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Ancient DNA, a Neolithic legging from the Swiss Alps and the early history of goat

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ABSTRACT

Ancient DNA from a Neolithic legging (1st half of the 3rd millennium BC) found at Lenk, Schnidejoch (2750 m a.sl.) in the Swiss Alps has demonstrated, that modern distribution of genetic variation does not reflect past spatio-temporal signatures. The legging was made from the skin of a domestic goat (*Capra hircus*), belonging to the caprine haplogroup B1, which is marginal in Europe today, but represents a third highly diverse goat haplogroup entering Europe already in the Neolithic. Population expansion of lineage B therefore happened more than 4500 years ago, but their members were at some point almost completely replaced by goats of today's common A and C haplogroups.

1. Introduction

Ancient DNA (aDNA) research revealed that modern genetic pattern do not necessarily reflect long term history of domestic animals and has changed our understanding of domestication (e.g. (Dobney and Larson, 2006; Edwards et al., 2007; Kavar and Dovc, 2008; Vigne and Darlu, 2008; Zeder et al., 2006; Zeder, 2008)) and subsequent past human mediated dispersal in plants and animals on a temporal and spatial level (Erickson et al., 2005; Larson et al., 2007). The advantage of aDNA compared to modern DNA is the direct assessment of genetic constitution at precise time points in the past.

Due to the recent glacial retreats in the Alps, one immaculately-preserved, almost complete left leg of a prehistoric leather legging was discovered on the Schnidejoch, Lenk (2750 m a.sl.) in the Bernese Alps of Switzerland (Grosjean et al., 2007; Suter et al., 2005). So far no human body has been found, but findings of a bow, arrows and arrow heads as well as a bark quiver found nearby suggests that a hunter might have died at the site.

Garments are socio-cultural indicators and demonstrate the technical choice and skills of the producers. Material evidence for leather clothing in European prehistory is generally rare, because taphonomic conditions are unfavourable for leather preservation, even at lake shore settlements with extremely well preserved organic remains. Prehistoric clothing is therefore largely unknown. The most famous and spectacular garments found so far have been those of Ötzi, the Ice Man, from the Hauslabjoch (3350–3300 cal BC) (Höpfel et al., 1992). His clothes provided a singular insight into prehistoric dress fashion.

Archaeological leather as a source for DNA has rarely been exploited; the potential information to be gained through aDNA investigations from processed animal skins was however recognized (Poulakakis, 2006; Vuissoz et al., 2006).

Although the leather from the Schnidejoch presents a well-preserved grain surface its provenance could not be accurately determined with morphological analyses, let alone further information extracted. In this case we used mitochondrial cytochrome *b* and control region markers to identify the species and the maternal haplogroup of the animal skin used for the legging, and also to infer to early animal history.

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2. Material and methods

2.1. The leather object

On September 17th 2004, a team of the Archaeological Service of the Canton Berne (ADB) discovered the legging as part of a group of artefacts spanning from the Neolithic to the Medieval period on the Schnidejoch pass (2750 m a.sl., Bernese Alps, Switzerland) (Suter et al., 2005) (Fig. 1). The artefact was ^{14}C dated to 4215 ± 55 BP (ETH-29692/UZ-5179: 2σ 2915–2627 cal BC (95.4%; 2915–2830 cal BC 31.4% and 2823–2627 cal BC 64.0%)), calibration curve IntCal04 (Reimer et al., 2004) and calibration program OxCal 4.1 (Bronk Ramsey, 2001).

The legging consists of an entire hide, an insert and some repair patches. It was used with the hairy side on the outside, with the animal's neck section at the wearer's foot and the rear area at the top. The seam ran from the back of the wearer's leg up to the outer side of the hip. The insert was included in the main seam near the top of the legging as a widener. Two small clusters of stitch holes at the front and rear of the top edge showed where suspension loops had been stitched on for use with a belt.

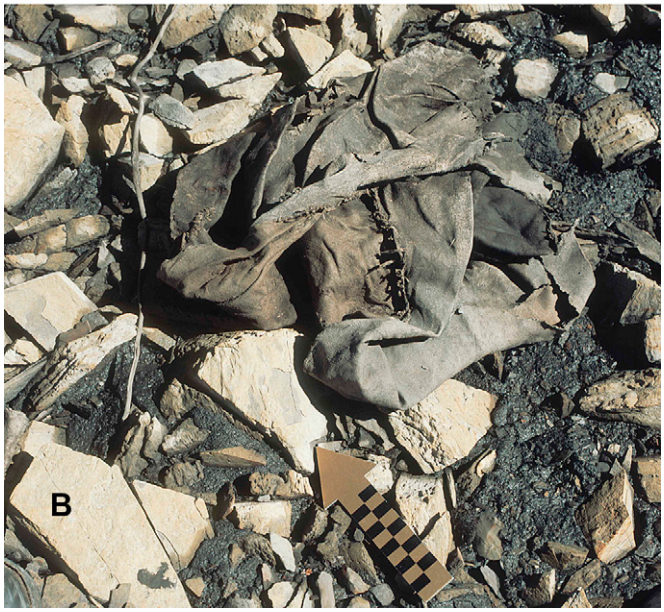

Fig. 1. A: Map of Switzerland showing the location of the site Lenk, Schnidejoch. B: The leather legging in situ after the discovery on the Schnidejoch pass.

Five days after the discovery and still mainly in frozen condition, the legging was stored at 45–50% r.h., at 12–15 °C, absence of light and non-stagnant air (passive conservation).

Three years after the discovery, a sample of ca. 1 cm^2 (code 103371) was excised and analysed for DNA. A second sample of 1 cm^2 (code 103547) was taken 4 months later and sent to Copenhagen for replication.

2.2. Morphological identification

In case of leather the species of the animal is identified by comparative observation of the leather grain (Haines, 1981). The arrangement of the pores and follicles is specific for each animal species. Reference samples are from modern domestic and wild species and usually taken from vegetable tanned leathers.

2.2.1. DNA extraction, amplification, cloning and sequencing, basel

No external treatment was performed. The leather was cut into small pieces with a scalpel and two sub samples (aliquots) were extracted with QiAmp DNA Mini Kit (tissue protocol) according to the manufacturer's instructions with the following exceptions: 3 volumes of buffer ATL were used and proteinase K digestion was overnight at 56 °C. Elution was with 2 × 200 µl buffer AE. Two extract blanks were processed accordingly.

Eluates were filtrated with Microccon 30KD following manufacturer's instruction with 2× washing with ddH_2O (Eppendorf water, molecular biology grade). Ca. 200 µl extract was recovered. These extracts were further diluted up to 1:100 to overcome inhibition.

Hotstart-PCR was carried out in an Eppendorf Mastercycler (Vaudaux Eppendorf, Allschwil, CH) using 25 µl volume containing 3 µl extract, 1× PCR buffer, 1 µM each primer, 2 mM $MgCl_2$, 250 µM each dNTP, 2.5–5 units AmpliTaq Gold DNA polymerase (Applied Biosystems, Rotkreuz, CH). After initial activation of polymerase for 11 min at 95 °C, 70 cycles of 1 min at 95 °C, 1 min 50 °C, 1 min 74 °C with 5 min final elongation at 74 °C was performed. Different primers (Table 1) which amplify short fragments of the mitochondrial cytochrome *b* gene and the control region were used.

PCR products were either eluted from the 3% NuSieve agarose gel using Qiagen MinElute Gel Extraction Kit or used directly for cloning with pGEM®-T Vector System (Promega, Dübendorf, CH). Plasmids were purified with QIAprep Spin Miniprep Kit, the correct insert was monitored by PVU II restriction enzyme analysis and sequenced by Microsynth, Balgach, CH, with standard primers.

Sequences were deposited with Genbank under accession numbers GQ342248–50.

2.2.2. DNA extraction, amplification, cloning and sequencing, Copenhagen

DNA was extracted with Qiagen's DNeasy Blood & Tissue Kit (Valencia, California) according to the manufacturer's instructions, followed by purification with the QIAquick PCR Purification Kit (protocol slightly modified). Two replicates and two blanks were extracted.

PCR amplification was performed in 25 µl volumes, using 1× PCR buffer, 2 mM of $MgSO_4$, 1.6 mg/ml Bovine Serum Albumine (BSA), 0.4 µM of each primer, 1 µM of dNTPs, 5 units of High Fidelity Platinum Taq (Invitrogen) and 1 µl of DNA. Cycling conditions were: 94 °C for 2 min; 60 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s followed by 72 °C for 7 min. PCR products were then cloned using TOPO TA cloning kit (Invitrogen) for sequencing.

2.3. DNA sequence analysis

Sequences were aligned with BioEdit and compared to entries from published data. For lineage identification BLAST search and