

# Using ancient DNA to identify aurochs (*Bos primigenius*) in ancient cattle remains from Belgium

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## Introduction

Aurochs (*Bos primigenius*) are the wild ancestors of the domesticated taurine cattle (*Bos taurus*). Their populations gradually declined during the Holocene, until their extinction at the turn of the 17th century.

Most aurochs can be distinguished from domestic cattle osteometrically, but large-sized domestic cattle may be misidentified as aurochs.

The mitochondrial genomes of aurochs and domestic cattle can be classified in 6 haplogroups:

- Haplogroup 'T' is found in most European taurine cattle. It descends from aurochs that were domesticated 10000-11000 years ago in the Near East and brought to Europe by the first farmers during the Neolithic period.

- Haplogroup 'P' was characteristic of European aurochs. Hybridization occurred more recently between domestic cattle and aurochs, with cases of introgression of haplogroup 'T' in wild aurochs, and of haplogroup 'P' in some East Asian cattle breeds (Mannen et al. 2020).

## Objectives

Determining the mitochondrial haplogroup of archaeological large bovid bones in order to document the presence of aurochs and large domestic cattle in Belgium in prehistory, Roman period and Middle Ages.

## Materials

Bone samples from 11 bovid specimens from Belgium (cf. table below), dating from the Epipaleolithic to the Middle Ages.

### Wet lab protocols

- All pre-PCR experiments performed in an ancient DNA lab, with negative controls.
- DNA extraction for short fragments (Dabney et al. 2013) and double-stranded DNA libraries using the single-tube protocol (Carøe et al. 2018).
- Paired-end sequencing using a NovaSeq instrument (Illumina) with 150 cycles.

### Bioinformatic pipeline

- Raw reads trimmed using AdapterRemoval, merged using PEAR, and classified using Kraken2 with all available mammal mitogenomes from GenBank as reference database.
- Reads mapped using Bowtie2 on the whole genomes of *Bos taurus*, *Bison bison*, *Homo sapiens* and *Mus musculus* (possible contaminants).
- Reads retained if mapping quality > 30 and size < 100 bp.
- Degradation pattern evaluated using PMDtools (Skoglund et al. 2014).
- Reads deduplicated using Picard, assembled and contigs compared using Geneious.

ID	LAST1	LAST2	LAST3	LAST4	LAST5	LAST6	LAST7	LAST8	LAST9	LAST10	LAST11	Neg
Picture												
Epoch (cal BP) <sup>1</sup>	Roman period	Roman period	Roman period	Roman period	Roman period	Roman period	Epipaleolithic (10940-10510)	Mesolithic (8980-8590)	Late Middle Ages (598-533)	Bronze Age (3980-3830)	Roman period	-
Sample <sup>2</sup>	hum R dist femur R dist	femur R dist	femur L	radius R	radius L	MC L	Ph1	MC	horn core	skull	radius L	-
Library ID	3	1	3	3	1	4	1	4	3	4	4	1,3,4
Data (Gb)	6.5	11.9	5.7	6.8	9.3	61.2	19.5	68	8.0	61.9	59.3	0.09-4.28
% mapped reads <sup>3</sup>	0.16	0.64	1.67	0.02	0.12	0.02	0.03 (0.04)	0.02	7.84	0.12	0.03	0.06-0.28
DNA size distribution (counts of read lengths in bp)												
Damage score <sup>4</sup>	1.14	0.92	1.04	0.32	0.58	0.47	0.88	0.20	0.32	0.37	0.51	-0.75-0.37
Closest bovid <sup>5</sup>	<i>Bos</i>	<i>Bos</i>	<i>Bos</i>	<i>Bos</i>	<i>Bos</i>	<i>Bos</i>	<i>Bison</i>	<i>Bos</i>	<i>Bos</i>	<i>Bos</i>	<i>Bos</i>	NA
Mitogenome coverage (%)	18.2	12.4	81.3	3.8	4.6	27.5	25.5	3.5	98.4	95.6	33.5	0 (0-0)
Depth <sup>6</sup>	0.2 (0-2)	0.1 (0-3)	1.9 (0-9)	0 (0-2)	0 (0-1)	0.3 (0-4)	0.6 (0-8)	0 (0-2)	9.1 (0-23)	3.3 (0-11)	0.4 (0-4)	0
% similarity <sup>7</sup>	98.8	99.2	98.3	97.1	99.0	98.6	97.8	99.2	98.8	98.6	99.3	-
Bos haplogroup	T	T	T	?	T	T	-	?	T	P	T	-

<sup>1</sup>cal BP: calibrated years before the present (95.4% probability) <sup>2</sup>hum=humeral, R=right, L=left, dist=distal, MC=metacarpus, Ph=phalanx <sup>3</sup>Percent of reads mapped on the whole genome of *Bos taurus* (or *Bison bison* for LAST7) <sup>4</sup>Post mortem damage score based on PMDtools (Skoglund et al. 2014) <sup>5</sup>Closest bovid estimated based on read classification and mitogenome similarity <sup>6</sup>Mean number of reads for each site (range) <sup>7</sup>Similarity (%) with the closest bovid species

## Results

Reads mapped to the whole genome of *Bos taurus* or *Bison bison* are shorter (cf. table above) and more damaged than those mapped to the other genomes. Their proportions suggest 0.02-10% of endogenous DNA. Negative controls contain 14-217 times less DNA despite a higher number of PCR enrichment cycles.

For three specimens, reads covered > 80% of the bovid mitogenome and enabled the identification of:

- haplogroup 'P' in one aurochs from the Bronze Age, and
- haplogroup 'T' in two bovinds from the Roman period and the Middle Ages.

Among the eight other specimens (covering 3.5-33.5% of the mitogenome):

- five additional Roman bovinds may be assigned to the haplogroup 'T' based on a few diagnostic positions,
- one was identified as bison based on read classification and mitogenome similarity.

## Discussion

→ Reads obtained and filtered here show characteristics of authentic ancient DNA. For some samples (LAST4 and 8), the smaller number of mapped reads indicates a higher risk of contamination from other samples.

→ The haplogroup 'P' recovered for the specimen of the Bronze Age is the first mitogenomic record for a Belgian aurochs. This haplogroup includes other aurochs from Sweden, Poland, the UK and Spain of 9100-403 years old.

→ The exceptionally large Roman bovinds attributed to haplogroup 'T' may relate to the already reported increased size of Roman domestic cattle.

→ These large Roman bovinds and the medieval bovid attributed to haplogroup 'T' may also result from the admixture between aurochs and domestic bovinds, a phenomenon which was suggested to occur repeatedly (Bro-Jørgensen et al. 2018).

→ The oldest and most fragmented bone sample of this study was identified as bison, a genus that was indeed present in Europe during the Epipaleolithic.

## References

- Bro-Jørgensen et al. 2018 J Archaeol Sci 99:47-54
- Carøe et al. 2018 Methods Ecol Evol 9(2):410-419
- Dabney et al. 2013 Proc Natl Acad Sci U S A 110(39):15758-63
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