Animal fibre use in the Keriya valley (Xinjiang, China) during the Bronze and Iron Ages: A proteomic approach

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Abstract

Textile technology strongly advanced with sedentism and pastoralism. During prehistory, many populations settled in central Eurasia, a place of extensive exchange and cultural contact. In the Taklamakan desert, the dry climate enabled good preservation of ancient textiles. The study presented here aimed to identify animal fibres from Bronze Age and Iron Age sites in the Keriya valley (Xinjiang, China) using proteomics. A large corpus of 109 keratin extracts obtained from raw fibres or textiles was analysed, enabling us to establish a corrected and improved list of peptide markers for the identification of species, not only among the family Bovidae, but also for camels and humans. In total, we were able to identify 97% of the sampled objects to the taxonomic level of tribe and 85% of caprines to the level of genus. The assemblage was dominated by hair of sheep (57.8%) followed by goat (16.5%), cattle (8.3%), camel (0.9%), human (0.9%) and non-differentiated Caprinae (sheep or goat) (12.8%). The study showed a continuity between the two sites in this respect. It revealed a choice of raw material linked to the function of the textile, with most woven textiles being made from sheep's wool and most pelt being obtained from goat. Comparison with the bone assemblage of one of the sites provided insight into the herd management strategies. The results confirm the heuristic potential of the proteomic approach for the determination of archaeological fibres and for textiles studies in general. Data are available via ProteomeXchange with identifier PXD012189.

Keywords: Proteomics, keratin, textiles, animal fibres, Xinjiang, Iron Age, Bronze Age

1. Introduction

Textile technology played a central role in the development of past societies (Good, 2001; Hardy, 2007). The first archaeological evidence for pelts dates back to 90,000–80,000 years ago, whereas the first evidence for twisted fibres appears as clay impressions 27,000 years ago (Hardy, 2007). The invention of looping and weaving opened up many possibilities for the creation of textile products, from nets to clothing. Starting in the Holocene, domestication and agriculture enabled the increase of textile production by supplying animal or vegetal fibres (Bender Jørgensen et al., 2018; Rast-Eicher, 2005). Therefore, the study of textiles can provide much information about resources, manufacturing techniques, trade, and culture through time. In contrast to other materials, such as ceramics, bone, or metal, textiles are very sensitive to degradation and are rarely preserved in archaeological contexts. Preservation occurs in specific environments, such as deserts, bogs, or permafrost (Gleba, 2011; Good,

2001; Hardy, 2007; Strand et al., 2010).

The preserved fibres are generally studied from the point of view of technology (of weaving, dyes, etc.), but their specific origin (vegetal or animal) is rarely studied in depth. A taxonomic determination of the animal or plant species at the origin of the textile is often made, but the rarity of the remains does not allow questions related to either the production methods of these fibres (organisation of livestock or crops) or the cultural choices underlying the activities to be addressed. Such questions are addressed using information from animal bones and teeth (often more numerous) or plant remains (generally rarer) found in archaeological sites, but it is difficult to use these proxies to define in detail the types of textiles produced and their use. When there are many textiles on a site, we can increase the number of taxonomic identifications and directly acquire information on the animals, plants, herds, and fields that are at their origin.

Generally, fibre identification is undertaken by microscopy. The morphological observation of animal fibres by optical and scanning electron microscopy normally permits specialists to clearly separate animal families. To achieve this, they focus on different parameters, such as the colour and diameter of the fibre, the morphology of the scales, and the structure of the medulla (Houck, 2010; Thomas et al., 2012; Rast-Eicher, 2016). However, alteration of the fibre due to diagenesis can make these techniques inapplicable to archaeological remains. In addition, these techniques cannot always differentiate between certain related species, such as sheep and goat. More importantly, wool characteristics have changed through time, so the general morphology of ancient fibre may not correspond to that of modern wool from the same species (Rast-Eicher, 2016). For any one of these reason, the differentiation of archaeological fibres can be very challenging.

Palaeoproteomics has recently emerged as a powerful method to characterise the fraction of proteins preserved over time, providing a molecular signature related to the nature and taxonomy of archaeological samples (Cappellini et al., 2014; Hendy et al., 2018). This approach involves the hydrolysis of protein extracts, followed by analysis by mass spectrometry. It can generate diagnostic peptides that help discriminate among animal species, through their *m/z* value and fragmentation pattern related to their amino acid sequence (Cleland and Schroeter, 2018). The main proteins present in hair and fur are keratins. They constitute a large family of proteins forming heteropolymeric filaments (Plowman, 2007; Schweizer et al., 2006) incrusted into a matrix of keratin-associated proteins (KAPs) that stabilise the filament structure through extensive disulphide bonding (Gong et al., 2012; Marshall et al., 1991). Keratins are classified into two families: type I keratins (40–55 kDa), which are acidic, and type II keratins (55–70 kDa), which are basic or neutral. The revised nomenclature of mammalian keratins names type I keratins from K31 to K40 and type II keratins from K81 to K87 (Schweizer et al., 2006). Keratin K33 generally exists as two isoforms, named K33a and K33b. However, the annotation of keratins in databases is often heterogeneous or related to previous classifications. Thus, wool microfibrillar keratins are often named based on the old nomenclature, which relies on electrophoresis separation: components 8C-1, 8C-2, 8A and 8B for type I and components 5, 7A, 7B, and 7C for type II (Plowman, 2003; Miao et al., 2018). A correspondence between the nomenclatures is provided in the Supplementary material Table S1. Proteomic analysis of hair, fur, and textiles was first conducted on modern samples to assess the quality of textile products (Hollemeyer et al., 2002; Hollemeyer and Heinzle, 2007; Clerens et al., 2010; Plowman et al., 2012; Paolella et al., 2013; Vineis et al., 2014; Li et al., 2016; Plowman et al., 2018). Analysis of ancient samples in order to characterise remains of Neolithic human hair and clothes (Hollemeyer et al., 2008, 2012; Fresnais et al., 2017) and for other, archaeozoological applications (Solazzo, 2017; Solazzo et al., 2017, 2014, 2013, 2011) has permitted researchers to refine taxonomic attributions, in some cases increasing knowledge of the fabrication of ancient textiles (Fresnais et al., 2017; Solazzo et al., 2011). The high sequence similarity between keratins of the same type and between proteins of closely related species makes keratin identification difficult (Plowman, 2007). Nevertheless, diagnostic

peptides have been proposed to differentiate between related animal species, such as a fragment of K33, which differs by only one amino acid between goat and sheep (Solazzo et al., 2014, 2013). These markers are not always detected in proteomic studies of archaeological fibres, which leaves many unidentified samples. New markers and identification keys are essential for the improvement of archaeological fibre identification.

Here we present the results of a proteomic study aimed at identifying ancient fibres from the Xinjiang region at the species level. Xinjiang is an arid region of northwestern China particularly rich in well-preserved organic remains, including textiles and furs (Wang, B., 1999; Good, 1998; Keller et al., 2001; Liu et al., 2011; Mallory and Mair, 2000; Zhao, 2004, 2002, 2001, 1999).

Located in the eastern part of Central Asia, Xinjiang was a nodal point within the large Eurasian sphere of contact and interaction known as the Silk Roads, and within their forerunners. Many sites from the Bronze and Iron Age have been discovered so far (Chen and Hiebert, 1995; Jia et al., 2007, 2010), the oldest dated around 2500-2000 BCE. Isotopic analyses on vegetal remains made it possible to enlighten us on human diet, subsistence strategies, and cross-cultural contacts from the Late Bronze Age to early historic times, both in northern and in southern Xinjiang (Wang, W. et al., 2017;. Wang, T. et al., 2017). However, the textile remains found to date come mainly from eastern Xinjiang (the Wupu cemetery, near Hami); from the Lopnor region (including the Xiaohe and Gumugou cemeteries) (Li, 2007; Liang et al., 2012; Qiu et al., 2014; Qu et al., 2017; Wang, 2014; Yang et al., 2014); and from the Tarim Basin, in both the Turfan region (including the Yanghai and Subeixi cemeteries) and the southern part of the Taklamakan desert (notably the Keriya Valley and the Chärchan area) (Wang, B., 1999; Good, 1998; Wang et al., 2016).

We focused on two sites in the Tarim basin (southern Xinjiang), where large corpuses of textiles and furs have been unearthed: Djoumboulak Koum (also called Yuansha gucheng), which is an Iron Age fortified settlement, and the Northern Cemetery, which dates from the Bronze Age (Figure 1) (Debaine-Francfort and Idriss, 2001; Debaine-Francfort, 2013). Discovered by the Sino-French Archaeological Mission in Xinjiang, both are located in the now-dry protohistoric delta of the Keriya River, which crosses the Taklamakan desert from south to north. Both have yielded large corpuses of textiles and furs that are being studied in a multidisciplinary way. Our research not only aims to discriminate between native and exogenous fauna, it also focusses on the methods used to process the fibres and initiates wider research on the evolution of fleeces and textiles from the Bronze Age to the Iron Age and a comparison with contemporaneous fleeces and textiles found in Europe. This information is cross-referenced with that provided by archaeozoology to supplement our knowledge on the history of breeding, transhumance practices, and hunting. This work, which has already revealed the complexity and diversity of weaving techniques and dying practices, suggests that the textile industry had a major importance in the ancient societies of the Keriya valley (and beyond) (Cardon et al., 2013; Debaine-Francfort and Idriss, 2001). The often poor state of preservation of the fibres, which is sometimes insufficient to enable their taxonomic determination using microscopy, has led us to adopt non-zooarchaeological analytical methods to overcome this taphonomic handicap and to thus address unanswered questions about the raw materials used in the manufacture of these textiles.

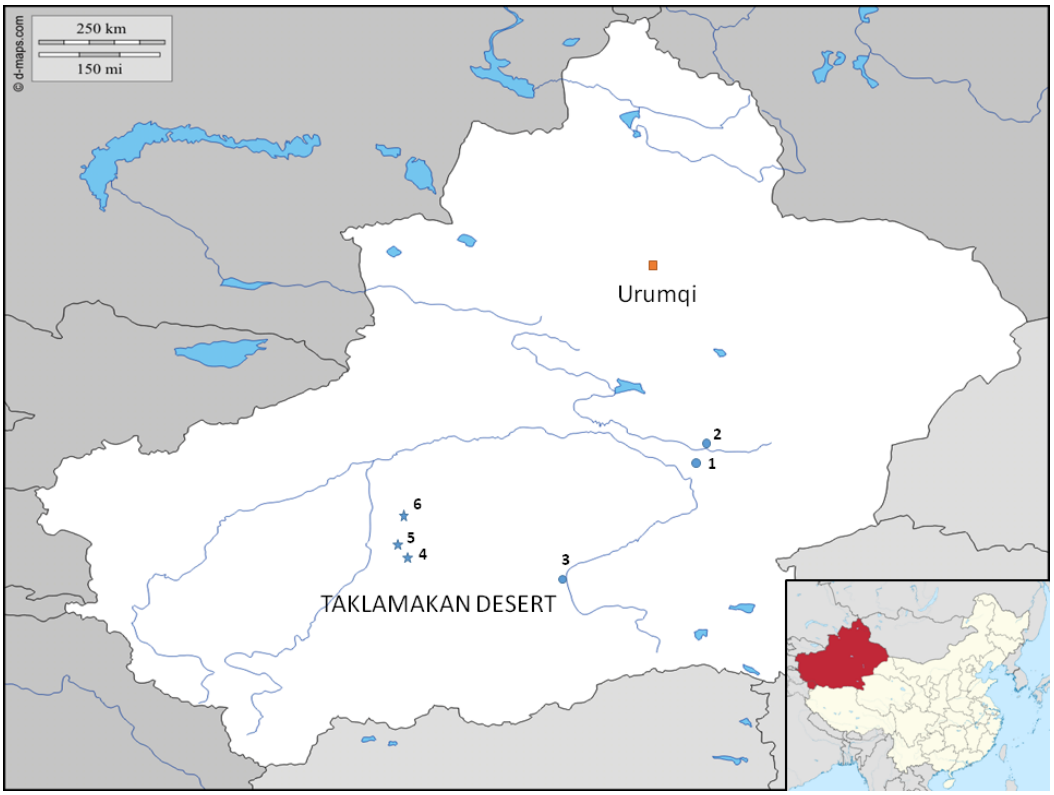

Figure 1. Map showing some of the archaeological sites in the Xinjiang Uyghur autonomous region –1: Xiaohe, 2: Gumugou, 3: Chärchan, 4: Karadong, 5: Djoumboulak Koum (Yuanshagucheng), 6: Northern Cemetery.

In this article, we report on how a proteomics approach was used on raw fibres and textiles in order to identify the origin of animal fibres from these two archaeological sites. The taxonomic specificity of keratins was evaluated on a large corpus of samples, with the aim to define new peptide markers for species differentiation. The fibre identification was used to figure out the relation between domestic animal species and type of textile produced and to document the diachronic development of textile use in Xinjiang from the Bronze Age to the Iron Age.

2. Material & Methods

2.1. Samples

The samples come from Djoumboulak Koum and the Northern Cemetery site. The Sino-French archaeological mission in Xinjiang found both sites, located in the protohistoric delta of the Keriya River, in the Taklamakan desert.

Djoumboulak Koum is an Iron Age settlement and the Northern Cemetery is a cemetery dating from the Bronze Age. Djoumboulak Koum (mid-1st millenium BCE), was found in 1994. The area enclosed by the walls covers 10 hectares and is surrounded by various cemeteries. The sedentary population practiced agriculture, producing millet, wheat, and barley thanks to an elaborate system of irrigation (Debaine-Francfort et al., 2010; Debaine-Francfort and Idriss, 2001). The Northern Cemetery dates from c. 1950–c. 1400 BCE (Debaine-Francfort, 2013, 2019; Cardon et al., 2013; Mair, 2014; Aoudia et al., 2016; Qu et al., 2017). Discovered in 2008, and unfortunately severely looted, it shows astonishing similarities with the famous and much better preserved cemetery of Xiaohe, located 600 km to the east (Bergman, 1939; Xinjiang wenwu kaogu yanjiusuo, 2003, 2007; Li, W., 2007; Mair, 2006). These two contemporary funerary sites belong to the same culture, but since the Northern

Cemetery is connected with remains of dwellings that are without equivalent, it is more than just the "twin" of Xiaohe: these remains (the earliest of which date back to about 2500 cal. BC) represent the earliest known human settlement in the Tarim basin.

The Northern Cemetery initially contained many graves divided into at least two levels, similar to those of Xiaohe, which has yielded 167 tombs in five levels. The structure of the tombs is regular. The deceased, wrapped in a shroud, were placed in a coffin with a lid covered with the pelts of sheep or cattle that had been freshly slaughtered. Naked, they wore only felt caps of various types, leather shoes, and a loincloth (for women) or a fringed belt (for men), a costume very different from the pants, skirts, and tunics worn by the Iron Age populations buried at Djoumboulak Koum.

A set of 109 sampled objects from the two Keriya valley sites was studied (Figure 2, Supplementary Material Table S2). The samples were grouped into the following categories: The category textile refers to all fibres transformed by weaving, twisting, and similar techniques (e.g., felt, clothing, shrouds). Within this category, we separated out the shoes. The category raw fibres refers to all furs, pelts, and other unprocessed raw fibres. A total of 93 sampled objects come from the Northern Cemetery (35 raw fibres, 56 textiles, 2 shoes) and 16 from Djoumboulak Koum (10 raw fibres, 5 textiles, 1 shoe).

In addition, we collected modern reference fibres in 2012, in the Arkhangai aimag, near Oziit, Mongolia, namely, one sample from goat and one from sheep.

Figure 2. Sampled objects from the Bronze Age site of the Northern Cemetery. Clockwise from top left: a dark cord, a woven textile, a pelt, and a shoe.

2.2. Sample preparation

First, the samples were washed with milliQ water + 2% DeconTM Decon 90 under stirring, until clear. Then they were washed 2 × 15 min in a MeOH/CHCl3 solution (1:2) under stirring and 15 min in milliQ water under sonication. During this protocol, textiles tend to disintegrate, contrary to unprocessed hair.

The sample preparation protocol was adapted from Paollela et al. (2013). Three mL of 25 mM Tris-HCl, 2.4 M thiourea, 5 M urea (pH 8.5), and dithiothreitol (DTT, 100 mM) were added to 20 mg of washed sample. The solution was left three days under stirring at 50°C, then filtered on a Buchner

funnel. A volume of 100 µL of protein extract was reduced and alkylated: (1) Reduction was performed by addition of 100 µL of NH_4HCO_3 (100 mM) and 5 µL of DTT (200 mM in the NH_4HCO_3 solution) followed by 1 h incubation at room temperature; (2) alkylation was performed by addition of 4 µL of a 1 M solution of iodoacetamide, followed by 1 h of incubation in the dark at room temperature. To this new solution, we added 20 µL of the 200 mM DTT solution, after which it was incubated for 1 h. Finally, 1 µL of trypsin gold® (1 g/L in 50 mM acetic acid) and 770 µL of milliQ water were added and the 1 mL solution was incubated at 37°C for 16 h. This step was followed by solid phase extraction on a C8 cartridge (Sep-Pak C8 Plus, 400 mg sorbent, 37–55 µm particle size, Waters). Conditioning of the cartridge was done with 8 mL HPLC-grade acetonitrile (AcN) and 4 mL milliQ-water + 0.1% formic acid (FA). After loading 1 mL of sample, the cartridge was washed with 1 mL H2O + 0.1% FA and eluted with 2 mL H2O + 0.1% FA / AcN 20:80 (v/v). The samples were dried under vacuum, after which they were resuspended in 1.5 mL H2O + 0.1% FA / AcN 70:30 (v/v).

2.3. Analysis of the trypsic digests by mass spectrometry

The trypsic digests were analysed by ultra-high-performance liquid chromatography–mass spectrometry (UHPLC-MS) on an Ultimate 3000-RSLC system (Thermo Scientific) connected to an electrospray ionization–quadrupole–time of flight (ESI Q-TOF) instrument (Maxis II ETD, Bruker Daltonics). The separation was achieved using a RSLC Polar Advantage II Acclaim column (2.2 µm, 120 Å, 2.1 × 100 mm, Thermo Scientific) with the following gradient of mobile phase A (milliQ water + FA 0.1%) and B (LC-MS grade AcN + FA 0.08%), at 300 µL/min: linear increase from 5% B to 60% B for 12 min, linear increase to 100% B for 0.5 min, plateau at 100% B for 0.5 min, decrease to 5% B for 0.5 min, equilibration for 4 min (total run time 17.5 min). The MS detection was carried out in positive mode in the range *m/z* 100–2000. The source parameters were as follows: nebulizer gas 35 psi, dry gas 8 L/min, capillary voltage 3500 V, end plate offset 500 V, temperature 200 °C. Analyses were performed using collision-induced dissociation in data dependent auto-MS/MS mode, using the following set-up: preferred charge states: 1–4, unknown charge states excluded, cycle time 3 s, MS spectra rate: 2 Hz, MS/MS spectra rate: 2 Hz at 16.000 counts increasing to 6 Hz at 160.000 counts or above. MS/MS active exclusion was set after one spectrum unless intensity increased fivefold. Collision energy was automatically calculated from *m/z* and charge states. The MS instrument was calibrated at each run start using a sodium formate solution consisting of 10 mM sodium hydroxide in isopropanol / 0.2% formic acid (1:1 v/v). Four samples (P189, P226, P227, and P239) were analysed on our previous LC-MS instrumentation, a U3000 micro-HPLC system connected to a QStar Pulsar ESI-Q-TOF-MS instrument, using the conditions described by Cersoy et al. (2019).

2.4. Data analysis

The LC-MS/MS data were converted to mgf files using DataAnalysis software (Bruker Daltonics). A database search was carried out against the SwissProt and NCBI databases simultaneously with Mascot (MatrixScience.com, using the online version or an in-house licence). We restricted the search to Mammalia (mammals) and allowed 2 missed cleavages. We considered carbamidomethylation of cysteine as a fixed modification and deamidation of glutamine and asparagine as well as oxidation of methionine as variable modifications. Charge states 1+, 2+, and 3+ were considered with 10 ppm tolerance for precursor ions and 0.05 Da tolerance for MS/MS fragments (25 ppm for precursor ions and 0.5 Da tolerance for MS/MS fragments for the 4 samples run on the QStar MS instrument). The statistic decoy was applied to minimise false-positive match. In addition, the samples with highest

scores were treated using the error-tolerant mode in order to identify potential additional modifications. A new search was then performed on the samples with ambiguous or failed identification, considering lysine carbamylation as variable modification or error-tolerant mode, against a restrained database constituted of the keratin sequences present in NCBI (50,422 sequences). Mascot results were inspected manually in the "protein family summary" mode in order to determine the most confident identification, paying attention to the section "subsets and intersections," which sometimes contained the best protein hits. The mass spectrometry proteomics data and Mascot results have been deposited in the ProteomeXchange Consortium via the PRIDE (Vizcaíno et al., 2016) partner repository with the dataset identifiers PXD012189 and 10.6019/PXD012189.

To be able to distinguish among animal species, a list of peptide markers was constituted based on the literature (Izuchi et al., 2013; Paolella et al., 2013; Solazzo et al., 2014, 2013, 2011) and the Mascot searches against the NCBI and Swissprot databases. In particular, unique peptides assigned by Mascot were assessed for their taxonomic potential. The specificity of the markers was checked by protein alignments generated with Muscle (https://www.ebi.ac.uk/Tools/msa/muscle) and Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The correspondence analysis was performed on the freely available R environment version 3.4.3 (www.r-project.org), using the package ade4 (Dray and Dufour, 2007).

3. Results

Protein inference is the process used to assemble identified peptides into a list of proteins that are believed to be present in a sample (He et al., 2016). This process is challenging in the case of keratins, given their high intra- and inter-specific sequence similarities. First, a systematic analysis of the best protein hits obtained for each sample was carried out (Supplementary material Table S3). Although a specific peptide can scarcely be assigned unambiguously to a specific keratin protein, this step permitted us to delineate the most confident taxonomic identifications as well as the most represented keratins. Type I keratins provided by far the highest scores, followed by type II keratins and, sometimes, KAPs. This step proposed sheep, goat, cattle, and, to a much lesser extent, camel and human as the best identifications. While identification of camel (1 sample) was straightforward, identification of Bovidae was sometimes ambiguous, due to the difficulty of distinguishing among the closely related sequences of goat, sheep, and cattle and to their high similarity with the sequences of certain Cervidae (deer family). Identification of human (2 samples) was also to some extent ambiguous, because it could indicate sample contamination and because there are high sequence similarities within primate keratins. The best protein matches were keratins K33b and K31 for sheep and cattle and K33a and K31 for goat (Supplementary material Table S4).

Then we searched for peptide markers permitting distinction among closely related species. These markers are listed in Table 1. Their distribution in the 109 archaeological and 2 reference samples is reported in Supplementary material Table S5, and their specificity is shown in Supplementary material Table S6. Alignment of representative sequences of type I keratins (Supplementary material Figure S1) showed that the C-terminal region is the most informative regarding species identification. The peptide at position 307-329 (given for type I K33B from *Ovis aries*, NP_001185999.1), already reported in the literature (Clerens et al., 2010; Solazzo et al., 2013), appears to be a good marker, presenting sequences specific to sheep (YSCQLSQVQSLIVNVESQLAEIR), goat (YSCQLNQVQSLIVNVESQLAEIR), undetermined caprine (YSCQLNQVQSLISNVESQLAEIR), human (primate, YSSQLSQVQRLITNVESQLAEIR), and camel (YGSQLSQVQGLITNVEHQLAEIR). In addition, we observed another informative peptide from keratin K31 or K33, with sequences specific to goat (CGPCSSYVR), non-goat Bovidae (sheep, cattle, deer), caprine, cattle, camel, and primate. Only the peptides specific to goat, non-goat Bovidae, and

289 camel were detected in our samples. The corresponding MS/MS spectra are shown ion Figure 3.
290 Although short (9 amino acids) and located at the C-terminus of the keratin proteins, these peptides
291 were detected in many samples (15 out of 17 goat samples), sometimes providing the only specific
292 clue to a goat taxonomic identification. A new non-cattle marker from keratin K31,
293 GDLERQNQEYQVLLDVR, was also identified (Supplementary material Figure S2A). This sequence
294 is present in various mammals but not in cattle species. It is derived from peptide
295 QNQEYQVLLDVR, reported in wool keratin (Cardamone et al., 2009), but this latter peptide was not
296 validated in our study as an informative marker since it was detected in caprine as well as cattle
297 samples. Type I keratins were revealed to be ambiguous for identifying cattle because the identified
298 peptide markers were generally shared with *Cervus elaphus hippelaphus* (Central European red deer)
299 and *Odocoileus virginianus texanus* (Texas white-tailed deer). A new marker,
300 ILERSQQQEPLLCPNYQSYFR, was identified from keratin 31 (Supplementary material Figure
301 S2B), but it, too, is shared with deer. Type II keratins were revealed to be more informative in that
302 respect. Alignment of representative sequences of type II keratins (Supplementary material Figure S3)
303 permitted us to identify new cattle peptide markers in the N-terminal and central region of the proteins,
304 such as AGYCSR, GLNMDNIVAEIK, and GLNMDNIVAEIKAQYDDIASR from keratin K83 and
305 ALPAFSCVSACGPRPGR from keratin K86 (Supplementary material Figure S4). In addition, the C-
306 terminal region constituted an informative region for differentiating between the other species. In
307 particular, region 448-487 (position relative to keratin K85 from *Ovis aries*, K2M3_SHEEP:
308 QIASGPVATGGSITVLAPDSCQPR) contains markers specific to caprine, goat, sheep, camelid, and
309 primate. While type I keratin markers identified for humans were common in certain non-human
310 primates, type II keratins provided one marker (VSSVPSNSNVVVGTTNACAPSAR) specific to
311 *Homo sapiens*. Finally, KAPs, although detected in the best-preserved samples only, provided
312 additional peptide markers. Most of the samples displayed non-discriminant KAPs peptides, such as
313 (R)FWPFALY, detected in 72 samples identified as caprine or cattle. However, we identified markers
314 from KAPs from cattle (SSCCQPCCLPIR), non-cattle Bovidae (SLCGSGYGYGSR), and camel
315 (TSTLSRPCQTTYSGSLGFGSR). These markers are shown on the alignment of representative KAPs
316 in Figure S5.

317 **Table 1.** List of peptide markers used to distinguish among species.

Attribution	Sequence	Best match keratin	Number of observations	Mean score ± sd	Reference
	Type I keratin markers				
sheep	YSCQLSQVQSLIVNVESQLAEIR	K33b	62	92.0 ± 14.6	(Clerens et al., 2010; Solazzo et al., 2013)
goat	YSCQLNQVQSLIVNVESQLAEIR	K31,	13	99.7 ± 13.8	(Clerens et al., 2010; Solazzo et al., 2013)
non-caprine (cattle, deer)	YSCQLAQVQGLIGNVESQLAEIR	K31,	9	127.7 ± 12.8	(Clerens et al., 2010; Solazzo et al., 2013)
caprine (sheep, goat)	YSCQLNQVQSLISNVESQLAEIR	K31	45	78.1 ± 24.1	(Clerens et al., 2010; Solazzo et al., 2013)
cattle, deer	ILERSQQQEPLLCPNYQSYFR	K31	4	28.5 ± 4.2	This study
non-cattle (various mammals)	GDLERQNQEYQVLLDVR	K31	81	33.2 ± 11.0	This study
non-cattle (various mammals)	ARLESEINTYR	K31, K33	90	39.3 ± 13.5	(Clerens et al., 2010; Solazzo et al., 2013)

non-cattle (various mammals)	LESEINTYR	K31, K33	97	70.9 ± 10.6	(Clerens et al., 2010; Solazzo et al., 2013)
goat	CGPCSSYVR	K31	15	41.2 ± 11.9	This study
non-goat (cattle, sheep, deer)	CGPCNSYVR	K31	33	31.8 ± 15.5	(Clerens et al., 2010; Solazzo et al., 2013)
camel	YGSQLSQVQGLITNVEHQLAEIR	K33	1	81	(Solazzo et al., 2013)
camel	CGPCNTFMR	K33a	1	32	This study
camelid	QTEELNKQVVSSSEQLQSNQAEIIELRR	K33a	1	37	This study
primate	YSSQLSQVQSLITNVESQLAEIR	K33a	2	99.5 ± 3.5	This study
primate	YSSQLSQVQR	K31	2	75.0 ± 11.3	This study
primate	LITNVESQLAEIR	K31	2	95.5 ± 9.2	This study
primate	DNAELENLIR	K31, K33a	2	60.0 ± 2.8	This study
	Type II keratin markers				
non-cattle (various mammals)	DLNMDCIVAEIKAQYDDIASR	K83	22	13.7 ± 10.0	(Clerens et al., 2010)
caprine (sheep, goat)	GGVACGGLTYSSTAGR	K85	64	60.6 ± 17.5	(Clerens et al., 2010; Solazzo et al., 2013)
non-caprine (cattle, deer)	GGVTCGGLTYSTTAGR	K85	9	78.0 ± 30.9	(Solazzo et al., 2013)
goat	(K)SDLEANAEALIQETDFLR(R)	K81	10	23.8 ± 12.5	This study
cattle and primate	(K)SDLEANVEALIQEIDFLR	K81	4	18.8 ± 6.2	(Solazzo et al., 2013)
cattle	AGYCSR	K83	2	16.0 ± 14.1	This study
cattle	GLNMDNIVAEIKAQYDDIASR	K83	3	45.3 ± 23.6	This study
cattle	GLNMDNIVAEIK	K83	4	86.3 ± 6.2	(Solazzo et al., 2013)
cattle	ALPAFSCVSACGPRPGR	K86	5	32.2 ± 12.7	(Solazzo et al., 2013)
goat, horse, deer	AFSCVSACGPRPSR	K81	17	15.7 ± 6.8	This study
sheep	QIASGPVATGGSITVLAPDSCQPR	K85	26	15.6 ± 14.2	(Clerens et al., 2010; Solazzo et al., 2013)
non-sheep	QIASGPVATGGSITVLAPDSCVPCQPR	K85	6	22.2 ± 16.0	(Solazzo et al., 2013)
camelid	DLNLDCIVAEIKEQYDDIAR	K81	1	54	This study
primate	GGVVCGDLCASTTAPVVSTR	K86	1	54	This study
human	VSSVPSNSNVVVGTTNACAPSAR	K86	1	39	This study
	KAPs markers				
caprine (sheep, goat) and cattle	(R)FWPFALY	-	72	15.1 ± 1.8	(Clerens et al., 2010)
cattle	SSCCQPCCLPIR	-	1	26	This study
non-cattle (caprine, deer, antelope)	SLCGSGYGYGSR	-	3	59.3 ± 11.2	(Clerens et al., 2010; Solazzo et al., 2013)
camel	TSTLSRPCQTTYSGSLGFGSR	-	1	39	This study

One important step to validate the peptide markers was to assess their presence or absence in each sample (Supplementary material Table S5). Indeed, database incompleteness and intra-specific sequence variability may alter their specificity. For example, sequence VLQAHISDTSVIVKMDNNR appeared to be specific to the species *Bubalus bubalis* (water buffalo) based on our database search. However, this peptide was detected not only in samples assigned to cattle, as one might expect, but also in those assigned to goat and sheep. In addition, this peptide was generally proposed with a low score (< 10) and high expectation value (~ 1000), indicative of a high chance of it being a random match. This sequence was therefore discarded from the list of peptide markers. Another example of a discarded sequence is LLEGQEQR, specific to sheep based on our database search but detected in our study in samples identified as goat, cattle, camel, and human. The specificity of Bovidae markers was

also revised. For example, sequence DLNMDCIVAEIKAQYDDIASR, assigned to diverse mammals including sheep, but not to cattle or goat, in the existing databases, was detected in samples identified in our study as both sheep and goat, and thus was proposed as a "non-cattle" marker. The high number of samples used in this study proved to be very efficient to test and establish a list of validated specific markers (Table 1). Note that certain of the marker peptides contain missed cleavages, as already reported (Solazzo, 2017). This may relate to protein modifications that compromise enzymatic digestion.

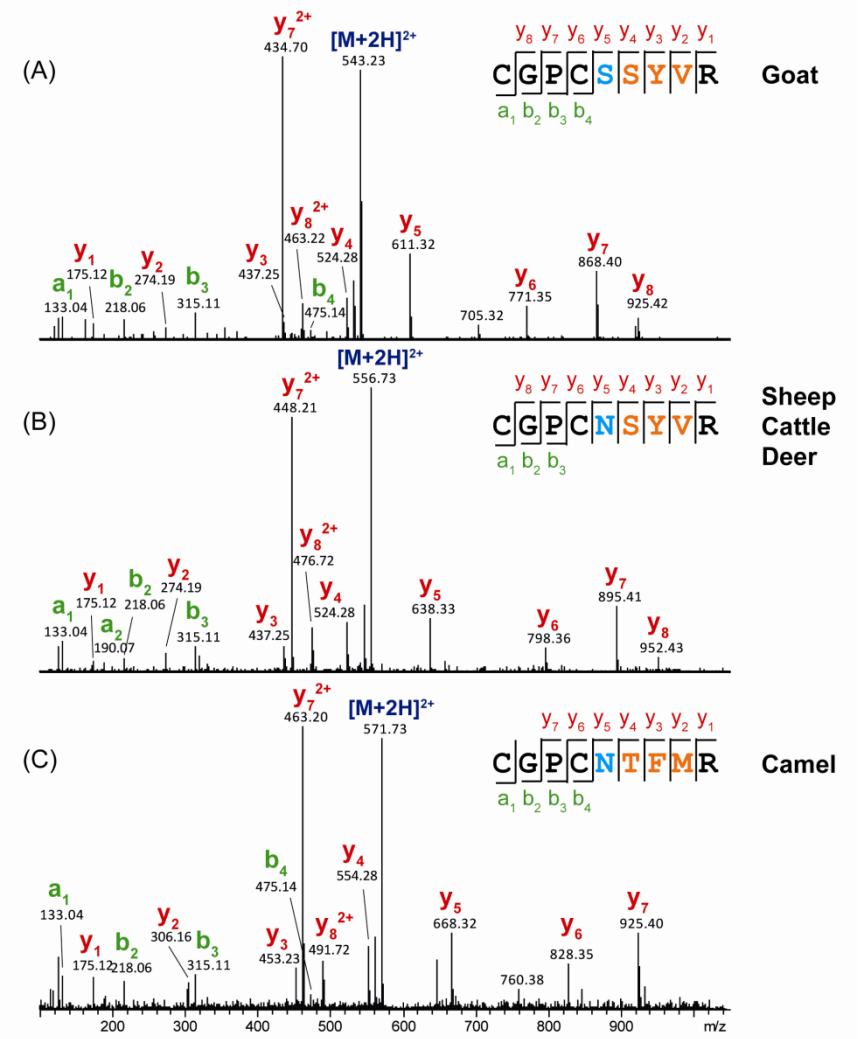

Figure 3. MS/MS spectra of the C-terminal type I keratin markers. (A) [M+2H]2+ species of peptide CGPCSSYVR, specific to goat (*m/z* 543.23, retention time 3.2 min, collision energy 22.6 eV), (B) [M+2H]2+ species of peptide CGPCNSYVR, specific to sheep, cattle, and deer (*m/z* 556.73, retention time 3.1 min, collision energy 23.4 eV), (C) [M+2H]2+ species of peptide CGPCNTFMR (*m/z* 571.73, retention time 3.9 min, collision energy 24.3 eV). Cysteine residues are carbamidomethylated.

These peptide markers permitted us to clearly distinguish among goat, sheep, and cattle samples, as illustrated in a correspondence analysis (Figure 4). Out of the 109 samples, 64 were identified as sheep, 17 as goat, 14 as undetermined caprine, and 9 as cattle based on Mascot and peptide marker searches (Table 2). In addition, one Djoumboulak Koum sample was identified as camel and a dark twisted cord from the Northern Cemetery (Figure 2) was characterised as human hair. Note that caprine samples P237 and P263 revealed a similar profile. Only these two samples showed peptide ILERSQQQEPLVCPNY**A**SYFR assigned to the ambiguous sequence ILERSQQQEPLVCPNY**X**SYFR, which is specific to goat based on our database search. They were designated as caprine due to their ambiguous marker profile.

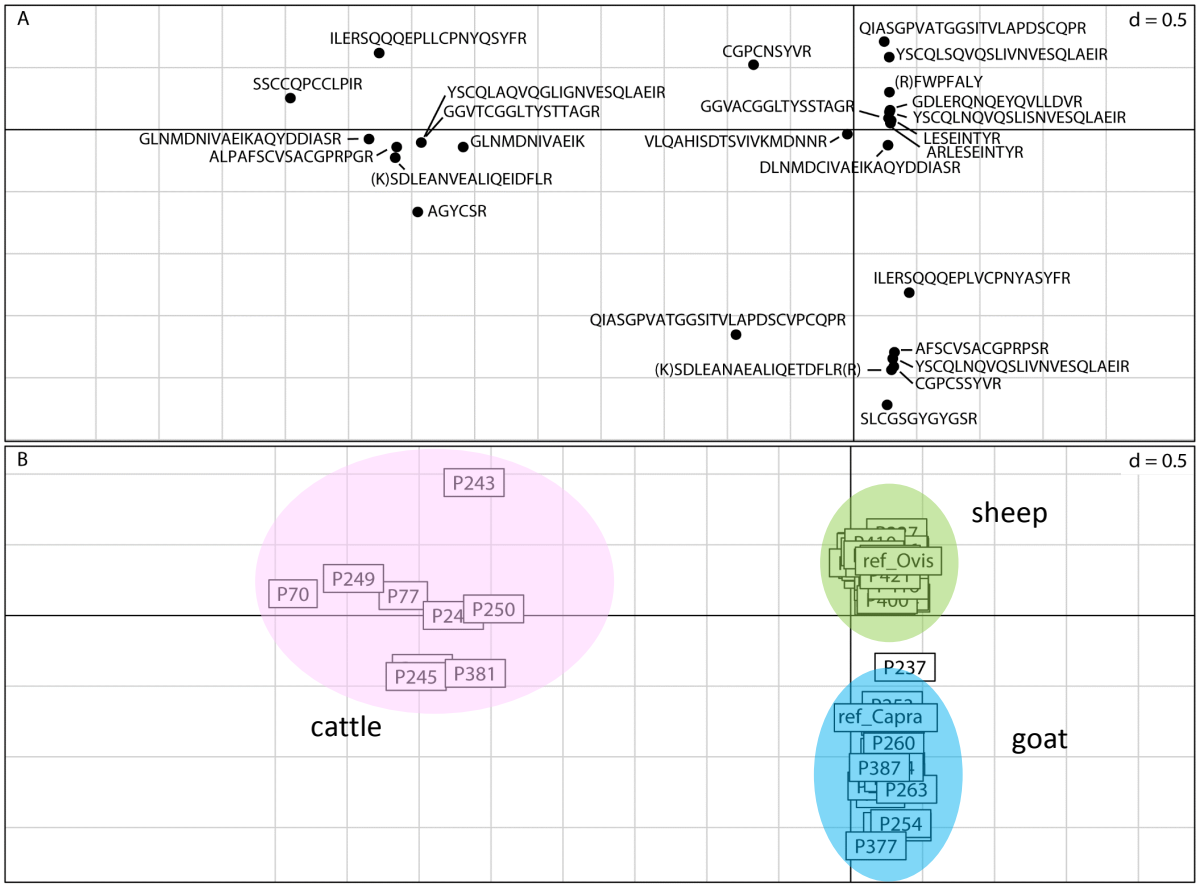

Figure 4. Correspondence analysis of Bovidae peptide markers–based identification, based on two axes. Scatter plots showing the peptide markers (A) and samples (B).

	Djoumboulak Koum	Northern Cemetery
Sheep	10	53
Goat	2	16
Caprine	2	12
Cattle	1	8
Camel	1	0
Unknown	0	3
Human	0	1
total	16	93

Table 2. Proteomic-based taxonomic identifications for the two sites.

The database search revealed important deamidation of asparagine and glutamine amino acids, as already reported (Solazzo et al., 2014) (Figure 5). Although the average percentage of deamidation sites of the archaeological sample (around 46.8%) was nearly double that of modern fibre (24.3% and 25.6% for goat and sheep, respectively), the maximum protein sequence coverage was generally similar. Indeed, the maximum percentage ranged between 14% and 62%, with a median of 47%, for the archaeological sampled objects and between 49% and 38% for the modern references used in this study.

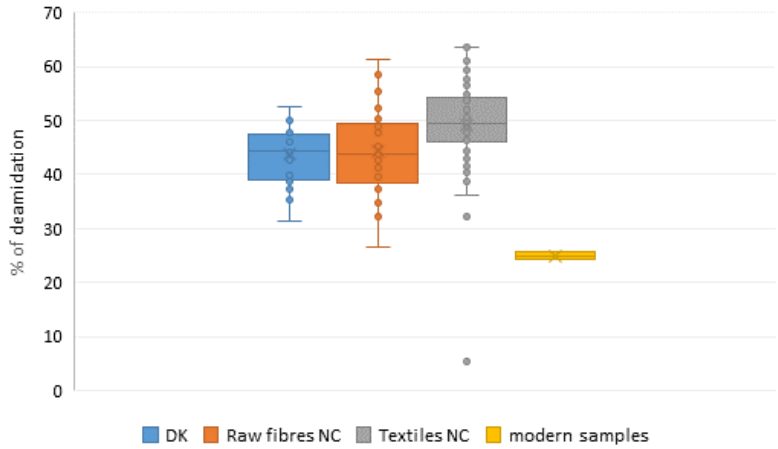

Figure 5. Percentage of deamidation sites for the modern and archaeological samples. DK = Djoumboulak Koum. NC = Northern Cemetery.

Finally, a database search in the error-tolerant mode revealed lysine carbamylation as a frequent modification, as already reported (Clerens et al., 2010; Solazzo et al., 2013). This modification may arise from sample treatment, which includes a long incubation under the presence of urea (Sun et al., 2014). However, if it increased the complexity of digests analysed, it did not affect protein/peptide identification since the peptides were generally present in both modified and unmodified forms.

4. Discussion

4.1. Keratins identification, variation and degradation

A high percentage of archaeozoological identifications was obtained due to good sample preservation, owing to the dry environment at the two sites. In total, 97% of the samples were identified to the tribe level or lower, and 85% of caprines were identified to the genus level. This result confirms that proteomics, when allied with detection of appropriate makers, is a powerful technique to differentiate ancient animal fibres. One limitation of this technique is that it requires complete and reliable sequence databases. Thus, a key challenge to extend the potential of palaeoproteomics is to enrich those protein sequence databases, particularly with ancient species and with amino acid variations resulting from single nucleotide polymorphism.

The best-hit proteins were generally the type I keratins K33 and K31. The representation of type II keratins and KAPs strongly differed depending on sample preservation. For keratin K33, sheep and cattle exhibited K33b as the most represented isoform, while goat exhibited K33a as the most represented isoform. In goat, keratin K33a is highly expressed in winter and constitutes a major component of cashmere (Seki et al., 2011), which explains its high representation in goat samples. In addition, single nucleotide polymorphisms have been reported in keratin K33a gene sequences among domestic and wild goats, resulting in amino acid differences among the individual goats (Seki et al., 2011) at positions 24 (S or P), 104 (Q or H), 202 (R or Q), and 271 (E or D). Position 104 corresponds to the peptide marker ILERSQQQEPLVCPNY**X**SYFR, where X denotes glutamine or histidine at position 104. In our study, we found two samples (P237 and P263) with an alanine at position 104, which suggests a new polymorphism in K33a keratin. Since these two samples did not exhibit any specific goat peptide markers, they were categorised as undetermined caprine. However, the presence of the peptide AFSCVSACGPRPSR in P263, which was detected in 16 out of 17 goat samples and could correspond to goat, horse, or deer based on the databases, strongly supports that they are goat

samples with a particular polymorphism in the gene encoding K33a. If the discrimination between goat and sheep was feasible in most of the cases, distinction among breeds appears challenging in the case of archaeological samples, since their distinction relies on different levels of KAPs or minor proteins (Li et al., 2018; Plowman et al., 2018).

The difference in the deamidation ratio between archaeological and modern samples was remarkably low (Figure 5). The hair of Andean mummies, also preserved under a dry climate, showed more than 85% deamidated sites according to Fresnais et al. (2017). A comparison of Djoumboulak Koum and the Northern Cemetery raw fibres and textiles shows that the difference in deamidation rates between those two categories is minor. The textiles seem slightly more degraded compared with the raw fibres – and they appeared more fragile during the wet pre-treatment steps – most probably due to alterations related to manufacturing and dying processes.

4.2. The use of animal fibres in textile manufacture in Xinjiang

Looking at the fibre identifications, it seems that not all animals present in this region were used for their fibres. A previous archaeozoological study showed that several bones of horse and dog were recovered at Djoumboulak Koum (Lepetz, 2001, Figure 8), but no fibre from these species was identified by our proteomics study. The number or choice of sampled objects may have influenced this result. We note that few horsehair hairnets were identified by the archaeologists, and it seems that horsehair was reserved for this specific use. An independence chi-square test was used to detect association between species distribution and collection site. It revealed that the two sites are independent and thus that their distribution cannot be considered identical (chi-square = 6.85, p-value = 0.33). However, both sites showed a high proportion of sheep (above 50%), and lower proportions of goat and cattle (Figure 6).

For Bronze Age cemeteries of the so-called Xiaohe culture, osteological data (Debaine-Francfort, 2019) show the frequent presence of bovine heads placed in the graves and attached to poles marking the presence of the graves. In addition, cattle hides were placed on the coffins. At Xiaohe, where the tombs are best preserved, painted bovine skulls with massive horns were also placed on the shroud (Debaine-Francfort, 2019). Cattle also appear in other forms, notably as cut ears and tendons, but also as cow dung, dairy products, cosmetic sticks, and glue (Qiu, Z. et al, 2014; Liang, Y. et al., 2012; Yang, Y., 2014; Rao et al., 2015). DNA study of the cattle indicates that they are *Bos taurus*, originally of West **A**sian origin (Cai, D. et al., 2014). This reveals the importance of this animal in Bronze Age cultures. But because these are very particular choices related to the ritual character of the site, it is not possible to use these osteological data to deduce in detail the use of animals in the economy of these societies and therefore to state, for example, that cattle were more numerous than sheep in the herds. Moreover, our study shows a clear preference for sheep wool in textile manufacturing at both sites. It can therefore be deduced that (1) there was a choice in the animals used in funeral practices; (2) sheep had an important place in artisanal activities; and (3) care must be taken to consider all these elements when addressing issues of breeding strategies and, more broadly, of the food economy.

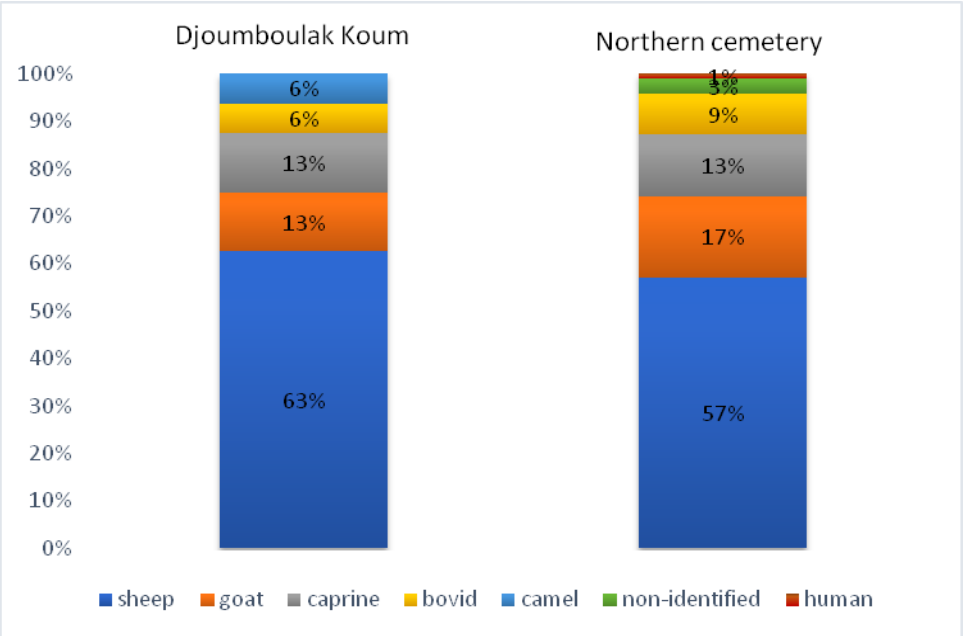

Figure 6. Taxonomic distribution of fibre origin at Djoumboulak Koum and the Northern Cemetery.

A human hair string was identified from the Northern Cemetery. Archaeologists have previously described braided or twisted human hair postiches from Djoumboulak Koum. These artefacts are often present in steppe traditions, and various examples have been found in Xinjiang and Altai graves (Debaine-Francfort and Idriss, 2001). Thus, this cord from the Northern Cemetery may be a postiche element that belongs to this tradition.

One hair sample collected in the F4 settlement unit at Djoumboulak Koum was identified as camel, whereas no camel fibre was identified in the 93 sampled objects from the Northern Cemetery. This difference in camel hair occurrence could result from the difference in social function between the two sites. But it comes from a refuse pit containing animal remains.

The large number of objects sampled at the Northern Cemetery (35 raw fibres and 58 textiles) permitted us to assess the preferential uses of each species (Figure 7). The species distribution revealed a strong difference between raw fibres and textiles. Indeed, 72% of the textile fibres were made from sheep's wool, whereas only 4% were made of goat hair. On the contrary, most of the goat fibres were found as raw material, mostly furs or pelts. The softness of sheep fibre compared with that of goat may explain this difference. Cattle fibres, like goat fibres, were mostly present as furs, but these were also used in the confection of shoes. This use most probably relied on their coarse, resistant, and thick skin. Shoelaces were made either from undetermined caprine or from sheep, revealing objects made from a mix of fibres. However, the number of shoe samples is too small to generalise about the use of cattle hides. The results from an ongoing study on a larger corpus from the Northern Cemetery, Djoumboulak Koum, and Xiaohe provide better insight into shoe manufacture, revealing that attention was paid to the choice of the raw fibre in the fabrication of textiles and the use of furs from the Keriya population.

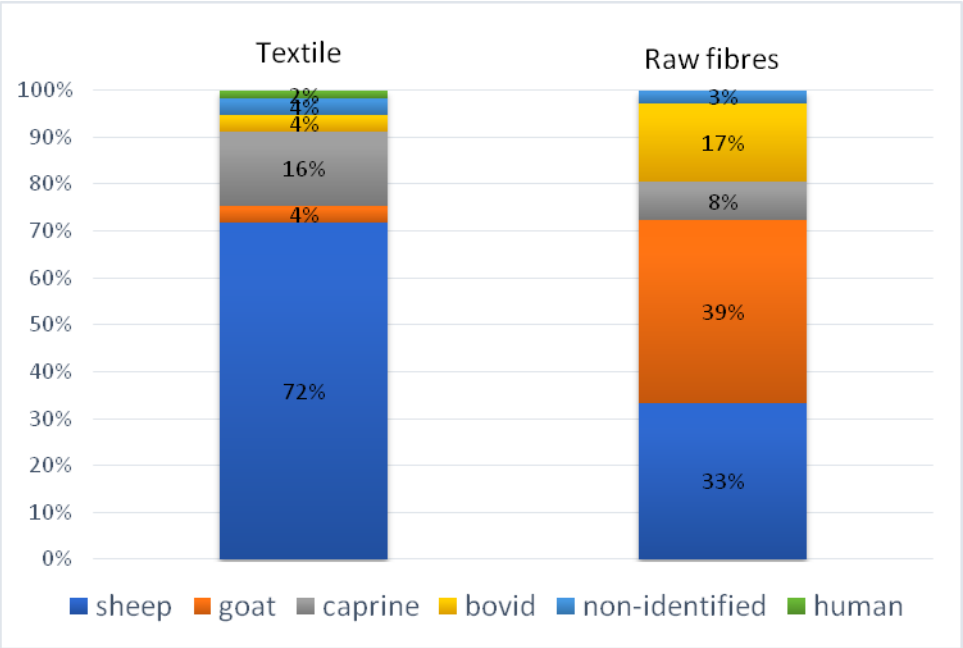

Figure 7. Species distribution by type of product found at the Northern Cemetery.

At Djoumboulak Koum, animal bones and teeth are mostly (99%) from domestic species (goat, sheep, dog, cattle, camel, horse). Only 1% was attributed to wild animals (Lepetz, 2001) (Figure 8), which is consistent with the fibre assemblage. However, the importance of each of these domestic species differs within the two sub-assemblages. Caprines represent only 31.5% of the bone remains, whereas they dominate (87.5%) the fibre assemblage. Cattle and camel are well represented (>25%) in the remains (NISP), while we identified only 1 item representing camel and 1 representing cattle from our 16 fibre samples. Finally, 14.3% of the bones come from equids, while no horse was found in our proteomics study. Regarding caprines, among the bones, the majority of those identified below the subfamily level are goat rather than sheep (76% vs. 24%), while we observe the opposite trend for the fibres (12.5% vs. 62.5%). These differences could be explained by the small number of fibre samples collected from Djoumboulak Koum (n=16), which may not be representative of the site as a whole. Alternatively, it might also indicate that animal fibre use was species-dependent and reflected the needs of the population. Sheep may well have represented a small minority of the farmed animals and were largely used for textile manufacture. The sheep herd would have been managed to provide a sufficient quantity of wool from live sheep. In contrast, obtaining goat pelts would have required the animals to be slaughtered.

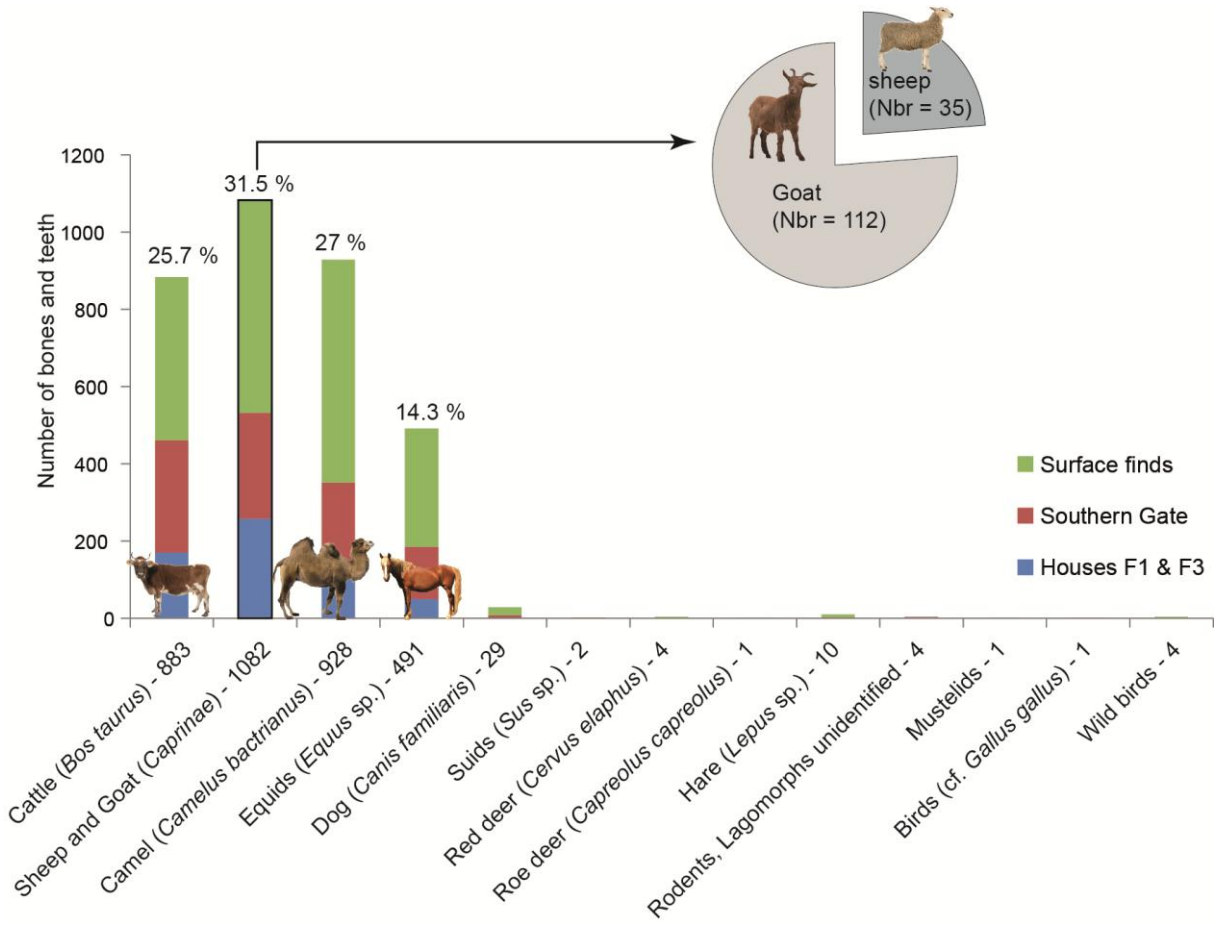

Figure 8. Animal taxa identified at Djoumboulak Koum. Nbr = Number. The remains of the South Gate and in houses F1 and F2 were found during the excavations. Manual collect is a collection from the surface of the ground during surveys (not excavation).

Many textiles remains have been found in Eurasia, in the form of fibres as well as ceramic impressions or carbonised fragments, for instance at Begash (Kazakhstan) (Doumani Dupuy et al., 2018; Doumani and Frachetti, 2012). Unfortunately, despite the numerous textiles found, their identification mostly remains at a very basic taxonomic level (i.e., animal vs. vegetal). When the issue of textiles is addressed, it is often from a technological point of view, through the analysis of weaving techniques or the identification of dyes (Keller et al., 2001; Kramell et al., 2014; Liu et al., 2011; Zhang et al., 2008). These studies only touch upon the link with pastoralism. It would be interesting to perform proteomics on the textiles from Chärchän (1000 BCE to 3rd century CE) and Karadong (3rd to early 4th century CE), where possible mohair goat wool textiles were described (Desrosiers and Debaine-Francfort, 2016; Good, 1998), to validate these results. If goat fibres were used, it would reveal an interesting difference in fibre use between different periods in the Taklamakan desert. The origin of mohair goat is still not fully understood (Ryder, 1993), and its presence at these sites could be evidence in favour of a central Asia origin. If we focus on the Keriya valley, the Karadong site revealed not only woollen but also silk and cotton textiles, as proof of the increase in exchange and the transfer of goods linked to the appearance of the Silk Road (Desrosiers and Debaine-Francfort, 2016). This change in textile fabrication habits certainly would have had an influence on herd management strategies.

5. Conclusion

The dry climate of the Tarim Basin and the Taklamakan desert is highly favourable to the preservation of animal fibre. The results of this study confirm the heuristic potential of the proteomic approach for the determination of archaeological fibres and for textiles studies in general, especially when the fibres are degraded and their distinctive features are no longer observable or interpretable by other methods. In combination with other analytical approaches, such as microscopy, as well as archaeozoological and palaeoenvironmental studies, it also holds more global potential for archaeology that goes far beyond a simple case study.

The good state of preservation and the use of proteomics enabled us to identify 97% of the remains to the tribe level or lower and 85% of the caprines to the genus level. This study permitted us to extend the list of peptide markers useful for species identification within Bovidae, but also for camel and human. The comparison between the two Keriya valley sites, Djoumboulak Koum and the Northern Cemetery, showed similarities in the fabrication and use of textiles, with a predominance of sheep and goat fibres, followed by cattle. Camel and human fibres were used only occasionally. During the Bronze Age, sheep were largely used for manufactured woollen textiles, whereas goats provided mostly furs and pelts. The few shoes analysed are made from cattle hide, while the shoelaces are made from sheep or undetermined caprine fibres. A comparison of the taxonomic composition of the fibre assemblage at Djoumboulak Koum and at the Northern Cemetery with the respective bone assemblage suggests the occurrence of specific herd management strategies according to the animal species, in order to provide enough raw material. Our analysis underlines the need to base argumentation on the widest possible range of data types if the question of the economics of these societies, and of their rites, is to be effectively addressed. It would be highly desirable to extend this approach to other sites that provided textiles, in order to further explore regional differences in the use of the animal fibres and the changes caused by the spread of cotton and silk in this region.

Acknowledgments

We thank the members of the Sino-French archaeological Mission in Xinjiang who provided the samples. All proteomics experiments were conducted in the bioorganic mass spectrometry platform at the National Natural History Museum in Paris. We are also thankfull for the critical comments of the three reviewers that greatly helped to improve this paper as well as S. Needs for English editing.

Funding: This work was supported by a grant from Agence Nationale de la Recherche under the LabEx ANR-10-LABX-0003-BCDiv, in the program “Investissements d’avenir” n\u0001 ANR-11-IDEX-0004-02.

Declarations of interest: none

References

Aoudia, L., Debaine-Francfort, C., Idriss, A., Hu, X., 2016. Quand la chaîne opératoire funéraire se dévoile : sépultures de l'âge du Bronze dans le désert du Taklamakan (Xinjiang, Chine du Nord-Ouest). BMSAP 28, 17–23. https://doi.org/10.1007/s13219-016-0155-8.

Bender Jørgensen, L., Sofaer, J., Stig Sørensen, M.L., 2018. Creativity in the Bronze Age: Understanding Innovation in Pottery, Textile, and Metalwork Production, first ed. Cambridge University Press. https://doi.org/10.1017/9781108344357.

Bergman, F., 1939. Archaeological Researches in Sinkiang, Reports from the Scientific Expedition to the North-Western Provinces of China under the Leadership of Dr. Sven Hedin, Stockholm, Bokförlags Aktiebolaget Thule, part II, 3/F, Cemetery 5 ('Ördek's necropolis'), 61-99.

Cai D., Sun Y., Tang, Z., Hu, S., Li, W., Zhao, X., Xiang, H., Zhou, H., 2014. The origins of Chinese domestic cattle as revealed by ancient DNA. J. Archaeol. Sci. 41, 423–434. https://dx.plos.org/10.1016/j.jas.2013.09.003.

Cappellini, E., Collins, M.J., Gilbert, M.T.P., 2014. Biochemistry. Unlocking ancient protein palimpsests. Science 343, 1320–1322. https://doi.org/10.1126/science.1249274.

Cardamone, J.M., Nuñez, A., Garcia, R.A., Aldema-Ramos, M., 2009. Characterizing Wool Keratin [WWW Document]. Adv. Mater. Sci. Eng. https://doi.org/10.1155/2009/147175.

Cardon, D., Debaine-Francfort, C., Abduressul, I., Anwar, K., Hu, X., 2013. Bronze Age textiles of the North Cemetery: Discoveries made by the Franco-Chinese Archaeological Mission in the Taklamakan desert, Xinjiang, China. Archaeol. Text. Rev. 68–85.

Cersoy, S., Zirah, S., Marie, A., Zazzo, A., 2019. Toward a versatile protocol for radiocarbon and proteomics analysis of ancient collagen. J. Archaeol. Sci. 101, 1–10. https://doi.org/10.1016/j.jas.2018.10.009.

Chen, K., Hiebert, F.T., 1995. The late prehistory of Xinjiang in relation to its neighbors. J. World Prehistory 9, 243–300. https://doi.org/10.1007/BF02221840.

Cleland, T.P., Schroeter, E.R., 2018. A comparison of common mass spectrometry approaches for paleoproteomics. J. Proteome Res. 17, 936–945. https://doi.org/10.1021/acs.jproteome.7b00703.

Clerens, S., Cornellison, C.D., Deb-Choudhury, S., Thomas, A., Plowman, J.E., Dyer, J.M., 2010. Developing the wool proteome. J. Proteomics 73, 1722–1731. https://doi.org/10.1016/j.jprot.2010.05.005.

Debaine-Francfort, C., 2019 (forthcoming). Sépultures d'Asie centrale : des corps, des représentations, des esprits. In Cahiers de la Villa 'Kérylos' n° 30, Des esprits et des dieux, Actes du XIXe colloque de la Villa Kérylos, 12–13 octobre 2018.

Debaine-Francfort, C., 2013. L'âge du Bronze dans le désert de Taklamakan: nouvelles découvertes dans les deltas fossiles de la rivière Keriya, Xinjiang, Chine. Comptes Rendus des Séances de l'Académie des Inscriptions et Belles-Lettres Jan-Mar, 303–331.

Debaine-Francfort, C., Idriss, A., 2001. Keriya, mémoires d'un fleuve: archéologie et civilisation des oasis de Taklamakan. Findakly, Suilly-la-Tour and Paris.

Debaine-Francfort, C., Debaine, F., Idriss, A., 2010. The Taklimakan Oases: An environmental evolution shown through geoarchaeology, in: Schneier-Madanes, G., Courel, M.-F. (Eds.), Water and Sustainability in Arid Regions. Springer Netherlands, Dordrecht, pp. 181–202.

Desrosiers, S., Debaine-Francfort, C., 2016. On textile fragments found at Karadong, a 3rd to early 4th century oasis in the Taklamakan Desert (Xinjiang, China). Textile Society of America Symposium Proceedings 958.

Doumani Dupuy, P.N., Spengler, R.N., Frachetti, M.D., 2018. Eurasian textiles: Case studies in exchange during the incipient and later Silk Road periods. Quat. Int., Early craft uses of plant and animal fiber 468, 228–239. https://doi.org/10.1016/j.quaint.2016.09.067.

Doumani, P.N., Frachetti, M.D., 2012. Bronze Age textile evidence in ceramic impressions: Weaving and pottery technology among mobile pastoralists of central Eurasia. Antiquity 86, 368–382. https://doi.org/10.1017/S0003598X00062827.

Dray, S., Dufour, A.-B., 2007. The ade4 package: Implementing the duality diagram for ecologists. J. Stat. Softw. 22, 1–20. https://doi.org/10.18637/jss.v022.i04.

609 Fresnais, M., Richardin, P., Sepúlveda, M., Leize-Wagner, E., Charrié-Duhaut, A., 2017. Omics for
610 precious rare biosamples: Characterization of ancient human hair by a proteomic approach.
611 OMICS J. Integr. Biol. 21. https://doi.org/10.1089/omi.2017.0067.
612 Gleba, M., 2011. Textiles studies: Sources and methods. Kubaba 2.
613 Gong, H., Zhou, H., McKenzie, G.W., Yu, Z., Clerens, S., Dyer, J.M., Plowman, J.E., Wright, M.W.,
614 Arora, R., Bawden, C.S., Chen, Y., Li, J., Hickford, J.G.H., 2012. An updated nomenclature
615 for keratin-associated proteins (KAPs). Int. J. Biol. Sci. 8, 258–264.
616 https://doi.org/10.7150/ijbs.3278.
617 Good, I., 2001. Archaeological textiles: A review of current research. Annu. Rev. Anthropol. 30, 209–
618 226.
619 Good, I., 1998. Bronze Age cloth and clothing of the Tarim Basin: The Chärchän evidence, in: Mair,
620 V.H. (Ed.), The Bronze Age and Early Iron Age Peoples of Eastern Central Asia. Institute for
621 the Study of Man, Inc., pp. 656–668.
622 Hardy, K., 2007. Where would we be without string?, in: Beugnier, V., Crombé, P. (Eds.), Plant
623 Processing from a Prehistoric and Ethnographic Perspective / Préhistoire et ethnographie du
624 travail des plantes, BAR International Series. Archaeopress, Oxford, pp. 9–22.
625 He, Z., Huang, T., Zhao, C., Teng, B., 2016. Protein inference, in: Mirzaei, H., Carrasco, M. (Eds.),
626 Modern Proteomics – Sample Preparation, Analysis and Practical Applications, Advances in
627 Experimental Medicine and Biology. Springer International Publishing.
628 Hendy, J., Welker, F., Demarchi, B., Speller, C., Warinner, C., Collins, M.J., 2018. A guide to ancient
629 protein studies. Nat. Ecol. Evol. 2, 791–799. https://doi.org/10.1038/s41559-018-0510-x.
630 Hollemeyer, K., Altmeyer, W., Heinzle, E., 2002. Identification and quantification of feathers, down,
631 and hair of avian and mammalian origin using matrix-assisted laser desorption/ionization
632 time-of-flight mass spectrometry. Anal. Chem. 74, 5960–5968.
633 https://doi.org/10.1021/ac020347f.
634 Hollemeyer, K., Altmeyer, W., Heinzle, E., Pitra, C., 2012. Matrix-assisted laser desorption/ionization
635 time-of-flight mass spectrometry combined with multidimensional scaling, binary hierarchical
636 cluster tree and selected diagnostic masses improves species identification of Neolithic keratin
637 sequences from furs of the Tyrolean Iceman Oetzi: Species identification: Neolithic keratin
638 sequences. Rapid Commun. Mass Spectrom. 26, 1735–1745.
639 https://doi.org/10.1002/rcm.6277.
640 Hollemeyer, K., Altmeyer, W., Heinzle, E., Pitra, C., 2008. Species identification of Oetzi's clothing
641 with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry based on
642 peptide pattern similarities of hair digests. Rapid Commun. Mass Spectrom. 22, 2751–2767.
643 https://doi.org/10.1002/rcm.3679.
644 Hollemeyer, K., Heinzle, E., 2007. Identification of furs of domestic dog, raccoon dog, rabbit and
645 domestic cat by hair analysis using MALDI-ToF mass spectrometry. Spectrosc. Eur. 19, 8–15.
646 Houck, M.M., 2010. Identification of textile fibers, Reprint. ed, Woodhead publishing series in
647 textiles. CRC Press, Boca Raton, FL.
648 Izuchi, Y., Tokuhara, M., Takashima, T., Kuramoto, K., 2013. Peptide profiling using matrix-assisted
649 laser desorption/ionization-time-of-flight mass spectrometry for identification of animal fibers.
650 Mass Spectrom. 2. https://doi.org/10.5702/massspectrometry.A0023.
651 Jia, P.W., Betts, A.V.G., 2010. A re-analysis of the Qiemu'erqieke (Shamirshak) cemeteries, Xinjiang,
652 China. J. Indo-Eur. Stud. 38, 275–317.
653 Jia, P.W., Betts, A.V.G., Wu, X., 2009. Prehistoric archaeology in the Zhunge'er Junggar) Basin,
654 Xinjiang, China. Eurasian Preh. 6 (1–2), 167–198.
655 Keller, D., Schorta, R., Abegg-Stiftung, 2001. Fabulous Creatures from the Desert Sands: Central
656 Asian Woolen Textiles from the Second Century BC to the Second Century AD. Abegg-
657 Stiftung.
658 Kramell, A., Li, X., Csuk, R., Wagner, M., Goslar, T., Tarasov, P.E., Kreusel, N., Kluge, R.,
659 Wunderlich, C.-H., 2014. Dyes of late Bronze Age textile clothes and accessories from the
660 Yanghai archaeological site, Turfan, China: Determination of the fibers, color analysis and
661 dating. Quat. Int., The Bridging Eurasia Research Initiative: Modes of mobility and
662 sustainability in the palaeoenvironmental and archaeological archives from Eurasia 348, 214–
663 223. https://doi.org/10.1016/j.quaint.2014.05.012.

Lepetz, S., 2001. Animaux et alimentation carnée à Djoumboulak koum, in: Kériya, mémoires d'un fleuve: archéologie et civilisation des oasis du Taklamakan. Findakly, Suilly-la-Tour and Paris, pp. 132–133.
Li, S., Zhang, Y., Wang, J., Yang, Y., Miao, C., Guo, Y., Zhang, Z., Cao, Q., Shui, W., 2016. Combining untargeted and targeted proteomic strategies for discrimination and quantification of cashmere fibers. PLOS ONE 11, e0147044. https://doi.org/10.1371/journal.pone.0147044.
Li, W., 2007. Xiaohe da faxian. Huaxia Dili Natl. Geogr. 8, 152–168.
Li, Y., Zhou, G., Zhang, R., Guo, J., Li, C., Martin, G., Chen, Y., Wang, X., 2018. Comparative proteomic analyses using iTRAQ-labeling provides insights into fiber diversity in sheep and goats. J. Proteomics 172, 82–88. https://doi.org/10.1016/j.jprot.2017.10.008.
Liang, Y., Yang, Y., Li, W., Li, X., Wang, C., 2012. Proteomic analysis of residues in grass basket excavated from Xiao-He graveyard. Sci. Conserv. Archaeol. 24, 81–85.
Liu, J., Guo, D., Zhou, Y., Wu, Z., Li, W., Zhao, F., Zheng, X., 2011. Identification of ancient textiles from Yingpan, Xinjiang, by multiple analytical techniques. J. Archaeol. Sci. 38, 1763–1770. https://doi.org/10.1016/j.jas.2011.03.017.
Mair, V.H., 2014. Northern Cemetery: Epigone or progenitor of Small River Cemetery No.5? In Mair, V.H., Hickman, J., Reconfiguring the Silk Road: New Research on East-West Exchange in Antiquity. The University of Pennsylvania Press, Philadelphia, 23–32.
Mair, V.H., 2006. The rediscovery and complete excavation of Ördek's Nekropolis. J. Indo-Eur. Stud. 34, 3-4, 2006, 273–318.
Mallory, J.P., Mair, V.H., 2000. The Tarim Mummies: Ancient China and the Mystery of the Earliest Peoples from the West, with 190 illustrations, 13 in color. Thames & Hudson, New York, NY.
Marshall, R.C., Orwin, D.F.G., Gillespie, J.M., 1991. Structure and biochemistry of mammalian hard keratin. Electron Microsc. Rev. 4, 47–83. https://doi.org/10.1016/0892-0354(91)90016-6.
Miao, C., Yang, Y., Li, S., Guo, Y., Shui, W., Cao, Q., 2018. Discrimination and quantification of homologous keratins from goat and sheep with dual protease digestion and PRM assays. J. Proteomics 186, 38–46. https://doi.org/10.1016/j.jprot.2018.07.010.
Paolella, S., Bencivenni, M., Lambertini, F., Prandi, B., Faccini, A., Tonetti, C., Vineis, C., Sforza, S., 2013. Identification and quantification of different species in animal fibres by LC/ESI-MS analysis of keratin-derived proteolytic peptides: Species identification in fibres by LC/ESI-MS. J. Mass Spectrom. 48, 919–926. https://doi.org/10.1002/jms.3222.
Plowman, J., Thomas, A., Perloiro, T., Clerens, S., de Almeida, A.M., 2018. Characterisation of white and black merino wools: A proteomics study. animal 1–7. https://doi.org/10.1017/S1751731118001647.
Plowman, J.E., 2007. The proteomics of keratin proteins. J. Chromatogr. B, Analytical Tools for Proteomics 849, 181–189. https://doi.org/10.1016/j.jchromb.2006.07.055.
Plowman, J.E., 2003. Proteomic database of wool components. J. Chromatogr. B, Proteomic Databases Part II 787, 63–76. https://doi.org/10.1016/S1570-0232(02)00211-8.
Plowman, J.E., Deb-Choudhury, S., Clerens, S., Thomas, A., Cornellison, C.D., Dyer, J.M., 2012. Unravelling the proteome of wool: Towards markers of wool quality traits. J. Proteomics 75, 4315–4324. https://doi.org/10.1016/j.jprot.2012.03.027.
Qiu, Z., Yang, Y., Shang, X., Li, W., Abuduresule, Y., Hu, X., Pan, Y., Ferguson, D.K., Hu, Y., Wang, C., Jiang, H., 2014. Paleo-environment and paleo-diet inferred from Early Bronze Age cow dung at Xiaohe Cemetery, Xinjiang, NW China. Quat. Int., Quaternary of East Asia and the Western Pacific: Part 2 349, 167–177. https://doi.org/10.1016/j.quaint.2014.03.029.
Qu, Y., Hu, Y., Rao, H., Abuduresule, I., Li, W., Hu, X., Jiang, H., Wang, C., Yang, Y., 2017. Diverse lifestyles and populations in the Xiaohe culture of the Lop Nur region, Xinjiang, China. Archaeol. Anthropol. Sci. 1–10. https://doi.org/10.1007/s12520-017-0520-7.
Rao, H. et al., 2015. Proteomic identification of adhesive on a bone sculpture-inlaid wooden artifact from the Xiaohe Cemetery, Xinjiang, China. J. Archaeol. Sci. 53, 2015, 148–155. https://doi.org/10.1016/j.jas.2014.10.010
Rast-Eicher, A., 2016. Fibres: Microscopy of archaeological textiles and furs, Archaeolingua. Archaeolingua, Budapest.

Rast-Eicher, A., 2005. Bast before wool: The first textiles, in: Bichler, P., Symposium Hallstatt-Textilien (Eds.), Hallstatt Textiles: Technical Analysis, Scientific Investigation and Experiment on Iron Age Textiles. Archaeopress, Oxford, pp. 117–131.

Ryder, M., 1993. The use of goat hair: An introductory historical review. Anthropozoologica 17, 37–46.

Schweizer, J., Bowden, P.E., Coulombe, P.A., Langbein, L., Lane, E.B., Magin, T.M., Maltais, L., Omary, M.B., Parry, D.A.D., Rogers, M.A., Wright, M.W., 2006. New consensus nomenclature for mammalian keratins. J. Cell Biol. 174, 169–174. https://doi.org/10.1083/jcb.200603161.

Seki, Y., Yokohama, M., Wada, K., Fujita, M., Kotani, M., Nagura, Y., Kanno, M., Nomura, K., Amano, T., Kikkawa, Y., 2011. Expression analysis of the type I keratin protein keratin 33A in goat coat hair. Anim. Sci. J. Nihon Chikusan Gakkaiho 82, 773–781. https://doi.org/10.1111/j.1740-0929.2011.00912.x.

Solazzo, C., 2017. Follow-up on the characterization of peptidic markers in hair and fur for the identification of common North American species. Rapid Commun. Mass Spectrom. RCM 31, 1375–1384. https://doi.org/10.1002/rcm.7923.

Solazzo, C., Fitzhugh, W., Kaplan, S., Potter, C., Dyer, J.M., 2017. Molecular markers in keratins from Mysticeti whales for species identification of baleen in museum and archaeological collections. PLOS ONE 12, e0183053. https://doi.org/10.1371/journal.pone.0183053.

Solazzo, C., Heald, S., Ballard, M.W., Ashford, D.A., DePriest, P.T., Koestler, R.J., Collins, M.J., 2011. Proteomics and Coast Salish blankets: A tale of shaggy dogs? Antiquity 85, 1418–1432. https://doi.org/10.1017/S0003598X00062141.

Solazzo, C., Rogers, P.W., Weber, L., Beaubien, H.F., Wilson, J., Collins, M., 2014. Species identification by peptide mass fingerprinting (PMF) in fibre products preserved by association with copper-alloy artefacts. J. Archaeol. Sci. 49, 524–535. https://doi.org/10.1016/j.jas.2014.06.009.

Solazzo, C., Wadsley, M., Dyer, J.M., Clerens, S., Collins, M.J., Plowman, J., 2013. Characterisation of novel α-keratin peptide markers for species identification in keratinous tissues using mass spectrometry: Novel α-keratin peptide markers for species identification. Rapid Commun. Mass Spectrom. 27, 2685–2698. https://doi.org/10.1002/rcm.6730.

Strand, E.A., Frei, K.M., Gleba, M., Mannering, U., Nosch, M.-L., Skals, I., 2010. Old Textiles – New Possibilities. Eur. J. Archaeol. 13, 149–173. https://doi.org/10.1177/1461957110365513.

Sun, S., Zhou, J.-Y., Yang, W., Zhang, H., 2014. Inhibition of protein carbamylation in urea solution using ammonium-containing buffers. Anal. Biochem. 446, 76–81. https://doi.org/10.1016/j.ab.2013.10.024.

Thomas, A., Harland, D.P., Clerens, S., Deb-Choudhury, S., Vernon, J.A., Krsinic, G.L., Walls, R.J., Cornellison, C.D., Plowman, J.E., Dyer, J.M., 2012. Interspecies comparison of morphology, ultrastructure, and proteome of mammalian keratin fibers of similar diameter. J. Agric. Food Chem. 60, 2434–2446. https://doi.org/10.1021/jf204811v.

Vineis, C., Tonetti, C., Paolella, S., Pozzo, P., Sforza, S., 2014. A UPLC/ESI–MS method for identifying wool, cashmere and yak fibres. Text. Res. J. 84, 953–958. https://doi.org/10.1177/0040517513512394.

Vizcaíno, J.A., Csordas, A., del-Toro, N., Dianes, J.A., Griss, J., Lavidas, I., Mayer, G., Perez-Riverol, Y., Reisinger, F., Ternent, T., Xu, Q.-W., Wang, R., Hermjakob, H., 2016. 2016 update of the PRIDE database and its related tools. Nucleic Acids Res. 44, D447–456. https://doi.org/10.1093/nar/gkv1145.

Wang, B., 2014. Gumumou. Urumqi, Xinjiang renmin chubanshe.

Wang, B., 1999. The ancient corpses of Xinjiang. Urumqi, Xinjiang renmin chubanshe.

Wang, B., Wang, M., Minawar, H., Lu, L., 2016. Textile Treasures of Zaghunluq. Abegg-Stiftung, Riggisberg and Cultural Relics Press, Beijing.

Wang, T., Wei, D., Chang, X., Yu, Z., Zhang, X., Wang, C., Hu, Y., Fuller, B.T., 2017. Tianshanbeilu and the Isotopic Millet Road: Reviewing the late Neolithic/Bronze Age radiation of human millet consumption from north China to Europe. Natl. Sci. Rev. nwx015. https://doi.org/10.1093/nsr/nwx015.

Wang, W., Wang, Y., An, C., Ruan, Q., Duan, F., Li, W., Dong, W., 2017. Human diet and subsistence strategies from the Late Bronze Age to historic times at Goukou, Xinjiang, NW China. The Holocene 0959683617735588. https://doi.org/10.1177/0959683617735588.

Xinjiang wenwu kaogu yanjiusuo, 2003. 2002 nian Xiaohe mudi kaogu diaocha yu fajue baogao (Investigation and excavation of the Xiaohe Cemetery in 2002), Xinjiang wenwu 2, 8–64.

Xinjiang wenwu kaogu yanjiusuo, 2007. Xinjiang Luobupo Xiaohe mudi 2003 nian fajue jianbao (A brief excavation report on Xiaohe graveyard located in Luobupo, Xinjiang Autonomous Region), Wenwu 10, 4–42.

Yang, R., Yang, Y., Li, W., Abuduresule, Y., Hu, X., Wang, C., Jiang, H., 2014. Investigation of cereal remains at the Xiaohe Cemetery in Xinjiang, China. J. Archaeol. Sci. 49, 42–47. https://doi.org/10.1016/j.jas.2014.04.020.

Yang, Y., Shevchenko, A., Knaust, A., Abuduresule, I., Li, Y., Hu, X., Wang, C., Shevchenko, A., 2014. Proteomics evidence for kefir dairy in Early Bronze Age China. J. Archaeol. Sci. 45, 178–186. https://doi.org/10.1016/j.jas.2014.02.005

Zhang, X., Good, I., Laursen, R., 2008. Characterization of dyestuffs in ancient textiles from Xinjiang. J. Archaeol. Sci. 35, 1095–1103. https://doi.org/10.1016/j.jas.2007.08.001.

Zhao, F., 2004. The evolution of textiles along the Silk Road, in: Watt, J.C.Y., Howard, J.C.Y., Marshak, B.I., Bai, S., Feng, Z. (Eds.), China: Dawn of a Golden Age, 200–750 AD. The Metropolitan Museum of Art.

Zhao, F. (Ed.), 2002. Recent Excavations of Textiles in China. ISAT/ Costume Squad Ltd, Hong Kong.

Zhao, F., 2001. Wang Xu and Textile Archaeology in China: In Memory of Wang Xu on the Third Anniversary of His Death. ISAT/Costume, Hong Kong.

Zhao, F., 1999. Treasures in Silk: An Illustrated History of Chinese Textiles. ISAT/Costume Squad, Hong Kong.