have as many joints affected by the condition: one  
240 had OA of the left temporomandibular joint, and the other had OA of the right shoulder and  
241 spine. OA was also evident on further individuals from the monument. An adult female  
242 (Individual F), had the condition in the right sternoclavicular joint, both shoulders, the spine and  
243 left foot. Several disarticulated bones, which could not be allocated to any particular individual,  
244 also exhibited osteoarthritic changes: another case of OA of the temporomandibular joint; 4  
245 vertebrae; six carpals and three finger bones; two further cases of OA of the hip; and one more of  
246 the knee. The prevalence of osteoarthritis in the assemblage as a whole is high compared to other  
247 sites of a comparable nature<sup>2</sup>. Evidence of OA was found in some individuals covering all joints  
248 across the assemblage except for the elbow. The difference is notable when comparing the  
249 Hazleton North remains with those from West Kennet, which has a similar MNI of 42 yet has far  
250 less evidence of the disease. The aetiology of OA is unclear but may be linked to age, activity,  
251 obesity, or trauma, among other factors<sup>21</sup>.

252 The re-analysis of the assemblage confirmed the diagnosis of Diffuse Idiopathic Skeletal  
253 Hyperostosis (DISH) for one individual, together with evidence of septic arthritis in the left foot.  
254 Both conditions are associated with individuals who also have diabetes<sup>27,28</sup>. Another individual  
255 exhibited two lesions on the anterior surface of the left humerus which may be indicative of a  
256 benign cartilaginous tumour called a chondroblastoma, but a differential diagnosis could include  
257 a simple cyst or tuberculosis. He also exhibited a lesion on the left navicular, a circulatory  
258 disorder condition called osteochondritis dissecans (OD) which only affects synovial joints,  
259 eroding a small area of the subchondral bone and the overlying cartilage<sup>29</sup>. Three further



260 examples of the condition were found on three disarticulated bones (a right trapezium, an axis,  
261 and the glenoid fossa of a left scapula), that could not be assigned to a particular individual. The  
262 presence of at least three individuals with OD is high compared to other contemporary sites in  
263 southern Britain: one case was found at Haddenham long barrow, Cambridgeshire, and at least  
264 one at Rodmarton long barrow in the Cotswolds<sup>2</sup>.

265 There were four cases of infectious disease among the assemblage, one of which came from an  
266 individual for whom we obtained aDNA (SE4m(D)). Three disarticulated lower limb bones (a  
267 right fibula, a right tibia and a left femur) from the north chamber all exhibited periosteal new  
268 bone formation on the diaphysis. Periosteal new bone formation is caused by inflammation of the  
269 periosteal membrane that covers the outside of the bone, resulting in an area of new woven bone  
270 that will eventually remodel into hardened lamellar bone<sup>30</sup>. However, the presence of periosteal  
271 new bone on an element may not always be an indication of an infectious process, but can also  
272 be linked to trauma, cancer, tearing events, or stretching<sup>31,32</sup>. A possible case of a viral infection  
273 was detected in Individual D (SE4m). This individual exhibited signs of poliomyelitis, a virus  
274 that is spread inter-personally by infected faeces and which causes permanent limb paralysis<sup>33,34</sup>.  
275 His skeleton has a noticeably more gracile forearm on the right side than on the left, and the limb  
276 has suffered disuse atrophy due to the underdevelopment of the muscles.

277 Two individuals had minor congenital disorders of the vertebral column, which is formed from  
278 three different structures. One adult from the south chamber had Klippel-Feil Syndrome, where  
279 two vertebrae fuse together (the second and third cervical vertebrae in this case)<sup>35</sup>. This condition  
280 is not life-threatening but may reduce the length of the neck and impair movement<sup>36</sup>. Individual  
281 D (SE4m, who may also have suffered from polio) had a unilateral and unsymmetrical  
282 sacralization of the fifth lumbar vertebra. This condition occurs when the fifth lumbar vertebra is  
283 fused to the sacrum, thereby reducing the lumbar spine by one vertebral body and increasing the  
284 height of the sacrum<sup>37</sup>. The fact that the fifth lumbar vertebra was unfused on the right side of the  
285 sacrum would have caused the bone to lean to the left, thereby causing the spine to twist  
286 (scoliosis). No vertebrae were recovered from this individual to confirm this, but the association  
287 of scoliosis with poliomyelitis has been attested<sup>38</sup>. Reported cases of poliomyelitis for the  
288 Neolithic period are very rare and only one other example of the disease has been described for  
289 an individual found at a causewayed enclosure at Cissbury, West Sussex<sup>39</sup>. Only one other  
290 example of sacralization of the fifth lumbar vertebrae has been noted among Cotswold long  
291 barrows, at Lanhill<sup>2</sup>.

292 Individuals A, D, F and Skeletons 1 and 2 (i.e., NE2m(A), SE4m(D), NC3f(F), NE4m(1) and  
293 NE1m(2)) had more severe pathology than others and may have been visibly and physically  
294 more frail than others: Individual A may have moved awkwardly due to DISH and septic arthritis  
295 in his foot; Individual D may have had polio and scoliosis of the spine, resulting in awkward  
296 movement; Individual F had severe OA in both shoulders, the spine, one sternoclavicular joint  
297 and the foot, and may have limped; Skeleton 1 had OA of the shoulder and spine, and a healed  
298 fracture of the fibula that may have caused a limp; and Skeleton 2 had widespread OA of the

299 spine and right lower limb, which would likely have affected movement. There is evidence of  
300 OA of the hip and knee at two other Neolithic sites which may have affected the movement of  
301 those suffering from the disease, but likely not to the extent of those from Hazleton. Similarly,  
302 two other individuals from different sites had evidence of a fractured fibula, but the individuals  
303 from Hazleton North appear to have had more serious and extensive conditions, which would  
304 likely have affected their range of movement<sup>2</sup>.

305 Traumata are evident on the remains of at least two individuals in Supplementary Table 1. One  
306 adult male had healing facial fractures<sup>40</sup> at the time of death, injuries which are often the result of  
307 assault<sup>41</sup>. Another adult male had a healed fracture to the left distal fibula, an injury associated  
308 with twisting and/or abduction<sup>42</sup>. A further individual from the assemblage, represented only by  
309 a right fibula, suffered a fracture to the neck and an avulsion fracture to the styloid process of the  
310 proximal shaft, resulting in remodelling and flattening of the head. Individual D had a well  
311 healed fracture to the right forearm (a parry fracture), often sustained by a direct blow to the  
312 forearm<sup>43</sup>. One example of a vertebral fracture was found, often sustained following vertical  
313 compression<sup>44</sup>. Three instances of soft tissue injury were found. A right fibula from the  
314 assemblage exhibited myositis ossificans traumatica. This condition occurs when a tendon or  
315 muscle attachment is injured, and the resulting haematoma calcifies then eventually ossifies,  
316 leaving an easily identifiable unorganised bony mass on the bone<sup>17</sup>. This condition was also  
317 found on a disarticulated left fifth metatarsal of an adult. A cortical defect was detected on a  
318 disarticulated left humerus of an adult, and is characterised by deep grooves on the proximal end  
319 of the bone where powerful muscles attach. This condition may be caused by several different  
320 actions such as repetitive stress on the muscles and trauma<sup>45</sup>. Whilst the fractures mentioned  
321 were not life-threatening (or uncommon), only two may have sustained trauma due to inter-  
322 personal violence, whilst the other cases were likely caused by accidents. The lack of evidence  
323 for trauma caused by inter-personal violence at Hazleton North is somewhat unusual: there is  
324 evidence of such trauma from one or more individuals from many of the Early Neolithic  
325 monuments in southern Britain, including perimortem blunt force trauma to the cranium, healed  
326 cranial trauma, and arrowheads lodged in bones<sup>2,11,13,46,47</sup>.

### 327 *Spatial summary*

328 The analysis of the human remains from Hazleton North reveal some differential mortuary  
329 treatments prior to deposition in the two sides of the tomb: individuals who were subjected to  
330 excarnation and cremation were predominately placed within the northern side of the monument.  
331 Those who suffered from the more severe health conditions (DISH, septic arthritis, widespread  
332 OA and other joint problems) were in the northern side of the monument with the exception of  
333 individual D (limb atrophy, possible polio, parry fracture) whose remains were placed in the  
334 south entrance where it met the passage. The majority of these were individuals who had lived  
335 into older adulthood. Four of seven cases of nutritional deficiency (57%) were interred within the  
336 northern side of the tomb but the majority of individuals with dental disease were placed within

337 the southern side which included a larger number of individuals overall. In general, there is no  
338 evidence for systematic differences in health among those buried on either side of the monument.

## 339 **SI Section 2: Genetic analysis of biological relatedness and family tree reconstruction**

### 340 **2.1. Introduction**

341 We took advantage of the high-quality genome-wide data generated for most individuals to study  
342 their biological relationships. We recovered information from the autosomes (chromosomes 1–  
343 22), which provide rich information about ancestry as they are a mosaic of DNA segments  
344 inherited from ancestors across the whole family tree. Therefore, our data allowed us to  
345 determine relationships among individuals through all lines of descent, unlike ancient DNA  
346 methodologies that predated the advent of Next-Generation technologies that only recover  
347 mitochondrial and/or Y-chromosome information and therefore only infer strictly matrilineal and  
348 patrilineal relationships, respectively. Our goal was to identify a unique family tree whose  
349 topology fits all types of genomic and anthropological evidence available for the Hazleton North  
350 individuals, while discarding all the other possible tree topologies that might be considered for  
351 relationships between these individuals. A summary of the process is as follows:

352 *-Section 2.2:* For each pair of individuals, we estimated the relatedness coefficients  $r$  that  
353 represents the fraction of the genome shared between 2 individuals. In the case of pairs identified  
354 as first-degree relatives ( $r \sim 0.5$ ), we also determined the type of relation (parent-offspring or  
355 siblings).

356 *-Section 2.3:* Using the pairwise degrees of relationship between all individuals, we followed a  
357 triangulation procedure to discard non-fitting tree topologies. To aid this process, we also  
358 incorporated information regarding the type of first-degree relationships, the mitochondrial and  
359 Y-chromosome lineages, the genetic evidence for inbreeding, and the age-at-death as determined  
360 through anthropological analysis. We arrived at two possible tree solutions fitting all the  
361 aforementioned pieces of evidence (Fig. 1c and Extended Data Fig. 4).

362 *-Section 2.4:* To disambiguate between the two possible tree topologies, we studied the co-  
363 localization of break points of shared DNA segments that inform about recombination events  
364 between the maternal and paternal chromosomes. This allowed us to obtain a unique family  
365 pedigree relating most of the Hazleton North individuals (Fig. 1c).

366 *-Section 2.5:* To further evaluate the validity of the proposed family tree structure, we used three  
367 lines of genetic evidence (not used in *sections 2.3 and 2.4*): 1) X-chromosome information which  
368 is completely independent from autosomal data, 2) number of shared segments between first and  
369 second-degree pairs, and 3) the software *NgsRelate v.2*<sup>48</sup> that estimates biological kinship using a  
370 different method as compared to that used in *section 2.2*.

### 371 **2.2. Estimation of pairwise relatedness coefficients ( $r$ )**

372 We began by estimating relatedness coefficients  $r$  that represents the fraction of the genome  
373 shared between 2 individuals. We estimated pairwise allelic mismatch rates in the autosomes<sup>49–51</sup>  
374 for each pair of libraries ( $n=156$ ) deriving from 66 different samples, randomly sampling one  
375 DNA sequence at each ‘1240k’ polymorphic position and masking the two terminal nucleotides

376 of each sequence to reduce the effects of post-mortem deamination. We then computed  
377 relatedness coefficients  $r$  for each pair (Supplementary Table 4):

378 
$$r = 1 - (2*(x-(b/2))/b)$$

379 with  $x$  being the mismatch rate of the pair under analysis and  $b$  the mismatch rate expected for  
380 two unrelated individuals from the same population. We also computed 95% confidence  
381 intervals using block jackknife standard errors over 5 Megabase (Mb) blocks<sup>52</sup>.

382 To estimate the constant  $b$ , we used genomic data from 53 Neolithic individuals from England,  
383 Scotland and Wales from previous publications<sup>53,54</sup>. This set (Supplementary Table 3) includes  
384 only individuals with the same type of data as our Hazleton North individuals (captured data and  
385 UDG-treated); individuals not identified as close relatives to others in previous publications; and  
386 individuals lacking recent Mesolithic hunter-gatherer admixture. This ensures that this set  
387 represents individuals with very similar ancestry background as Hazleton North individuals  
388 (Extended Data Fig. 9a). We then computed allelic mismatch rates for all pairwise comparisons  
389 between the 53 Neolithic individuals from Britain (1,378 pairs) and also comparisons between  
390 these 53 individuals and Hazleton samples (3,498 pairs; using only one library per sample).  
391 Including the comparisons between Hazleton samples (2,145 pairs; using only one library per  
392 sample) yields a total of 7021 comparisons, of which 4,528 had more than 100,000 overlapping  
393 SNPs. We computed the median mismatch rate among this set of 4,528 pairs (of which 1,280 are  
394 Hazleton-Hazleton pairs) and obtained a value of 0.2504 that we used to represent  $b$ , the value  
395 expected for unrelated pairs. Even if there were plenty of related pairs among the Hazleton-  
396 Hazleton pairs and plausibly some among Hazleton-Other Sites pairs (although close relatives  
397 across sites are extremely rare in the ancient DNA literature) or among the 53 Neolithic  
398 individuals from Britain that went undetected in previous publications, by using the median  
399 value we ensured that the lower mismatch rate in these related pairs had minimal impact on the  
400 estimate of mismatch rate in unrelated pairs. For these related pairs to have an impact in the  
401 median value, they would need to account for at least half of the 4,528 comparisons and this  
402 would imply more than a thousand closely related pairs across sites around Britain, which is  
403 exceptionally unlikely. Computing the median value using only across-site comparisons yielded  
404 a very similar value of 0.2507, and only for Hazleton-Hazleton pairs yields a slightly lower value  
405 of 0.2488, which could be affected by the presence of a large number of closely related pairs in  
406 Hazleton (see below). Therefore, we keep for analysis the value obtained using all comparisons  
407 (0.2504).

408 Using  $b=0.2504$ , we computed relatedness coefficients for all pairs ( $n=12,090$ ) of Hazleton  
409 libraries (Supplementary Table 4). A total of 105 pairs of libraries stemming from 44 pairs of  
410 samples had relatedness coefficients larger than 0.85, indicating that they share their entire  
411 genome and that they derived from the same individual. This is not surprising given that human  
412 remains in both chambers were commingled and given the large number of samples we analyzed.  
413 To increase resolution in the kinship analysis, we merged the data from samples deriving from  
414 the same individual (as well as data from libraries deriving from the same sample), keeping 35

415 unique individuals for further analysis. We gave a unique identifier to each of these 35  
416 individuals (Supplementary Table 1) based on their burial location and genetic sex (e.g., NC1m  
417 = male individual 1 from the north chamber), and use this identifier through the supplementary  
418 materials and main text.

419 We recomputed the mismatch rates and relatedness coefficients  $r$  on the merged dataset and  
420 annotated degrees of relationship (Supplementary Table 5 and Extended Data Fig. 2). Following  
421 a similar approach as in Monroy Kuhn *et al.* 2018<sup>55</sup>, we used cutoffs lying halfway between the  
422 expected relatedness coefficients for different degrees of genetic relationships: 1 for identical  
423 twins or samples deriving from the same individuals, 0.5 for first-degree relationships (parent-  
424 offspring and siblings), 0.25 for second-degree relationships (grandparent-grandchild,  
425 uncle/aunt-nephew/niece, half-siblings, double cousins), 0.125 for third-degree relatives (first  
426 cousins, great-grandparent-great-grandchild, half uncle/aunt-nephew/niece, etc) and 0.0625 for  
427 fourth-degree relationships. The cutoffs are the following:

428 -We annotated a pair as first-degree biological relatives if the 95% confidence interval of their  
429 relatedness coefficient overlapped the range (0.375-0.75].

430 -We annotated a pair as second-degree biological relatives if the 95% confidence interval of their  
431 relatedness coefficient overlapped the range (0.1875–0.375].

432 -We annotated a pair as third-degree biological relatives if the 95% confidence interval of their  
433 relatedness coefficient overlapped the range (0.09375–0.1875].

434 -We annotated a pair as fourth-degree or more distant biological relatives if the 95% confidence  
435 interval of their relatedness coefficient overlapped the range (0-0.09375].

436 -If the 95% confidence interval of the relatedness coefficient of a given pair overlapped more  
437 than one of these ranges, we annotated multiple degrees of relationships as possible for this pair.

438 -We annotated a pair as biologically unrelated within resolution if the 95% confidence interval of  
439 their relatedness coefficient overlapped 0.

440 A total of 8 individuals did not yield any close biological kin relationship to other Hazleton  
441 North individuals within the limits of our resolution (Extended Data Fig. 2). The remaining 27  
442 individuals were connected through close biological kinship relationships and were part of a  
443 large family.

444 Additionally, we determined the type of relationship (siblings or parent-offspring) connecting  
445 first-degree relatives based on uniparental markers (mtDNA and Y-chromosome) and the DNA  
446 sharing along the chromosomes: biological siblings present ~25% of the genome consistent with  
447 two chromosomes being identical by descent (IBD2), ~25% of the genome consistent with zero  
448 chromosomes being identical by descent (IBD0) and ~50% of the genome consistent with one  
449 chromosome being identical by descent (IBD1), whereas parent-offspring pairs share one  
450 chromosome across all the autosomal chromosomes. To analyse DNA sharing patterns along the  
451 chromosomes, we computed allelic mismatch rates patterns across sliding windows of 20 Mb,

452 moving by 1 Mb each step (Supplementary Table 6), and visually identified the presence or  
453 absence of regions with 0 or 2 chromosomes sharing for each first-degree relative pair with  
454 sufficient coverage. We illustrate this approach in Extended Data Fig. 3a and annotate the type of  
455 relationship for each first-degree pair (Supplementary Table 5).

### 456 **2.3. Family tree reconstruction**

457 In this section, we attempt to reconstruct the family tree relating 27 individuals from Hazleton  
458 North using the pairwise degrees of genetic relatedness (Extended Data Fig. 2) through a process  
459 of triangulation that allows us to discard most tree topologies relating these individuals. To aid  
460 this process, we also incorporated information regarding:

- 461 - The types of first-degree relationships (Supplementary Table 5).
- 462 - The mtDNA and Y-chromosome lineages transmitted through maternal and paternal lines  
463 (Supplementary Table 1).
- 464 - Genetic sex (Supplementary Table 1).
- 465 - Presence or absence of runs of homozygosity (ROH) indicative of inbreeding (see Methods  
466 section and Extended Data Fig. 9b).
- 467 - Age-at-death as determined through osteological analysis (Supplementary Table 1).

468 In what follows, we start by working out the biological relationships among different sets of  
469 individuals.

#### 470 **2.3.1. Tree topology relating males NC1m, SC2m, SP1m, SC3m, NC4m, NE2m**

471 The core of the family is formed by 6 males (NC1m, SC2m, SP1m, SC3m, NC4m, NE2m) who  
472 are all either first- or second-degree relatives and who all have different mitochondrial lineages,  
473 with the exception of **SC2m** and **SP1m** who share the same maternal lineage. Genetic data  
474 shows that:

- 475 1) **SC2m** and **SP1m** are first-degree relatives (Extended Data Fig. 2) via a **sibling**  
476 relationship (Supplementary Table 5), and both are first-degree relatives of male **NC1m**  
477 (Extended Data Fig. 2) with different mitochondrial lineages, who can only be their  
478 **father**.
- 479 2) **NC4m** is a first-degree relative of male **NC1m** (Extended Data Fig. 2) with a different  
480 mitochondrial lineage, who can be only be **NC4m's father** because if **NC1m** were  
481 **NC4m's son**, **NC2f** (as mother of **NC4m**; see section 2.3.3) would be grandmother of  
482 **NC1m** and therefore his second-degree relative, but **NC2f** is clearly unrelated to **NC1m**  
483 (Extended Data Fig. 2).
- 484 3) **NE2m** is a first-degree relative of male **NC1m** (Extended Data Fig. 2) with a different  
485 mitochondrial lineage, who can only be **NE2m's father** because if **NC1m** were **NE2m's**  
486 son, **NC3f** (as mother of **NE2m**; see section 2.3.4) would be grandmother of **NC1m** and

487 therefore his second-degree relative, but **NC3f** is clearly unrelated to **NC1m** (Extended  
488 Data Fig. 2).

489 4) **NC4m** and **NE2m** are second-degree relatives of each other and both also second-degree  
490 relatives of brothers **SC2m** and **SP1m** (Extended Data Fig. 2). Since they all are sons of  
491 **NC1m**, **NC4m** and **NE2m**, they must be **paternal half-brothers** and also **paternal half-**  
492 **brothers** of **SC2m** and **SP1m**.

493 5) **SC3m** is a first-degree relative of male **NC1m** and second-degree relative of **SC2m**,  
494 **SP1m**, **NC4m** and **NE2m** (Extended Data Fig. 2). Thus, **SC3m** can be either **NC1m's**  
495 **father** and **paternal grandfather** of **SC2m**, **SP1m**, **NC4m** and **NE2m**, or **NC1m's son**  
496 and **half-brother** of **SC2m**, **SP1m**, **NC4m** and **NE2m**.

497 **Conclusions:** Male **NC1m** is the **father** of brothers **SC2m** and **SP1m**, **father** of **NC4m** with a  
498 **different woman**, **father** of **NE2m** with a yet **different woman** and either **father** of **SC3m** with  
499 yet a **different woman** or **SC3m's son**.

### 500 2.3.2. Tree topology relating males **SC2m**, **SP1m**, **SC6m**, **SC7m**, **NC9m** and female 501 **SC1f**

502 Genetic data shows that:

503 1) Female **SC1f** is a first-degree relative of brothers **SC2m** and **SP1m** (Extended Data Fig.  
504 2) related to them through a **parent-offspring** relationship (Supplementary Table 5) and  
505 sharing with them the same mitochondrial lineage. Also, she is not related to **SC2m's** and  
506 **SP1m's father NC1m**. Thus, **SC1f** can only be **SC2m's** and **SP1m's mother**.

507 2) Males **SC6m** and **SC7m** are first-degree relatives (Extended Data Fig. 2) via a **sibling**  
508 relationship (Supplementary Table 5). They are both second-degree relatives of brothers  
509 **SC2m-SP1m**, and both second-degree relatives of **SC2m's** and **SP1m's parents SC1f**  
510 and **NC1m** (Extended Data Fig. 2). With these constraints, the only possible topology is  
511 one where **SC6m** and **SC7m** are indeed **siblings, grandsons** of **SC1f** and **NC1m** and  
512 **nephews** of brothers **SC2m-SP1m**.

513 3) Male **NC9m** is a second-degree relative of **SC2m** with a different mitochondrial lineage,  
514 and also a third-degree relative of **SC2m's brother SP1m** and parents **SC1f-NC1m**  
515 (**Extended Data Fig. 2**). This allows us to discard **NC9m** as **SC2m's half-brother**,  
516 nephew, double cousin, grandfather or uncle, as all these scenarios predict a second-  
517 degree relation between **NC9m** and **SP1m** and either first-degree, second-degree or no  
518 relationship between **NC9m** and both **SC1f** and **NC1m**. Thus, the only possible  
519 relationship is **SC2m** as paternal **grandfather** of **NC9m**; **he cannot be the maternal**  
520 **grandfather** of **NC9m**, because he would then also be the maternal grandfather of **NC7f-**  
521 **SP3m-NC8m**, which he is clearly not since he is not their second-degree relative.



522 **Conclusions:** NC1m and SC1f are **parents** of brothers SC2m-SP1m and **grandparents** of  
523 brothers SC6m-SC7m through an unsampled brother of SC2m-SP1m. SC2m is NC9m's  
524 paternal **grandfather**.

### 525 2.3.3. Tree topology relating female NC2f and males NC4m, SE1m and SP2m

526 Genomic data shows that:

527 1) NC4m and NC2f are first-degree relatives (Extended Data Fig. 2) with a parent-offspring  
528 relationship (Supplementary Table 5) and with the same mitochondrial lineage. NC2f can  
529 only be NC4m's **mother** because if NC2f were NC4m's daughter, she would be  
530 granddaughter of NC1m, but NC2f is clearly **not related** to NC1m (Extended Data Fig.  
531 2).

532 2) SE1m and NC2f are first-degree relatives (Extended Data Fig. 2) with a **parent-**  
533 **offspring relationship** (Supplementary Table 5). SE1m is also a second- or third-degree  
534 relative of NC4m (Extended Data Fig. 2). Given that NC4m's, SE1m's and NC2f's  
535 mtDNA lineage U8b1b is rare in Neolithic Britain (only one other individual out of 82  
536 published Neolithic individuals (1.2%) belongs to this maternal lineage, I2935 from  
537 Scotland<sup>53</sup>), and that we did not find any sign of genetic inbreeding in NC2f (see Methods  
538 section and Extended Data Fig. 9b), SE1m as a father of NC2f is very unlikely because  
539 fathers very rarely share their mtDNA lineage with their daughters in outbred  
540 populations, even more so when the mtDNA lineage is rare. Thus, we conclude that  
541 SE1m is **most likely** to be the **son** of NC2f and **maternal half-brother** of NC4m.

542 3) SE1m and SP2m are first-degree relatives (Extended Data Fig. 2) with a **father-son**  
543 relationship based on different mtDNA lineages (Supplementary Table 1). Given that  
544 NC4m and SE1m are maternal half-brothers sharing mother NC2f, SP2m **cannot** be the  
545 father of SE1m because SP2m would in this case not be biological relative of NC2f and  
546 NC4m, which is contradicted by the data. Specifically, SP2m is clearly second-degree  
547 relative of NC2f and third or more distant relative of NC4m, which means that the order  
548 of the relationship is SE1m as the father and SP2m as the son (Extended Data Fig. 2).

549 4) NC1m and SE1m are **fourth-degree or more distant** relatives (Extended Data Fig. 2),  
550 **more likely fifth** given their relatedness coefficient of 0.027 (Supplementary Table 1).  
551 Since SE1m's mother NC2f is not related to NC1m, SE1m's relation to NC1m must run  
552 through SE1m's father, who was likely NC1m's **fourth-degree relative** (one degree  
553 closer than SE1m-NC1m). This agrees with SE1m and NC1m sharing the same Y-  
554 chromosome lineage (Supplementary Table 1).

555 **Conclusions:** NC2f and NC1m are the **parents** of NC4m. SE1m is **most likely** the **son** of  
556 NC2f, **father** of SP2m and **maternal half-brother** of NC4m. SE1m's unsampled father U1m is  
557 **likely a fourth-degree** relative of NC1m.

558 **2.3.4. Tree topology relating NC3f, NE2m, NE1m, SC5m, NC7f, SP3m, NC8m and**  
559 **NC6m**

560 Genomic data shows that:

- 561 1) **NE2m** and **NC3f** are **first-degree relatives** (Extended Data Fig. 2) with a parent-  
562 offspring relationship (Supplementary Table 5) and with the same mitochondrial lineage.  
563 **NC3f** can only be **NE2m's mother** because if **NC3f** were **NE2m's daughter**, she would  
564 be granddaughter of **NC1m**, but **NC3f** is clearly **not related** to **NC1m** (Extended Data  
565 Fig. 2).
- 566 2) **NC7f, SP3m and NC8m** are all **first-degree relatives** (Extended Data Fig. 2) with the  
567 same mitochondrial lineage, and they are also **NE2m's first-degree relatives** (Extended  
568 Data Fig. 2). Since **NE2m** belongs to a different mitochondrial lineage (Supplementary  
569 Table 1), he can only be the **father** of **siblings NC7f, SP3m and NC8m**.
- 570 3) **SC5m** is also a **first-degree relative** of **NE2m** (Extended Data Fig. 2) again with a  
571 different maternal lineage to that of **NE2m** and **NC7f-SP3m-NC8m**. Thus, **SC5m** can  
572 only be **NE2m's son** and **paternal half-brother** of **NC7f-SP3m-NC8m**.
- 573 4) **NE1m** and **NC3f** are first-degree relatives (Extended Data Fig. 2) with a **parent-**  
574 **offspring relationship** (Supplementary Table 5) and sharing the same maternal lineage.  
575 **NE1m** is also a second-degree relative of **NE2m** (Extended Data Fig. 2). Given that  
576 **NE1m's, NC3f's** and **NE2m's** mtDNA lineage K1a3a1 is rare in Neolithic Britain (only  
577 one other individual out of 82 published Neolithic individuals (1.2%) belongs to this  
578 maternal lineage, I0518 from Northampton, England<sup>53</sup>), **NE1m** as a father of **NC3f** is  
579 very unlikely because fathers very rarely share their mtDNA lineage with their daughters  
580 in outbred populations, even more so when the mtDNA lineage is rare. Thus, we  
581 conclude that **NE1m** is **most likely** the **son** of **NC3f** and **maternal half-brother** of  
582 **NE2m**.
- 583 5) **NC9m** is a second-degree relative of siblings **NC7f, SP3m and NC8m** (Extended Data  
584 Fig. 2) sharing the same mtDNA lineage. The most likely scenario is that **NC7f-SP3m-**  
585 **NC8m's** mother (unsampled woman U6f) is also the **mother** of **NC9m** with a different  
586 male (**SC2m's** unsampled son U11m) who is third-degree relative of **NC7f-SP3m-**  
587 **NC8m's** father **NE2m**. Having woman U6f as a sister of **NC9m** (and **NC9m** as a  
588 maternal uncle of **NC7f-SP3m-NC8m**) predicts that **NC7f-SP3m-NC8m's** parents are  
589 fourth-degree relatives and that **NC7f, SP3m and NC8m** have several long runs of  
590 homozygosity. The expected length of ROH would be intermediate between that  
591 characteristic of offspring of first cousins (third-degree) and offspring of second cousins  
592 (fifth-degree) (see Extended data Fig. 9b), but these individuals clearly lack any long  
593 ROH (Extended data Fig. 9b) making this scenario **very unlikely**. Having **NC9m** as  
594 double cousin of **NC7f-SP3m-NC8m** is **impossible** because **NC9m** is not a second-  
595 degree relative of **NC3f** (Extended Data Fig. 2).

596 6) **NC1m** and **NE1m** are **fourth-degree or more distant** relatives (Extended Data Fig. 2),  
597 **more likely fourth-degree** given their relatedness coefficient of 0.062 (Supplementary  
598 Table 1). Since **NE1m**'s mother **NC3f** is not related to **NC1m** (**Extended Data Fig. 2**),  
599 **NE1m**'s relation to **NC1m** must run through **NE1m**'s father, who was likely **NC1m**'s  
600 **third-degree relative** (one degree closer than **NE1m-NC1m**). This agrees with **NE1m**  
601 and **NC1m** sharing the same Y-chromosome lineage (Supplementary Table 1).

602 7) **NE1m** and **SE1m** are **fourth-degree or more distant** relatives (Extended Data Fig. 2),  
603 **more likely fifth-degree** given their relatedness coefficient of 0.038 (Supplementary  
604 Table 1). Since **SE1m**'s mother **NC2f** is not related to **NE1m**'s mother **NC3f** (**Extended**  
605 **Data Fig. 2**), **SE1m**'s relation to **NE1m** must run through their **fathers**, who were **likely**  
606 **third-degree relatives** (two degrees closer than their sons' pairwise relationship). This  
607 agrees with **SE1m** and **NE1m** sharing the same Y-chromosome lineage (Supplementary  
608 Table 1).

609 8) **NC6m** is **NC3f**'s **second- or third-degree relative, more likely second-degree** given  
610 their relatedness coefficient of 0.22 (Supplementary Table 1). **NC6m** is also **third-**  
611 **degree or more distant** relative of **NC3f**'s sons **NE2m** and **NE2m**, **more likely third-**  
612 **degree** given their relatedness coefficient of 0.13 (Supplementary Table 1). Depending  
613 on which generation **NC6m** is placed, we could have:

- 614 • **NC3f** as niece of **NC6m**, daughter of **NC6m**'s brother.
- 615 • **NC3f** as paternal half-sister of **NC6m**.
- 616 • **NC3f** as paternal aunt of **NC6m**.
- 617 • **NC3f** as paternal grandmother of **NC6m**, through a reproductive union  
618 between **NC3f** and a different male (not **U2m** or **NC1m**).

619 Given that there are different topologies relating **NC6m** with his close relatives, we  
620 connect them in the trees with dotted lines without implying any specific topology.

621 **Conclusions:** **NC3f** and **NC1m** are the **parents** of **NE2m**. **NE2m** is the **father** of **siblings**  
622 **NC7f-SP3m-NC8m** and also the **father** of **SC5m** with a **different woman**. **NE1m** is **most**  
623 **likely** the **son** of **NC3f**, and **maternal half-brother** of **NC4m**. **NC9m** is **most likely** the  
624 **maternal half-brother** of **siblings NC7f-SP3m-NC8m**. **NE1m**'s unsampled father **U2m** is  
625 **likely** a **third-degree** relative of both **NC1m** and **SE1m**'s unsampled father **U1m**.

### 626 2.3.5. Tree topology relating **SC3m**, **SC4f**, **SE3m**, **SC8m**, **SC9f**, **SP4m**, **NC5m** and 627 **SE2m**

628 Genomic data shows that:

629 1) **SC4f** and **SE3m** are **first-degree** relatives (Extended Data Fig. 2) with a **parent-**  
630 **offspring** relation (Supplementary Table 1). Given that **SC4f**'s and **SE3m**'s mtDNA  
631 lineage K1d is rare in Neolithic Britain (no other individual belongs to this maternal

632 lineage), **SE3m** as a father of **SC4f** is **very unlikely** because fathers very rarely share  
633 their mtDNA lineage with their daughters in outbred populations, even more so when the  
634 mtDNA lineage is rare. Thus, we conclude that **SC4f** is **most likely** the mother of **SE3m**.

635 2) **SC8m** and **SC9f** are **first-degree** relatives (Extended Data Fig. 2) with a **sibling**  
636 relationship (Supplementary Table 1).

637 3) **SC4f** and **SC3m** are not close relatives but they are both second-degree relatives of  
638 siblings **SC8m-SC9f** and first degree relatives of **SE2m** (**Extended Data Fig. 2**).  
639 Therefore, **SC4f** and **SC3m** are **SE2m**'s parents and **paternal grandparents** of siblings  
640 **SC8m-SC9f**. We **confirm** this scenario by comparing allelic mismatch rates along the  
641 chromosomes (Supplementary Table 6) between **SC9f** and **SC4f/SC5m**. In regions of the  
642 genome where **SC9f** is consistent with 1 chromosome being shared with **SC4f**, **SC9f** does  
643 not share any chromosome with **SC3m**, and vice-versa (Extended Data Fig. 3b). This is  
644 the expected pattern when comparing an individual with his two paternal grandparents (or  
645 maternal grandparents) because either the father's paternal chromosome or the father's  
646 maternal chromosome is inherited at a given location of the genome, but never both at the  
647 same time.

648 4) **SP4m** is a third-degree relative of **SC4f**, a third-degree or more distant relative of **SC3m**  
649 and a second- or third-degree relative of **SE2m** (Extended Data Fig. 2), more likely third-  
650 degree given their relatedness coefficient of 0.15. Since **SC4f** and **SC3m** are not  
651 themselves related, **SP4m** must be their **descendant** through a sibling of **SE3m**,  
652 specifically their great-grandson either through two male steps or one male and one  
653 female (more likely two male steps given that **SP4m** and **SC3m** share the same Y-  
654 chromosome lineage).

655 5) **SE2m** is a first degree relative of **SC3m**, **SC4f**, **SC8m** and **SC9f** (Extended Data Fig. 2),  
656 and thus he can only be the father of **SC8m** and **SC9f**, and the son of **SC3m** and **SC4f**.

657 6) **NC5m** is a third or more distant relative of **SP4m** (Extended Data Fig. 2), most likely  
658 fourth relative given their relatedness coefficient of 0.077 (Supplementary Table 1). They  
659 also share the same mitochondrial lineage. One possibility is that **NC5m** is **SP4m**'s half-  
660 grand-uncle, a half-brother of his maternal grandmother. However, since there are other  
661 possible topologies relating these two individuals such as brother of his great-  
662 grandmother or son of his maternal female cousin (less likely given a relatively early  
663 radiocarbon date for **NC5m**), we connect them in the trees with a dotted line without  
664 implying any specific topology.

665 **Conclusions:** **SC4f** and **SC3m** are the **parents** of **SE2m**, who is the **father** of siblings **SC8m**  
666 and **SC9f**. **SE3m** is **most likely** the **son** of **SC4f** with a **different male (not SC3m)**. **SP4m** is the  
667 **great-grandson** of **SC4f** and **SC3m** through a different son (not **SE2m**). **NC5m** is **likely** a  
668 **maternal fourth-degree** relative of **SP4m**.

669 **In summary, we have retained two main possible tree topologies relating individuals in this**  
670 **large family. They differ based on whether SC3m is NC1m's son (Tree in Fig. 1c) or father**  
671 **(Extended Data Fig. 4).**

#### 672 *2.4. Disambiguation between the two possible tree topologies*

673 In this section, we compare the location of IBD segment breakpoints, that is, points where an  
674 IBD segments begins or ends, between different pairs of individuals.

675 Specifically, we compare IBD breakpoint locations between **SC3m-SC2m**, **SC3m-SP1m**,  
676 **SC3m-NC4m** and **SC3m-NE2m**. Recombination events in the paternal gamete (within NC4m's  
677 father's testis) that eventually led to **NC4m** will produce a change in the paternal chromosome  
678 that is inherited by **NC4m** (from inheriting the paternal grandmother's chromosome to inheriting  
679 the paternal grandfather's chromosome or vice-versa).

680 In the **tree in Fig. 1c**, since **SC3m** is **paternal half-brother** of **SC2m**, **SP1m**, **NC4m** and  
681 **NE2m**, recombination events in **SC3m's** gamete will **break IBD segments** between SC3m and  
682 his four half-brothers at **the exact same position**, and we will therefore observe that in several  
683 locations of the genome (where these recombination events in SC3m occurred), the allelic  
684 mismatch rate between SC3m and each of his four half-brothers **SC2m** will change from 0  
685 chromosome shared to one chromosome shared or vice-versa.

686 In contrast, in **the alternative tree** (Extended Data Fig. 4), since **SC3m** is the **paternal**  
687 **grandfather** of **SC2m**, **SP1m**, **NC4m** and **NE2m**, recombination events in **SC3m's** gametes  
688 (within his mother's and father's bodies) would determine what combination of paternal and  
689 maternal chromosomes he inherited, but would be invisible when comparing mismatch rates  
690 between **SC3m** to each of his grandsons because we would not be able to tell whether a  
691 chromosomal segment shared between **SC3m** and one of his grandsons is derived from **SC5's**  
692 maternal or paternal chromosome. The recombination events that **would** be visible when  
693 comparing **SC3m** to each of his grandsons are the ones happening in the gametes leading to each  
694 of his grandsons (within **SC3m's** son's testis). However, the recombination events in **SC2m's**  
695 gamete would **produce a change** in the allelic mismatch rate between **SC2m** and **SC3m**, from 0  
696 chromosome shared to one chromosome shared or vice-versa, but they **will not be observed**  
697 **when looking at the sharing pattern between SC3m and each of his other three grandsons**  
698 (see Extended Data Fig. 5 for the rationale behind this approach). The same logic applies to the  
699 recombination events in the gametes leading to **SP1m**, **NE2m** or **NC4m**. In this scenario, the  
700 **only possibility** for observing a **change in the allelic mismatch rate patterns at the same**  
701 **location** when comparing **SC3m-SC2m**, **SC3m-SP1m**, **SC3m-NC4m** and **SC3m-NE2m** pairs  
702 is the occurrence of **four independent recombination events at the same genomic location**,  
703 one in each of the gametes leading to **SC2m**, **SP1m**, **NC4m** and **NE2m**, **which is extremely**  
704 **unlikely**. Thus, if we detected **several cases of IBD breaking points at the same genomic**  
705 **locations** for **SC3m-SC2m**, **SC3m-SP1m**, **SC3m-NC4m** and **SC3m-NE2m** comparisons, this

706 would strongly support **the tree in Fig. 1c** (SC3m, SC2m, SP1m, NC4m and NE2m as paternal  
707 half-brothers) over **tree in Extended Data Fig. 4** (SC3m as their paternal grandfather).

708 Indeed, we observe such cases, for instance in chromosome 3 (Extended Data Fig. 5;  
709 Supplementary Table 6) where we detect two IBD break points between SC3m-NE2m at 130  
710 Mb and 175 Mb. These two break points are detected at the exact same locations in SC3m-  
711 SC2m, SC3m-SP1m and SC3m-NC4m comparisons. If SC3m is a **paternal half-brother** of  
712 NE2m, SC2m, SP1m and NC4m (Fig. 1c), this is easily explainable **by two recombination**  
713 **events in SC3m's gamete** at chromosome 3, one at 130 Mb and other at 180 Mb (Extended Data  
714 Figure 5). If SC3m is the paternal grandfather of NE2m, SC2m, SP1m and NC4m, we **would**  
715 **need two recombination** events at 130 Mb and 175 Mb in NE2m's gamete to produce this  
716 pattern, **two independent recombination events** at the same locations in SC2m's gamete, **two**  
717 **independent recombination events** at the same locations in SP1m's gamete and **two**  
718 **independent recombination events** at the same locations in NC4m's gamete (Extended Data  
719 Figure 5). This scenario is **extremely unlikely** and therefore we keep **one feasible tree** in which  
720 **SC3m is paternal half-brother of NE2m, SC2m, SP1m and NC4m (Fig. 1c).**

## 721 *2.5. Evaluating the validity of the proposed family pedigree with other lines of evidence.*

722 In this section, we report on three independent lines of evidence (not used in previous sections)  
723 that we used to validate the family tree in Fig 1c.

### 724 **2.5.1. X-chromosome information**

725 In previous sections, we have reached a unique tree structure using exclusively genomic data  
726 from the autosomes, Y-chromosome and mitochondrial genome. If this tree structure is correct, it  
727 should also be consistent with the X-chromosome data.

728 For female-female and female-male comparisons, we computed pairwise mismatch rates and  
729 relatedness coefficients on the X-chromosome (Supplementary Table 5) following the same  
730 formula as in **section 2.2**. For male-male comparisons, we adjusted the formula as follows:

$$731 \quad r = 1 - (x/b)$$

732 to account for the fact that males have one X-chromosome as compared to two sets of  
733 autosomes, and that two samples from the same male individual would yield 0 mismatch rate on  
734 the X-chromosome as compared to  $b/2$  on the autosomes (when comparing two samples from the  
735 same individual in the autosomes, the same homologous chromosome will be sampled only half  
736 of the time).

737 We again estimated the mismatch rate value expected for unrelated pairs  $b$  using the median  
738 value of all comparisons between Hazleton North individuals and the set of 53 Neolithic  
739 individuals from Britain. Restricting to comparisons with more than 5,000 overlapping SNPs, we  
740 obtained a value of 0.1978.

741 We plotted relatedness coefficients in the X-chromosome for first and second-degree pairs  
742 (Extended Data Fig. 6a), grouping these pairs based on whether they are expected to share X-  
743 chromosome DNA according to the tree structure proposed in the previous section (Fig. 1c):

744 -The following relationships in the proposed tree are not expected to share DNA in the X-  
745 chromosome: Father-son, grandchild-paternal grandfather, paternal half-brothers, paternal half  
746 siblings (male-female) and nephew-paternal uncle.

747 -The following relationships in the proposed tree must share DNA in the X-chromosome:  
748 mother-son and father-daughter ( $r=1$  as the males in these pairs share their whole X-chromosome  
749 with the females), and paternal grandmother-granddaughter ( $r=0.5$  as fathers pass their entire X-  
750 chromosome from their mothers directly to their daughters).

751 -The following relationships in the proposed tree can (but will not necessarily) share  
752 DNA in the X-chromosome: Brothers, brother-sister, maternal half-brothers, maternal half  
753 siblings (male-female) and niece-paternal uncle. All these pairs can have a  $r$  coefficient in the X-  
754 chromosome between 0 to 1.

755 We found that X-chromosome sharing patterns perfectly fit the proposed tree structure (Extended  
756 Data Fig. 6a).

### 757 **2.5.2. Number of IBD segments shared for second-degree relatives**

758 Second-degree relatives share 25% of their genomes, but the number of chromosomal segments  
759 shared from a very recent common ancestor varies depending on the type of relationship due to  
760 the different number of meioses separating both individuals. Grandparent-grandchildren relations  
761 are separated by two meioses (although only the one in the father is visible in our data when  
762 comparing grandparents and their grandchildren), while avuncular relationships (uncle/aunt-  
763 nephew/niece) are separated by three meioses, resulting in more recombination events splitting  
764 up shared DNA segments. As a consequence, avuncular relationships show a higher number of  
765 shorter IBD segments as compared to grandparent-grandchildren relationships<sup>56</sup>. Half-siblings  
766 are separated by two meioses, but since recombination rate in females is higher than in males<sup>57</sup>,  
767 paternal half-siblings resemble grandparent-grandchildren relationships in the number of IBD  
768 segments shared, while maternal half-siblings resemble avuncular relationships.

769 For each first- or second-degree pair with more than 100,000 overlapping SNPs, we computed  
770 allelic mismatch rate values across sliding windows of 20 Mb, moving by 1 Mb each step  
771 (Supplementary Table 6). We plotted these values along the chromosomes and visually identified  
772 contiguous regions where the allelic mismatch rate is consistent with one shared chromosome.  
773 For example, at chromosome two we count two IBD segments between SC9f and his grandfather  
774 SC3m, and one IBD segment between SC9f and his grandmother SC4f (Extended Data Fig. 3b).  
775 We annotated in Supplementary Table 5 the number of such segments identified for each first  
776 and second-degree relative pair. In the future, algorithms recovering the haplotype sequences

777 through imputation and phasing in ancient DNA capture data will allow a more accurate  
778 detection of IBD segments and thus a more accurate estimation of the number of IBD segments.

779 We next plotted the number of IBD segments for first- and second-degree relationships  
780 (Extended Data Fig. 6b), again grouping the pairs according to their type of relationship in the  
781 proposed tree (Fig. 1c). We recover the expected pattern of a higher number of IBD segments in  
782 avuncular and maternal half-sibling pairs as compared to grandparent-grandchild and paternal  
783 half-sibling pairs, adding further support to the proposed tree structure. Furthermore, these data  
784 add further evidence supporting the placement of NE1m and SE1m as maternal half-brothers of  
785 NE2m and NC4m, respectively (40 and 30 shared segments), rather than as their maternal  
786 grandfathers.

### 787 **2.5.3. Concordance with *NgsRelate* kinship estimates**

788 In this section, we replicated our results using the software *NgsRelate* v.2<sup>48</sup> that estimates  
789 biological kinship using genotype likelihoods and population allele frequencies to estimate  
790 Cotterman coefficients  $k_0$ ,  $k_1$  and  $k_2$ , which correspond to the probability of sharing 0, 1 and 2  
791 alleles in IBD. From these coefficients, the software computes the Theta coefficient ( $\theta$ ) which is  
792 equivalent to the relatedness coefficient  $r$ .

793 To run *NgsRelate*, we first created genotype likelihoods directly from the bam alignment files  
794 using ANGSD<sup>58</sup>. We included Hazleton North individuals as well as the set of 53 Neolithic  
795 individuals from other sites in Britain. We then ran *NgsRelate* providing as input the genotype  
796 likelihood file and allele frequencies estimated only on the Neolithic set from Britain, to avoid  
797 possible bias in allele frequencies stemming from the presence of a high number of closely  
798 related individuals at Hazleton North.

799 Pairwise coefficients computed with *NgsRelate* are included in Supplementary Table 5. To  
800 visualize the correspondence between the two methodologies, we plotted for each pair the Theta  
801 coefficient ( $\theta$ ) and the relatedness coefficient ( $r$ ) from section 2.2 (Extended Data Fig 7a). We  
802 observe a striking correlation between both estimates and a good correspondence between the  
803 theta coefficients and the degrees of relationship in the proposed tree structure. We also plotted  
804 Cotterman coefficients  $k_0$  and  $k_2$  for first and second-degree pairs (Extended Data Fig 7b), again  
805 showing a good correspondence with their type of relation in the proposed tree in Fig. 1c. As  
806 expected, parent-offspring pairs have  $k_0$  and  $k_2$  values close to 0, sibling relationships have  $k_0$   
807 and  $k_2$  close to 0.25, and second-degree pairs have  $k_0$  values close to 0.50 and  $k_2$  values close to  
808 0 (with the exception of some pairs that are related through both their maternal and paternal lines  
809 and are therefore expected to present slightly elevated  $k_2$  values).

### 810 **Conclusion**

811 Based on the previous sections, we conclude that the tree in Fig. 1c is the only one strongly  
812 supported by all lines of evidence. We therefore use this tree across the paper, but also highlight



813 that the main findings about the social organization of the group at Hazleton do not significantly  
814 change (Supplementary Table 7) when using the alternative tree (Extended Data Fig 4).

815

816

817 **SI Section 3: Statistical testing of kinship patterns**

818 Taking advantage of the large pedigree reconstructed in previous sections, we statistically tested  
819 several pattern/rules of social organization in the human group buried at Hazleton  
820 (Supplementary Table 7).

821 We designed the following tests:

822 1) *Sex bias among the individuals buried at Hazleton*

823 We tested whether the number of genetic males and females buried at Hazleton was  
824 significantly different (Supplementary Table 7).

825 **Result:** The number of males buried at Hazleton was significantly higher than the number of  
826 females, both in all individuals and in individuals from the large pedigree. **This implies a**  
827 **deliberate sex bias against the burial of women at Hazleton.**

828 2) *Patrilineality versus matrilineality*

829 We tested whether there was a significant difference in the number of patrilineal and  
830 matrilineal genealogical transmissions between the founding male NC1m and his  
831 descendants (both biological and through adoption) in generations 3–5. Our strategy for  
832 counting genealogical transmissions was as follows:

833 - To establish whether a genealogical transmission between a first-generation individual  
834 and one of his or her descendants runs through a male or a female, we need at least one  
835 generation in between the two. Thus, we only consider descendants in generations 3–5.

836 - Males who do not biologically descend from NC1m but who are sons of women  
837 reproducing with him or his sons (SE1m, NE1m and SE3m) were treated in the same way as  
838 their maternal-half-brothers.

839 - If a genealogical transmission has already been traversed when analysing a different  
840 descendant of NC1m, we do not count this transmission again. For example, siblings SC8m  
841 and SC9f are both connected with NC1m through two male transmissions (U13m and  
842 SC3m). However, the U13m to NC1m connection running through SC3m is shared by both  
843 SC8m and SC9f and thus we count only 3 male transmissions connecting SC8m and SC9f  
844 with NC1m.

845 **Result:** Inclusion in the Hazleton North tomb for lineage members is **strictly patrilineal**,  
846 with all 15 genealogical transmissions between the founding male NC1m and his descendants  
847 running through male individuals (Supplementary Table 7).

848 3) *Sex bias among adult offspring*

849 We tested whether there was a significant sex bias among the adult offspring of all  
850 reproductive unions in the pedigree (including the ones between females and males not  
851 descending from the founding male NC1m), either including missing individuals who we

852 know reached adulthood because they have descendants buried in the tomb, or without  
853 including these individuals.

854 **Result:** There is a **complete absence** (0 females versus 14 males) of adult females  
855 descending from reproductive unions in the pedigree (Supplementary Table 7). This strongly  
856 suggests either that female descendants left the Hazleton community as they reached  
857 reproductive age and were buried elsewhere, or that they were given a different type of  
858 mortuary treatment which did not result in the inclusion of their remains in the tomb and  
859 potentially did not result in the archaeological survival and recovery of their remains.

#### 860 4) Association between female lineages and burial location

861 We tested whether there was a significant association between the female sub-lineage each  
862 individual belonged to and burial location in the north or south side of the tomb. Four female  
863 sub-lineages are evident in the tree: NC2f, NC3f, SC1f and U3f. Individuals were included in  
864 a female sub-lineage if they descended from that female or if they reproduced with a  
865 descendant of that female (the founding females themselves are included as well). NC9m can  
866 be either included in SC1f's sub-lineage as great-grandson of SC1f or in NC3f's sub-lineage  
867 as step-son of NC3f's son. NC9m was buried in the north chamber together with two of his  
868 maternal half-siblings (themselves NC3f's grandchildren), and not with his closest paternal  
869 relatives (all members of SC1f's lineage) who were all buried in the southern chambered  
870 area. This suggests that he was viewed as a member of NC3f's lineage, and we thus  
871 considered him as member of NC3f's sub-lineage for this analysis. Considering NC9m as a  
872 member of SC1f's lineage (P=0.009318) or removing this individual for this analysis  
873 (P=0.002392) still yields a significant association between female sub-lineages and burial in  
874 the north or south of the tomb.

875 **Result:** We find a **significant association between** female lineages and burial location  
876 (Supplementary Table 7), with members of females SC1f's and U3f's sub-lineages **being**  
877 **exclusively buried in the south** chambered area and members of females NC2f's and NC3f's  
878 lineages preferentially in the north chambered area.

#### 879 5) Temporal signal in the burial location of members of females NC2f's and NC3f's lineages

880 *As explained below (SI section 4), the collapse of the north passage prevented continued use*  
881 *of the north chamber and passage some time during the period 3660–3630 cal. BC, and this*  
882 *may have played a factor in the shift in deposition of some individuals descended from NC2f*  
883 *and NC3f.*

884 We tested whether the burial location among members of females NC2f's and NC3f's sub-  
885 lineages changed over time from occurring preferentially in the north to occurring  
886 preferentially in the south. To that end, we divide the members of these two sub-lineages into  
887 groups based on the generation they belong to and their age of death:

888 - A group with a likely earlier date of death: members of generation 1 (NC2f and NC3f),  
889 members of generation 2 who died as young adults (NC4m), and members of generation 3  
890 who died as infants or young children (NC7f and NC8m).

891 - A group with a likely later date of death: members of generation 2 who died as old adults  
892 and members of generations 3-4 who died as teenagers or as adults.

893 **Result:** We found a **significant temporal pattern** (Supplementary Table 7), with individuals  
894 in the first group being buried exclusively in the north chamber, and individuals in the second  
895 group being buried in other spaces outside of the north chamber with the exception of NC9m.  
896 This suggests that the south vs north duality in burial location between members of females  
897 SC1f's and U3f's lineages and members of females NC2f's and NC3f's lineages was broken  
898 due to the collapse of the north chamber, rather than through renegotiation of kinship and  
899 burial rules.

900

901

902 **SI Section 4: Comparison of generational reconstruction with Bayesian modelling of**  
903 **radiocarbon dates from Hazleton North**

904 Here we consider the implications of the family tree presented in Fig 1c in comparison with the  
905 Bayesian model of burials from Hazleton North presented by Meadows *et al.*<sup>59</sup>

906 In a comparative anthropological analysis, Fenner concluded that the reproductive interval  
907 between generations averages at least 20 years for women and at least 28 for men for diverse  
908 societies<sup>60</sup>. If this is the case, then conservatively the five generations detected in the genetic  
909 analysis in Figure 1 span at least four intergenerational intervals and thus at least 80 years. This  
910 counts the space between births whereas radiocarbon dating, and thus the Bayesian model of  
911 radiocarbon dates at Hazleton North set out by Meadows<sup>59</sup>, calculates the dates at which each  
912 death occurred. The overall timespan for the Bayesian model is 15–75 years for the ‘first phase’  
913 of tomb use, from which all of the dated samples derive, then a hiatus, then a few further burials  
914 after 3515 cal. BC. We also have new dates for ten individuals from four of the five generations  
915 of the main lineage identified by the aDNA analysis, and since the generations are continuous,  
916 we conclude their deaths all occurred in or prior to this first phase of activity. At first glance this  
917 seems to suggest the genetic model of generations is not consistent with the Bayesian model, but  
918 a closer analysis suggests that both are compatible if either (a) the Bayesian model dates only the  
919 first three generations of the lineage or (b) different rates of reproduction applied in one or both  
920 sets of sub-lineages. We explore this below, but note that the new dates obtained on individuals  
921 from the north chamber are consistent with the existing Bayesian model, while some of those  
922 from the southern chambered area suggest that the period of use modelled for that area needs to  
923 be extended slightly later. All the dates used by Meadows *et al.*<sup>59</sup> were on samples taken from  
924 human femora, so are directly comparable with one another and likely to relate to the formation  
925 of bone within the last ten years of life, but some of them have a wide range of deviation (e.g.  
926  $\pm 70$  years). Their modelling used the IntCal04 calibration curve. The new dates are from petrous  
927 (in the case of SC9f and SC6m only) or teeth, have only a  $\pm 25$  year range of deviation, and have  
928 been calibrated to 2 sigma using OxCal v4.4.2 and the IntCal20 calibration curve. Petrous bone  
929 does not remodel after early childhood, while teeth form in utero, in infancy and in childhood,  
930 depending on which tooth is dated<sup>61</sup>. During this time period the two calibration curves IntCal04  
931 and IntCal20 are almost identical, so it is not inappropriate to jointly discuss dates obtained  
932 based on the different calibration curves. All the dates in italics below are from Meadows *et*  
933 *al.*<sup>59</sup>’s model 1:

934 *Generation 1:* NC2f(C) has provided two radiocarbon dates: 3950–3630 cal. BC, which was  
935 modelled to a date of death within the period 3685–3640 cal. BC by Meadows *et al.*, and 3761–  
936 3637 cal. BC (this paper). This individual died aged 17–25, so could have been born as early as  
937 3720 cal. BC and still fit the Bayesian model. Her remains were exposed to the elements and  
938 scavengers, so may even have been located outside the chamber prior to tomb construction.  
939 There are other dated remains which, on the basis of the Bayesian modelling, would likely derive  
940 from individuals living in generation 1, particularly femurs 11035 and 9554 in the south

941 chamber, which derive from an adult woman who died *c.* 3685–3640 *cal. BC*; we cannot  
942 associate her with an aDNA sample at present.

943 *Generation 2:* NC4m, a son of NC2f(C), has now been dated to 3774–3650 *cal. BC*: he died aged  
944 17–25. NE1m(2) died 3655–3630 *cal. BC* aged 45+, and so could have been born as early as  
945 3700+ *cal BC* to as late as 3675+ *cal. BC*. NE2m(A) died 3650–3620 *cal. BC* aged 23–57, and  
946 could have been born in the last decade or so of the 3700s or as late as 3643 *cal. BC*. These two  
947 half-brothers were entombed after the collapse of the walling at the junction of the North  
948 entrance and North passage. This collapse is dated in Meadows *et al.* model to 3660–3630 *cal.*  
949 *BC, probably 3640s*, on the basis that it physically and chronologically separates the placement  
950 of the dated individuals in the north chamber, including NC2f(C), and these two individuals. Our  
951 results are still compatible with that model. The passage collapse prevented further access to the  
952 north chamber, which may also explain why several subsequent individuals from this sub-lineage  
953 were buried in the southern passage or southern entrance rather than joining their lineage  
954 predecessors in the northern chambered area (see SI section 3). NC5m(G), who died in infancy,  
955 also likely lived during this generation but died before NE1m(2) and NE2m(A), and his remains  
956 were placed in the north chamber. NE4m(1), who was not a biological lineage member, could  
957 also be contemporary with this generation or with generation 3 (he died 3645–3615 *cal. BC* aged  
958 *c.* 40). SE4m(D), who was not biologically related to the main lineage, died in adulthood 3685–  
959 3635 *cal. BC* so may also have been a contemporary of generation 2 or 3.

960 *Generation 3:* SC5m(E) died 3680–3625 *cal. BC* aged 9–15, and was the son of NE2m(A) who  
961 Meadows *et al.*<sup>59</sup>'s model suggests died 3650–3620 *cal. BC* aged 23–57. The son therefore likely  
962 died before his father, and was likely born as early as 3695 *cal. BC* or as late as 3634 *cal. BC*.  
963 SP2m(vi) died within the period 3632–3380 *cal. BC*, aged 25–35 years old. There are some other  
964 Bayesian modelled dates that would fall within this generation, notably two dated femurs from  
965 the southern chamber.

966 *Generation 4:* Siblings SC8m and SC9f died 3624–3374 aged 23–35 and 3632–3380 *cal. BC*  
967 aged 6–9 respectively. It is possible that no individuals from this generation were dated in  
968 previous studies, but we note that a femur with the bone number 7835 was included in the  
969 Meadows *et al.*<sup>59</sup> analysis, modelled at 3640–3615 *cal. BC*, and considered to be later than the  
970 rest of the activity in the southern chambered area. The generation 3 results discussed above  
971 suggest there was no hiatus in activity, and that deposition in the southern chambered area  
972 continued later than that model suggested.

973 *Generation 5:* We only have one individual from this generation and it does not seem to have  
974 been dated by Meadows *et al.*<sup>59</sup>, so this generation lies outside their model. He remains undated.

975 *NC5m(G):* The death of NC5m(G) aged 3–4 circa 3685–3640 *cal. BC* suggests he was a member  
976 of generation 2, or possibly generation 3. He was likely a fourth-degree relative of SP4m from  
977 generation 5, sharing the same mitochondrial lineage, and potentially his great grand-uncle  
978 (brother of his great-grandmother through the maternal line). From a genetic perspective he  
979 could also have lived in generation 3 as the maternal half-brother of the maternal grandmother of

980 SP4m, in generation 4 as the maternal cousin of the mother of SP4m, or in generation 5 as  
981 maternal half-cousin of SP4m, although the Bayesian model suggests these are less likely. The  
982 remains of NC5m(G) were gnawed, indicating excarnation, and were placed in a discrete pile, so  
983 may have spent some time outside the tomb.

984 The collapse of the northern passage would have cut off access to the north chamber. It might be  
985 argued that this influenced where the ‘southern branch’ of the lineage could place their dead,  
986 affecting our conclusion that choice of north versus south side of the tomb related to maternal  
987 sub-lineages. While we cannot model with precision whether SC2m, SP1m, SC4f and SC3m and  
988 some of their offspring died prior to or after the collapse of the northern passage, it is notable  
989 that no individuals descending from SC1f or U3f were ever buried in the north side of the tomb,  
990 including the north entrance which clearly remained open for use.

991 Meadows *et al.*<sup>59</sup> suggested the use of the southern chambered area ended in the 3640s, but all of  
992 our four new dates from generation 3 and 4 samples have start dates later than 3641 cal. BC at 2  
993 sigma. It is possible that Meadows *et al.*<sup>59</sup> were missing samples from generations 4 and 5, so  
994 their model dates the peak period of tomb use but underestimates the tail end of this activity. The  
995 Bayesian model and the lineage tree are technically compatible, therefore, but the estimated  
996 overall timespan in the Bayesian model would be too short if it does not include any samples  
997 from generations 4 and 5, at a time when we suggest tomb use was dwindling.

998 Meadows *et al.*<sup>59</sup> model the construction of the tomb to within the period 3695–3650 cal. BC,  
999 and note that some of the disarticulated remains might have been those of ‘ancestors’ who had  
1000 died before the tomb was completed, though they felt there was not strong archaeological  
1001 evidence for this<sup>59</sup>. Cuthbert’s osteological analysis suggests that some of the remains in the  
1002 north chamber were exposed to scavengers and/or the weathering prior to being placed in the  
1003 tomb. While this might result from the introduction of remains from those who had died some  
1004 time before tomb construction, it could potentially also result from a repeated mortuary practice  
1005 whereby the bodies of the dead were exposed to the elements or stored somewhere less well-  
1006 sealed than the tomb chambers prior to being installed in the tomb. It is attested in several  
1007 generations.

1008 Finally, it is worth noting that Fenner’s data on reproductive intervals is based on a survey of  
1009 westernized industrialized societies and hunter-gatherers. It is possible that the adults buried at  
1010 Hazleton North reproduced more frequently than in the communities considered by Fenner, in  
1011 which case this interval may have been lower and a greater number of generations would fit  
1012 within the Bayesian modelled timespan.

1013

1014 **Supplementary References**

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