

A Mid-Holocene reindeer antler from Regstrup, Sjælland, Denmark

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ABSTRACT

A small, shed antler fragment of a reindeer from Zealand, Denmark has been dated to the Mid-Holocene, c.4700 cal BC. Reindeer was an important component of the Lateglacial fauna in Denmark, and the species survived for c.1400 years into the Holocene. However, we consider it highly unlikely that the species lived in Denmark during the Mid-Holocene, when dense forests characterized the vegetation and summer temperatures were slightly higher than at present. We suggest that the reindeer antler came to Zealand from Norway or Sweden as a result of trade, perhaps involving flint.

ARTICLE HISTORY

Received

19 February 2021;

Accepted

20 October 2021

KEYWORDS

Reindeer; Rangifer tarandus; Ertebølle Culture; Stone age; Trade; Denmark

Introduction

During the Lateglacial period, the vegetation of Denmark was dominated by dwarf-shrub heaths and the cold-adapted reindeer (*Rangifer tarandus*) was the most common large mammal. During the earliest Holocene, temperatures were increasing but this was interrupted by the cold Preboreal oscillation at c.9400 cal BC (Björck et al. 1997). Denmark was covered by open forests and reindeer continued to live in the area for some time. The latest dated reindeer specimen from Denmark gave an age of c.8300 cal BC (Aaris-Sørensen et al. 2007), corresponding to c.1400 years into the Holocene.

During the Mid-Holocene, Denmark was covered by dense forests and the fauna of large, terrestrial mammals was dominated by red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*; Aaris-Sørensen 2009). At this time, the flora and fauna in Denmark included several warmth-demanding species such as the water plants *Trapa natans* and *Najas minor* and the woody plant mistletoe (*Viscum album*; Iver-

sen 1973; Bennike et al. 2001) as well as the pond turtle (*Emys orbicularis*; Degerbøl and Krog 1951). These species no longer live in Denmark and Iversen (1973) concluded that the mean July temperature was 2-3°C higher than at present during the Holocene thermal maximum, which is in good accordance with more recent estimates based on pollen data (Brown et al. 2011).

During excavations at Regstrup on Zealand, Denmark, a shed antler fragment of a reindeer was found; it was dated to the Mid-Holocene, c.4700 cal BC. The aim of this paper is put this surprising find on record.

Materials and methods

Prior to the coming highway between Holbæk and Kalundborg, Museum West Zealand conducted archaeological excavations following the Danish Museum's Act of developer-funded excavations. Trenches were excavated and the profiles mapped and described by archaeologists from Museum West Zealand. Samples were collected from open



Figure 1. Map of Denmark showing the location of Regstrup (Re) between Holbæk (Ho) and Kalundborg (Ka) on Zealand.

sections. The samples consisted of sediment monoliths and kg-large bulk sediment samples. The excavations and later analyses revealed the presence of Lateglacial sediments in the area and a shed antler of a reindeer was also found.

The excavated area is located near Regstrup, ca. 9 km south-west of Holbæk (Figure 1), and the antler was found at ca. 55.66°N, 11.61°E, 32 m above sea level. The antler was found in a layer of brownish-grey silty clay. The age of the clay layer is unknown, it did not contain any macroscopical remains of terrestrial plants that could be used for radiocarbon dating. The clay layer is underlain by a layer with a beaver (*Castor fiber*) bone dated to the Younger Dryas and overlain by a layer with artefacts dated to the late Neolithic or Bronze Age.

A sample from the antler was submitted to the Ångström Laboratory, University of Uppsala for radiocarbon dating, using accelerator mass spec-

trometry (AMS). The collagen fraction of the antler was used for dating. Two dates were obtained. The radiocarbon dates were calibrated to calendar years before Christ (BC) using the CALIB program version 8.20 (Stuiver et al. 2021) and the INTCAL20 calibration curve (Reimer et al. 2020).

The antler gave a surprising age and therefore we decided to check the species identification using protein sequencing by liquid chromatography tandem mass spectrometry (LC-MS/-MS). For extraction and digestion, we sampled approximately 15 mg of bone powder from the antler. Protein extraction was performed as described by Jensen et al. (2020; extraction 1). An extraction control was processed in parallel to the sample. Briefly, 100 μ L of 50 mM NH_4HCO_3 (Sigma) was added to the sample followed by incubation at ambient temperature for 16 h, after this time the sample was centrifuged at 10,000 \times g for 10 minutes and the supernatant was removed and discarded. The



Figure 2. Antler fragment of reindeer (*Rangifer tarandus*) dated to c.4700 cal BC. Sample no. MHO1082x200. The antler was treated with Paraloid B72 after sample was taken for dating. The specimen is housed in the Zoological Museum, University of Copenhagen (ZMK 1/2017).

purpose of this pretreatment was to reduce surface contamination. Subsequently, proteins were extracted by the addition of 100 μ L of 50 mM NH_4HCO_3 and incubation at 65°C for 1 hour. After centrifugation at 10,000 \times g for 10 minutes, the supernatant was removed and quantified using the BCA assay (Thermo Fischer) according to the manufacturer's instructions. A volume equivalent to 20 μ g of protein was placed into a fresh protein LoBind tube (Eppendorf) to which 0.4 μ g of sequencing grade trypsin (Promega) was added. The digestion was allowed to proceed at 37°C for 18 hours. The digestion was terminated by the acidification of the samples to <pH 2 using 10% (vol/vol) trifluoroacetic acid (TFA, Sigma Aldrich). After acidification the sample was centrifuged at 10,000 \times g for 10 minutes. Peptide purification was performed using C18 reverse-phase resin Zip-Tips (Pierce™) according to the manufacturer's instruction. Peptides were eluted with 50 μ L of 50% acetonitrile (ACN; Sigma Aldrich)/0.1% TFA (vol/vol).

For LC-MS/MS a volume containing approximately 2 μ g of protein was placed in separate wells on a new 96-well plate and topped up to 30 μ L using 40% (ACN) and 0.1% FA. The sample and a blank were then vacuum centrifuged to approximately 3 μ L and resuspended with 10 μ L of 0.1% TFA, 5% (ACN) and 5 μ L of sample analysed by LC-MS/MS. The LC-MS/MS parameters were the

same as previously used for palaeoproteomic samples (Jensen et al. 2020; Mackie et al. 2018), in short: MS1: 120 k resolution, maximum injection time (IT) 25 ms, scan target 3E6. MS2: 60 k resolution, top 10 mode, maximum IT 118 ms, minimum scan target 3E3, normalized collision energy of 28, dynamic exclusion 20 s, and isolation window of 1.2 m/z.

We used MaxQuant (v.1.6.3.4; Cox and Mann 2008) to search the generated Thermo RAW files. We prepared a FASTA database containing published collagen type I alpha 1 and alpha 2 (COL1 α 1 and COL1 α 2) sequence data from reindeer, red deer (*Cervus elaphus*), elk (*Alces alces*) and roe deer (*Capreolus capreolus*). MaxQuant settings were as follows: Digestion mode was set to specific for Trypsin. Variable modifications were: oxidation (M), Acetyl (Protein N-term), Deamidation (NQ), Gln \rightarrow pyro-Glu, Glu \rightarrow pyro-Glu, and Hydroxyproline. Fixed modifications were: Carbamidomethyl (C). The remaining settings were set to the program defaults, apart from Min. score for unmodified and modified peptides searches, which were both set to 60.

Results

The antler fragment consists of the proximal part of a dextral antler, with only the proximal part of

Laboratory number	Age (¹⁴ C years BP) ¹	Cal. age (years BC) ²	Cal. age (years BC) ³	Cal. age (years BC) ⁴
Ua-59941a	5853 ± 33	4788–4719	4837–4688	4756
Ua-59941b	5775 ± 99	4723–4498	4842–4369	4626

¹ Radiocarbon ages are reported in conventional radiocarbon years BP (before present = AD 1950; Stuiver and Polach (1977)).

² Calibration to calendar years BC (1 sigma) is according to the INTCAL20 data (Reimer et al. 2020).

³ Calibration to calendar years BC (2 sigma)

⁴ Median probability ages.

Table 1. Radiocarbon dates of the reindeer antler from Regstrup, Zealand, Denmark.

the brow tine preserved. The beam is fairly straight. We don't know if the distal part of the antler was broken off naturally, or artificially cut off. The fragment is 16.5 cm long and it measures 2.34 cm × 2.03 cm in cross section and represents a small, slender shed antler of a female reindeer (Figure 2). A sample from the antler was dated after conventional acid/alkali pretreatment and the result was 5853 ± 33 ¹⁴C years BP (Table 1), which is much later than anticipated. Therefore, it was dated a second time, following ultrafiltration and using the fraction > 30 kD, which precludes contamination by small molecules. The result of 5775 ± 99 ¹⁴C years BP is in agreement with the first date and it was noted that the collagen in the antler was well preserved and of good quality (Göran Possnert, personal communication, 2019). The dates are calibrated to c.4700 cal BC (Table 1).

A too late date may result from the use of preservative materials, but the sample was not treated with such material. A late date could also result from recent plant roots or humic acid, but no roots were observed and humic acids were removed during the chemical preparation. Therefore, we con-

sider the date correct and conclude that the age of the antler corresponds to the Mid-Holocene Ertebølle Culture (5300-3950 cal BC).

This is a most surprising age for a Danish find of reindeer, because reindeer disappeared from Denmark in the Early Holocene (discussed previously). To confirm the identification, we performed species identification by LC-MS/MS to validate whether this was indeed a reindeer antler. We found three single amino acid substitutions (SAPs) unique to reindeer that we used to distinguish this species from three closely related species: red deer, elk and roe deer. The SAPs are located at: position 396 D (*Rangifer*) or A (*Cervus/Alces/Capreolus*) on the COL1α1 chain, at position 223 D (*Rangifer*) or N (*Cervus/Alces/Capreolus*) on the COL1α2 chain, and at position 691 S (*Rangifer*) or P (*Cervus/Alces/Capreolus*) on the COL1α2 chain. All three peptides were detected and were matched in the sample multiple times (Table 2). Additionally, y and b ion series provided good coverage of phylogenetically important sites. All three peptides appeared and were matched in the sample multiple times (Table 2), with both y and b ions covered

Sequence ¹	Length	Da	Q	Highest MQ score	Matched spectra
GEPGPPGAGAAGPAGNPGADGQPGGK	27	2237.0533	2:3	501.24	12
GEVGIPGISGVPVPPGNPGADGIPGAK	27	2366.2302	2:3	401.44	5
GENGPVGPVGAAGPSGPNPSPGAGSR	30	2554.216	2:4	544.03	16

¹ Single amino acid substitutions used to discriminate between reindeer and related taxa within the clade Cervidae are marked in bold.

Table 2. Identified reindeer peptides from published collagen sequences (Welker et al. 2016).

along both sides of the peptide backbone. This allowed us to identify with high confidence that the antler indeed derived from a reindeer. Two examples of reindeer-specific peptides with well-covered y and b ion series used to identify the antler are shown in the appendix.

Other finds of exotic mammals

There are a few other reports of reindeer antlers dated to the Mid-Holocene from the region. A decorated antler hammer dated to the Mid-Holocene and recovered during peat cutting at Vedbæk in northeast Zealand was identified as reindeer (*Rangifer tarandus*; Mathiassen 1941; Troels-Smith 1941). However, the identification has been changed to elk (*Alces alces*; Vang Petersen 1982; Brinch Petersen 2015).

A decorated antler object from an early Mesolithic refuse layer in central Scania was identified as a reindeer antler (Larsson 1976). However the identification has been questioned, it might be an antler from an elk (Larsson a and b in print). Due to a low collagen content a ZooMS analysis failed. However, the discussion about the origin of the object is still valid as it has elements well known from the Mesolithic in south-western Norway.

A worked antler identified as reindeer from Husum harbour in north-west Germany that was found in 1881 yielded a date of *c.*4000 cal BC (KIA-17652; Fischer and Jensen 2018). This date may be influenced by preservative material, but the dating laboratory put a lot of effort into removing such material and the date is probably correct. However, it is possible that the antler comes from a red deer (*Cervus elaphus*). The artefact was found together with other cultural material, which from a typological point of view, would fit perfectly with the radiocarbon date. At 4000 cal BC red deer was common in Germany. Fischer and Jensen (2018) mentioned that “it may also be that both the radiocarbon date and the original species identification are correct, in which case this find would represent an archaeologically very important example of an imported object from the extreme north of Europe, where reindeer were present at that time”.

From central Poland a decorated reindeer antler was reported by Osipowicz et al. (2017), this

find was dated to *c.*7900 cal BC, corresponding to the Early Mesolithic. Based on analyses of stable isotopes, it was concluded that the antler probably came from northern Karelia or southern Lapland.

Remains of other exotic mammals from Mid-Holocene deposits on Zealand were discussed by Vang Petersen (1990), Fisher (2003), Price et al. (2007) and Brinch Petersen (2015, 140-147). Artefacts and pearls made of teeth of elk (*Alces alces*), aurochs (*Bos primigenius*) and brown bear (*Ursus arctos*) have been reported from sites that post-date the disappearance of these species from the island. However, the species were present in nearby Skåne in southern Sweden, so it is not so surprising to find remains of them on Zealand and Brinch-Petersen (2015) concluded that the remains of the exotic species indicate a lively traffic across Øresund.

Isotope proveniencing of remains of elk and brown bear from Kainsbakke, Djursland, eastern Jutland indicate contact across the Kattegat in the Middle Neolithic (Price et al. 2021). However, the reindeer antler from Regstrup must have been brought to Zealand from a more distant source, probably northern Scandinavia. Flint was exported from Denmark to flint-less regions in the rest of Scandinavia during the Stone Age (Mathiassen 1934; Becker 1959), and we suggest that the reindeer antler from Regstrup came from Norway or Sweden, perhaps in connection with flint trade. Long-distance contact among Mesolithic hunter-gatherers in northern Europe has also been proposed by Fuglestedt (1999, 2017), based on analyses of rock art.

Acknowledgement

We thank two anonymous referees for comments on the manuscript.

Declaration of interest statement

The authors declare no conflict of interests.

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Supplementary

Two examples of peptides with well-covered y and b ion series used to identify the antler as deriving from reindeer.

see pdf-attachment.