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# The last Viking King: A royal maternity case solved by ancient DNA analysis

Jørgen Dissing <sup>a,\*</sup>, Jonas Binladen <sup>b</sup>, Anders Hansen <sup>b,c</sup>, Birgitte Sejrsen <sup>d</sup>, Eske Willerslev <sup>b,e</sup>, Niels Lynnerup <sup>f</sup>

a Research Laboratory, Institute of Forensic Medicine, University of Copenhagen, Frederik V Vej 11, DK-2100 Copenhagen, Denmark
b Department of Evolutionary Biology, Biological Institute, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark
C Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Frederik V Vej 11, DK-2100 Copenhagen, Denmark
d Department of Forensic Pathology, Institute of Forensic Medicine, University of Copenhagen, Frederik V Vej 11, DK-2100 Copenhagen, Denmark
C Center for Evolutionary Genetics, Niels Bohr Institute, University of Copenhagen, Juliane Maries Vej 30, DK-2100 Copenhagen, Denmark
f Antrophological Laboratory, Institute of Forensic Medicine, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark

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#### **Abstract**

The last of the Danish Viking Kings, Sven Estridsen, died in A.D. 1074 and is entombed in Roskilde Cathedral with other Danish kings and queens. Sven's mother, Estrid, is entombed in a pillar across the chancel. However, while there is no reasonable doubt about the identity of Sven, there have been doubts among historians whether the woman entombed was indeed Estrid. To shed light on this problem, we have extracted and analysed mitochondrial DNA (mtDNA) from pulp of teeth from each of the two royals. Four overlapping DNA-fragments covering about 400 bp of hypervariable region 1 (HVR-1) of the D-loop were PCR amplified, cloned and a number of clones with each segment were sequenced. Also a segment containing the H/non-H specific nucleotide 7028 was sequenced. Consensus sequences were determined and D-loop results were replicated in an independent laboratory. This allowed the assignment of King Sven Estridsen to haplogroup H; Estrid's sequence differed from that of Sven at two positions in HVR-1, 16093T  $\rightarrow$  C and 16304T  $\rightarrow$  C, indicating that she belongs to subgroup H5a. Given the maternal inheritance of mtDNA, offspring will have the same mtDNA sequence as their mother with the exception of rare cases where the sequence has been altered by a germ line mutation. Therefore, the observation of two sequence differences makes it highly unlikely that the entombed woman was the mother of Sven. In addition, physical examination of the skeleton and the teeth strongly indicated that this woman was much younger (approximately 35 years) at the time of death than the 70 years history records tell. Although the entombed woman cannot be the Estrid, she may well be one of Sven's two daughters-in-law who were also called Estrid and who both became queens.

Keywords: Ancient DNA; Haplogroup; D-loop; Teeth; Viking King; Skeletal remains

#### 1. Introduction

The Scandinavian Viking Era from the 8th century to well into the 11th century has been endowed with much aura. At the peak of their influence the Vikings controlled or raided large parts of Western Europe from Russia in east, England in west to France in south and they sailed the European rivers to Constantinople and to the Mediterranean shores. Being excellent navigators they roamed the seas of the North Atlantic

from Europe to North America. However, during the reign of the last of the Danish Viking Kings, Sven Estridsen (AD 1047–1074) the Nordic dominance in Europe dwindled considerably. The Danes had generally adopted christianity at the turn of the first millennium [1]. Sven's mother, Estrid, was of tremendous importance to the christianity in Denmark and she initiated the construction of a travertine church in Roskilde (30 km to the west of Copenhagen), which preceded the brick cathedral that was build about A.D. 1225. This is where the Danish royal dynasty buries its dead. Both Sven Estridsen and his mother were entombed in the earlier church but they were subsequently moved to the pillar tombs in the cathedral when the chancel was completed [2]. This is at least

<sup>\*</sup> Corresponding author. Tel.: +45 35326125; fax: +45 35326150. E-mail address: joergen.dissing@forensic.ku.dk (J. Dissing).



Fig. 1. Skulls of King Sven Estridsen (left) and Estrid (right). Left canine and right wisdom tooth from the maxilla were used for extraction of mtDNA from Sven, and left canine and right first premolar from the maxilla were used from Estrid. These teeth (removed on the photograph) were all sitting firmly in place in the jaw.

what history records tell, but some historians had doubts whether the woman entombed was indeed Estrid because several other royals at that time, especially two of King Sven's daughters in-law, were also called Estrid. In contrast, it is fairly certain that the other tomb contains the remains of Sven Estridsen. To shed light on this identity problem the curator of Roskilde Cathedral Museum asked whether a DNA analysis might be possible.

Successful extraction and analysis of authentic mitochondrial DNA (mtDNA) from human fossil remains has been accomplished in several instances [3–7]. This is facilitated by the presence of thousands of mtDNAs in each human cell [8]. The almost exclusive maternal inheritance of mtDNA is an additional advantage in the investigation of mother/child relationships since offspring is expected to have exactly the same mtDNA sequence as the mother [9]. We have previously mtDNA typed remains from nine Vikings excavated from an early christian cemetery outside Roskilde [10], and the Church Council reluctantly gave permission that an ancient DNA analysis of dental pulp material from Estrid and Sven be performed, provided the teeth to be used were restored and replaced in the skulls.

### 2. Materials and methods

Since the present work concerns the analysis of ancient DNA a number of special measures were crucial to ensure that reliable results were obtained (see e.g. [11,12]). The various elements are described in the following:

#### 2.1. Human remains

The skeletons of King Sven Estridsen and Estrid were retrieved from the pillar tombs by two of us (JD, NL); full body suits, facemasks and sterile gloves were worn throughout. The skeletons showed excellent physical preservation; this is important since a correlation between physical preservation and the amount of contaminating DNA molecules that has penetrated into bone has been reported [13]. Two teeth (canines, premolars or molars) were extracted from each of the skulls (Fig. 1) and stored in sterile plastic tubes. Sexing and ageing of the skeletons were performed using standard physical anthropological criteria, relying mainly on pelvic and cranial traits and femoral head diameter for sexing [14–16], and dental status and wear for ageing [17].

# 2.2. Chemicals, reagents, PCR and centrifuge tubes, pipette tips

All chemicals and reagents were of analytical grade or the highest purity available. PCR tubes and micro centrifuge tubes for extracts and primers were free of human DNA as guaranteed by the manufacturer ("PCR-clean" or "Biopure" tubes, Eppendorf). Pipette tips were aerosol resistant and certified pure and free of DNA (Molecular BioProducts).

#### 2.3. Clean-laboratory (clean-lab)

All manipulations of teeth, extraction of DNA, and mixing of reactions for PCR were performed in a clean-lab dedicated solely to aDNA work [10]; this laboratory is situated in a

<sup>&</sup>lt;sup>1</sup> Curator Annette Kruse, personal communication.

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