Peer Review File

Manuscript Title: A high-resolution picture of kinship practices in an Early Neolithic tomb

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referee #1 (Remarks to the Author):

A. Summary of the key results
This original and highly significant paper investigates the genetic and social relations of skeletal remains (MNI= 41) that were successively placed within a Neolithic chambered tomb (long barrow) at Hazleton North during a 75-100 year long period around 5700 years ago. The authors analyse the genomes of 25 of these individuals and integrates these alongside the archaeological and osteological data from the site to reveal their treatment in relation to their biological relatedness. They use this information to construct the first extended pedigree spanning five generations and infer significant new details about the kinship practices associated with this monument. This shows that burial in this tomb was closely associated with descent from a specific male lineage, but that matrilineal descent was also a factor. Through its unparalleled integration of genetic and archaeological data, this paper presents ground-breaking new empirical evidence for adoption, social fatherhood, mating and other social practices relating to kinship in the deep past. This will be of very significant interest to many archaeologists, anthropologists, geneticists and several other disciplines. However, some readers would observe that the paper's approach and its discussion of the results prioritise biological relatedness in ways that are problematic and can easily be resolved (see comments in Section B and especially Section C on validity of approach) .

B. Originality and significance: if not novel, please include reference
Previously, archaeogenetics focused on the identification of genetic variability across vast swathes of time and space (e.g. Olalde et al 2018). More recently, there has been a turn towards more micro-scale analyses of aDNA based upon multiple samples from fewer sites. This has provided significant new information about people's mobility, chromosomal sex, genetic ancestors and biological relatedness including (re)constructions of prehistoric kinship (e.g. Sánchez-Quinto et al 2019; Cassidy et al 2020; Knipper et al. 2017; Mittnik et al. 2019; Sjögren et al. 2020).

This paper considerably advances the contribution of aDNA to understanding social relations in the deep past. By analysing the genomes of a large number of individuals from a single cemetery/tomb across a century, it provides a uniquely high resolution multi-generational spatio-temporal analysis of the biological and social relations of those who were co-interred. This yields novel insights into the complex character of intimate relations within this and potentially other Neolithic tombs.

Archaeologists have widely suggested that the placement of human remains in Neolithic tombs was one of the ways in which kinship was made/marked. For example, it has previously been suggested that the human remains in the Hazleton North tomb came from "a single, closely interconnected community that was based predominantly around biological kinship/descent " (Thomas & Ray 2018, 215). This study supports this by showing that 19 of the 25 individuals analysed from this tomb were close genetic relations. Furthermore, it reveals that many of these were paternally related (agnatic)to the first or second degree indicating that burial in this tomb was closely associated with descent from a specific male lineage.

The results also indicate a patrilocal (virilocal) residence and burial pattern which suggests female exogamy: the absence of any adult lineage daughters in conjunction with the presence of two child lineage daughters implies that women moved away from their paternal kin at marriage, while the presence of adult non-lineage women indicates that they moved into the community at marriage

and were subsequently buried with their husband's kin. Although this has long been suspected for the LBK Neolithic (e.g. Whittle and Bickle 2013, 390–91), this is the first clear indications of this in a British context.

The results also indicated that marriage/mating practices may not have been monogamous: males and females each had children with multiple others suggesting polygamy. Related to this, there is evidence for the adoption of biologically unrelated males into the patriline indicating that social fatherhood seems to have been important. This is very notable because it confirms that kinship here was not fixed, but rather it emerged from a range of social practices. This is really important because this contribution does much excellent work that avoids many of the problems highlighted in recent critiques of aDNA-based kinship reconstructions, most notably bio-determinism (e.g. Brück 2021a; Carlin & Cooney 2020; Crellin & Harris 2020).

Significantly, despite the evidence for patrilineal descent and the presence of more genetic males than females among the analysed burials, the results reveal that matrilineal descent was also considered highly significant. Indeed, the choice to construct the tomb so that it comprised two separate chambers and the subsequent deposition of burials within these seems to have been guided by different maternal relationships. This is evidenced by the fact that all those who were descended from two particular maternal lineages were found in one chamber, while most of those from a different maternal lineage were in the other chamber. These results are unprecedented and without direct parallel in European prehistory. Significantly, this results seem to suggest that the tomb's architecture and the location of burials within it were both directly related to kinship negotiations. This provides empirical evidence that kinship cannot be viewed as either social or biological (instead it is a mix of both). Unfortunately, despite the current paper's laudably integrative and balanced approach, the authors decline the opportunity to make this very important point. This needs to be addressed and doing so will further enhance the paper (see below) .

C. Data & methodology: validity of approach, quality of data, quality of presentation
I fully recommend this paper's publication in Nature. There are no significant flaws that would prevent its publication, but there are areas relating to the approach taken (and its limits) that could usefully be clarified that would significantly improve its strong contribution to the field and reduce the potential of future negative critiques.
Although the authors do acknowledge (lines 98-99) that "Hazleton North was also characterized by complex kinship practices some of which extend beyond biological (agnatic) kinship.", they still display a slight tendency to operate within a nature/culture binary and give primacy to the former over the latter (see Crellin & Harris 2020). The authors choose not to fully highlight or discuss other aspects of their results which further indicate that kinship was not exclusively determined biologically. For example, the presence of non-genetically related (male and female sexed) individuals in close spatial proximity within the chambers e.g. I13899, I13893, I13897, I13889, I21391, I20818. These beings seem to have been considered as significant kin for reasons that go beyond sexual reproduction or parentage (adoptive or otherwise). Indeed, the presence of these unrelated individuals in the tomb shows that kinship here was not exclusively patrilineal. Strangely these important points are not made in the paper. This also needs to be addressed and doing so will further enhance the paper.

Given the longstanding and recent critiques of biological approaches to kinship(see Schneider 1984 & Bruck 2021a & b), it is necessary for me as a reviewer to ask does this paper sufficiently avoid rooting kinship primarily in the human body? My view is that the authors would improve their paper by doing more to take account of the published critiques of recent attempts to reconstruct ancient kinship systems e.g. Brück 2021a , Bickle 2020, Crellin 2021; Frieman 2021; Furholt 2021. The paper would benefit from more explicitly acknowledging that in many societies, genetics, blood or biology are neither a determining or necessary factor of relatedness: people make their kin through cultural practices conducted within the particular context of their society like living or working or burying their dead together (e.g. Carsten 2004; Brück 2021a). This requires a

statement that while the genome is a powerful tool for revealing biological relationships, the authors have chosen an integrative approach to establish how such relationships were socially understood because kinship is more than biological.
Similarly, family trees or genealogies like that which the paper reconstructs have been recognised as being a very narrow way of thinking about kinship which limit our ability to recognise or include other forms of relatedness (for example, see Ingold 2009). The authors could also usefully state that they are focused on a particular form of kinship which is anthropocentric and does not attempt to fully account for non-biological forms of interconnection and relatedness 'between humans and non-humans (e.g. Sahlins 2013). One potential solution to this is to insert some sentences defining what is meant by kinship in this paper while clearly stating that it is not a biological fact (with appropriate references). In addition to this, the authors could usefully attend to the question of how their findings inform us about how particular forms of kinship were naturalised in the Neolithic (I would argue that the evidence presented here is highly suggestive of attempts to naturalise kinship by making the different familiar).

The authors need to be more open about the fact that they are weaving together a genetics driven narrative from incomplete data. They need to be more explicit about the potential for sampling bias: does the sample size and the exclusion of the cremated human remains from the tomb result in the over representation of some and under-representation of others? This is especially pertinent given that the study shows that some women were treated differently at death. If more females than males were cremated, might that result in female under representation in the study?

The authors recognise that social organisation and kinship organisation are not always the same (Godelier 2009) and they avoid jumping to problematically andocentric conclusions about the social role of people in the Neolithic (see Bickle 2020, see Frieman et al 2019). However the paper seems to assume that sex is biological or chromosomal and that gender was a fixed/essentialised entity which can be chromosomally determined. This is indicated by the author's consistent use of terms like male and female as if these binaries were valid and neutral categories of analysis. The paper insufficiently attends to the fact that gender is constructed relationally in ways that are historically and contextually specific. There also seems to be an assumption that the family and marriage was universally heterosexual/heteronormative. These are recognised issues that have previously been critiqued (e.g. Bickle 2020 Robb & Harris 2017; Frieman et al 2019, Geller 2008; 2017). Therefore, it is necessary for the authors to critically reflect on their chosen categories of analysis and their impact on their conclusions and then to explicitly state these in text. It would also be good to explicitly define what is meant or not meant by marriage to avoid colonising the past with our own present-day western concepts of hetero-normative families (see Furholt 2021)
The authors seem to conflate value with status in Lines 107-109. This overlooks the fact that there are multiple reasons relating to the social identify of the deceased for why adopted sons were not buried in the inner chambers. Indeed on Lines 126-7, the authors themselves state that the "arrangement of chambers at this Neolithic tomb was related to negotiations of kinship". There is no supporting evidence provided to indicate that the values associated with a particular spatial location for burial were understood in relation to status. Any assumption that this is the case would rest upon a number of highly problematic assumptions that would ignore the last 30 years of archaeological thought including the work of one of the authors (e.g. Fowler 2013).

The authors suggest that the practice of adopting people into the patriline "may have been advantageous during the first agricultural expansions in Britain" (Line 116-118). This statement is problematic. Firstly, we know that the adoptions occurred between 3700-3600 BC, at least two or three centuries after the first agricultural expansions to south central England c. 3900-3800 BC (see Whittle et al. 2011, fig. 15.8), so it is more than a reach to try to link the evidence for this social practice to the latter. Secondly, for all we currently know (given that this is the first study of this kind), adoption may well have been a standard practice that was not exclusive to Earlier Neolithic Britain. Thirdly, this represents an unduly instrumentalist interpretation of an act of social kinship that may have been conducted for a thousand other reasons and falls into many of the traps of evolutionary psychology.

More information on the site itself could usefully be included in the SI.
A descriptive who's who outlining all the relevant info for each person in the SI would be very informative. I found it necessary to complete my own version of this to be able to review the paper.
Fig 1C could be improved by making it easier to identify and differentiate first degree, second degree and third degree relationships

D. Appropriate use of statistics and treatment of uncertainties
, methodology, statistical analysis, clarity of the text and
Given the fuzzy nature of the data, the statistical tests conducted are appropriate and I see no issues with these. There are a few areas where minor clarifications would improve the text.

The authors could usefully clarify for the less specialist reader whether the aDNA analysis conducted here is based upon the sequencing of whole genomes, rather than the targeting of a limited number of DNA sequences.

The word 'large' is used quite a few times in the paper e.g. "few large sample size studies from individual cemeteries" or "our ability to construct a very large family tree" but nowhere is 'large' explicitly defined.

E. Conclusions: robustness, validity, reliability
strength of the conclusions, Do you find that the conclusions and data interpretation are robust, valid and reliable?
The conclusions are very strong but their robustness and validity will be greatly increased by addressing the issues highlighted above in Sections B, C & D. Here are a few additional comments relating to other aspects
It seems that the results indicate a reduction in Y chromosomal diversity over time compared to a high level of mt DNA diversity indicating the movement of women into the population as observed in other aDNA studies like we see in Sjögren et al. 2020. The lack of comment on this seems odd as it would lend support for patrilocal exogamy.
The paper states (line 80-81) that "each individual whose lineage we can trace through the second generation to the first is connected entirely through males: thus, all 14 of the parent-to-child linkages are through fathers". While the broad thrust this seems to require more clarification. I21388 is a son of female I21390 & son of male ?1 (whose remains were not identified) and I13895 is a son of female I21390 &'adoptive son' of male ?1 (whose remains were not identified). This seems to contradict/ invalidate the statement above. Notably there is no star to indicate the parentage of these two individuals in Fig 1C
Lines 46-8: ". Strontium and oxygen stable isotopes on teeth were similar for 18 of 22 sampled individuals suggesting they had spent some time on the geology local to the tomb and some on geology at least 40 km away during their childhood". The authors did to make it much clearer that they are referring here to 22 individuals from a completely different previous study and that only 10 of those individuals with isotopic data from that study overlap with 10 (3 women & 7 men) of the 25 individuals with genetic data in the present study. This is necessary for the full and fair consideration of subsequent statements relating the results of both studies to each other as discussed in Lines 110-115.
Lines 110-115: "Two of three individuals with local stable isotope signatures from childhood were not part of the biological patriline while the third was the child of a man adopted into the patriline, and all three were buried in the periphery (entrance or passage). This contrasts with five individuals born into the lineage and buried in the chambers with stable isotope signatures indicating mobility across geological zones, suggesting that members of the patriline had a mobility pattern in earlier life not shared by all those buried in the tomb".
This contrast highlighted in lines 110-115 "that members of the patriline had a mobility pattern in earlier life not shared by all those buried in the tomb" does not fully incorporate the chronological and generational differences between the different individuals. For example, the non-lineage

individual I13893 in the periphery of the north chamber is-chronologically later according to Bayesian modelling, 3645–3615 BC. The authors neglect to mention that two non-lineage individuals (I21391 & I21395) show cross-geology subsistence, thereby indicating that either they were considered part of the patriline and/or that this mobility pattern was shared by the patriline with other members of the community. It is also notable that this cross-geology mobility pattern was present across multiple generations of the lineage. However, the authors have either not identified or highlighted the evidence for this intergenerational practice.
F. Suggested improvements: experiments, data for possible revision
See previous comments

G. References: appropriate credit to previous work?
Following on from my comments in Section C, the manuscript could usefully reference previous literature relating to the limits of their approach
Line 75, the authors state their study to be "more than twice as large as the largest pedigree reconstructed from ancient DNA data to date", but they do not provide a reference to that other study here.
H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
The overall text is very clear and accessible but the abstract could be rewritten to make it easier for a wider audience to understand and appreciate the paper's significance.
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Peer review by Neil Carlin

Referee #2 (Remarks to the Author):

Review of Fowler et al. 2021

Fowler and colleagues present the largest pedigree yet reconstructed for a set of prehistoric individuals. This consists of 19 individuals sampled from a single Megalithic tomb. This is a very impressive piece of work, based almost entirely off a single measure of genetic distance (the allelic mismatch rate), which is leveraged to estimate kinship coefficients and local patterns of IBD-sharing between pairs of samples. The authors combine these values with standard aDNA and osteological metrics (sex, uniparental markers, age-at-death) to meticulously reconstruct the best fitting pedigree through a manual process of elimination. From this, the authors are able to draw several conclusions about the community's kinship practices, including a descent system that emphasized the patriline and potential adoptive practices. The latter is an exciting find and demonstrates the use of genetic data in the detection of diverse forms of kinship.

Overall, the manuscript is a wonderful example of the power of ancient DNA to inform on social organization, which has in recent years become the new frontier of the field. What makes this study stand out from others of its kind is the level of detail provided for a single extended family, which gives us unprecedented insight into the cultural practices, values and lifeways of a Neolithic community. The use of ancient DNA to potentially inform on different aspects of prehistoric architecture is also noteworthy, made possible through adept integration of archaeological data (e.g Bayesian modelling of RC dates).

I believe this study has the potential to draw interest from a wide readership, however I do have some concerns. Most generally, I feel that the broader significance of Hazleton North is left unclear to the non-specialist. Do the authors believe their results have altered our understanding of the Early Neolithic of Britain in any major way, or do they see their study as a proof-of-principle? They seem to suggest (Line 152) that it would be inappropriate to extrapolate about kinship practices in Early Neolithic Britain from the Hazleton North pedigree, even for chambered tombs built within the same region, although I might be misreading this? However, in the abstract they suggest this sort of kinship system may have promoted agricultural expansion across Britain. This seems contradictory. They also do not integrate results from other genomic surveys of megalithic tombs

into the discussion section, including evidence for patrilineality, which could enhance their interpretations. Finally, more context could be provided on the demographic and cultural landscape of Britain at the time (e.g. large scale influxes of new people, construction boom of chambered tombs ~3700-3500 BC in certain regions, relatively rapid spread of the Neolithic package). At times it feels that Hazleton North is being considered in a vacuum, which may lessen the manuscript's overall impact.

I also have a number of more specific concerns and suggestions, which I detail in the below comments.

COMMENT 1: THE NORTH WALL COLLAPSE

The idea that the architecture of the tomb was at least in part guided by kinship is an exciting one. However, the authors also concede there is a likely temporal component when it comes the placement of individuals in the tomb. Specifically, the north passage-entrance wall collapse c. 3660–3630 cal. BC, which may have led to the abandonment of northern compartments in favour of the southern one (Lines 133-135). I am concerned that this may be a confounder in their tests of significance for the north-south differentiation of maternal lineages. No significance tests were carried out to detect north-south differentiation on the basis of date of death.

I was curious and put together a rough spreadsheet (attached) estimating these dates from the individual's approx. age-at-death and generation in Tree1. I made the assumption that individuals in each generation were born at approximately the same time with a 25 year gap between generations. If we assume a birth date for generation 1 individuals at 3,710 BC (the earliest date for I21390 that still fits the Bayesian model, supp line 604) and a wall collapse at ~3,640 BC, we get a significant differentiation between individuals that died pre- and post- collapse ($p=0.0034$) with respect to north and south placement. I think this deserves more attention in the text, which discusses the collapse only in terms of disrupting the duality (lines 130-132), rather than as a potential driver of this perceived pattern. The authors could also include an analysis similar to that provided in the spreadsheet.

The wall collapse may have also impacted the placement of the possible adoptees. I note the authors' tests for both adoptee and maternal lineage differentiation produce p-values close to the threshold of significance (~0.025) and are based on small sample sizes. For this reason, I believe more caution is needed in interpretation. e.g. in lines 10-11, 108-110 and 152.

COMMENT 2: ANCESTRAL MAKE-UP OF THE HAZLETON NORTH POPULATION

I understand that this is a study on kinship, rather than deep ancestry. However, the ancestral profile of the 15 newly reported individuals (line 57) is of relevance to both the field and to the interpretation of the pedigree. Specifically, are there any individuals with an atypical profile? Do any individuals show evidence of recent European HG ancestry or appear more closely related to a Neolithic population outside Britain and Ireland? Lines 228-233 in the supplement suggest not, but I think it is important to provide the actual data underlying this statement in the online tables and distinct supplementary section.

COMMENT 3: ATYPICAL MATING PATTERNS

The authors assume a relatively simple pedigree structure due to the lack of evidence for inbreeding across individuals. However, I was surprised to see no consideration of other possible confounders, such as double cousins. Marriages between multiple siblings from different families is a common practice in some societies. Skimming through the data, I believe this can be ruled out as an explanation for the 2nd degree relationships presented here, but it might be useful to be explicit about this.

COMMENT 4: THRESHOLDS FOR DEFINING RELATIONSHIPS

How did the authors assign the degree of relationship between two samples? I assume threshold values of r were chosen, but I can't spot any mention of this in the text. How confident are the authors in their assignment of 3rd and 4th degree relationships? I ask, because these relationships are often presented as "very likely" or "likely" in Supplementary section 2 (see COMMENT 5), but I'm unaware of the exact justifications behind these statements.

For example, two possible pedigrees are excluded for [I12437, I12438, I12440, I21388], where I12437 is a paternal grandfather or uncle to the other three, given that I13898 is a second degree relative of I12437 and either "likely" or "very likely" more distant than 4th degree with the other three (lines 273—296). However, I estimate from Online Table 3 that a coefficient of relatedness of ~0.035 is required to be classified as possible 4th degree relatives. I13898 has r ± SE of 0.026-0.046, 0.012-0.04 and 0.011-0.031 for I12440, I21388 and I12438 respectively. I understand from the later lines 349-353 that this pedigree is also excluded as it would require I12437 to be a grandfather of I13898 with the same mtDNA haplogroup, which is also unlikely. In the next paragraph I13898 is said to be "likely a fifth-degree relative of I12440", criteria again unclear.

Could the authors provide more clarity and consistency in this regard? Perhaps a list of statements at the start of the supplementary section detailing their main assumptions and their confidence in these (e.g. thresholds of r, ruling out male ancestor and female descendent relationships if the individuals share the same mtDNA haplogroup, number of expected shared IBD segments etc.)

COMMENT 5: PRESENTATION OF THE PEDIGREE RESULTS

This paper is centered on a single landmark result, the reconstruction of a five-generation extended pedigree from aDNA data. This was very carefully done, using all available lines of evidence and a logical process of elimination. However, the presentation of this process (SI Section 2) and the figures detailing the results are at times difficult for the reader to follow.

Suggested improvements -

1: Labels
Please use a simplified set of individual labels in the main text, figures and supplementary info, with a key to the longer Lab IDs provided in the online tables. It is impossible to keep track of multiple similar six character IDs. Also, please find another way to represent unsampled individuals without the use of a preceding "?". To the unfamiliar reader, it continuously registers as punctuation or font error.

A possible labelling system could be based on sample location in the tomb, with an additional qualifier for sex. Having the sex information in the ID would make the supplement a lot easier to follow, without having to continuously refer back to the figures and tables. For example, mNE1, mNE2, mNE3, fNC1, mNC2, fNC3, mSP1 and so on. Unsampled individuals could then be denoted with an "X" (e.g. mX1, fX2). This would also help simplify Figure 1, which is very busy and whose fonts are too small to read (see next).

2: Figure 1
This figure is dense with interesting information, but it is not the easiest to digest. Some suggestions to improve things

1. Increase font sizes and remove unnecessary text (e.g. the locational data box, the title "uniparental genetic markers")
2. Are both the blue and yellow stars and dots necessary?
3. Use consistent line thickness and distances between double lines on pedigree

4. It is hard to quickly differentiate between sampled and inferred individuals. Perhaps the inferred individuals symbols could be reduced size? The dotted line types are also different for inferred males and females.
5. Align the generation labels on the left or right side of the plot.
6. Standardise the text and placement of RC dates.

3: Simplifying the Supplementary Text
As the paper is entirely dependent on the logic outlined in SI Section 2, more needs to be done to make this section clear, concise and accessible to the reader. Better use of headers, bold fonts and bullet points/numbers (rather than large blocks of text) would be welcome, or even embedded tables if useful. An initial summary of the logical progression detailed in subsections 1-8 maybe useful at the start to orientate the reader.

There are a lot of repetitive statements in SI2 subsections 1-5 that could be condensed or relegated to Online Table 3, especially when it comes to definitive relationships (e.g. siblings). One example of repetition is the section on I12437, I12438, I12440, and I21388 (line 256), all pedigrees involving maternal half-brothers and maternal uncles can be instantly ruled out, given that none share an mtDNA haplogroup. However, these pedigrees are ruled out separately in lines 268 and 307. Pedigrees involving two or more paternal uncles of a single individual can also be easily ruled out as they require first degree relationships. However, these are discussed separately in lines 303-306 and 328-336. Lines 328-336 also rule out pedigrees in which three individuals are nephews to a fourth, which seems confused as two of the five plausible pedigrees are this structure (Lines 263-264). Please order this section in a more logical and concise manner and double-check all statements are consistent. Also double-check grammar and spelling (e.g. line 347 is missing a word I think.

The authors also need to be more explicit as to the meaning of the phrases used in SI2 (see also COMMENT 4). For example, one header (line 267) states that all other possible relationships listed below are "ruled out". However, below this header we see both "ruled out" scenarios and "extremely unlikely" ones. The "extremely unlikely" ones are dependent on the exclusion of fourth degree relationships, which is deemed either "likely" or "very likely". Similarly, the header of line 337, uses the definitive phrase "can only be related in a single way". However, this is again dependent on probability (that it is unlikely two unrelated individuals would share the same mtDNA haplogroup by chance and that the inference of a 5th degree relationship is correct – lines 356-360). In other parts, what I would consider definite relationships are presented in a way that implies potential uncertainty (e.g. line 340 – "indicating a sibling relationship")

To be clear, I am happy with logic used to eliminate different relationships from the pedigree and that tree1 is by far the best fit, but more needs to be done to differentiate statements of truth from those of high probability. Condensing statements on clear-cut relationships (e.g. in concise bullet point list) may help focus the reader's attention on more important passages that require some weighing up of probabilities or more complex deduction.

COMMENT 6: INFERENCE OF KINSHIP PRACTICES

The inferences drawn from the pedigree with respect to kinship practices are for the most part robust and carefully considered. However, I find some parts of the discussion a little unbalanced (e.g. too much speculation on adoption), ambiguous or contradictory. I've identified three main issues.

1: Definitions of kinship and complexity

The title of the study is vague. It is unclear what defines a "complex kinship practice" and how it differs from a "non-complex" practice. In fact, I'm not sure of any society whose kinship practices can be regarded as simple. I suppose a kinship system itself can be considered in terms of

complexity (e.g. number of kin terms and rules of descent). However, the authors cannot make much comment in this regard. Indeed, the fact that they were able to provide such strong evidence for "a central role for patrilineality" could suggest relatively simple unilineal rules of descent, although the possible differentiation of maternal sub-lineages is interesting.

Also, it seems problematic to conflate the complexity of a community's kinship practices with the presence of adoption, which is a relatively common phenomenon (Line 98). The concept of fatherhood can be just as complex in societies where adoptive practices are absent.

To increase clarity, I suggest the authors provide a more explicit title that refers to the main inferences drawn from the pedigree. If the word "complex" is to be used, I suggest it is qualified. It also may be useful to differentiate between the genetic definition of kinship (degree of biological relatedness) and the anthropological one (e.g. the values a society may or may not ascribe to biological relatedness) early in the text for the general reader. Anthropological kinship is by definition more complex than genetic kinship, regardless of whether a biological relationship is actually present.

2: Evidence for adoption

I believe the authors have provided good evidence for the adoption of non-lineage males into the Hazleton North patriline. However, I do not think they have provided definitive evidence and they need to temper their discussion to reflect this. For example, the term "adopted" is used with certainty at various points (e.g. line 111). The abstract states, rather than suggests, that "husbands commonly adopted their wives' children into the patriline. (line 13). The conclusion (151) states that the "acceptance of step-sons into the patriline" has been revealed. Online table 1 also describes both adoption and marriage in certain terms (column Z).

The evidence for adoption comes from three women (?5, I21390, I21387), who each reproduced with both a non-lineage and lineage male, and whose non-lineage adult sons and one grandson are present in the tomb. The authors also argue that there may have been some status differentiation between "adoptees" and biological descendants, on the basis of the placement of these four males outside the chambers (although see COMMENT 1).

There are, however, explanations alternative to adoption, that should be considered in the text.

1. If there is a potential status difference, it is also possible that the "adoptees" were not actually viewed as members of the patriline. It is not definite that everyone interred within the tomb was a member of the patriline. Indeed there are six unrelated "non-lineage" individuals interred within both chambers and entrances, whose presence remains unexplained.

2. I am surprised to see no consideration of infidelity or rape, given the prevalence of both across diverse societies. It is problematic to assume that men in the Neolithic had complete knowledge of their partners' sexual and reproductive lives. There are many reasons why a woman may conceal paternity information.

3: Discussion of marriage practices

While I find the manuscript overconfident in its discussion of adoption, it is perhaps a little overcautious in its discussion of patrilineality, patrilocality and potential polygamy. Specifically, relevant data from both the anthropological and ancient DNA literature are not considered in the authors' discussion, which may have helped to enhance their conclusions.

1. The ancient DNA literature. Two studies on megalithic tombs in Britain and Ireland have provided evidence for patrilineality (Sanchez-Quinto et al. 2019; Cassidy et al. 2020). Most relevant to the current manuscript is the Irish survey, which took sizeable samples from a

chambered tomb and a neighbouring portal tomb (lines 25-26). While no close relatives were found, they did observe a significant differentiation between the tombs with respect to a rare Y haplogroup. They interpreted this as social differentiation between the tomb users with an emphasis on patrilineal descent. This result should be noted in the introduction and integrated into the discussion. This study also reported elite inbreeding in a megalithic context, a practice that strongly associates with patrilineality and polygyny. Thus, there is already a precedent for the Hazleton North results in the literature, which is not clear in the text.

2. The anthropological literature. The authors have provided convincing evidence that patrilineal descent played a central role at Hazleton North (Line 151). There is also evidence for patrilocal exogamy (line 91). However, the authors go on to state that it is inappropriate to make any inferences about other aspects of the underlying kinship system and cite a single cautionary case study as justification for this (Lines 93-97). This does not seem well-reasoned. Cross-cultural comparisons have consistently shown correlation between patrilineal descent, patrilocality and other male-biased features of kinship systems. Most recently, Surowiec et al. (2019) considered ~1000 populations and demonstrated patrilineal descent was strongly correlated with patrilineal inheritance of property, political succession and patrilocal residence, and negatively correlated with the female-based equivalents. I think it is reasonable to suggest that the discovery of patrilineal descent at Hazleton North increases the likelihood that the community's kinship system had other male biases. Of course, the authors are not obliged to include such a statement in their manuscript. However, lines 94-97 seem almost imply the opposite (that the likelihoods have not changed in view of these new results). Could the authors soften this a little?

The authors also present serial monogamy, polygyny, polyandry and extra-marital relations as equally plausible explanations for the instances of multiple reproductive partners in their pedigree (Lines 118-120), again without reference to the comparative anthropological literature. However, polyandry is rarely observed and non-fraternal polyandry can violate the rules of a patrilineal descent system. Suroweic et al. show positive correlation between patrilineal descent and polygamy, and confirm its strong correlation with cattle and large animal domestication. This and related literature (e.g. Holden and Mace 2003 on the Bantu expansions) may be of interest to the authors, given they discuss polygamy as a potential strategy to rapidly grow a patrilineage during the agricultural expansions across Britain, but provide no references (lines 116-118).

MINOR NOTES

1. Can X chromosome mismatch rates be provided in a table?
2. Typos in online table 3: 4rd ->4th; I21395 is a lineage descendent, but not marked as one.
3. Lines 47 and 110 are a little hard to parse. Line 47 states that 18/22 individuals have isotopic signatures suggesting mobility during childhood. However, Line 110 states only three individuals have local signatures from childhood. Also the overlap between ancient DNA samples and isotope samples is not clear
4. Given the potential for female exogamy, do the authors note any difference in female isotopic signatures relative to males?

REFS

Surowiec A, Snyder KT, Creanza N. A worldwide view of matriliny: using cross-cultural analyses to shed light on human kinship systems. Philosophical Transactions of the Royal Society B: Biological Sciences. 2019;374(1780):20180077.

Holden CJ, Mace R. 2003 Spread of cattle led to the loss of matrilineal descent in Africa: a coevolutionary analysis. Proc. R. Soc. B 270, 2425 – 2433. (doi:10.1098/rspb.2003.2535

Cassidy, L. M. et al. A dynastic elite in monumental Neolithic society. Nature 582, 384-

388, doi:10.1038/s41586-020-2378-6 (2020).

Sánchez-Quinto, F. et al. Megalithic tombs in western and northern Neolithic Europe were linked to a kindred society. Proceedings of the National Academy of Sciences of the USA 116, 9469-9474, doi:10.1073/pnas.1818037116 (2019).

Referee #3 (Remarks to the Author):

The work by Fowler and Olalde et al. is an elegant example of use of aDNA in studying past human social traditions and dynamics. The authors construct a pedigree using multiple types of information collected from the Hazleton North skeletal collection, and using this data they test a number of hypotheses on kinship and burial patterns. They show strong patrilineal burial customs in this community, and at the same time, that adoptive sons (who may have been recognized as such, given differences in their burial context) were included in the patrilineage, at least with respect to their burial location.

The most significant novelty is the size of the ancient pedigree constructed, and some of the approaches used in the process are also innovative in being applied in kinship analysis of ancient individuals (e.g. use of IBD segment sharing to distinguish kinship types). That European Neolithic and BA communities frequently adopted patrilineal burial patterns has been shown in other work using isotopes and DNA (e.g. Bentley et al 2012 PNAS; Sánchez-Quinto et al 2019 PNAS; Mittnick et al 2019 Science), but the question is studied here at additional depth and new angles.

I enjoyed the manuscript. I would like to share some suggestions that should be simple to address.

Methodologically the approach used to construct the pedigree appears sound, but I have to admit I had difficulty following SI Section 2. Perhaps this could be written in a way to aid the reader follow the logic and analyses. Additional visualisation or more frequent references to tables/statistics (like that provided in some external data figures) would be extremely helpful.
I would also recommend adding more methodological detail in general: for instance, how ROH analysis was conducted is not mentioned, when and where chrX data was used was unclear, etc.

Another suggestion would be adding analyses to demonstrate how well the data fit the pedigree. For instance, the authors neatly use IBD segment sharing information in Supplemental Section 7, but only for a subset of cases. Why not compare the expected and observed number of segments for all pairs in a tree? This could provide a level of confidence in the tree, beyond the qualitative description (somewhat analogous to qpGraph).
The authors could also run a ML-based model such as NgsRelate on their pairs (at least the pairs in their final tree). This estimates both theta, but also Cotterman coefficients, and F, and could help improve confidence in the estimated relationships. Using chrX vs autosome comparisons could also be used more systematically.
Small errors in the pedigree would likely not alter the main conclusions (as the authors also explicitly show for their Trees 1-5), but I would still feel more safe if some additional sanity checks were conducted.

There could be further joint interpretation of the genetic and anthropological data, at least in the Supplemental section but possibly also briefly in the main text. I feel that it would be important for the authors to help the reader (especially those alien to British archaeology) to assess what type of prehistorical community this was. For instance, do the dietary patterns or frequencies of pathologies distinguish this group from its contemporaries? I think the paper should convey some idea of whether these individuals represented a commoner or an elite group.

Finally, I would recommend submitting the data to SRA in advance of publication and providing a

reviewer link.

Minor points and suggestions:

Fig 1: Double horizontal lines connecting parents are usually reserved for inbreeding in pedigrees, but in the figure they seem to be used to describe all types of matings. This could be confusing and I would recommend using a single line.

L12: "A striking finding is that four males descend from a non-lineage father but a mother who also reproduced with a lineage male" - this reads like all four were brothers. Perhaps using plural (fathers and mothers) would help?

L93: "lack of long runs of homozygosity indicate that inbreeding was effectively avoided." It is not clear where is this shown. Refer to OT1 and perhaps provide context in the supplement for readers less familiar with ROH distributions.

L103: "Our results show that social fatherhood in this Neolithic community could be as important as biological fatherhood"
Just saying "social fatherhood existed" may be more prudent.

L159: "the genotype dataset is available as supplementary material". I did not find it in the SI.

The authors could add a figure to show how pairwise mismatch patterns vary within and across sites, given that the British Neolithic population is treated as homogeneous (if I understand correctly). It could also help to show that using only the median or most distant pair from Hazleton North doesn't change the estimates.

The authors could test if the multiple partners of the same women tended to be more related to each other, than the multiple partners of the same man. This may be obvious but still, could be an option.

SI L31: "remained"

SI L92: ". They did not survive past six and nine years of age respectively." - not clear who "they" refers to

SI Section 1: It was difficult for me to interpret the data without further information, such as comparison with other contemporary groups. The main question is how representative were these individuals for their time with respect to health and diet parameters, and the answer to me was unclear. Even if this is difficult to conclude, it would be helpful to explicitly state so.

SI Section 1: Scurvy, cribra orbitalia and porotic hyperostosis, osteoarthritis - please present proportions (perhaps relative to MNI), and also comparison with other contemporary groups.

OT1: "Shotgun percentage of sequences mapping to human reference genome hg19" - should be "proportion".

SI Section 2: The discussion on possible relationships is interesting but hard to follow, with sentences such as "I21389 should be first-degree relative of I12437’s second-degree relatives, but he is third-degree-relative." (L343) with no reference to which third-degree-relatives are being considered.
Referring to figures (e.g. Ext Fig 3) and tables (e.g. OT3) more frequently would also help.

SI L350: "because individuals do not usually share their mtDNA lineage with their grandfathers" -

can the authors predict the frequency of that mtDNA haplotype in the population, to assign a probability (e.g. doi.org/10.1016/j.cub.2021.03.050)?

OT3: I21387-I21395 have r = 0.17 and SE 0.025, but are assigned 2nd degree. The table could indicate why is a 3rd degree relationship is implausible.

OT3: 8 of 9 first degree relatives have r < 0.5, somewhat unexpected. Any reasons for a bias? If there is a known reason, it would be helpful to mention in the SI.

SI L367: "I13895 and I21390 are first-degree relatives with a parent-offspring relation based on the absence of genomic regions where 2 or 0 chromosomes are shared." I did not find where this data was shown. I would recommend present a graph for each such case. This could also be uploaded in an external database.

X-chromosome relatedness information is used in some cases (L390), but (1) this is not mentioned in the introduction of SI Section 2, and (2) not used systematically (e.g. to confirm other grandmother-grandson relationship of I21390-I20821). The authors could at least add the average chrX estimate separately from those of autosomes in OT3. Also would be helpful to mark that OT3 data is only based on autosomes.

SI L399: "(...) predicts that I13888 and I21393's parents are fourth-degree relatives and that I13888 and I21393 have several long runs of homozygosity, but they lack these types of segments making this scenario very unlikely." How long would be expected and what was observed?

SI L402: "I13891 is a second-degree relative of I12440, third-degree relative of I12438-I21388-I12437 and likely fourth-degree of I12439. Depending on the topology relating I12438-I21388-I12437-I12440, I13891 can be nephew, grandson or paternal half-brother of I12440." Perhaps it would be better to show these relatively ambiguous parts of the pedigree in dots in Fig 1, instead of full lines.

SI L620: "a radiocarbon date of 4950±70 3950–3630 cal."

Extended Data Figure 3: Pairs with insufficient data (if they exist) could be shown with a different color.

Author Rebuttals to Initial Comments:

General note to all referees: We have made some additional changes to the text, images and supplementary materials because we have generated data on another 10 individuals from Hazleton North since our original submission, six of whom are part of the large pedigree. The new data increase our confidence in the pedigree reconstruction without changing any of the inferred relationships. They also provide additional details enriching understanding of the family structure at Hazleton North without changing any qualitative conclusions.

Referee #1 (Remarks to the Author):

A. Summary of the key results

This original and highly significant paper investigates the genetic and social relations of skeletal remains (MNI= 41) that were successively placed within a Neolithic chambered tomb (long barrow) at Hazleton North during a 75-100 year long period around 5700 years ago. The authors analyse the genomes of 25 of these individuals and integrates these alongside the archaeological and osteological

data from the site to reveal their treatment in relation to their biological relatedness. They use this information to construct the first extended pedigree spanning five generations and infer significant new details about the kinship practices associated with this monument. This shows that burial in this tomb was closely associated with descent from a specific male lineage, but that matrilineal descent was also a factor. Through its unparalleled integration of genetic and archaeological data, this paper presents ground-breaking new empirical evidence for adoption, social fatherhood, mating and other social practices relating to kinship in the deep past. This will be of very significant interest to many archaeologists, anthropologists, geneticists and several other disciplines. However, some readers would observe that the paper's approach and its discussion of the results prioritise biological relatedness in ways that are problematic and can easily be resolved (see comments in Section B and especially Section C on validity of approach) .

B. Originality and significance: if not novel, please include reference
Previously, archaeogenetics focused on the identification of genetic variability across vast swathes of time and space (e.g. Olalde et al 2018). More recently, there has been a turn towards more micro-scale analyses of aDNA based upon multiple samples from fewer sites. This has provided significant new information about people's mobility, chromosomal sex, genetic ancestors and biological relatedness including (re)constructions of prehistoric kinship (e.g. Sánchez-Quinto et al 2019; Cassidy et al 2020; Knipper et al. 2017; Mittnik et al. 2019; Sjögren et al. 2020).

This paper considerably advances the contribution of aDNA to understanding social relations in the deep past. By analysing the genomes of a large number of individuals from a single cemetery/tomb across a century, it provides a uniquely high resolution multi-generational spatio-temporal analysis of the biological and social relations of those who were co-interred. This yields novel insights into the complex character of intimate relations within this and potentially other Neolithic tombs.

Archaeologists have widely suggested that the placement of human remains in Neolithic tombs was one of the ways in which kinship was made/marked. For example, it has previously been suggested that the human remains in the Hazleton North tomb came from "a single, closely interconnected community that was based predominantly around biological kinship/descent " (Thomas & Ray 2018, 215). This study supports this by showing that 19 of the 25 individuals analysed from this tomb were close genetic relations. Furthermore, it reveals that many of these were paternally related (agnatic)to the first or second degree indicating that burial in this tomb was closely associated with descent from a specific male lineage.

The results also indicate a patrilocal (virilocal) residence and burial pattern which suggests female exogamy: the absence of any adult lineage daughters in conjunction with the presence of two child lineage daughters implies that women moved away from their paternal kin at marriage, while the presence of adult non-lineage women indicates that they moved into the community at marriage and were subsequently buried with their husband's kin. Although this has long been suspected for the LBK Neolithic (e.g. Whittle and Bickle 2013, 390–91), this is the first clear indications of this in a British context.

The results also indicated that marriage/mating practices may not have been monogamous: males and females each had children with multiple others suggesting polygamy. Related to this, there is evidence for the adoption of biologically unrelated males into the patriline indicating that social fatherhood seems to have been important. This is very notable because it confirms that kinship here was not fixed, but rather it emerged from a range of social practices. This is really important because this

contribution does much excellent work that avoids many of the problems highlighted in recent critiques of aDNA-based kinship reconstructions, most notably bio-determinism (e.g. Brück 2021a; Carlin & Cooney 2020; Crellin & Harris 2020).

Significantly, despite the evidence for patrilineal descent and the presence of more genetic males than females among the analysed burials, the results reveal that matrilineal descent was also considered highly significant. Indeed, the choice to construct the tomb so that it comprised two separate chambers and the subsequent deposition of burials within these seems to have been guided by different maternal relationships. This is evidenced by the fact that all those who were descended from two particular maternal lineages were found in one chamber, while most of those from a different maternal lineage were in the other chamber. These results are unprecedented and without direct parallel in European prehistory. Significantly, this results seem to suggest that the tomb's architecture and the location of burials within it were both directly related to kinship negotiations. This provides empirical evidence that kinship cannot be viewed as either social or biological (instead it is a mix of both). Unfortunately, despite the current paper's laudably integrative and balanced approach, the authors decline the opportunity to make this very important point. This needs to be addressed and doing so will further enhance the paper (see below).

-We have added a statement about kinship being both social and biological in the introductory paragraph of the main text, and highlighted the importance of both social and biological kinship throughout the text.

C. Data & methodology: validity of approach, quality of data, quality of presentation
I fully recommend this paper's publication in Nature. There are no significant flaws that would prevent its publication, but there are areas relating to the approach taken (and its limits) that could usefully be clarified that would significantly improve its strong contribution to the field and reduce the potential of future negative critiques.

Although the authors do acknowledge (lines 98-99) that "Hazleton North was also characterized by complex kinship practices some of which extend beyond biological (agnatic) kinship.", they still display a slight tendency to operate within a nature/culture binary and give primacy to the former over the latter (see Crellin & Harris 2020). The authors choose not to fully highlight or discuss other aspects of their results which further indicate that kinship was not exclusively determined biologically. For example, the presence of non-genetically related (male and female sexed) individuals in close spatial proximity within the chambers e.g. I13899, I13893, I13897, I13889, I21391, I20818. These beings seem to have been considered as significant kin for reasons that go beyond sexual reproduction or parentage (adoptive or otherwise). Indeed, the presence of these unrelated individuals in the tomb shows that kinship here was not exclusively patrilineal. Strangely these important points are not made in the paper. This also needs to be addressed and doing so will further enhance the paper.

-We have added a new paragraph to the main text discussing the individuals who are not close biological relatives of the core lineage and yet were buried in a tomb clearly structured around concepts of biological kinship. We highlight how these observations make it plausible that these

individuals were considered kin as well, even if the kinship links may not have been biological in nature (line 216).

Given the longstanding and recent critiques of biological approaches to kinship(see Schneider 1984 & Bruck 2021a & b), it is necessary for me as a reviewer to ask does this paper sufficiently avoid rooting kinship primarily in the human body? My view is that the authors would improve their paper by doing more to take account of the published critiques of recent attempts to reconstruct ancient kinship systems e.g. Brück 2021a , Bickle 2020, Crellin 2021; Frieman 2021; Furholt 2021. The paper would benefit from more explicitly acknowledging that in many societies, genetics, blood or biology are neither a determining or necessary factor of relatedness: people make their kin through cultural practices conducted within the particular context of their society like living or working or burying their dead together (e.g. Carsten 2004; Brück 2021a). This requires a statement that while the genome is a powerful tool for revealing biological relationships, the authors have chosen an integrative approach to establish how such relationships were socially understood because kinship is more than biological.

-We have added a statement about variation in how kinship is reckoned in the introductory paragraph of the main text, and highlighted the importance of both social and biological kinship throughout the text.

Similarly, family trees or genealogies like that which the paper reconstructs have been recognised as being a very narrow way of thinking about kinship which limit our ability to recognise or include other forms of relatedness (for example, see Ingold 2009). The authors could also usefully state that they are focused on a particular form of kinship which is anthropocentric and does not attempt to fully account for non-biological forms of interconnection and relatedness ’between humans and non-humans (e.g. Sahlins 2013). One potential solution to this is to insert some sentences defining what is meant by kinship in this paper while clearly stating that it is not a biological fact (with appropriate references). In addition to this, the authors could usefully attend to the question of how their findings inform us about how particular forms of kinship were naturalised in the Neolithic (I would argue that the evidence presented here is highly suggestive of attempts to naturalise kinship by making the different familiar).

-We have added a statement about kinship being both social and biological in the introductory paragraph of the main text, emphasizing that we are exploring one specific aspect of kinship (human biological relatedness) (lines 49-57). We also emphasize throughout the text cases where our data provide evidence of non-biologically-based kinship practices, such as three cases of possible adoption of males into the patriline (due to their mother having other children with patriline males), an inclusion in the tomb of 10 individuals with no evidence of close biological relatedness to the main lineage.

The authors need to be more open about the fact that they are weaving together a genetics driven narrative from incomplete data. They need to be more explicit about the potential for sampling bias: does the sample size and the exclusion of the cremated human remains from the tomb result in the

over representation of some and under-representation of others? This is especially pertinent given that the study shows that some women were treated differently at death. If more females than males were cremated, might that result in female under representation in the study?

-We discuss the possibility that women's remains were likely to have been disposed of outside tombs such as Hazleton North at a greater rate than men's through means such as cremation or exposure to the elements, which could explain the observation of significantly fewer female than male remains in Hazleton North similar to other Neolithic tombs from this time and region (Line 159).

- The bias in our data associated with not being able to genetically analyse cremated individuals is negligible given that there are only three cremated individuals known from the site - we have now explicitly stated that there are only three (line 82).

- We now explicitly acknowledge how genetic analysis can never provide a complete picture of kinship, writing: "Such reconstructions are necessary in order to explore the genealogical aspects of kinship in past societies and also to identify kinship practices that extend beyond genealogical descent. Anthropological studies have made it clear that kinship—the relationships of family connection and belonging that play a central role in organizing societies—varies dramatically across cultures. Biological relatedness may be of greater or lesser importance in determining kinship; kin need not be biological relatives (and need not even be human), and child rearing is not always centered on the relationship between biological father and mother. Funerary practices often play an important role in the social negotiation of connections and divisions between kin, and here we use this insight, along with the ability of ancient DNA to document biological relatedness, to provide a window into the role of biology in determining kinship among people who buried their dead in Neolithic chambered tombs."

- We have added more explicit discussion of how the sample size for genetic analysis compares with the osteological indication of the minimum number of individuals in the tomb to the supplementary text, section 1, and main text (line 235).

The authors recognise that social organisation and kinship organisation are not always the same (Godelier 2009) and they avoid jumping to problematically andocentric conclusions about the social role of people in the Neolithic (see Bickle 2020, see Frieman et al 2019). However the paper seems to assume that sex is biological or chromosomal and that gender was a fixed/essentialised entity which can be chromosomally determined. This is indicated by the author's consistent use of terms like male and female as if these binaries were valid and neutral categories of analysis. The paper insufficiently attends to the fact that gender is constructed relationally in ways that are historically and contextually specific. There also seems to be an assumption that the family and marriage was universally heterosexual/heteronormative. These are recognised issues that have previously been critiqued (e.g. Bickle 2020 Robb & Harris 2017; Frieman et al 2019, Geller 2008; 2017). Therefore, it is necessary for the authors to critically reflect on their chosen categories of analysis and their impact on their

conclusions and then to explicitly state these in text. It would also be good to explicitly define what is meant or not meant by marriage to avoid colonising the past with our own present-day western concepts of hetero-normative families (see Furholt 2021)

-We have added a discussion of how our analysis is based on chromosomal sex which we accept is only one element in how both sex and gender are contextually and culturally defined. Thus, we have added the sentence: "In this study we use "m/male/man" to indicate an individual with an X and a Y chromosome, and "f/female/woman" to indicate an individual with two X chromosomes, recognizing that chromosomal sex is only one element in how sex and gender are contextually and culturally defined."

- A fuller interpretation of the implications of these findings for theorizing gender in the Neolithic is important but lies outside the scope of the current article. We have suggested that the combined acknowledgement of patrilineal descent and maternal lineage for each individual will be of interest in further interpretations of personhood and gender, citing Robb and Harris 2017.

-We agree with the referee that the term 'marriage' has present-day and historical connotations that might have not applied in the society where the Hazleton North individuals were integrated. Thus, we have replaced the use of the term 'marriage' with terms such as 'reproductive partnership'.

The authors seem to conflate value with status in Lines 107-109. This overlooks the fact that there are multiple reasons relating to the social identify of the deceased for why adopted sons were not buried in the inner chambers. Indeed on Lines 126-7, the authors themselves state that the "arrangement of chambers at this Neolithic tomb was related to negotiations of kinship". There is no supporting evidence provided to indicate that the values associated with a particular spatial location for burial were understood in relation to status. Any assumption that this is the case would rest upon a number of highly problematic assumptions that would ignore the last 30 years of archaeological thought including the work of one of the authors (e.g. Fowler 2013).

-We agree with the referee and have removed the reference to status. Furthermore, given that the north chamber was blocked at some point, adoptive sons might well have been interred outside the chamber for this reason, and we discuss this explicitly (see below).

The authors suggest that the practice of adopting people into the patriline "may have been advantageous during the first agricultural expansions in Britain" (Line 116-118). This statement is problematic. Firstly, we know that the adoptions occurred between 3700-3600 BC, at least two or three centuries after the first agricultural expansions to south central England c. 3900-3800 BC (see Whittle et al. 2011, fig. 15.8), so it is more than a reach to try to link the evidence for this social practice to the latter. Secondly, for all we currently know (given that this is the first study of this

kind), adoption may well have been a standard practice that was not exclusive to Earlier Neolithic Britain. Thirdly, this represents an unduly instrumentalist interpretation of an act of social kinship that may have been conducted for a thousand other reasons and falls into many of the traps of evolutionary psychology.

-We have removed the reference to a link between our observations and agricultural expansions.

More information on the site itself could usefully be included in the SI.
A descriptive who's who outlining all the relevant info for each person in the SI would be very informative. I found it necessary to complete my own version of this to be able to review the paper.

-We have added a new table (Extended Data Table 1) with an entry outlining these details for each individual identified through the genetic analysis. We have also created a new nomenclature for naming the individuals that is much easier to understand (the identifier gives information on their location within the tomb and their biological sex).

Fig 1C could be improved by making it easier to identify and differentiate first degree, second degree and third degree relationships

Degrees of relationships in the tree can be identified by counting the genealogical steps separating two individuals. For instance, grandfather and grandsons are separated by two steps, hence second degree. We believe that including explicit information about the degree of relationship between all pairs in the tree, such as lines with different colours, will make the figure unreadable.

D. Appropriate use of statistics and treatment of uncertainties, methodology, statistical analysis, clarity of the text and Given the fuzzy nature of the data, the statistical tests conducted are appropriate and I see no issues with these. There are a few areas where minor clarifications would improve the text.

The authors could usefully clarify for the less specialist reader whether the aDNA analysis conducted here is based upon the sequencing of whole genomes, rather than the targeting of a limited number of DNA sequences.

The aDNA analysis is based on targeted enrichment of DNA libraries for DNA molecules overlapping 1.2 million polymorphic positions in the nuclear genome, and sequencing of those DNA molecules. Hence, it is not based on the sequencing of whole genomes. We have clarified this throughout the main text and supplementary materials, for instance in the main text "We extracted DNA, generated double- and single-stranded libraries, enriched for molecules

overlapping approximately 1.2 million polymorphic positions in the nuclear genome as well as mitochondrial DNA, and sequenced these libraries".

The word 'large' is used quite a few times in the paper e.g. "few large sample size studies from individual cemeteries" or "our ability to construct a very large family tree" but nowhere is 'large' explicitly defined.

-We have removed the term "large".

E. Conclusions: robustness, validity, reliability
strength of the conclusions, Do you find that the conclusions and data interpretation are robust, valid and reliable?

The conclusions are very strong but their robustness and validity will be greatly increased by addressing the issues highlighted above in Sections B, C & D. Here are a few additional comments relating to other aspects

It seems that the results indicate a reduction in Y chromosomal diversity over time compared to a high level of mt DNA diversity indicating the movement of women into the population as observed in other aDNA studies like we see in Sjögren et al. 2020. The lack of comment on this seems odd as it would lend support for patrilocal exogamy.

While the mtDNA diversity at Hazleton North is high, the Y chromosome diversity is very low, with all males (including non-lineage males) belonging to the same paternal lineage with the exception of male SE2m. However, we do not see any signal of decreasing Y chromosomal diversity over time in the site as it basically just one lineage from the outset. The low Y-chromosomal diversity is an island-wide and indeed Europe-wide phenomenon already documented in the literature on Neolithic Britain (e.g. Brace et al. 2019 or Olalde et al. 2018), and is a consequence of expansions of a narrow pool of very successful male lineages, but does not provide evidence of patrilocal exogamy at a site-specific level. For instance, the paternal lineage of Hazleton North males is quite common in Neolithic Britain, so even if males not closely related to the Hazleton North individuals joined the Hazleton community from nearby regions, they could well present the same paternal lineage as the Hazleton males to the limits of our resolution, as they shared common patrilineal ancestors hundreds of years in the past. The high mtDNA diversity indeed supports the movement of women, but in our study this evidence is superseded by the actual direct finding of women incorporated in the Hazleton community who reproduced with males in the patriline, and the recovery of genome-wide data (not just mtDNA) from these women that allows us to test directly their relationship with other individuals at the site.

The paper states (line 80-81) that "each individual whose lineage we can trace through the second generation to the first is connected entirely through males: thus, all 14 of the parent-to-child linkages

are through fathers". While the broad thrust this seems to require more clarification. I21388 is a son of female I21390 & son of male ?1 (whose remains were not identified) and I13895 is a son of female I21390 &'adoptive son' of male ?1 (whose remains were not identified). This seems to contradict/ invalidate the statement above. Notably there is no star to indicate the parentage of these two individuals in Fig 1C

- We now write: "Firstly, each third-, fourth- or fifth-generation individual whose lineage we can trace through the second generation to the first is connected to NC1m entirely through males. Specifically, all 15 of the genealogical connections are through fathers (13 cases) or stepfathers (2 cases) (P=0.000061 from a two-side binomial test; Fig. 1c, Table 1), providing the first direct evidence that patrilineal descent was a primary determinant of who was interred with whom in the Neolithic."

The reason we can only make statements about patrilineality (or matrilineality) when we study pedigrees of at least three generations is that what we are studying in this analysis is connections between individuals with at least one generation in between, so it is possible to determine whether the connection is through a male or through a female. Specifically, we are connecting individuals in the family tree to male NC1m, and thus we start in generation 3 (it does not make sense to study for instance whether the connection between SC2m in generation 2 and his father NC1m runs through a male or female because there is no generation in between the two).

To clarify this, we have drawn marks (black if biological father, blue if adoptive father) on the corner of individuals to represent how many genealogical transmissions traverse through each individual up to male NC1m. In the case of male NC5m (generation 2), SE1m (generation 2) and SP1m (generation 3), we draw one black mark on SE1m to represent that there is one genealogical transmission between SP1m and NC1m running through SE1m, who is the biological (hence black) father of SP1.

Lines 46-8: ". Strontium and oxygen stable isotopes on teeth were similar for 18 of 22 sampled individuals suggesting they had spent some time on the geology local to the tomb and some on geology at least 40 km away during their childhood". The authors did to make it much clearer that they are referring here to 22 individuals from a completely different previous study and that only 10 of those individuals with isotopic data from that study overlap with 10 (3 women & 7 men) of the 25 individuals with genetic data in the present study. This is necessary for the full and fair consideration of subsequent statements relating the results of both studies to each other as discussed in Lines 110-115.

Lines 110-115: "Two of three individuals with local stable isotope signatures from childhood were not part of the biological patriline while the third was the child of a man adopted into the patriline, and all three were buried in the periphery (entrance or passage). This contrasts with five individuals born into the lineage and buried in the chambers with stable isotope signatures indicating mobility across geological zones, suggesting that members of the patriline had a mobility pattern in earlier life not shared by all those buried in the tomb".

This contrast highlighted in lines 110-115 “that members of the patriline had a mobility pattern in earlier life not shared by all those buried in the tomb” does not fully incorporate the chronological and generational differences between the different individuals. For example, the non-lineage individual I13893 in the periphery of the north chamber is-chronologically later according to Bayesian modelling, 3645–3615 BC. The authors neglect to mention that two non-lineage individuals (I21391 & I21395) show cross-geology subsistence, thereby indicating that either they were considered part of the patriline and/or that this mobility pattern was shared by the patriline with other members of the community. It is also notable that this cross-geology mobility pattern was present across multiple generations of the lineage. However, the authors have either not identified or highlighted the evidence for this intergenerational practice.

-We have revised the passage on interpreting the stable isotope data, as the results are not in fact definitive given our limited sample size of individuals with overlapping genetic data. We write simply: “We have too few measurements of stable isotopes on the individuals we analysed to be able to study correlations to cross-geology mobility but isotopic analyses of additional individuals with genetic data could reveal undetected patterns.” (lines 246-248).

F. Suggested improvements: experiments, data for possible revision
See previous comments

G. References: appropriate credit to previous work?
Following on from my comments in Section C, the manuscript could usefully reference previous literature relating to the limits of their approach
Line 75, the authors state their study to be “more than twice as large as the largest pedigree reconstructed from ancient DNA data to date”, but they do not provide a reference to that other study here.

We reference two studies describing pedigrees of 9 (Mittnik et al 2019) and 10 (Amorim et al 2018) individuals. With the addition of our new data, this pedigree has 26 individuals.

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions

The overall text is very clear and accessible but the abstract could be rewritten to make it easier for a wider audience to understand and appreciate the paper’s significance.

-We have edited the abstract which we believe highlights the key conclusions of the article.

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Peer review by Neil Carlin

nature portfolio

Referee #2 (Remarks to the Author):

Review of Fowler et al. 2021

Fowler and colleagues present the largest pedigree yet reconstructed for a set of prehistoric individuals. This consists of 19 individuals sampled from a single Megalithic tomb. This is a very impressive piece of work, based almost entirely off a single measure of genetic distance (the allelic mismatch rate), which is leveraged to estimate kinship coefficients and local patterns of IBD-sharing between pairs of samples. The authors combine these values with standard aDNA and osteological metrics (sex, uniparental markers, age-at-death) to meticulously reconstruct the best fitting pedigree through a manual process of elimination. From this, the authors are able to draw several conclusions about the community's kinship practices, including a descent system that emphasized the patriline and potential adoptive practices. The latter is an exciting find and demonstrates the use of genetic data in the detection of diverse forms of kinship.

Overall, the manuscript is a wonderful example of the power of ancient DNA to inform on social organization, which has in recent years become the new frontier of the field. What makes this study stand out from others of its kind is the level of detail provided for a single extended family, which gives us unprecedented insight into the cultural practices, values and lifeways of a Neolithic community. The use of ancient DNA to potentially inform on different aspects of prehistoric architecture is also noteworthy, made possible through adept integration of archaeological data (e.g Bayesian modelling of RC dates).

I believe this study has the potential to draw interest from a wide readership, however I do have some concerns. Most generally, I feel that the broader significance of Hazleton North is left unclear to the non-specialist. Do the authors believe their results have altered our understanding of the Early Neolithic of Britain in any major way, or do they see their study as a proof-of-principle? They seem to suggest (Line 152) that it would be inappropriate to extrapolate about kinship practices in Early Neolithic Britain from the Hazleton North pedigree, even for chambered tombs built within the same region, although I might be misreading this? However, in the abstract they suggest this sort of kinship system may have promoted agricultural expansion across Britain. This seems contradictory.

-We have added the following to the concluding paragraph: "Nonetheless, this analysis advances our understanding of kinship and chambered tomb construction in Neolithic Britain. Future research carrying out similar studies in additional tombs both in a Neolithic context in northern Europe and in other cultural contexts has the potential to test alternative theories of kinship in past societies."

-We have removed the statement concerning agricultural expansion.

They also do not integrate results from other genomic surveys of megalithic tombs into the discussion section, including evidence for patrilineality, which could enhance their interpretations.

- We now add a reference to Sanchez-Quinto et al. 2019 and Cassidy et al 2020 when explaining the results on patrilineality. We clarify that a unique contribution of our study is that it is the first to provide direct evidence on patrilineality in the Neolithic British Isles. Previous studies have relied on indirect evidence such us biased sex ratios among burials (a type of inference that has also come from physical anthropological surveys) or on observations of the same Y-chromosome haplogroups reoccurring over time at the same site (Sanchez-Quinto et al. and Cassidy et al.). Specifically, we write: "These observations are consistent with circumstantial evidence of patrilineality in prehistoric Britain and Ireland based on the persistence of rare Y-chromosome haplotypes over time among individuals from the same Neolithic tombs (although such patterns can also arise due to single reproductively highly successful males in small endogamous communities in the absence of patrilineality)." In our revision, we clarify that what we add in this study to document patrilineality is pedigree reconstruction, which can only distinguish between patrilineality and matrilineality with pedigrees of at least three generations since the first two generations by themselves are uninformative (see response to referee 1).

Finally, more context could be provided on the demographic and cultural landscape of Britain at the time (e.g. large scale influxes of new people, construction boom of chambered tombs ~3700-3500 BC in certain regions, relatively rapid spread of the Neolithic package). At times it feels that Hazleton North is being considered in a vacuum, which may lessen the manuscript's overall impact.

-We have added a few lines at the start of paragraph 2 explaining the Early Neolithic context of this site in Britain.

I also have a number of more specific concerns and suggestions, which I detail in the below comments.

COMMENT 1: THE NORTH WALL COLLAPSE

The idea that the architecture of the tomb was at least in part guided by kinship is an exciting one. However, the authors also concede there is a likely temporal component when it comes the placement of individuals in the tomb. Specifically, the north passage-entrance wall collapse c. 3660–3630 cal. BC, which may have led to the abandonment of northern compartments in favour of the southern one (Lines 133-135). I am concerned that this may be a confounder in their tests of significance for the north-south differentiation of maternal lineages. No significance tests were carried out to detect north-south differentiation on the basis of date of death.

I was curious and put together a rough spreadsheet (attached) estimating these dates from the individual's approx. age-at-death and generation in Tree1. I made the assumption that individuals in each generation were born at approximately the same time with a 25 year gap between generations. If we assume a birth date for generation 1 individuals at 3,710 BC (the earliest date for I21390 that still fits the Bayesian model, supp line 604) and a wall collapse at ~3,640 BC, we get a significant differentiation between individuals that died pre- and post- collapse (p=0.0034) with respect to north and south placement. I think this deserves more attention in the text, which discusses the collapse only

in terms of disrupting the duality (lines 130-132), rather than as a potential driver of this perceived pattern. The authors could also include an analysis similar to that provided in the spreadsheet.

-This is fully addressed in SI section 7, and we have added further discussion of the collapse event and its impact on the duality of deposition at the site in the main text (197-203).

The wall collapse may have also impacted the placement of the possible adoptees. I note the authors' tests for both adoptee and maternal lineage differentiation produce p-values close to the threshold of significance (~0.025) and are based on small sample sizes. For this reason, I believe more caution is needed in interpretation. e.g. in lines 10-11, 108-110 and 152.

-We agree with the referee that the placement of adoptees outside the North chamber is plausibly related to its blockage. We have re-evaluated this analysis and removed it from the article.

COMMENT 2: ANCESTRAL MAKE-UP OF THE HAZLETON NORTH POPULATION

I understand that this is a study on kinship, rather than deep ancestry. However, the ancestral profile of the 15 newly reported individuals (line 57) is of relevance to both the field and to the interpretation of the pedigree. Specifically, are there any individuals with an atypical profile? Do any individuals show evidence of recent European HG ancestry or appear more closely related to a Neolithic population outside Britain and Ireland? Lines 228-233 in the supplement suggest not, but I think it is important to provide the actual data underlying this statement in the online tables and distinct supplementary section.

We now include a Principal Component Analysis (PCA) of the Hazleton individuals (SI Section 6 and Extended Data Figure 9a). They all have the typical profile for Neolithic individuals from Britain, with no evidence of recent hunter-gatherer related admixture in any individual.

COMMENT 3: ATYPICAL MATING PATTERNS

The authors assume a relatively simple pedigree structure due to the lack of evidence for inbreeding across individuals. However, I was surprised to see no consideration of other possible confounders, such as double cousins. Marriages between multiple siblings from different families is a common practice in some societies. Skimming through the data, I believe this can be ruled out as an explanation for the 2nd degree relationships presented here, but it might be useful to be explicit about this.

We now discuss the possibility of double cousins throughout SI Section 4, for instance in 4.3.2 or 4.3.4. We can rule out double cousins in all cases.

COMMENT 4: THRESHOLDS FOR DEFINING RELATIONSHIPS

How did the authors assign the degree of relationship between two samples? I assume threshold values of r were chosen, but I can't spot any mention of this in the text. How confident are the authors in their assignment of 3rd and 4th degree relationships? I ask, because these relationships are often presented as "very likely" or "likely" in Supplementary section 2 (see COMMENT 5), but I'm unaware of the exact justifications behind these statements.

We now explain in detail the assignment of degrees of relationships in SI section 4.2.

For example, two possible pedigrees are excluded for [I12437, I12438, I12440, I21388], where I12437 is a paternal grandfather or uncle to the other three, given that I13898 is a second degree relative of I12437 and either "likely" or "very likely" more distant than 4th degree with the other three (lines 273—296). However, I estimate from Online Table 3 that a coefficient of relatedness of ~0.035 is required to be classified as possible 4th degree relatives. I13898 has r ± SE of 0.026-0.046, 0.012-0.04 and 0.011-0.031 for I12440, I21388 and I12438 respectively. I understand from the later lines 349-353 that this pedigree is also excluded as it would require I12437 to be a grandfather of I13898 with the same mtDNA haplogroup, which is also unlikely. In the next paragraph I13898 is said to be "likely a fifth-degree relative of I12440", criteria again unclear.

Could the authors provide more clarity and consistency in this regard? Perhaps a list of statements at the start of the supplementary section detailing their main assumptions and their confidence in these (e.g. thresholds of r, ruling out male ancestor and female descendent relationships if the individuals share the same mtDNA haplogroup, number of expected shared IBD segments etc.)

The logical process of discarding tree topologies is now much easier due to the many additional constraints provided by the new individuals, and we have also amended Supplementary Information section 4.3 to increase clarity and consistency as the referee suggests.

COMMENT 5: PRESENTATION OF THE PEDIGREE RESULTS

This paper is centered on a single landmark result, the reconstruction of a five-generation extended pedigree from aDNA data. This was very carefully done, using all available lines of evidence and a logical process of elimination. However, the presentation of this process (SI Section 2) and the figures detailing the results are at times difficult for the reader to follow.

Suggested improvements -

1: Labels
Please use a simplified set of individual labels in the main text, figures and supplementary info, with a key to the longer Lab IDs provided in the online tables. It is impossible to keep track of multiple similar six character IDs. Also, please find another way to represent unsampled individuals without the use of a preceding "?". To the unfamiliar reader, it continuously registers as punctuation or font error.

A possible labelling system could be based on sample location in the tomb, with an additional qualifier for sex. Having the sex information in the ID would make the supplement a lot easier to follow, without having to continuously refer back to the figures and tables. For example, mNE1, mNE2, mNE3, fNC1, mNC2, fNC3, mSP1 and so on. Unsampled individuals could then be denoted with an "X" (e.g. mX1, fX2). This would also help simplify Figure 1, which is very busy and whose fonts are too small to read (see next).

-We very much appreciated this comment and have revised the labelling system throughout the article and supporting materials. The new system is inspired by the one suggested by the referee, providing information on the location, the number of that individual among those at that location and their sex: e.g. NC1m = North chamber individual 1 who is male. Unsampled individuals are identified with an "U".

2: Figure 1
This figure is dense with interesting information, but it is not the easiest to digest. Some suggestions to improve things

1. Increase font sizes and remove unnecessary text (e.g. the locational data box, the title "uniparental genetic markers")
2. Are both the blue and yellow stars and dots necessary?
3. Use consistent line thickness and distances between double lines on pedigree
4. It is hard to quickly differentiate between sampled and inferred individuals. Perhaps the inferred individuals symbols could be reduced size? The dotted line types are also different for inferred males and females.
5. Align the generation labels on the left or right side of the plot.
6. Standardise the text and placement of RC dates.

We thank the referee for these suggestions and have implemented most of the proposed modifications to the figure.

3: Simplifying the Supplementary Text
As the paper is entirely dependent on the logic outlined in SI Section 2, more needs to be done to make this section clear, concise and accessible to the reader. Better use of headers, bold fonts and bullet points/numbers (rather than large blocks of text) would be welcome, or even embedded tables if useful. An initial summary of the logical progression detailed in subsections 1-8 maybe useful at the start to orientate the reader.

We appreciate these suggestions and have implemented them in SI Section 4.

There are a lot of repetitive statements in SI2 subsections 1-5 that could be condensed or relegated to Online Table 3, especially when it comes to definitive relationships (e.g. siblings). One example of

repetition is the section on I12437, I12438, I12440, and I21388 (line 256), all pedigrees involving maternal half-brothers and maternal uncles can be instantly ruled out, given that none share an mtDNA haplogroup. However, these pedigrees are ruled out separately in lines 268 and 307. Pedigrees involving two or more paternal uncles of a single individual can also be easily ruled out as they require first degree relationships. However, these are discussed separately in lines 303-306 and 328-336. Lines 328-336 also rule out pedigrees in which three individuals are nephews to a fourth, which seems confused as two of the five plausible pedigrees are this structure (Lines 263-264). Please order this section in a more logical and concise manner and double-check all statements are consistent. Also double-check grammar and spelling (e.g. line 347 is missing a word I think.

We believe this revised section (SI Section 4) is now simplified and easier to follow.

The authors also need to be more explicit as to the meaning of the phrases used in SI2 (see also COMMENT 4). For example, one header (line 267) states that all other possible relationships listed below are "ruled out". However, below this header we see both "ruled out" scenarios and "extremely unlikely" ones. The "extremely unlikely" ones are dependent on the exclusion of fourth degree relationships, which is deemed either "likely" or "very likely". Similarly, the header of line 337, uses the definitive phrase "can only be related in a single way". However, this is again dependent on probability (that it is unlikely two unrelated individuals would share the same mtDNA haplogroup by chance and that the inference of a 5th degree relationship is correct – lines 356-360). In other parts, what I would consider definite relationships are presented in a way that implies potential uncertainty (e.g. line 340 – "indicating a sibling relationship")

This has now been amended.

.
To be clear, I am happy with logic used to eliminate different relationships from the pedigree and that tree1 is by far the best fit, but more needs to be done to differentiate statements of truth from those of high probability. Condensing statements on clear-cut relationships (e.g. in concise bullet point list) may help focus the reader's attention on more important passages that require some weighing up of probabilities or more complex deduction.

COMMENT 6: INFERENCE OF KINSHIP PRACTICES

The inferences drawn from the pedigree with respect to kinship practices are for the most part robust and carefully considered. However, I find some parts of the discussion a little unbalanced (e.g. too much speculation on adoption), ambiguous or contradictory. I've identified three main issues.

1: Definitions of kinship and complexity

The title of the study is vague. It is unclear what defines a "complex kinship practice" and how it differs from a "non-complex" practice. In fact, I'm not sure of any society whose kinship practices can be regarded as simple. I suppose a kinship system itself can be considered in terms of complexity (e.g. number of kin terms and rules of descent). However, the authors cannot make much comment in this regard. Indeed, the fact that they were able to provide such strong evidence for "a central role for patrilineality" could suggest relatively simple unilineal rules of descent, although the possible

differentiation of maternal sub-lineages is interesting.

Also, it seems problematic to conflate the complexity of a community's kinship practices with the presence of adoption, which is a relatively common phenomenon (Line 98). The concept of fatherhood can be just as complex in societies where adoptive practices are absent.

To increase clarity, I suggest the authors provide a more explicit title that refers to the main inferences drawn from the pedigree. If the word "complex" is to be used, I suggest it is qualified. It also may be useful to differentiate between the genetic definition of kinship (degree of biological relatedness) and the anthropological one (e.g. the values a society may or may not ascribe to biological relatedness) early in the text for the general reader. Anthropological kinship is by definition more complex than genetic kinship, regardless of whether a biological relationship is actually present.

-We have removed the term 'complex' from the title and the article and have also added several additional lines discussing social kinship practices and emphasizing that they can be as important as biologically-determined kinship.

2: Evidence for adoption

I believe the authors have provided good evidence for the adoption of non-lineage males into the Hazleton North patriline. However, I do not think they have provided definitive evidence and they need to temper their discussion to reflect this. For example, the term "adopted" is used with certainty at various points (e.g. line 111). The abstract states, rather than suggests, that "husbands commonly adopted their wives' children into the patriline. (line 13). The conclusion (151) states that the "acceptance of step-sons into the patriline" has been revealed. Online table 1 also describes both adoption and marriage in certain terms (column Z).

-We are cautious in our phrasing when discussing the inference of adoption and have added additional qualifiers.

The evidence for adoption comes from three women (?5, I21390, I21387), who each reproduced with both a non-lineage and lineage male, and whose non-lineage adult sons and one grandson are present in the tomb. The authors also argue that there may have been some status differentiation between "adoptees" and biological descendants, on the basis of the placement of these four males outside the chambers (although see COMMENT 1).

There are, however, explanations alternative to adoption, that should be considered in the text.

1. If there is a potential status difference, it is also possible that the "adoptees" were not actually viewed as members of the patriline. It is not definite that everyone interred within the tomb was a member of the patriline. Indeed there are six unrelated "non-lineage" individuals interred within both chambers and entrances, whose presence remains unexplained.

-We have added a new paragraph on the 'non-lineage' individuals and the implications of their presence for understanding kinship (line 216).

2. I am surprised to see no consideration of infidelity or rape, given the prevalence of both across diverse societies. It is problematic to assume that men in the Neolithic had complete knowledge of their partners' sexual and reproductive lives. There are many reasons why a woman may conceal paternity information.

- We state that 'We cannot determine whether this was an instance of serial monogamy or polygyny, and we cannot exclude the possibility of progeny from unions that were not socially sanctioned in any of the six instances." We do not have any data that would allow us to be more specific or speculate on the nature or disclosure of parentage.

3: Discussion of marriage practices

While I find the manuscript overconfident in its discussion of adoption, it is perhaps a little overcautious in its discussion of patrilineality, patrilocality and potential polygamy. Specifically, relevant data from both the anthropological and ancient DNA literature are not considered in the authors' discussion, which may have helped to enhance their conclusions.

1. The ancient DNA literature. Two studies on megalithic tombs in Britain and Ireland have provided evidence for patrilineality (Sanchez-Quinto et al. 2019; Cassidy et al. 2020). Most relevant to the current manuscript is the Irish survey, which took sizeable samples from a chambered tomb and a neighbouring portal tomb (lines 25-26). While no close relatives were found, they did observe a significant differentiation between the tombs with respect to a rare Y haplogroup. They interpreted this as social differentiation between the tomb users with an emphasis on patrilineal descent. This result should be noted in the introduction and integrated into the discussion. This study also reported elite inbreeding in a megalithic context, a practice that strongly associates with patrilineality and polygyny. Thus, there is already a precedent for the Hazleton North results in the literature, which is not clear in the text.

- We now discuss these studies in the introduction and discussion, but at the same time highlight the novelty of the genetic evidence presented in our study as compared to previous ones.

2. The anthropological literature. The authors have provided convincing evidence that patrilineal descent played a central role at Hazleton North (Line 151). There is also evidence for patrilocal exogamy (line 91). However, the authors go on to state that it is inappropriate to make any inferences about other aspects of the underlying kinship system and cite a single cautionary case study as justification for this (Lines 93-97). This does not seem well-reasoned. Cross-cultural comparisons have consistently shown correlation between patrilineal descent, patrilocality and other male-biased features of kinship systems. Most recently, Surowiec et al. (2019) considered ~1000 populations and demonstrated patrilineal descent was strongly correlated with patrilineal inheritance of property,

political succession and patrilocal residence, and negatively correlated with the female-based equivalents. I think it is reasonable to suggest that the discovery of patrilineal descent at Hazleton North increases the likelihood that the community's kinship system had other male biases. Of course, the authors are not obliged to include such a statement in their manuscript. However, lines 94-97 seem almost imply the opposite (that the likelihoods have not changed in view of these new results). Could the authors soften this a little?

- While patrilineality does broadly correlate with the factors described by the referee, those do not by themselves give an understanding of the role of women in those patrilineal societies (as discussed by some of the sources cited by referee 1), and it is also important to bear in mind the evidence for maternal sub-lineages at Hazleton North. We are therefore cautious about drawing broad inferences about gender relations at Hazleton North based on the cross-cultural comparative source cited (which does not seem to include the polgynous patrilineal Nuer in its analysis, for instance, among whom maternal ancestry is also important). We are only seeing patrilineal descent in death (and not for all individuals in the tomb) and mortuary practice is only one context so we are cautious about making wider generalizations about gender relations based on this evidence.

We have, however, acknowledged the reviewer's point, stating:

"These results show that patrilineal descent played an important role in shaping social relations—a finding that provides some insight into the nature of the community at Hazleton North (especially given the associations between patrilineal descent, virilocality, polygyny and cattle husbandry documented in ethnographically diverse cultures)—but as we show below, the spatial organization of the dead, and the inclusion of individuals who were not part of the biological patriline, indicate that other considerations also had significant influence on burial patterns. (lines 169-175).

The authors also present serial monogamy, polygyny, polyandry and extra-marital relations as equally plausible explanations for the instances of multiple reproductive partners in their pedigree (Lines 118-120), again without reference to the comparative anthropological literature. However, polyandry is rarely observed and non-fraternal polyandry can violate the rules of a patrilineal descent system. Suroweic et al. show positive correlation between patrilineal descent and polygamy, and confirm its strong correlation with cattle and large animal domestication. This and related literature (e.g. Holden and Mace 2003 on the Bantu expansions) may be of interest to the authors, given they discuss polygamy as a potential strategy to rapidly grow a patrilineage during the agricultural expansions across Britain, but provide no references (lines 116-118).

- We have removed the reference to agricultural expansions and polyandry, and have flagged the common association of polygyny with patriliny and cattle husbandry (see above).

MINOR NOTES

1. Can X chromosome mismatch rates be provided in a table?

- We now provide X-chromosome mismatch rates in OT5 and show their concordance to the pedigree reconstruction in Extended Data Figure 6a.

2. Typos in online table 3: 4rd ->4th; I21395 is a lineage descendent, but not marked as one.

- Fixed

3. Lines 47 and 110 are a little hard to parse. Line 47 states that 18/22 individuals have isotopic signatures suggesting mobility during childhood. However, Line 110 states only three individuals have local signatures from childhood. Also the overlap between ancient DNA samples and isotope samples is not clear

4. Given the potential for female exogamy, do the authors note any difference in female isotopic signatures relative to males?

We have revised the passage on interpreting the stable isotope data, as the results are not definitive given our limited sample size of individuals with overlapping genetic data. We write simply: "We have too few measurements of stable isotopes on the individuals we analysed to be able to study correlations to cross-geology mobility but isotopic analyses of additional individuals with genetic data could reveal undetected patterns." (lines 246-248).

REFS

Surowiec A, Snyder KT, Creanza N. A worldwide view of matriliny: using cross-cultural analyses to shed light on human kinship systems. Philosophical Transactions of the Royal Society B: Biological Sciences. 2019;374(1780):20180077.

Holden CJ, Mace R. 2003 Spread of cattle led to the loss of matrilineal descent in Africa: a coevolutionary analysis. Proc. R. Soc. B 270, 2425 – 2433. (doi:10.1098/rspb.2003.2535

Cassidy, L. M. et al. A dynastic elite in monumental Neolithic society. Nature 582, 384-388, doi:10.1038/s41586-020-2378-6 (2020).

Sánchez-Quinto, F. et al. Megalithic tombs in western and northern Neolithic Europe were linked to a kindred society. Proceedings of the National Academy of Sciences of the USA 116, 9469-9474, doi:10.1073/pnas.1818037116 (2019).

nature portfolio

Referee #3 (Remarks to the Author):

The work by Fowler and Olalde et al. is an elegant example of use of aDNA in studying past human social traditions and dynamics. The authors construct a pedigree using multiple types of information collected from the Hazleton North skeletal collection, and using this data they test a number of hypotheses on kinship and burial patterns. They show strong patrilineal burial customs in this community, and at the same time, that adoptive sons (who may have been recognized as such, given differences in their burial context) were included in the patrilineage, at least with respect to their burial location.

The most significant novelty is the size of the ancient pedigree constructed, and some of the approaches used in the process are also innovative in being applied in kinship analysis of ancient individuals (e.g. use of IBD segment sharing to distinguish kinship types). That European Neolithic and BA communities frequently adopted patrilineal burial patterns has been shown in other work using isotopes and DNA (e.g. Bentley et al 2012 PNAS; Sánchez-Quinto et al 2019 PNAS; Mittnick et al 2019 Science), but the question is studied here at additional depth and new angles.

I enjoyed the manuscript. I would like to share some suggestions that should be simple to address.

Methodologically the approach used to construct the pedigree appears sound, but I have to admit I had difficulty following SI Section 2. Perhaps this could be written in a way to aid the reader follow the logic and analyses. Additional visualisation or more frequent references to tables/statistics (like that provided in some external data figures) would be extremely helpful.
I would also recommend adding more methodological detail in general: for instance, how ROH analysis was conducted is not mentioned, when and where chrX data was used was unclear, etc.

- We appreciate the referee's comments and have implemented all the suggestions.

Another suggestion would be adding analyses to demonstrate how well the data fit the pedigree. For instance, the authors neatly use IBD segment sharing information in Supplemental Section 7, but only for a subset of cases. Why not compare the expected and observed number of segments for all pairs in a tree? This could provide a level of confidence in the tree, beyond the qualitative description (somewhat analogous to qpGraph).

The authors could also run a ML-based model such as NgsRelate on their pairs (at least the pairs in their final tree). This estimates both theta, but also Cotterman coefficients, and F, and could help improve confidence in the estimated relationships. Using chrX vs autosome comparisons could also be used more systematically.

Small errors in the pedigree would likely not alter the main conclusions (as the authors also explicitly show for their Trees 1-5), but I would still feel more safe if some additional sanity checks were conducted.

- Aided by the additional constraints provided by the new individuals, we now reconstruct the family tree without using X-chromosome information or information from the number of shared segments (SI Section 4.2), and use these two pieces of evidence as well as NgsRelate results (SI Section 4.5) to evaluate the validity of the proposed tree structure (e.g. Extended Data Figures 6-7).

There could be further joint interpretation of the genetic and anthropological data, at least in the Supplemental section but possibly also briefly in the main text. I feel that it would be important for the authors to help the reader (especially those alien to British archaeology) to assess what type of prehistorical community this was. For instance, do the dietary patterns or frequencies of pathologies distinguish this group from its contemporaries? I think the paper should convey some idea of whether these individuals represented a commoner or an elite group.

-We have added further text contextualizing the Hazleton North population to both the main manuscript and SI section 2.

Finally, I would recommend submitting the data to SRA in advance of publication and providing a reviewer link.

- We provide the genotype files as supplementary materials and have submitted the sequence data to ENA to be available upon publication (the European Nucleotide Archive – which is the same type of respository as SRA and has become the primary place where ancient DNA raw data are deposited). The accession number is PRJEB46958.

- We have also created a Dropbox folder with the raw sequence data and the reviewer can access it through the link here:

https://www.dropbox.com/sh/o8u3rgcpqztmrqw/AADBBqt3S9HmgF-oNLBlHt5Fa?dl=0

Minor points and suggestions:

Fig 1: Double horizontal lines connecting parents are usually reserved for inbreeding in pedigrees, but in the figure they seem to be used to describe all types of matings. This could be confusing and I would recommend using a single line.

- We have implemented this suggestion.

L12: "A striking finding is that four males descend from a non-lineage father but a mother who also

reproduced with a lineage male" - this reads like all four were brothers. Perhaps using plural (fathers and mothers) would help?

- We have amended this.

L93: "lack of long runs of homozygosity indicate that inbreeding was effectively avoided." It is not clear where is this shown. Refer to OT1 and perhaps provide context in the supplement for readers less familiar with ROH distributions.

- We have incorporated a section in the supplementary documents (SI section 6) describing the ROH analysis and a figure (Extended Data Fig. 9b).

L103: "Our results show that social fatherhood in this Neolithic community could be as important as biological fatherhood"
Just saying "social fatherhood existed" may be more prudent.

- Sentence edited

L159: "the genotype dataset is available as supplementary material". I did not find it in the SI.

- We now include the genotype dataset as supplementary material.

The authors could add a figure to show how pairwise mismatch patterns vary within and across sites, given that the British Neolithic population is treated as homogeneous (if I understand correctly). It could also help to show that using only the median or most distant pair from Hazleton North doesn't change the estimates.

- In SI section 4.2 we now justify in detail the choice of a baseline mismatch rate for unrelated individuals, which addresses the referee's suggestion. We do not include a figure due to the limited number of Extended Data items allowed by the journal.

The authors could test if the multiple partners of the same women tended to be more related to each other, than the multiple partners of the same man. This may be obvious but still, could be an option.

- The was an excellent question that we had not thought to look into before, and indeed there is a signal of the male reproductive partners of a female tending to be more closely related than the female reproductive partners of a male. We have included a figure (Extended Data Fig. 8) addressing this topic, and also now mention it in the main text.

SI L31: "remained"

- Fixed

SI L92: ". They did not survive past six and nine years of age respectively." - not clear who "they" refers to

- Fixed

SI Section 1: It was difficult for me to interpret the data without further information, such as comparison with other contemporary groups. The main question is how representative were these individuals for their time with respect to health and diet parameters, and the answer to me was unclear. Even if this is difficult to conclude, it would be helpful to explicitly state so.

SI Section 1: Scurvy, cribra orbitalia and porotic hyperostosis, osteoarthritis - please present proportions (perhaps relative to MNI), and also comparison with other contemporary groups.

-We have added text contextualizing the rates of these conditions in Hazleton North compared to the more general population to both the main manuscript (line 85) and SI section 2.

OT1: "Shotgun percentage of sequences mapping to human reference genome hg19" - should be "proportion".

- Fixed.

SI Section 2: The discussion on possible relationships is interesting but hard to follow, with sentences such as "I21389 should be first-degree relative of I12437's second-degree relatives, but he is third-degree-relative." (L343) with no reference to which third-degree-relatives are being considered. Referring to figures (e.g. Ext Fig 3) and tables (e.g. OT3) more frequently would also help.

- We have amended this section (now SI Section 4) according to this suggestion.

SI L350: "because individuals do not usually share their mtDNA lineage with their grandfathers" - can the authors predict the frequency of that mtDNA haplotype in the population, to assign a probability (e.g. doi.org/10.1016/j.cub.2021.03.050)?

- We include throughout SI Section 4 the frequencies of mtDNA lineages in the British Neolithic population, whenever relevant.

OT3: I21387-I21395 have r = 0.17 and SE 0.025, but are assigned 2nd degree. The table could indicate why is a 3rd degree relationship is implausible.

- With the new additional data from I21395 (SC9m), we obtained a 95% CI of 0.166–0.231 for the relatedness coefficient between SC4f (I21387) and SC9m, compatible with second- and third-degree relationships. In SI Section 4.3.5 we explain why a third-degree relation is not possible.

OT3: 8 of 9 first degree relatives have r < 0.5, somewhat unexpected. Any reasons for a bias? If there is a known reason, it would be helpful to mention in the SI.

- With new data, 21 out of 26 first degree pairs have a 95% CI overlapping 0.5, so we believe that even if there is slight bias, it does not have any impact on our results. One possible reason is the use of a baseline mismatch rate for unrelated individuals that is slightly lower than the true value for unrelated Hazleton individuals.

SI L367: "I13895 and I21390 are first-degree relatives with a parent-offspring relation based on the absence of genomic regions where 2 or 0 chromosomes are shared." I did not find where this data was shown. I would recommend present a graph for each such case. This could also be uploaded in an external database.

- The underlying data used to check for the presence of these regions is included in OT6, and we annotate the presence or absence of these regions in OT5. We have made this clear throughout the SI section 4. In the revised manuscript, these inferences are independently confirmed by NgsRelate Cotterman coefficients that are also included in OT5.

X-chromosome relatedness information is used in some cases (L390), but (1) this is not mentioned in the introduction of SI Section 2, and (2) not used systematically (e.g. to confirm other grandmother-grandson relationship of I21390-I20821). The authors could at least add the average chrX estimate separately from those of autosomes in OT3. Also would be helpful to mark that OT3 data is only based on autosomes.

- We now use X-chromosome data to check the validity of the tree structure (SI section 4.5 and Extended Data Figure 6a). We also include X-chromosome estimates in OT5.

SI L399: "(...) predicts that I13888 and I21393's parents are fourth-degree relatives and that I13888 and I21393 have several long runs of homozygosity, but they lack these types of segments making this scenario very unlikely." How long would be expected and what was observed?

- This is now implemented in SI section 4.3.4.

"The expected length of ROH would be intermediate between that characteristic of offspring of first cousins (third degree) and offspring of second cousins (fifth degree) (see Extended Data Fig. 9b), but these individuals clearly lack any long ROH (Extended Data Fig. 9b) making this scenario very unlikely".

SI L402: "I13891 is a second-degree relative of I12440, third-degree relative of I12438-I21388-I12437 and likely fourth-degree of I12439. Depending on the topology relating I12438-I21388-I12437-I12440, I13891 can be nephew, grandson or paternal half-brother of I12440." Perhaps it would be better to show these relatively ambiguous parts of the pedigree in dots in Fig 1, instead of full lines.

- This particular part of the pedigree is no longer ambiguous thanks to the new data, but we show less certain degrees of relationships with dotted lines in Fig. 1c

.
SI L620: "a radiocarbon date of 4950±70 3950–3630 cal."

-Edited

Extended Data Figure 3: Pairs with insufficient data (if they exist) could be shown with a different color.

- We now incorporate "*" symbols for low coverage comparisons. (This is now Extended Data Figure 2).

Reviewer Reports on the First Revision:

Referee #1 (Remarks to the Author):

I have read over the revised version of the manuscript and associated documents.

The points made by all three reviewers have been taken into consideration and the manuscript has been adjusted accordingly. In a few cases, the authors have appropriately rebutted some of the reviewer's comments.

I do have one tiny suggestion that would serve to maintain the consistently high levels of accuracy displayed throughout the paper.
At lines 67-69: change the words "roughly a hundred years" to "at least a hundred years" in the sentence "The tomb was constructed in the thirty-seventh century BC 19,20, roughly a hundred years after cattle and cereal cultivation had been introduced to Britain along with the construction of megalithic monuments". This change would more accurately reflect the fact that the construction of the tomb between c.3695–3650 BC occurred at least 100 years after the introduction of cattle and cereal cultivation to many parts of the island of Britain and this this occurred between 3900-3800 BC in the region where the tomb is located (see Whittle et al. 2011,

fig. 15.8).

Overall, the reviewers points have been very satisfactorily addressed and the paper is now even stronger than it was previously.I have no hesitation in recommending this paper for publication. Well done to the authors!

Referee #2 (Remarks to the Author):

Fowler and colleagues have resubmitted a much-improved version of the manuscript, with welcome new date from an additional ten individuals. I thank the authors for their careful consideration of my comments. Overall, I am happy with the revised version and congratulate the authors on a wonderful body of work. However, I still have a few outstanding concerns that I do not believe have been fully addressed.

1. The North Wall Collapse

The authors did not provide a full response to my comments on the potentially confounding impact of the North Wall Collapse on the perceived separation of maternal lineages. I was directed to SI7, but I am unclear exactly what passages address my concerns. The authors show there is indeed significant temporal differentiation in tomb placement of the descendants of NC2f and NC3f based on date-of-death. This is of interest and could also have impacted the choice of placement of the "Southern Branch" descendants, if their deaths also post-dated the collapse. Why is this not considered as a potential confounder? For example, it is worth noting that the two offspring of the unsampled U1f lived to middle age, while their sampled offspring also lived to adulthood. All could have died after the wall collapse.

To be clear, I feel the authors are justified in their discussion of architectural division based on maternal sub-lineages, given the spatial patterning. However, I'd like this caveat to be highlighted to the reader. Otherwise, if the authors do not think there is any possibility that the dates-of-death of the southern branch individuals have influenced their tests of statistical significance, could they please explain why. My opinion is that it would require more precise osteological ages of death and denser RC dating across the pedigree to resolve, but please correct me if I'm missing something here.

Also, I do not agree with the reasoning that child NC8m should placed in the NC3f lineage of the pedigree due to his burial location (line 1421) and then using this grouping to test the relationship between burial location and maternal lineage. It seems circular. He is most likely a direct descendent of SC1f through the male line and not related to NC3f. Does the removing this individual from the dataset or placing him within the SC1f lineage meaningfully impact the significance tests?

2. Integration of results from the published literature into discussion

A) Lines 42-44: As Reviewers 1 and 3 both highlight, there have been a growing number of impactful studies over the past few years that have densely sampled small regions to inform on past social phenomena. This is not clear from these introductory lines.

B) I agree with the authors that this study provides the first direct evidence of patriliny in a Neolithic population - an exciting and novel result worthy of publication. However, I still do not feel previous results from Britain and Ireland have been properly incorporated into discussion. For example, I do not think lines 153-157 accurately describe the results from early megalithic tombs in Ireland. There is no evidence of endogamy or inbreeding in these populations as the authors suggest might be the case. Like Hazleton North, they were reported to

be generally outbred, a similarity perhaps worthy of note. The results from Sanchez-Quinto and Cassidy are also conflated, which I don't think accurately represents the findings of either paper (Line 154). Most relevant is the differentiation of Y haplotypes between neighbouring tombs.

C) I am happy with the updated discussion on adoption of non-lineage males. However, I am not convinced there is any strong evidence to suggest the ten unrelated individuals, who make up ~30% of the sampled population, were considered kin and believe lines 39-40 in the abstract are overly speculative. Given that the other major genomic surveys of megalithic tombs in Ireland and Britain have yielded mostly unrelated individuals deriving from outbreeding communities, it is quite possible that social bonds or phenomena beyond the realm of kinship (biological or otherwise) informed some co-interments (e.g. ritual sacrifice; prestige derived from occupation, skill, political or religious role). Importantly, these tombs were constructed by individuals who shared recent ancestral and cultural origins with the builders of Hazleton North and should be considered in the authors' interpretations.

The possible explanations given for the presence of non-related individuals (starting Line 220) could be improved upon. I find the argument of insufficient sampling weak, given that four individuals are adult males in a community suggested to practice virilocal burial, while three(?) are juvenile, the implication being that none of the six parents were closely related to any individual in the extended pedigree. Also, please note that the age-at-death info in OT1 does not seem to match up with this discussion. Furthermore, I see that one unrelated individual yielded the latest RC date of all samples (NE3m; 3500–3340 cal BC), while another (NE4m) overlaps with this range (3650–3380*), although the reason for the asterisks is unclear in OT1. Six of the unrelated individuals are undated. Could there be a temporal effect here, given that the scheme from Meadows et al. underestimates the tail end of the tomb's usage?

In sum, I believe the presence of unrelated individuals raises questions beyond the mechanisms of kin determination (lines 146-147) and it is not clear why these are avoided. I think discussion of fictive kinship is important, as requested by Reviewer 1, however it is also important to offer alternative interpretations and justify why fictive kinship is most likely if that is indeed the authors' argument.

OTHER MINOR COMMENTS

1. Given almost all male individuals in this study belong to Y haplogroup I2a1b1a1a1, the most commonly observed lineage in the British and Irish Neolithic, it seems improper to use shared Y haplogroup membership to inform on relationships within the pedigree. For example, lines 1072-1073 state "grandsons and maternal grandfathers do not usually share the Y-chromosome lineage". Lines 1180-1181 follow a similar logic. Please remove or amend these sections to reflect the fact that in this population, it is quite likely that a maternal grandfather and grandson would share this particular Y lineage.

2. The sharing of a rare(?) mtDNA haplogroup between two fourth degree relatives very likely reflects some female-to-female transmissions in the pedigree and indeed the authors think it most likely that SP3m is related to NC4m through this maternal side (lines 1212). This indicates that matrilocality was not completely absent from the site and might be useful to highlight in the discussion?

Referee #3 (Remarks to the Author):

I commend the authors for improving the manuscript by including additional data and analyses. The supplementary information section is now much clear and easy to follow. Using both chrX mismatch rates, NgsRelate and IBD segment number comparisons to test confidence in the data has also greatly improved the reliability of the pedigree. The authors have also done an great job

in addressing the other two reviewers' points, especially those regarding interpretations of the findings.

Some minor points:

L112: The formula seems to have an error; it should be r = 1 – ((x-(b/2))/(b/2)), as in L931.

L490: "genotyped the"

L685-686: "and is crucial" should perhaps be "which is crucial"?

L1283: The authors could aid the reader by reminding that for male-female comparisons, the earlier formula of r is valid.

L1317-1323: "meioses" (plural) instead of "meiosis"

L1401: "The Hazleton North group is strictly patrilineal, with all 15 genealogical transmissions between the founding male NC1m and his descendants running through male individuals (Online Table 7)."
If I understand the pedigree and supplemental information correctly, the genetic relationship between NC4m and SP3m (in Fig 1c) implies a *matrilineal* connection between these two individuals via U16f. This is a minor exception and with ambiguous context, but still, the authors could prefer to describe Hazleton North as "mainly patrilineal" instead of "strictly patrilineal".

OT6: The numbers in the table following the underscore are most likely SNP numbers, but would be better to indicate in the legend.

Mehmet Somel

Author Rebuttals to First Revision:

Referee #1 (Remarks to the Author):

I have read over the revised version of the manuscript and associated documents.

The points made by all three reviewers have been taken into consideration and the manuscript has been adjusted accordingly. In a few cases, the authors have appropriately rebutted some of the reviewer's comments.

I do have one tiny suggestion that would serve to maintain the consistently high levels of accuracy displayed throughout the paper.
At lines 67-69: change the words "roughly a hundred years" to "at least a hundred years" in the sentence "The tomb was constructed in the thirty-seventh century BC 19,20, roughly a hundred years after cattle and cereal cultivation had been introduced to Britain along with the construction of megalithic monuments". This change would more accurately reflect the fact that the construction of the tomb between c.3695–3650 BC occurred at least 100 years after the introduction of cattle and cereal cultivation to many parts of the island of Britain and this this occurred between 3900-3800 BC in the region where the tomb is located (see Whittle et al. 2011, fig. 15.8).

Overall, the reviewers points have been very satisfactorily addressed and the paper is now even stronger than it was previously.I have no hesitation in recommending this paper for publication. Well done to the authors!

We have made the minor change to line 68 and thank referee 1 for their comments throughout the review process.

Referee #2 (Remarks to the Author):

Fowler and colleagues have resubmitted a much-improved version of the manuscript, with welcome new date from an additional ten individuals. I thank the authors for their careful consideration of my comments. Overall, I am happy with the revised version and congratulate the authors on a wonderful body of work. However, I still have a few outstanding concerns that I do not believe have been fully addressed.

1. The North Wall Collapse

The authors did not provide a full response to my comments on the potentially confounding impact of the North Wall Collapse on the perceived separation of maternal lineages. I was directed to SI7, but I am unclear exactly what passages address my concerns. The authors show there is indeed significant temporal differentiation in tomb placement of the descendants of NC2f and NC3f based on date-of-death. This is of interest and could also have impacted the choice of placement of the "Southern Branch" descendants, if their deaths also post-dated the collapse. Why is this not considered as a potential confounder? For example, it is worth noting that the two offspring of the unsampled U3f lived to middle age, while their sampled offspring also lived to adulthood. All could have died after the wall collapse.

To be clear, I feel the authors are justified in their discussion of architectural division based on maternal sub-lineages, given the spatial patterning. However, I'd like this caveat to be highlighted to the reader. Otherwise, if the authors do not think there is any possibility that the dates-of-death of the southern branch individuals have influenced their tests of statistical significance, could they please explain why. My opinion is that it would require more precise osteological ages of death and denser RC dating across the pedigree to resolve, but please correct me if I'm missing something here.

We have further modified the text in SI4 to raise and address the referee's concern. It is notable that none of the 'southern branch' were ever placed in the north side of the tomb. Even after the north passage collapse the north entrance remained accessible, and was used for burial of members of the 'north branch'. There was ample opportunity to place 'south branch' descendants here, but this was never taken up. It is not possible at present to resolve the issue of precise dates of death in relation to the passage collapse any further than we have. The added text in SI4 reads:

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

Also, I do not agree with the reasoning that child NC9m should placed in the NC3f lineage of the pedigree due to his burial location (line 1421) and then using this grouping to test the relationship between burial location and maternal lineage. It seems circular. He is most likely a direct descendent of SC1f through the male line and not related to NC3f. Does the removing this individual from the dataset or placing him within the SC1f lineage meaningfully impact the significance tests?

NC9m can be either included in SC1f's sub-lineage as great-grandson of SC1f or in NC3f's sub-lineage. The latter is not because of his burial location but because he is the step-son of NE2m, a key member of NC3f's lineage. In deciding which of the 2 possible lineages for this individual to use in the test, we opted for NC3f's lineage because NC9m's burial location suggests he was viewed as member of this lineage. To address the reviewer's point we have now added to SI3 that the test of the spatial pattern of the maternal sub-lineages is still significant if we were instead to consider NC9m as a member of SC1f's lineage (P=0.009318), or removing this individual from the test as the referee suggests (P=0.002392).

We write in SI3, test 4: "*NC9m can be either included in SC1f's sub-lineage as great-grandson of SC1f or in NC3f's sub-lineage as step-son of NC3f's son. NC9m was buried in the north chamber together with two of his maternal half-siblings (themselves NC3f's grandchildren), and not with his closest paternal relatives (all members of SC1f's lineage) who were all buried in the southern chambered area. This suggests that he was viewed as a member of NC3f's lineage, and we thus considered him as member of NC3f's sub-lineage for this analysis. Considering NC9m as a member of SC1f's lineage (P=0.009318) or removing this individual for this analysis (P=0.002392) still yields a significant association between female sub-lineages and burial in the north or south of the tomb.*"

We have also colour-coded NC9m in Figure 1 to show their membership of both lineages.

2. Integration of results from the published literature into discussion

A) Lines 42-44: As Reviewers 1 and 3 both highlight, there have been a growing number of impactful studies over the past few years that have densely sampled small regions to inform on past social phenomena. This is not clear from these introductory lines.

We have edited these introductory lines to reflect this. Our revised text reads as follows:

"In studies of Neolithic chambered tombs in Britain and Ireland, relatedness patterns documented to date include cases of first- or second- degree relative pairs within or across

tombs[18], persistence of particular Y-chromosome lineages in the same tombs[18]; two brothers in the same chamber in England[19], and an absence of biological kin within the third degree among 11 and 15 sampled individuals at two tombs in Ireland[8].”

Here, ref. 8 is Cassidy et al. Nature 2020, ref. 18 is Sánchez-Quinto et al. PNAS 2019, and ref. 19 is Scheib et al. Annals of Human Biology 2019.

B) I agree with the authors that this study provides the first direct evidence of patriliny in a Neolithic population - an exciting and novel result worthy of publication. However, I still do not feel previous results from Britain and Ireland have been properly incorporated into discussion. For example, I do not think lines 153-157 accurately describe the results from early megalithic tombs in Ireland. There is no evidence of endogamy or inbreeding in these populations as the authors suggest might be the case. Like Hazleton North, they were reported to be generally outbred, a similarity perhaps worthy of note. The results from Sanchez-Quinto and Cassidy are also conflated, which I don't think accurately represents the findings of either paper (Line 154). Most relevant is the differentiation of Y haplotypes between neighbouring tombs.

We have amended these lines to distinguish the findings of Sanchez-Quinto and Cassidy and to highlight the finding of persistence of the Y-lineages over time within the same tombs.

C) I am happy with the updated discussion on adoption of non-lineage males. However, I am not convinced there is any strong evidence to suggest the ten unrelated individuals, who make up ~30% of the sampled population, were considered kin and believe lines 39-40 in the abstract are overly speculative. Given that the other major genomic surveys of megalithic tombs in Ireland and Britain have yielded mostly unrelated individuals deriving from outbreeding communities, it is quite possible that social bonds or phenomena beyond the realm of kinship (biological or otherwise) informed some co-interments (e.g. ritual sacrifice; prestige derived from occupation, skill, political or religious role). Importantly, these tombs were constructed by individuals who shared recent ancestral and cultural origins with the builders of Hazleton North and should be considered in the authors' interpretations.

To address the reviewer's point we have modified the last line of the abstract to read as follows (the underling is added here to show the key change – emphasizing that this is a hypothesis but other scenarios are possible).

“Eight individuals were not close biological relatives of the main lineage, raising the <u>possibility</u> that kinship also encompassed social bonds independent of biological relatedness.”

Later in the text we unpack this, writing:

“The reconstructed pedigree includes a sufficiently rich network of relationships to identify kinship practices that would be invisible in smaller datasets (Table 1 and Supplementary

Table 7), while the inclusion in the tomb of eight individuals without evidence of close biological relationships or reproductive partnerships with others in the pedigree suggests either that kinship did not always depend on such relations or that kinship may not have been the only criterion for inclusion in the tomb throughout its use."

The possible explanations given for the presence of non-related individuals (starting Line 220) could be improved upon. I find the argument of insufficient sampling weak, given that four individuals are adult males in a community suggested to practice virilocal burial, while three(?) are juvenile, the implication being that none of the six parents were closely related to any individual in the extended pedigree. Also, please note that the age-at-death info in OT1 does not seem to match up with this discussion. Furthermore, I see that one unrelated individual yielded the latest RC date of all samples (NE3m; 3500–3340 cal BC), while another (NE4m) overlaps with this range (3650–3380*), although the reason for the asterisks is unclear in OT1. Six of the unrelated individuals are undated. Could there be a temporal effect here, given that the scheme from Meadows et al. underestimates the tail end of the tomb's usage?

We have edited the text to acknowledge that these individuals were not close biological kin and may not have been considered kin, stating '*it is possible that reasons other than kinship were a factor in their inclusion in the tom*b'. There is not enough evidence at present to consider change over time a factor as there are both lineage and non-lineage individuals with early dates and later dates.

In sum, I believe the presence of unrelated individuals raises questions beyond the mechanisms of kin determination (lines 146-147) and it is not clear why these are avoided. I think discussion of fictive kinship is important, as requested by Reviewer 1, however it is also important to offer alternative interpretations and justify why fictive kinship is most likely if that is indeed the authors' argument.

As above, we have edited the text around these lines, and the abstract, to highlight that it is possible some of the biologically unrelated individuals were not considered kin.

OTHER MINOR COMMENTS

1. Given almost all male individuals in this study belong to Y haplogroup I2a1b1a1a1, the most commonly observed lineage in the British and Irish Neolithic, it seems improper to use shared Y haplogroup membership to inform on relationships within the pedigree. For example, lines 1072-1073 state "grandsons and maternal grandfathers do not usually share the Y-chromosome lineage". Lines 1180-1181 follow a similar logic. Please remove or amend these sections to reflect the fact that in this population, it is quite likely that a maternal grandfather and grandson would share this particular Y lineage.

The referee is right, we have removed these sections.

2. The sharing of a rare(?) mtDNA haplogroup between two fourth degree relatives very

likely reflects some female-to-female transmissions in the pedigree and indeed the authors think it most likely that SP4m is related to NC5m through this maternal side (lines 1212). This indicates that matrilocality was not completely absent from the site and might be useful to highlight in the discussion?

This case reflects a maternal connection between two males on the pedigree, but does not necessarily reflect matrilocality at the site, and we think the latter is unlikely. Given that NC5m (who died aged 3-4) was not able to leave descendants, and that his relatively early radiocarbon date places him alongside the earliest generations of the tree, the two most likely topologies relating these individuals are: 1. NC5m as a brother of SP4m's maternal great-grandmother, or; 2. NC5m as a half-brother of SP4m's maternal grandmother. This implies that there were very likely either three or two females connecting NC5m and SP4m, all of them missing in our sampling of the tomb. One possibility is that they were buried at the site but we have missed them in our sampling, which will indicate matrilocality at the site, but given that we see a complete absence of adult lineage daughters at the tomb, a plausible scenario is that NC5m's sister or half-sister left the Hazleton North community, and her granddaughter or daughter (U15f) came back to Hazleton North and reproduced with one of the lineage males. This latter scenario would not imply matrilocality at the site, but rather maternal lineages leaving the community and reappearing after some generations.

Referee #3 (Remarks to the Author):

I commend the authors for improving the manuscript by including additional data and analyses. The supplementary information section is now much clear and easy to follow. Using both chrX mismatch rates, NgsRelate and IBD segment number comparisons to test confidence in the data has also greatly improved the reliability of the pedigree. The authors have also done an great job in addressing the other two reviewers' points, especially those regarding interpretations of the findings.

Some minor points:

L112: The formula seems to have an error; it should be r = 1 – ((x-(b/2))/(b/2)), as in L931.

Thanks, we have amended this error.

L490: "genotyped the"

Thanks, we have amended this error.

L685-686: "and is crucial" should perhaps be "which is crucial"?

We have corrected this, thanks.

L1283: The authors could aid the reader by reminding that for male-female comparisons, the earlier formula of r is valid.

We thank this referee for this suggestion, which we have implemented.

L1317-1323: "meioses" (plural) instead of "meiosis"

Thanks, we have amended this error.

L1401: "The Hazleton North group is strictly patrilineal, with all 15 genealogical transmissions between the founding male NC1m and his descendants running through male individuals (Online Table 7)."
If I understand the pedigree and supplemental information correctly, the genetic relationship between NC5m and SP4m (in Fig 1c) implies a *matrilineal* connection between these two individuals via U15f. This is a minor exception and with ambiguous context, but still, the authors could prefer to describe Hazleton North as "mainly patrilineal" instead of "strictly patrilineal".

We thank the referee for this suggestion. As we explain in response to one of the referee 2's comments above, we don't believe that the case of NC5m and SP4m is clear evidence for matrilineality at the site, but given that maternal sub-lineages are important at the site and that there are eight individuals with no biological relation to the patriline we have amended that sentence which now reads: "*Inclusion in the Hazleton North tomb for lineage members is strictly patrilineal*".

OT6: The numbers in the table following the underscore are most likely SNP numbers, but would be better to indicate in the legend.

We thank this referee for this suggestion, which we have implemented.

Mehmet Somel