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## Poor DNA preservation in bovine remains excavated at Pre-Pottery Neolithic Göbekli Tepe (Southeast Turkey): Brief communication

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sites in the Near East may help to characterize the original mtDNA haplotypes. The preliminary results obtained for bovine remains excavated at the Early Neolithic site Göbekli Tepe, however, indicate a poor DNA preservation.

### Abstract

With regard to the domestication history of cattle the molecular genetic investigation of bovid remains excavated at Neolithic

### Keywords

Göbekli Tepe, domestication, *Bos*, ancient DNA, mtDNA

### Introduction

Recent studies indicate that members of the extinct Near Eastern aurochs (*Bos primigenius*) were the progenitors of European domesticated cattle (e.g. Troy et al., 2001; Bollongino et al., 2006; Edwards et al., 2007; Achilli et al., 2009).

In this context, the molecular genetic investigation of bovine remains excavated at Neolithic sites in the Near East may help to characterize the original Near Eastern mtDNA haplotypes. Our molecular genetic analyses were applied to bovine remains excavated at the Early Neolithic site Göbekli Tepe, which is located about 15 km northeast of the town Şanlıurfa (often known simply as Urfa) in South-eastern Turkey. The site is situated on the highest point of a mountain ridge and consists of a number of megalithic structures which date to the period between 9,300 BC and 8,000 BC. The earlier levels contain many pillars decorated with animal depictions. Illustrations include wild boar, snakes, red fox, crane, wild cattle, ducks and vultures (Peters and Schmidt, 2004; Schmidt, 2007).

### Material and methods

A first set of bovine tooth and bone samples excavated at Göbekli Tepe were washed on location, dried in the sun and subsequently stored conventionally. A second set of additional *Bos* remains were freshly excavated (excavation campaign in April 2009) and continuously cooled. Sample preparation, DNA extraction and PCR set-up were carried out in laboratories dedicated to ancient DNA analysis. Amplification and the analysis of amplification products were performed in separate laboratories.

After removal of the bone and tooth surface, the samples were UV-irradiated on all sides (254 nm, 30 min) and subsequently ground. DNA extraction was performed following the silica-based extraction protocol C developed by Yang and colleagues (1998). Each extraction series consisted of five samples and was accompanied by an extraction control. With regard to the highly variable region of the bovine mtDNA control region, four sets of overlapping primers were designed with the aid of the software component *Primer3* (Rozen and Skaletsky, 2000). The lengths of the amplification products vary between 102 bp and 152 bp and the overall framed mtDNA HVRI region contains 281 bp (nucleotide positions

16031 to 16311, GenBank accession number V00654.1, [Anderson et al., 1982]). Each PCR analysis consisted of five *Bos* samples and was accompanied by the associated extraction control and a PCR control (no template controls). In order to avoid contamination of the ancient samples, no positive control (modern cattle DNA) was processed with the samples. The amplification products obtained were purified and then directly sequenced on an Applied Biosystems 3730 DNA analyzer.

In addition to the molecular genetic investigation, collagen was extracted from 27 bovine remains. The collagen yield was calculated as the percentage of whole bone.

## Preliminary results

All extraction and PCR controls processed with the samples remained free of amplification products, except primer dimers. Unfortunately, most of the 40 *Bos* samples under study remained free of amplification products, too. Amplification products were obtained for five conventionally treated *Bos* specimens (washed on site and sun-dried) only. The DNA sequences showed a high similarity with the reference sequence (GenBank accession number V00654.1) which is classified as haplotype T3. However, in almost all cases, sequences obtained in a single PCR attempt were not reproducible in a second one. The amount of collagen measured for 17 conventionally stored *Bos* specimens excavated at Göbekli Tepe was very low. Nine samples revealed 0.0% collagen and seven samples yielded a collagen content below 0.4%. One *Bos* specimen contained 0.91% collagen, but also failed to show any PCR products on a silver-stained polyacrylamide gel.

The collection of freshly excavated and continuously cooled *Bos* remains gave rise to great expectations. But, no amplicons have as yet been obtained using this sample material. The amount of collagen measured for 10 freshly excavated *Bos* specimens was very low, too. Six samples revealed a collagen content below 0.2% and four samples had a collagen content of below 0.4%.

## Discussion

Our preliminary results indicate that DNA preservation in the bovine remains from this important excavation site is poor, and the few mtDNA results that were obtained showed a low reproducibility. Reproducibility (from the same extract and separate extractions), however, is the most important criterion for the authenticity of ancient DNA results (Edwards et al., 2004).

Poor DNA preservation in skeletal remains from Neolithic sites in the Near East has already been demonstrated in other studies (Edwards et al., 2004; Edwards et al., 2007; Bollongino and Vigne, 2008; Bollongino et al., 2008). This poor biomolecular preservation is due to the climatic conditions in the Near East, which can be adequately summarized as hot and dry summers and humid and rainy winters. In this context, the climate feature of the Şanlıurfa Province is characterized by extremely hot, dry summers and cool, moist winters (semi-arid). According to Smith and colleagues (2003), the thermal history of a fossil is a key parameter for the survival of biomolecules. In general, fossils from cold environments will have better biomolecular preservation rates than those from hot climates (Smith et al., 2003). High temperatures promote DNA degradation, especially DNA depurination. Conventional excavation techniques (i.e. water cleaning and sun drying) subject the samples to additional thermal stress (Bollongino and Vigne, 2008).

With regard to the specimens under study, another biomolecule, collagen, was detectable only in small quantities. This is a further indication of poor sample preservation at this excavation site. Several studies have shown at least a weak correlation between DNA amplification and protein content (Colson et al., 1997; Poinar and Stankiewicz, 1999). Götherström and colleagues (2002) showed that the presence of DNA is strongly related to the crystallinity in the hydroxyapatite and to the amount of collagen. They suggested that DNA is adsorbed to and stabilized by hydroxyapatite, and that collagen is part of the complex system that preserves DNA in bone tissue (Götherström et al., 2002).

With regard to the Early Neolithic site Göbekli Tepe, the investigation of animal remains originating from deeper levels might produce better results, since these finds may have been better protected against adverse conditions of fluctuating levels of humidity and other abiotic factors. As suggested by Pruvost et al. (2007) and Bollongino and Vigne (2008), the freshly excavated bones should not be washed, but continuously cooled.

## Acknowledgement

I would like to thank Prof. Dr. K. Schmidt (DAI Berlin) for the opportunity to analyze the bovid remains discussed in this study. This work was financially supported by the Deutsche Forschungsgemeinschaft (DFG project PE 424/9-1: Ungulate domestication and early animal husbandry in the Upper Euphrates basin [Prof. J. Peters, Prof. G. Grupe, Prof. H.-J. Uerpmann]).

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