

ABSTRACT BOOKLET



SPECIALIST WORKSHOP

Tuesday 10th March 2026 - Online (Teams)

Maximising data from Palaeolithic bone: methods, integration and application

Bone from Palaeolithic excavations is often very fragmented, limiting its interpretive value. However, recent **methodological advances in archaeological science, proteomics and genetics** can now help maximise the biological, environmental and behavioural data that can be extracted from these non-diagnostic bones and teeth.

This **free, online workshop** will explore strategies for extracting maximal information from Palaeolithic bone collections. It will include **short (10 min) talks** on either a specific method or case study, followed by an **open discussion** on current issues (e.g. sampling strategies, method integration, collagen preservation, ethical considerations, open science).

This workshop is part of the UKRI-funded project COEXIST at the University of Reading

Organisers: Karen Ruebens, Geoff M Smith, Frankie Tait, Anna Wagner

For further information visit our workshop website:
<https://research.reading.ac.uk/coexist/workshop>



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Timetable

Tuesday 10th March 2026 (GMT)

Time (GMT)	Speaker	Title
09:20	Karen Ruebens	Introduction
Chronology and dating		Chair: Karen Ruebens
09:30	Adams, Neil	Dating historically collected material in museums: the case of the Upnor elephant
09:40	Talamo, Sahra	Back to Zero: Where do we stand with the Châtelperronian?
09:50	Casaccia, Nicole	A multidisciplinary approach redefining Central Europe's earliest complex toolmaking: the case of the ivory boomerang from Obłazowa Cave (Poland)
10:00	Discussion	
10:30	Coffee break (20 mins)	
Taphonomy & human behaviour		Chair: Anna Wagner
10:50	Raymond, Pauline	Integrating ZooMS and taphonomy further highlights differential butchery strategies and Neandertal cannibalism in the Quina Mousterian of Les Pradelles (southwest France)
11:00	Jallon, Anaëlle	Don't judge a bone by its color! A taphonomical investigation of bone faunal material from Amud Cave (Israel)
11:10	Peters, Carli	The impact of heating on bone protein preservation and its implications for palaeoproteomics
11:20	Brotos, Lisa	Applying palaeoproteomics to Middle Palaeolithic faunal assemblage from the Adriatic Region
11:30	Discussion	
12:00	Lunch break (50 mins)	
Method advancements		Chair: Frankie Tait
12:50	Collins, Matthew	A High-Fidelity Language for Mapping the Modified Proteome in Ancient Bone
13:00	Tait, Frankie	Assessing the effect of NaOH for humic acid removal in palaeoproteomic analyses of archaeological bone
13:10	Rimon, Oded	Optimising methods for maximising evolutionary information content in palaeoproteomics
13:20	Discussion	
13:50	Coffee break (20 mins)	
Subsistence and environment		Chair: Geoff M Smith
14:10	Wagner, Anna	Refining Taxonomic Resolution in an Upper Palaeolithic Faunal Assemblage at Korman' 9 (Ukraine): An Integrated ZooMS Approach
14:20	Agras, Kenan	A Geometric Morphometric Approach to Assessing Morphological Variation in Fallow Deer Astragali from Üçağızlı I and Üçağızlı II Caves
14:30	Briatico, Giuseppe	From fossil teeth to environments and climate: implications for the Pleistocene archaeological records of the Ethiopian highlands
14:40	McCartin, Madison	Early human subsistence in northeast Asia: Preliminary results from Tolbor-17 and Tarvagataiin Am (Mongolia)
14:50	Discussion	
15:15	Conclusion	End 15:45



Dating historically collected material in museums: the case of the Upnor elephant

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A great deal of faunal material from historical excavations at Palaeolithic and Pleistocene sites can be found in museum collections, including non-diagnostic bone and tooth fragments from sites that were destroyed entirely long ago or are no longer accessible. New methods and approaches applied to this fragmentary material can help improve our understanding of associated more-complete material that is less amenable to destructive sampling. Here, we discuss the example of the Upnor elephant in the Fossil Mammal collection at the Natural History Museum, London.

A remarkably complete skeleton of *Palaeoloxodon antiquus* was excavated in 1915 on the banks of the River Medway near Upnor in Kent and was mounted for display in NHM public galleries shortly afterwards. It is one of the only mature adult male skeletons known from Britain and Europe. The Upnor elephant is therefore uniquely placed to provide insights into elephant life history and evolution during the Pleistocene. However, its potential is limited by its unresolved geological age, and correlations to marine isotope stage (MIS) 5e and MIS 7 have been proposed.

Our new analyses using amino acid racemisation (AAR) and Uranium-series dating on small samples from the elephant itself and importantly from associated fragmentary mammalian remains, coupled with more traditional biostratigraphic and morphometric methods, provide compelling evidence to discount an Ipswichian (MIS 5e) age and favour an older age (MIS 7 or MIS 9). We also present new statistical approaches to multivariate analysis of AAR data that may prove useful for colleagues working with similar datasets.



Back to Zero: Where do we stand with the Châtelperronian?

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The Châtelperronian remains one of the most debated techno-complexes of the Middle-to-Upper Palaeolithic transition. It is currently the only so-called “transitional” industry for which direct associations with Neanderthal remains have been documented¹⁻³, giving rise to two main interpretative frameworks: as an independent Neanderthal innovation or as the result of acculturation through contact with incoming *Homo sapiens* groups.

Recent discoveries and direct dating of *Homo sapiens* remains associated with the IUP⁴ and the LRJ⁵, chronologically older than the Châtelperronian, have reshaped the comparative framework, leaving the Châtelperronian as the only transitional techno-complex still linked to Neanderthals. This has prompted increased scrutiny of its archaeological record, with several studies highlighting issues of stratigraphic integrity, post-depositional disturbance, and uncertain associations at key sites such as Saint-Césaire and the Grotte du Renne^{6,7}.

This paper offers a reassessment of the Châtelperronian through a critical review of radiocarbon evidence and directly dated human remains. Its chronological framework remains largely structured around a limited number of well-documented reference sites, most notably Arcy-sur-Cure, which continues to provide the backbone for regional and supra-regional correlations. Within this framework, securely identified human remains from Châtelperronian contexts are Neanderthals, and all of them fall within the Châtelperronian chronological range.

Recent methodological advances are expanding the available dataset and encouraging a careful re-evaluation of long-standing associations. This contribution aims to clarify which aspects of the Châtelperronian are supported by robust chronological evidence and to define a new starting point for moving beyond inherited debates.

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A multidisciplinary approach redefining Central Europe's earliest complex toolmaking: the case of the ivory boomerang from Obłazowa Cave (Poland)

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The beginning of the Upper Palaeolithic (ca. 50,000 to 30,000 years ago) marks a turning point in human evolution, associated with the arrival of *Homo sapiens* in Europe and the emergence of new symbolic and technological behaviors (Picin *et al.* 2023). A remarkable example of such innovation is the mammoth ivory boomerang recovered from layer VIII of Obłazowa Cave (Poland), discovered in association with a human phalanx (Valde-Nowak *et al.* 1987). Although directly dated to 18,160 ±260 BP (Housley 2003), this age has long been considered anomalously young in light of the stratigraphic context, raising concerns about contamination and chronological reliability.

This study provides the first robust chronological reassessment of layer VIII through a multidisciplinary and minimally destructive approach. Thirteen faunal remains and the associated human phalanx were analyzed using an integrated set of methods, including ancient DNA analysis, ZooMS, stable isotope analysis, Near Infrared spectroscopy with hyperspectral imaging for collagen assessment (Malegori *et al.* 2023), and high precision radiocarbon dating combined with Bayesian modelling. This strategy also allowed for indirect dating of the mammoth tusk artefact while preserving its physical integrity.

The resulting chronological model indicates a significantly earlier occupation phase, between 42,810 and 38,550 cal BP (95.5% probability), with the boomerang likely dating to 42,290–39,280 cal BP (95.5% probability). These results place the Obłazowa boomerang among the earliest known examples of complex projectile technology in Europe and showcases the power of interdisciplinary methods in helping our understanding of early *Homo sapiens* behavior, offering new perspectives on Upper Paleolithic cultural evolution.

Acknowledgments

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Integrating Zooarchaeology by Mass Spectrometry (ZooMS) and taphonomic analyses further highlights differential butchery strategies and Neandertal cannibalism in the Quina Mousterian site of Les Pradelles (southwest France)

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The shelter of Les Pradelles (Marillac-le-Franc, France) was repeatedly visited by Neandertals (~55,000 BP), where they processed reindeer and human carcasses. Excavated in two campaigns with differing recovery methods, 1967-1980 by B. Vandermeersch and 2001-2013 by B. Maureille and A.E. Mann, the site produced rich faunal assemblages (>18,000 bones). In this study, we apply ZooMS (Zooarchaeology by Mass Spectrometry) and taphonomic analyses to morphologically unidentifiable bones from both collections. Fragments from the Vandermeersch collection ($n = 762$) were targeted to investigate taphonomic patterns and depositional contexts. Sieve-recovered fragments from the Maureille/Mann collection ($n = 377$) were analysed to discuss the underrepresentation of human articular extremities previously linked to cannibalism. Our results indicate excellent collagen preservation (99.2 % identification rate) and consistent glutamine deamidation. A relatively low amount ($n = 5$) of Neandertal bones were identified supporting the sporadic nature of cannibalism. Abundant human bone surface modifications (25%) were observed on the ZooMS-identified fauna, but not the new Neandertal remains. While reindeer dominate the ZooMS fraction, increases in bovid and equid remains may relate to differential processing of trabecular elements (e.g. trunk, articular extremities). Our study confirms the use of Les Pradelles as a reindeer hunting camp alongside the occasional processing of human bodies.



Don't judge a bone by its color! A taphonomical investigation of bone faunal material from Amud Cave (Israel)

Anaëlle Jallon, doctoral student at the Hebrew University of Jerusalem under the direction of Erella Hovers and Rivka Rabinovich

First studies of Amud cave's faunal assemblage report particularly high proportions of burnt remains. Various scenarios including anthropic actions (e.g. related to food processing, fuel source or waste management), incidental causes, or any combination of both can be considered to explain the high occurrence and spatial repartition of these materials. However, the fragmented nature of this assemblage makes it challenging to detect and interpret visual and spatial burning patterns on the remains and to distinguish them from those related to other, geogenic or chemical post-depositional processes. Our study aims to re-evaluate the criteria used to distinguish burnt elements in the assemblage, by supplementing the visual evaluation of bone fragments' surface appearance and color – largely used by zooarcheologists as an indicator of direct or indirect exposition to fire – with quantifiable data. This study integrates spectrophotometric colorimetry measurements with Fourier transform infrared spectroscopy (FTIR) analyses to detect the presence of oxides, and evaluate the temperature and conditions of the burning events impacting the bone remains. Our results challenge previous interpretations of the taphonomical history of Amud cave's faunal remains. We show that color and surface appearance are poor indicators of burning episodes on the Amud bone assemblage, likely leading to the overestimation of burnt bone remains proportions in past studies. This raises the discussion on the share of human behavior vs diagenesis in the current aspect of the remains.



The impact of heating on bone protein preservation and its implications for palaeoproteomics

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In the field of palaeoproteomics, there is a general assumption that ancient proteins do not preserve in burned materials, due to the susceptibility of proteins to degradation at high temperatures. However, the successful recovery of ancient proteins from archaeological burned bone specimens has already been reported in a handful of studies. While many studies have focused on the structural, mechanical, and chemical change in bone upon heating, investigations into the degradation and recovery of bone protein from heated bones are more limited. Since the initial proteomics study on experimentally burned bone by Fellows-Yates¹ in 2013, new extraction methods and analytical workflows have been developed that have yet to be tested on burned bones. Here, we tackle one main question: Is it possible to extract and identify proteins from burned bones, and, if so, until what temperatures? We experimentally heated modern pig rib fragments in temperature increments up to 750°C. Fourier Transform Infrared Spectroscopy (FTIR) is used to assess structural and chemical changes to the bones. ZooMS and Species by Proteome INvestigation (SPIN) are then applied to qualitatively assess the degradation of proteins at different temperatures. We show that bones can be taxonomically identified with a proteomics-approach up to burning temperatures of ~200°C, regardless of the extraction protocol used.

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Applying palaeoproteomics to Middle Palaeolithic faunal assemblage from the Adriatic Region.

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Zooarchaeology by Mass Spectrometry (ZooMS) is a well-established proteomic analytical method involved in the taxonomic determination of highly fragmented Palaeolithic faunal assemblages, providing molecular evidence to support traditional zooarchaeological analyses. Aside from species identification, its application can reflect the heterogeneous palaeoenvironmental dynamics and complex depositional settings. This contribution presents the ongoing palaeoproteomics investigation on Middle Palaeolithic faunal remains from the Adriatic region, a key biogeographical area that experienced significant ecological variability throughout this period. ZooMS analyses were conducted under the framework of the ERC Last Neanderthals on samples recovered from the Mousterian layers in three different sites – Ždriilo (Croatia), Bioče (Montenegro), and Uluzzo C (Italy) – based on a destructive protocol developed by Buckley et al. (2009). This study aims to evaluate the contribution of peptide mass fingerprinting to taxonomic identification and assess the biomolecular preservation across the various depositional contexts. Using a comparative approach across the different faunal assemblages, preliminary ZooMS results highlight site-specific molecular degradation processes, expressed by peptide representation patterns and collagen preservation indicators such as glutamine deamidation. These findings contribute to ongoing methodological discussions on the applicability and interpretative limits faced by ZooMS, underlining its potential as an integrative tool within multi-proxy zooarchaeological research on Middle Palaeolithic faunal assemblages.



A High-Fidelity Language for Mapping the Modified Proteome in Ancient Bone

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Traditional proteomics workflows often encounter a fundamental disjunction between the molecular complexity of ancient samples and the restrictive "language" of standard sequence files. In Palaeolithic bone, where proteins are highly fragmented and chemically altered, standard formats act as a lossy filter that strips away essential variability. This software solution addresses the "linguistic crisis" by reimagining the protein sequence as a conditionally structured record that preserves site-specific modifications and evolutionary context.

The approach utilises an expanded alphabet to create a descriptive map of the modified proteome. By distinguishing between biologically mediated modifications and chemical degradation markers—such as deamidation—the system restores information typically lost in traditional searches. This "compact grammar" provides the necessary high-fidelity input for Large Language Models (LLMs) to accurately identify highly degraded proteins that standard tools cannot comprehend. By integrating gene architecture and evolutionary history directly into the workflow, this method maximises the biological and environmental data recoverable from even the most non-diagnostic archaeological assemblages.



Assessing the effect of NaOH for humic acid removal in palaeoproteomic analyses of archaeological bone.

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Humic acids (HAs) are a complex mixture of organic compounds formed through plant and animal tissue decomposition, found in soils and skeletal remains (van Klinken and Hedges, 1995). As HAs are soluble in alkaline solutions, sodium hydroxide (NaOH) is routinely used as a wash step in collagen pretreatment protocols before radiocarbon dating and stable isotope analysis where exogenous carbon from HAs can impact results (Szpak et al., 2017). Due to the potential for HAs to act as an inhibitor during proteomic analysis, an NaOH wash is subjectively applied in ZooMS protocols – however, its impacts are still poorly understood and require systematic testing (Brown et al., 2020).

This study assesses the effect of an NaOH wash on 47 archaeological bone fragments of varying preservation states from six Palaeolithic contexts. The bones were sampled in duplicate and collagen was extracted using the standard HCl ZooMS protocol (Buckley et al., 2009; Welker et al., 2015), with one set receiving the NaOH wash and the other retaining HAs.

The resulting peptides were analysed on a Bruker MALDI-TOF, a high resolution Waters MRT-MALDI, and an EvoSep LC coupled to a Bruker timsTOF MS/MS instrument to investigate the effect of the NaOH wash on the extracted collagen and the proteomic identification. The concentration of HA removed was determined using a SpectraMax i3x against a calibration curve of technical grade Humic Acid.

In this talk, we will discuss the impact of the NaOH wash on collagen degradation, sample loss and improvements to taxonomic resolution.

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Optimising methods for maximising evolutionary information content in palaeoproteomics

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Proteins extracted from bones and teeth are used for a wide range of archaeological applications, from radiocarbon dating [1] to zooarchaeological species identification [2, 3] to individual sexing [4] and protein sequencing [5]. Increasingly, proteins are being extracted from hominin fossils to assign them to a specific hominin clade and learn more about our ancestors and relatives. However, by analysing the state-of-the-art of palaeoproteomics with modern statistical techniques, we determine that most current datasets lack the power to distinguish hominin species, and none can reliably discern neandertals from *Homo sapiens*. By translating thousands of modern human and high-coverage archaic hominin genomes, we show that increasing the number of different proteins observed is key to increasing the chances of capturing evolutionarily informative variation. In modern samples, different extraction methods have been shown to exhibit widely different profiles of the extracted proteome [6]. We observe the same in ancient samples, and show that the extraction step can have a major effect on the amount of evolutionary information which can be derived from a given sample. The breadth of coverage does not directly correlate with protein yield and inversely correlates with collagen 1 purity, leading to a clear distinction between optimal extraction methods for maximising information content and those best for other downstream applications such as radiocarbon dating or ZooMS. Here, we will present our re-evaluations of published data, and suggest criteria for assessing wet-lab and computational methods in terms of the evolutionary information content of their output.

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Refining Taxonomic Resolution in an Upper Palaeolithic Faunal Assemblage at Korman' 9 (Ukraine): An Integrated ZooMS Approach

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Zooarchaeology by Mass Spectrometry (ZooMS) identifies faunal remains through collagen fingerprinting, using the most abundant protein preserved in animal tissues. This method enables taxonomic identification at the genus, family, or occasionally species level and is particularly valuable for highly fragmented assemblages that cannot be identified morphologically. When integrated with zooarchaeological and taphonomic analyses, ZooMS provides a powerful approach for recovering taxonomic information from Palaeolithic faunal assemblages, where unidentifiable fragments often dominate.

In this study, we analysed the small faunal fraction retrieved from wet-sieving from the Upper Palaeolithic open-air site of Korman' 9, located in the Middle Dniester valley, Ukraine. Material was examined using a combined framework of morphological assessment, taphonomic analysis, and ZooMS-based identification. Of the 238 remains 102 were subjected to ZooMS-analyses. Collagen preservation was relatively high for an open-air context, with 74.5% of samples successfully identified using the acid extraction protocol. Overall, species composition between methods was congruent, showing a dominance of horse and reindeer complemented with red deer, hare and fox. Integrative morphological and ZooMS analyses proved especially helpful to narrow-down the identification of cervidae/saiga in the assemblage and identified an additional large carnivore. To throw light on the exploitation of juveniles and their species composition, part of the ZooMS analyses was geared toward unfused (and otherwise unidentifiable) diaphysis, the majority of which turned out to be reindeer, adding both to the MNI and providing a more well-rounded view of the demography of selected prey at the site.



A Geometric Morphometric Approach to Assessing Morphological Variation in Fallow Deer (*Dama mesopotamica*) Astragali from Üçağzılı I and Üçağzılı II Caves

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This study examines the morphological variation of fallow deer (*Dama mesopotamica*) astragali recovered from Üçağzılı II Cave, dated to the Middle Paleolithic, and Üçağzılı I Cave, dated to the Early Upper Paleolithic. Bone shape was analyzed using Geometric Morphometrics (GM), while metric variation was assessed through the Logarithmic Size Index (LSI). The assemblage derives from excavations conducted between 1999 and 2024. Three Middle Paleolithic layers (B1, B2, B3) were identified at Üçağzılı II Cave, whereas nine layers associated with the Ahmarian, Ahmarian Tradition Transition, and Initial Upper Paleolithic were defined at Üçağzılı I Cave. GM analyses revealed no statistically significant differences at the assemblage level; however, shape variation on the articular surfaces was detected at the individual level. These variations are concentrated in eight specimens attributed to specific periods and stratigraphic layers. LSI results indicate a preference for smaller-bodied individuals during the Ahmarian and Ahmarian Tradition Transition phases, while larger-bodied individuals were favored during the Late Middle Paleolithic and the Initial Upper Paleolithic. The findings suggest that fallow deer morphology was influenced not only by environmental changes but also by human hunting strategies, which may have exerted selective and evolutionary pressures on fallow deer populations.



From fossil teeth to environments and climate: implications for the Pleistocene archaeological records of the Ethiopian highlands

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For more than 30 years, stable carbon and oxygen isotopic analyses of herbivore skeletal tissues have been widely used to reconstruct animal life history and behavior, including diet, physiology, mobility, and past climate and environmental conditions. Tooth enamel is particularly well-suited for such investigations because its high crystallinity and low organic content confer exceptional resistance to diagenetic alteration, thereby preserving primary isotopic signals over geological timescales. Moreover, because enamel forms incrementally, it enables the extraction of isotopic time-series information that reflects the period of tooth mineralization.

Here, I explore the potential of stable isotope analysis to maximize paleoecological data retrieval from Paleolithic faunal assemblages, using the archaeological sites of Melka Kunture, Melka Wakena, and Gadeb as a case study. These three sites are located on the Ethiopian highlands at elevations of 2000–2300 m a.s.l. and chronologically span between ~2,000,000 and 600,000 years ago. Together, they provide a rare opportunity to investigate high-elevation past ecosystems in the tropics, where collagen preservation is often poor and enamel-based approaches are particularly valuable. By combining bulk and intra-tooth isotope analyses of herbivore tooth enamel with faunal, pollen, and phytolith evidence, I assess the complementarity of proxies for detecting climatic and environmental variability through time and for evaluating its implications for human and mammalian evolution. The results demonstrate how stable isotope analysis can substantially enhance the interpretation of Paleolithic records, particularly in ecologically marginal settings. Finally, I highlight the value of integrated, multi-proxy approaches when reconstructing past ecosystems.



Early human subsistence in northeast Asia: Preliminary results from Tolbor-17 and Tarvagataiin Am (Mongolia)

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In northern Mongolia, the open-air sites of Tolbor-17 (T17) and Tarvagataiin Am (T-Am) contain some of the only Initial/Early Upper Paleolithic (~45–30 ka cal BP) faunal assemblages in the region. This period is characterized by the first dispersals of modern humans (*Homo sapiens*) into northern Asia. Although significant, little is known about the nature of these dispersals and the subsistence strategies that facilitated humans' success in new environments. This talk presents the preliminary analyses of the T17 and T-Am faunal assemblages, highlighting challenges that limit our understanding of human behavior, as well as potential solutions.

Initial results show that T17 is one of the largest Initial/Early Upper Paleolithic faunal assemblages in the region (n = ~1000), representing a palimpsest of human habitations, characterized by ungulate butchery and osseous tool production, as well as large carnivore occupations. Despite promising results, interpretations are limited by significant post-depositional alterations (e.g., fragmentation, root etching, deflation, solifluction). Future work will disentangle this complex taphonomic history and increase taxonomic resolution via high-throughput ZooMS (Zooarchaeology by Mass Spectrometry). Conversely, the faunal assemblage of T-Am is smaller (NISP = ~400) but exceptionally well preserved. Although the assemblage is still under analysis, the near-complete carcasses of a horse and bovid suggest the assemblage represents an isolated hunting event. T-Am thus provides a unique, temporally constrained perspective on hunting and butchery practices in northern Mongolia. Together, T-Am and T17 offer novel and complementary data on the type and range of subsistence behaviors practiced by the earliest inhabitants of northeast Asia.