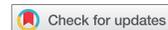


RESEARCH ARTICLE



The Maglemosian skeleton from Koelbjerg, Denmark revisited: identifying sex and provenance

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ABSTRACT

The Koelbjerg individual, dated c. 8500 cal BC, represents the earliest human skeletal remains described from Scandinavia. Based on ancient DNA, strontium isotope and statistical anthropological analyses the individual's sex, haplogroup and geographical provenance are here analysed and discussed. In contrast to previous claims, our genetic and anthropological analyses show that this individual was a male. Additionally, the strontium isotope ratio of one of his first molars indicates that he most likely grew up locally.

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Introduction

The bones of the Koelbjerg individual from Funen, Denmark (Figures 1 and 2), are the oldest human remains presently known from Scandinavia. For this reason, the relatively intact skeleton has received significant scientific and public attention since it was salvaged during peat digging in 1941; see Henriksen and Hansen (2017), for a brief summary of the earlier studies. The general consensus is that the skull and scattered skeletal remains belong to the same individual. This conclusion is based on the observation that there are no duplicated bones, all the bones have the same colouring and a similar state of preservation, and to the extent that they convey information on the person's age at death, they are in solid agreement. There are therefore no scientific arguments for considering the skeletal parts to represent more than one individual (Bröste and Fischer-Møller 1943, Bennike 1986).

The individual's sex, however, has repeatedly been debated since the first preliminary anthropological analysis of the skeleton. The remains were, with some ambiguity, interpreted as those of a c. 155–158-cm tall woman in her 20s, characterised by masculine neurocranial traits (Figure 3), rather androgynous facial features and gracile

extremities. The fragmented pelvic bones were, after some consideration, interpreted as that of a female (Bröste and Fischer-Møller 1943, Bennike 1986).

The dating of the Koelbjerg skeleton was for decades based solely on pollen analyses, which indicated that it belonged in an early stage of the Early Holocene when birch and pine forests had already covered the moraines of the latest Ice Age, and shrubs of hazel began to spread across the landscape (Troels-Smith 1943). This pollen date has more recently been accompanied by two radiocarbon dates, both based on the right femur: K-4063, 9250 ± 85 BP and AAR-8613, 9285 ± 50 BP (Tauber 1986, Fischer *et al.* 2007, Henriksen and Hansen 2017). Calibrated to calendar years, these dates show that the skeleton is c. 10,500 years old and thus belongs to the early stage of the Maglemose Culture. Apart from the Koelbjerg skeleton, this period (c. 10,500 BP) is yet only represented in the local archaeological record by a few single finds, as the majority of Funen finds from the early Maglemosian period are estimated to be younger than the skeleton from Koelbjerg, Figure 4 (Henriksen and Hansen 2017).

In Denmark in general, contemporaneous settlement assemblages etc. are well known, however,



Figure 1. Northwestern Europe c. 10,500 BP. The star marks the Koelbjerg find (Graphic: Simon Nissen; based on Moree and Sier 2015).

exemplified by the habitation site of Barmose (Johansson 1990), and the aurochs' skeleton from Vig, penetrated by flint projectile points (Fischer 1996), both found on present-day Zealand.

New technologies provide the opportunity not only to venture into new research areas but also to revisit old unresolved questions, and the Koelbjerg skeleton is no exception in this respect. In 2000, researchers from the Institute for Zoology and Anthropology, University of Göttingen, Germany, sampled material from the Koelbjerg skeleton's rear molar in the lower jaw (right side), and two samples from, respectively, the upper and lower part of the right femur, in order to test for DNA preservation. However, the technology available at the time failed to produce evidence of authentic ancient DNA in the samples. The analyses were therefore concluded with a short report (pers. comm. S. Hummel) to Odense City Museums that offered no clear results.

Over the last couple of decades, a number of scientific methods have been introduced, prompting new and exciting opportunities in archaeological research. Several of these technologies have the potential to settle the controversies surrounding the Koelbjerg skeleton. Next-generation sequencing, for example, has revolutionised molecular biology including the field of ancient DNA, making it possible to sequence many millions of DNA fragments in parallel. Importantly, this technology also allows for the sequencing of very short DNA fragments, which is crucial when working with highly degraded ancient DNA (e.g. Allentoft *et al.* 2012). Similarly, methodological improvements in the field of

strontium isotopic tracing have provided important information on individual mobility (Haak *et al.* 2008, Frei *et al.* 2015). DNA and Sr are excellent complimenting information, not comparable but instead giving each other context, with DNA showing heritage (genetic ancestry) and Sr geographical location, that is, where a person actually lived. Osteological methods for sex estimation have steadily improved since the first thorough measuring and publication of the Koelbjerg skeleton conducted by Bröste and Fischer-Møller (1943), and especially statistical methods such as discriminant analysis have been developed. The so-called DSP method (Diagnose Sexuelle Probabiliste, Murail *et al.* 2005, Brůžek *et al.* 2017) is a case in point. Based on reference data from more than 2000 skeletons (pelves), it provides a reliable sex estimation tool. Finally, the transition analysis method for age-at-death estimation (Boldsen 2002, Milner and Boldsen 2012) as well as a host of regression methods for stature estimation (see further down) has also been developed.

These recent advances have allowed us to reanalyse the Koelbjerg skeleton and address some of the fundamental enigmas. In this study, we combine classical and statistically based osteological methods and DNA analyses to determine the individual's sex. Further DNA analyses are employed to determine the individual's mitochondrial DNA haplogroup, while strontium isotope analyses are carried out to establish if the Koelbjerg individual was likely a local inhabitant of the hilly Maglemosian landscape that is now Funen. We will describe the methods and results from each discipline individually, followed by a brief overall discussion. The uniqueness of the find in question has led us to minimise the use of destructive sampling and thereby ensures the presence of sample material for future analyses.

Osteological anthropological analyses

Sex revision of Upper Palaeolithic and Mesolithic skeletons

For many years in palaeoanthropological analyses, the general robusticity of the skull and infracranial skeleton was commonly employed as indicator of the sex. As already mentioned by Weiss (1972), and reiterated by Donlon (1993) and Walker (1995), there appears to be a systematic bias in sexing skeletal collections from archaeological sites, with some



Figure 2. Most of the remaining parts of the Koelbjerg skeleton are well preserved. Only the bones of the pelvis are somewhat fragmented. The right femur is a cast of the bone which was used for ^{14}C -dating in the mid-1980s (photo: Jens Gregers Aagaard).



Figure 3. (a) The Koelbjerg skull, lateral view. (b) Anterior view (photo: Jens Gregers Aagaard).

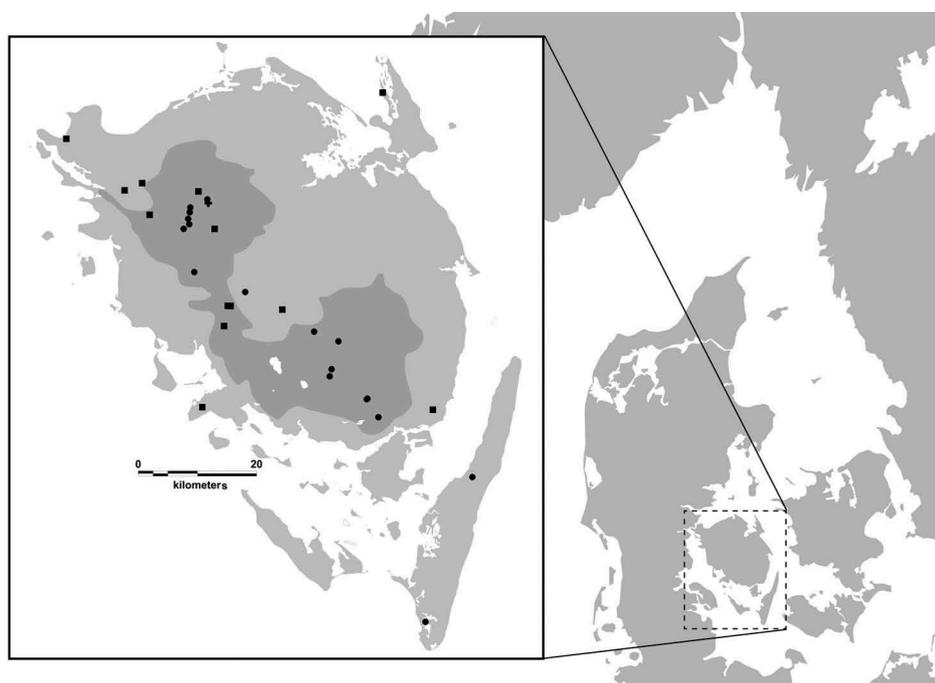


Figure 4. Present-day Funen and the surrounding islands with archaeological finds from the early Maglemosian period, c. 11,000–9800 cal BP. Cross: Koelbjerg. Circle: Flint. Square: Single finds (drawing is based on the national heritage database; Fund og Fortidsminder, data retrieved 29. August 2017).

female skeletons being estimated to represent males, the reason being that the qualitative traits employed as sexual indicators in extant populations are not necessarily suitable for past populations. In case of Upper Palaeolithic skeletons for instance, this bias will often result in most of the specimens being estimated to be males as already mentioned by Bröste and Fischer-Møller (1943).

It is difficult to infer cranial sexual dimorphism in past populations, due both to sample-size limitation

and the often poor state of preservation of ancient skeletal remains. With regard to cranial morphology, a failure to take age changes and especially populational variation into account can also introduce significant biases in sex estimation. Heavy masticatory and physical activity may influence the appearance of the skull and skeleton to appear more masculine. There is general agreement among scholars that skeletal morphological differences between the sexes, and consequently the reliability of sex estimation, are most

pronounced in the adult pelvis. In the last decade, a highly reliable method, based on discriminant function analysis including a probabilistic approach for sex estimation (DSP) of the hipbone, has been developed (Murail *et al.* 2005, Brůžek *et al.* 2017) and employed (e.g. Bruzek and Murail 2006, Gambier *et al.* 2006). The method can also be applied to incomplete pelvis but the estimation becomes uncertain when sexually dimorphic functional parts or segments of the os coxae are not well preserved.

The DSP procedure was recently applied to a large sample of European Upper Palaeolithic and Mesolithic skeletons in order to reassess previous sex estimates (Villotte 2009). In the review of these specimens, an evaluation of pelvic morphological traits and the pelvic discriminant function analysis (DSP) were employed. Out of 40 specimens, the DSP procedure arrived at different sex estimations in five cases, relative to the conclusion reached in the primary publications.

Over the years, comparative studies of Upper Palaeolithic and Mesolithic skeletal remains have included the Koelbjerg individual as a female, for example Constandse-Westermann (1974), Henke (1987) and Gerasomova and Pežemskij (2005), essentially without questioning the sexing, and based on the original publications of the skeleton (Bröste and Fischer-Møller 1943, Bröste and Balslev-Jørgensen 1956). In the latter, the authors acknowledge that the sex estimation posed problems. The cranium was regarded as presenting traits indicating that it represents a male, while the gracile impression of the long bones indicates a female. Bröste and Fischer-Møller (1943) also conclude that the pelvis, despite being incomplete, suggests that the skeleton represents a female. In a later publication, Bröste and Balslev-Jørgensen (1956) reiterate their arguments and conclusion, and the same applies to a more recent paper by Bennike (1986).

This generally accepted estimation of the Koelbjerg skeleton as a female has previously been questioned by Petersen (1998, 2004), primarily because the pelvis was deemed to show male characteristics, and by Newell *et al.* (1979). Based on the reservations presented, and the frequency of revised sex estimations in other cases in recent years (e.g. Gejvall 1970, Sommer 2007, Ahlström 2013), the Koelbjerg remains have been restudied to revise the

sex estimation of this individual (preliminary results in Petersen *et al.* 2009).

The Koelbjerg skeleton – a brief description

The Koelbjerg skeleton is fairly well preserved (see above and illustrations in Henriksen and Hansen 2017), with the cranium, including the upper face and most of the base and mandible. The dentition is represented by 11 teeth (6 upper molars and premolars, 5 lower molars). Substantial portions of the infracranial skeleton are preserved: parts of upper limbs (right humerus, both ulnae, left radius), left and right femora, tibiae and fibulae. The hand, foot and vertebra bones are missing. The lack of these relatively inconspicuous skeletal elements is most likely to be a result of the finds circumstances: lay persons' peat digging.

The pelvic remains are incomplete, consisting of the upper part of the sacrum and a large part of the right innominate bone. The pubic bone is nearly complete, lacking a portion of the inferior ramus, but with the pubic symphysis intact. The iliac wing lacks its superior part, while the auricular surface is partly preserved, as is the acetabular region. The sciatic notch is incompletely preserved and the ischium is absent (Figures 5 and 6). Even so, the anatomical regions present are sufficient to enable reconstruction of the overall shape of the pelvis, several sexually diagnostic regions are represented and a number of variables employed in the DSP procedure can be ascertained.

As the distal humerus carries information for sex estimation (Rogers 1999), two different aspects of this skeletal region from the right humerus are presented in Figure 7.

Transition analysis (Boldsen 2002, Milner and Boldsen 2012, ADBOU 2016), based on 5 out of 5 bilateral cranial traits and 10 out of 14 pelvic traits (only one side recorded), was used for evaluating the age at death of the Koelbjerg individual, employing the procedure developed for archaeological male specimens with unknown ancestry. The scores for the anatomical features employed are given in Table 1. The age at death can be estimated to be in the interval 21–33 years, with a most probable age of c. 27 years, with similar age-at-death estimates for pelvis and cranium. The result is broadly in accordance with age-at-death estimations based on more qualitative methods (Bröste and Fischer-Møller 1943, Bennike 1986).



Figure 5. (a) The remains of the right innomiate bone, medial view. (b) Lateral view (photo: Jens Gregers Aagaard).



Figure 6. (a) The preserved parts (fragment of right innomiate bone and fragment of os sacrum) of the pelvis, frontal view. (b) Inlet view (photo: Jens Gregers Aagaard).



Figure 7. (a) The Koelbjerg distal humerus, posterior view. (b) Inferior view (photo: Jens Gregers Aagaard).

Table 1. Scores of anatomical features used in the age-at-death estimation by transition analyses.

Anatomical feature	Score
Cranial sutures	
Coronal pterica	4
Sagittal obelica	2
Lambdoidal asterica	1
Interpalatine suture	2
Zygomaticomaxillary suture	2
Pubic symphysis	
Topography	3
Texture	2
Superior protuberance	2
Ventral margin	3
Dorsal margin	3
Auricular area	
Superior topography	2
Inferior topography	2
Superior characteristics	4
Apical characteristics	3/4
Superior exostoses	2

For cranial sutures right and left scores were equal.

The osteological assessment presented here was initiated because the conclusions in the primary publications state ambiguity concerning the sex estimation, where the cranium and long bones present conflicting evidence, and especially because no description is given of the anatomical details of the pelvis. The strongest previously presented evidence for the skeleton representing a female is the gracility of the limb bones and some of the cranial features. They are indeed very gracile relative to standard robusticity indices, and even when compared to female skeletons from the Upper Palaeolithic and Early Mesolithic (Jacobs 1985). However, as a large part of the pelvis is present, this part of the skeleton could be analysed in further detail.

Sex estimation

In the following, we present the results of three different morphological and osteometric approaches to the sex estimation of the Koelbjerg skeleton, all based on infracranial material. The cranial morphology was not included due to the reservations and uncertainties mentioned in the introduction to this section. Exemplifying this, Mesolithic crania, both males and females, are often very robust compared to present-day populations, as already noticed by Bröste and Fischer-Møller (1943). Moreover, a preliminary multivariate discriminant analysis based on more than 40 Mesolithic crania yielded an inconclusive sex classification for the Koelbjerg skeleton (results not shown).

The first analysis consists of scoring a set of classical morphological features of the pelvis used for sex estimation (Phenice 1969, Bruzek 2002, Rösing *et al.* 2007). The state of preservation of the Koelbjerg pelvis (Figure 5) limits these traits to the following: ventral arch, composite arch, subpubic concavity, medial aspect of pubic bone, subpubic angle and crista phallica. Dorsal pitting of the pubic bone at the symphysis was also recorded (Stewart 1970).

Second, we present the results of a morphometric study of the pelvis, using the DSP method and software, version DSP2 (Brůžek *et al.* 2017; <http://projets.pacea.u-bordeaux.fr/logiciel/DSP2/dsp2.html>). Measurements were performed by the second and fourth author, with an accuracy of 0.5 mm. Measurements showing a greater than 1 mm difference between the values obtained by the second and fourth author were repeated by the fifth author.

Finally, we briefly discuss some indices of infracranial robusticity such as diaphysical robusticity indices, relative size of the femoral head and the cnemic index (Jacobs 1985), as well as the morphology of the distal humerus (trochlear asymmetry and constriction), shape and perforation of the fossa olecrani and angle of epicondylis medialis (Rogers 1999) (Figure 7).

Table 2 shows the observations of morphological characters on the pelvis (Figures 5 and 6) and most of these were indicative of a male. The presence of a crista phallica cannot be confirmed with certainty, as the region of the pubis is not entirely preserved. The absence of dorsal pitting of the pubis is, in itself, not an indicator of the sex, but presence of such pitting has been claimed to indicate a female (Stewart 1970), although males can also show this trait (see Ahlström 2013, and references herein).

The measurements for the DSP method are given in Table 3. In the DSP analysis, the means of the two, respectively three, measurements were used.

Table 2. Observations on pelvic morphological characters and their resulting sex indication.

Trait	Status	Indicated sex
Ventral arch (P)	Absent	♂
Composite arch (B)	Absent	♂
Subpubic concavity (P)	Absent	♂
Medial aspect (P)	Relatively broad	♂
Subpubic angle (R)	80–84°	♂
Crista phallica (B)	Present (?)	♂ (?)
Dorsal pitting at pubis (S)	Absent	♂ (?)

Traits following: B: Bruzek (2002), P: Phenice (1969), R: Rösing *et al.* (2007); S: Stewart (1970).

Table 3. Measurements for the DSP analysis, all in millimetres.

Variable	Author		
	2nd	4th	5th
Acetabulo-symphyseal pubic length (PUM)	70.0	71.0	–
Cotylo-pubic width (SPU)	24.5	25.0	–
SS length	71.0	69.0	70.0
SA length	67.5	68.0	–
VEAC	59.0	61.0	63.0

SS: Spino-sciatic; SA: spino-auricular; VEAC: vertical acetabular diameter.

The following posterior probabilities were obtained: $p(\text{male}) = 0.980$, $p(\text{female}) = 0.020$.

Regarding infracranial robusticity, we acknowledge that the long bones of the Koelbjerg skeleton show a very gracile morphology when assessed according to the classical indices of diaphysis robusticity and compared to the sex-specific averages for Late Glacial and Early Postglacial European populations, as shown in Jacobs (1985). However, the relative size of the femoral head (105.8) and the cnemic index (63.3) are both closer to the male averages than to the female averages for these populations. It should also be mentioned that essentially all the infracranial indices indicating female are dependent on long bone lengths and will thus be highly correlated. Long bone gracility can essentially in this respect be regarded as a single trait. For a list of these indices and the definitions, see Appendix. Morphological traits of the distal humerus, discussed by Rogers (1999), also indicate a male-like pattern: an asymmetric and only slightly constricted trochlea, a triangular shape of the imperforated fossa olecrani and a flat angle of the medial epicondyle (Figure 7). Given that the pelvis and the long bone epiphyses show male traits and the posterior probability for male in the DSP analysis is close to unity, the conclusion of the osteological–anthropological analysis is that the skeleton represents a male with gracile long bone epiphyses.

Based on the results of the anthropological analyses, we must conclude, that the skeleton represents a relatively slightly built person, most likely to be a man and in his late-20s at the time of his death. Further, estimates of the living stature of the Koelbjerg man, comprising a range of different models (e.g. Pearson 1899, Sjøvold 1990), indicate that he was most likely just above 160 cm.

Clearly, our result contradicts most of the earlier attempts to estimate the sex of the Koelbjerg skeleton. This calls for a need to confirm the result with several independent methods.

Ancient DNA analysis

Laboratory work and methods

The DNA analysis of the Koelbjerg skeleton was conducted in a dedicated ancient DNA laboratory at the Centre for GeoGenetics, Natural History Museum, University of Copenhagen, according to strict aDNA guidelines (e.g. Willerslev and Cooper 2005). The right side third upper molar was selected for DNA extraction and sampled from the root by removing the inner dentine layer, thereby enriching for the outer DNA-rich cementum layer, as outlined previously (Damgaard *et al.* 2015). Approximately, 200-mg cementum was crushed and DNA extracted using a silica-in-solution protocol, with a 15-min predigestion step (Damgaard *et al.* 2015) and a binding buffer optimised for retaining very short DNA molecules (Allentoft *et al.* 2015). Following extraction, 20 μl of DNA extract was built into a blunt-ended, double-stranded library using the NEBNext DNA Sample Prep Master Mix Set 2 (E6070) and a pool of four Illumina-specific adapters (Meyer and Kircher 2010). The library preparation protocol was similar to that used by Allentoft *et al.* (2015) apart from the amplification step, described here: 1 μl of library was first amplified with qPCR and SYBR Green detection chemistry and the CT values recorded. The entire remaining DNA library (c. 24 μl) was then amplified and indexed in a 50- μl PCR reactions containing 1 \times KAPA HiFi HotStart Uracil + ReadyMix (KAPA Biosystems, Woburn, MA, USA) and 200 nM of each of Illumina's Multiplexing PCR primer in PE1.0. Thermocycling conditions were 1 min at 94 °C, followed by 10 cycles (determined by qPCR) of 15 s at 94 °C, 20 s at 60 °C and 20 s at 72 °C, and a final extension step of 1 min at 72 °C. The amplified DNA library was profiled on an Agilent Bioanalyzer 2100, and shotgun sequenced (100 bp, single read) on an Illumina HiSeq 2500 platform at the National High Throughput DNA Sequencing Centre (University of Copenhagen).

Bioinformatics

A total of 243,911,574 DNA sequences were generated from the tooth sample. The sequences were base-called using CASAVA v.1.8.2 and de-multiplexed with a requirement of full match of the six nucleotide indexes. The adapter sequences were

removed using AdapterRemoval2 (Schubert *et al.* 2016), retaining 239,583,126 reads with a minimal length of 30 bp. The trimmed sequences were then mapped against the human mitochondrial reference genome (rCRS, NC_012920.1) using bwa (v.0.7.5) aln (Li and Durbin 2009) with mapping quality >30 and seeding disabled. Duplicate reads were removed with Samtools v.0.1.19 (Li *et al.* 2009), resulting in a total of 9401 human mtDNA sequences with an average length of 55.3 bp and a complete mitochondrial genome of 31.4-fold coverage (31.4X).

Haplogroup

The mitochondrial sequences were imported into Geneious v.8.1.7 for manual inspection and the consensus sequence was generated using sites with a minimum of 5X coverage and >75% concordance between the reads. The complete mitochondrial sequence of the Koelbjerg skeleton is available from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>)

under accession number MF992925. The mtDNA haplogroup was determined using HaploGrep2 (<http://haplogrep.uibk.ac.at/>) to U5a2c with a quality score of 100. U5 haplogroups were common in the Mesolithic populations of Europe but became rare in the Early Neolithic times (Brandt *et al.* 2013, Posth *et al.* 2016). In the most comprehensive analysis of the European pre-Neolithic mitochondrial gene pool conducted to date (based on complete mitochondrial genomes), Posth *et al.* (2016) observe variants of U5 in 31 of 55 individuals. The same study reports a U5a2c haplogroup (same as Koelbjerg) in a Mesolithic individual from Felsdach Inzigkofen in Germany (Figure 8), dated to 8680 cal BP. Our observation of an U5 haplogroup variant supports the contention that the Koelbjerg individual belonged to the European Mesolithic gene pool, testifying to both the great age of the skeleton and the authenticity of the analysed DNA. However, in-depth analyses of the nuclear genome are needed to provide more details into the population affinity of this individual. Such data will be presented elsewhere.

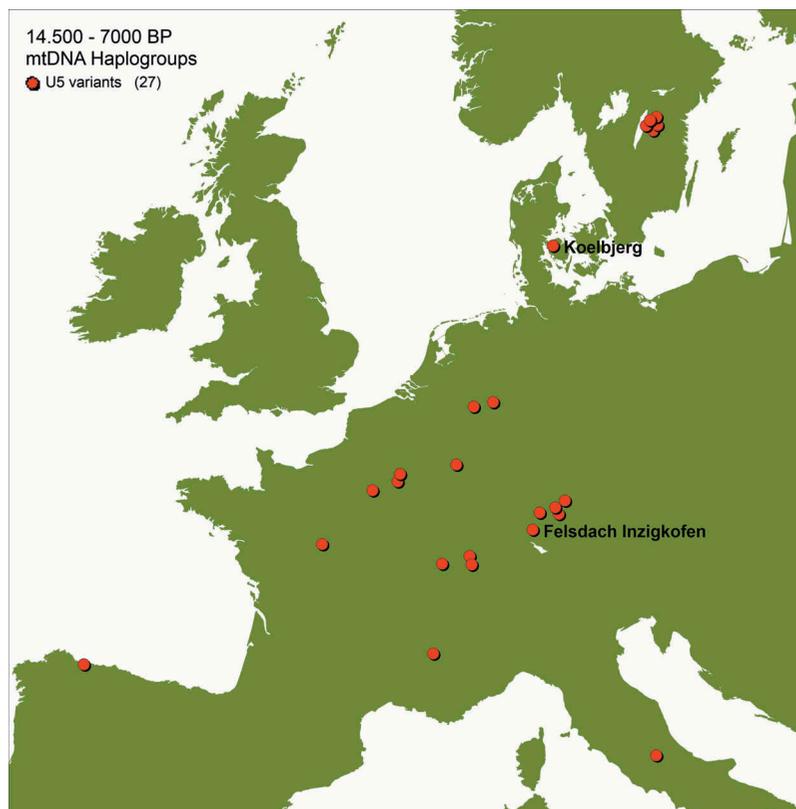


Figure 8. Geographical distribution of Mesolithic individuals displaying mitochondrial haplogroup variants within the U5 clade. All observations except for Koelbjerg are from Posth *et al.* (2016), which is the largest analysis of the European pre-Neolithic mitochondrial gene pool conducted to date. This study identifies U5 variants in 26 of 29 Mesolithic individuals. The same study reports a U5a2c haplogroup (same as Koelbjerg) in a Mesolithic individual from Felsdach Inzigkofen in Germany (map: Kristine Stub Precht).

Authenticity

To address the authenticity of the DNA molecules, we conducted analyses of DNA damage using MapDamage v.2.0.5 and recorded a C–T transition damage rate of 33.9% at position 1 in the 5' end. This is a very high damage level, reflecting the great age of the molecules and comparable to that observed in previous ancient DNA studies (e.g. Allentoft *et al.* 2015). To further assess the authenticity, we conducted an analysis of contamination based on all the mtDNA reads using ContamMix (Fu *et al.* 2013). ContamMix estimates how well all the mtDNA sequences match the Koelbjerg consensus sequence compared to a collection of 311 different mitochondrial genomes from human populations worldwide. With an estimated contamination level below 0.1% (MAP authentic = 0.09%, 95% CI = 0.01–2.05%), the result shows that the Koelbjerg DNA extract does not contain human DNA from more than one source.

Sex determination

Finally, we determined the sex of the individual. To do this, we performed a full genomic mapping of our shotgun data against the human reference genome HG19 build 37.1 with settings as above and recorded the number of reads assigned to the X and Y chromosomes, respectively. Skoglund *et al.* (2013) define a value R_Y as $R_Y = \text{ChrY}/(\text{ChrY} + \text{ChrX})$, with ChrY and ChrX being sequences assigned to chromosomes Y and X, respectively. Based on empirical sequencing data for modern and ancient individuals of known sex, the authors conclude that if the lower 95% confidence interval boundary of R_Y is >0.077 (assuming a normal approximation), then the individual is a male, and if the upper interval limit is <0.016 , then the individual is a female. In the case of the Koelbjerg skeleton, we obtained a lower boundary on the R_Y confidence interval of 0.087 (Table 4), securely identifying this individual as a male.

Having concluded that the Koelbjerg individual was a male, most likely in his late 20s and belonging to the common Mesolithic mtDNA haplogroup

U5a2c, leaves us with the final question of whether he was local to the area where he died or if he was more likely to be an itinerant hunter who originated far away from present-day Funen.

Strontium isotope analyses

Laboratory work and methods

In order to investigate the provenance of Koelbjerg Man, we conducted strontium isotope analysis of tooth enamel from one of his first molars. The sample consisted of a small piece of enamel collected previously, as tensions in the tooth had caused a few fragments to split off from one of the first molars of the upper jaw (Figure 9); hence, no additional handling was necessary.

In humans, tooth enamel of permanent teeth mineralises during early childhood with the exception of the third molar (wisdom tooth). The first molar mineralises between the perinatal stage (before birth) and 3–4 years of age and it does not remodel thereafter, thereby carrying childhood information on geographic origin (Hillson 1996). The strontium isotope signature of the enamel of the human first molar is consequently used in archaeology as a proxy for the place of origin of the individual being investigated (Montgomery 2010).

The sample of tooth enamel from the Koelbjerg individual was mechanically pre-cleaned with a dental diamond drill and subsequently washed ultrasonically in ultrapure (MilliQ™) water. The clean sample was introduced into a 7-ml pre-cleaned Teflon beaker (Saville™) and dissolved in a 1:1 solution of 0.5 ml 6 N·HCl (Seastar) and 0.5 ml 30% H₂O₂ (Seastar). The sample decomposed rapidly after approximately 5 min, and the solution was dried down on a hotplate at 80 °C.

The sample was taken up in a few drops of 3N·HNO₃ and then loaded on a disposable extraction column with a 0.2-ml stem volume charged with intensively pre-cleaned mesh 50–100 SrSpec™ (Eichrome Inc.) resin. The elution recipe essentially followed that by Horwitz *et al.* (1992) scaled to our needs. Strontium was subsequently eluted/stripped by pure deionised water and then the eluate was dried on a hotplate. The strontium sample was dissolved in 2.5 µl of a Ta₂O₅–H₃PO₄–HF activator solution and directly loaded onto previously outgassed 99.98% single rhenium filament. The sample was measured at 1250–1300 °C in dynamic multi-collection mode on a VG Sector 54 IT mass spectrometer

Table 4. Number of sequences assigned to the sex chromosomes and used to calculate R_Y as defined above.

ChrY + ChrX	ChrY	R_Y	95% CI	Sex
210,707	18,544	0.088	0.087–0.089	XY

The 95% lower boundary on the R_Y values is >0.077 , which securely identifies this skeleton as a male (XY).



Figure 9. Detail of the Koelbjerg skull, upper jaw, inferior view (photo: Jens Gregers Aagaard).

(TIMS) equipped with eight faraday detectors (at the Department of Geoscience and Natural Resource Management, University of Copenhagen). An amount of 5 ng loads of the NBS 987 Sr standard gave $^{87}\text{Sr}/^{86}\text{Sr} = 0.710237 \pm 0.00001$ ($n = 10, 2\sigma$).

Strontium isotope results

The strontium isotope bioavailable range for Denmark, the so-called baseline or isoscape, has previously been established for surface waters and fauna yielding a combined $^{87}\text{Sr}/^{86}\text{Sr}$ range of $\sim 0.708\text{--}0.711$ (excluding Bornholm) (Frei and Frei 2011, Frei and Price 2012, Frei 2013). The strontium isotope analysis of the tooth enamel sample from Koelbjerg Man's first molar yielded a $^{87}\text{Sr}/^{86}\text{Sr} = 0.71005$ ($\pm 2\sigma$ 0.00004). Consequently, this individual's value falls clearly within the Danish baseline range (Figure 10).

Previous stable isotope investigations have suggested that marine food was neglectable or completely missing in this individual's diet during adulthood (Fischer *et al.* 2007). Hence, based on the assumption that his diet did not change drastically between childhood and adulthood, we conclude that the strontium isotope value that we measured in the first molar was not affected by a marine signature.

In conclusion, it seems most likely that this individual was of local provenance; however, several other areas with similar bioavailable strontium isotopic ranges cannot be excluded. Areas with such ranges can be found for example in Britain (Evans

et al. 2010) and in northern Germany (Voerkelius *et al.* 2010, Price *et al.* 2017).

Discussion and conclusion

An unusually fortunate combination of circumstances resulted in the Koelbjerg skeleton coming to Odense City Museums about 75 years ago, accompanied by detailed contextual information. In the 1940s, nobody anticipated the invention of methods that would allow for an ancient individual's precise age and sex to be established and reveal details of the person's diet, place of origin and genetic ancestry.

With this article, several fundamental questions concerning the *c.* 10,500-year-old skeleton from Koelbjerg have been reappraised and new conclusions drawn. As a result of the coinciding results based on the anthropological and ancient DNA analyses, we conclude that the skeleton is that of a man. Moreover, based on our combined ancient DNA and strontium isotope analyses, it seems as this individual was local and has a genetic profile very typical for the European Mesolithic population.

The new study of the Koelbjerg individual clearly demonstrates the importance of allowing to perform reevaluation of old results and how new interpretations are possible as methods advance.

With the results presented here, we have significantly increased our knowledge on the Koelbjerg skeleton but there are ample opportunities to

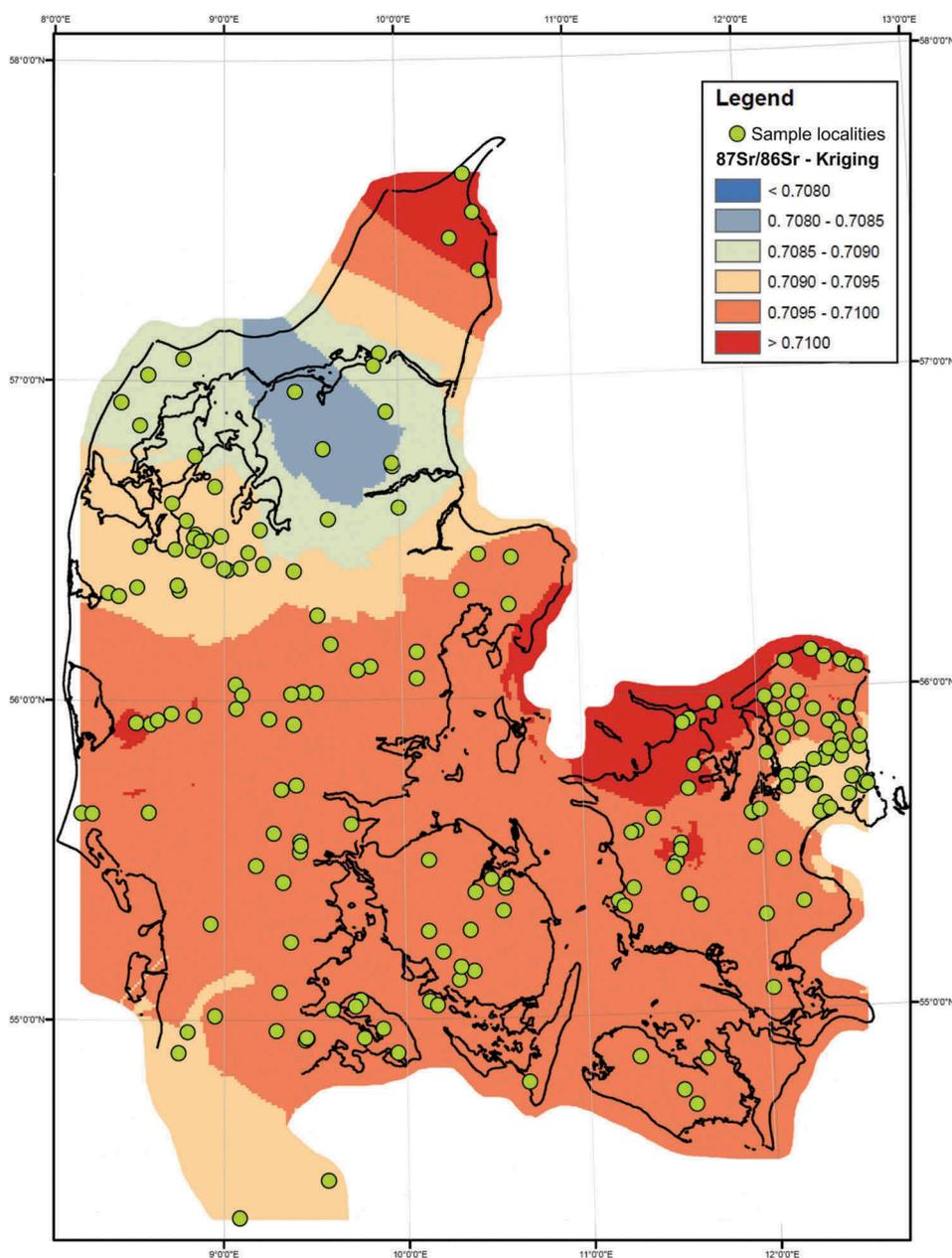


Figure 10. Map depicting the bioavailable strontium isotope ranges as measured in surface waters from Denmark (excluding Bornholm), after Frei and Frei (2011).

continue the scientific work. For example, dietary and provenance studies could proceed on the basis of methods within the continuously developing field of stable isotope analysis. Furthermore, on the subject of diet, a remarkable diversity of information can often be obtained by analysing the calculus found on the teeth of prehistoric skeletons (e.g. Weyrich *et al.* 2017) and these are present in abundance on the teeth of the Koelbjerg Man (Figure 11).

The obvious gracility of the long bone diaphyses also needs to be investigated in a broader Mesolithic context. Lastly, further genomic analyses of the

Koelbjerg skeleton are ongoing in order to provide a more detailed understanding of the Mesolithic gene pool in Denmark.

Although advanced modern analytical methods have been able to close several important gaps in the Koelbjerg Man's life history, they cannot explain why he ended up at the bottom of a small lake. In a Neolithic, Bronze and Iron Age context, it is commonly suggested that at least the carefully placed bog bodies are result of a planned chain of events as part of either offerings, punishment of criminals or regular burials (e.g. Van Der Sanden 1996, Bennike



Figure 11. The lower jaw with calculus on the teeth (photo: Jens Gregers Aagaard).

1999, Ravn 2010). This however contrasts with the Koelbjerg skeleton, as the bones were found scattered both vertically and horizontally in the mire deposits, as much as 10 m apart.

Already in the first publication by Troels-Smith (1943), this was interpreted as a consequence of the corpse floating in the water, with appendages and body parts gradually falling apart in the process. Modern forensic studies have given us a considerable insight into the processes that take place when a corpse decomposes in water (Haglund and Sorg 2002) and on this basis, the

original interpretation seems very likely. In that light, it seems plausible that the corpse entered the water during the warmer months, when development of gases in the abdomen resulted in it rising to the surface, where decomposition and decay took place. If the corpse had entered the water in winter, it would probably have become incorporated into the lake deposits as an articulated skeleton. This sequence of events does, however, leave open the possibility that parts of the skeleton may still be preserved in the wetland sediments at Koelbjerg (Figure 12).



Figure 12. The findspot near Koelbjerg, on central Funen. Missing parts of the skeleton could possibly still be found in nowadays bog/lake (photo: Anders Fischer).

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Appendix

Mean robusticity and relative indices for postglacial European sample from Jacobs (1985), female values adjusted for Koelbjerg not being a female. M no. indicates measurement number/definition in Martin and Saller (1957).

Bone and index	Males	Females	Koelbjerg
Humerus			
Robusticity $(M7 \times 100)/M1$	20.6	18.5	17.4
Relative head height $(M10 \times 100/M1)$	15.3	15.3	14.2
Relative biepicondylar breadth $(M4 \times 100/M1)$	19.8	18.9	16.8
Femur			
Robusticity $(\sqrt{(M6 \times M7)}/M1)$	0.067	0.063	0.060
Pilastric index $(M6 \times 100/M7)$	114.7	106.1	108.3
Metric index $(M10 \times 100/M9)$	80.0	76.3	71.0
Relative head diameter $(M18 \times 1000/M1)$	107.0	101.8	105.8
Relative distal epiphyseal breadth $(M21 \times 100/M1)$	19.2	18.2	17.8
Tibia			
Robusticity $(\sqrt{(M8 \times M9)}/M1a)$	0.076	0.069	0.061
Cnemic index $(M9a \times 100/M8a)$	63.9	62.4	63.3
Relative proximal epiphyseal breadth $(M3 \times 100/M1a)$	21.4	21.0	19.3