An investigation into diet and economy in ancient Mongolia through multiple biomolecular datasets

Dissertation

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By Wilkin, Shevan M.A.

born on 08 April, 1981 in Palatine, Illinois, USA
Gutachter:

1. Prof. Dr. Nicole Boivin
2. Prof. Dr. Erika Kothe
3. Prof. Dr. Matthew Collins

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1. Introduction

Studying the dietary diversity of past populations has the power to reveal how varied environments, population dynamics, and cultural practices have influenced human subsistence patterns. Expanding our understanding of the ways in which populations responded to environmental and political changes in the past can offer insights into the development of modern responses to similar changes. This is particularly relevant in Mongolia, an area often characterized as homogenous terrain occupied by specialized pastoralists, from prehistory to the present day; which resides within an environment increasingly threatened by climate change, pollution, and the demands of an extensively capitalist world system. However, due to a lack of solid stratigraphy, an overreliance on historical documentation, and limited application of archaeological science methodologies, our understanding of dietary and economic diversity in this part of the world has remained remarkably lacking, despite popular imaginations of Mongolian subsistence and mobility [1–4]. In this thesis, I apply stable isotope analysis and proteomic analysis to archaeological samples from Mongolia, spanning between Neolithic and the Mongol Empire periods (ca. 3000 BCE - 1400 CE) in order to determine the arrival of ruminant dairying on the eastern steppe, the origins of horse milk traditions, and the incorporation of grain agriculture into Mongolia’s early economies. The three resulting studies of Mongolia’s varied ancient and historic dietary traditions, across diverse environmental zones, offer a unique window into how eastern steppe populations subsisted through major ecological and political transitions.

Dairy pastoralism is a common global subsistence strategy that is especially prevalent across the Eurasian steppe and Mongolia today, however the date of emergence and the varied histories of this practice remain unclear. Economic and subsistence strategies in ancient Mongolia have been challenging to uncover, largely due to the paucity of excavated domestic sites prior to the Iron Age (ca. 800 BCE). Nevertheless, while occupation sites are seldom excavated, the prehistoric archaeological landscape is dominated by ritual stone monuments that often contain human burials. The recent rise and combination of a diversity of biomolecular methods that can reveal direct insights into foods consumed by an individual, mean that it is possible to draw out insights into changes in subsistence from funerary contexts.
To identify trends in dairying this thesis contains a study combining ancient protein analysis of human dental calculus in tandem with ancient DNA (aDNA) analysis of individuals from a Late Bronze Age (c. 1300 BCE) site in northern Mongolia (Manuscript 1) and an ancient protein analysis of an extended sample set of individuals spanning multiple time periods (3000 BCE – 1400 CE) and regions across Mongolia (Manuscript 2). To discover dietary trends related to C₃ (wheat, barley) and C₄ (millet, maize) grain consumption, we include a carbon and nitrogen stable isotope analysis of human bone collagen and carbon stable isotope analysis of dental enamel bioapatite (Manuscript 3). The use of these two methods allows us to investigate dairy intake through the identification of taxa-specific milk peptides in concert with C₃ and C₄ plant intake through stable carbon isotope ratios. Through the multi-proxy datasets produced in these studies, we create a timeline of changing subsistence practices and detail the earliest direct evidence for the consumption of dairy on the Eastern Eurasian Steppe (ca. 3000 BCE), explore the incorporation of horse milk into diets in the Late Bronze Age (ca.1200 BCE), and examine the diversification of diets during the Xiongnu and Mongol Empires (ca. 200 BCE - 1400 CE). Our results illustrate how early pastoral populations transitioned into diverse, imperial agro-pastoral dietary economies catalysed by the incorporation of horses, and horse riding, into existing herding practices.

1.1 The origins and spread of dairying

Today, worldwide milk production forecasts for 2019 are over 850 millions tons, as the consumption of milk and dairy products are an essential part of global subsistence strategies. The exact total differs from country to country, with individual consumption varying between 10 and 300 kg annually [5]. The initial suggestion for the origin of the dairying, among other secondary products (wool, traction), was put forth by Sherratt in the early 1980’s [6]. The idea was that dairying did not begin until the Chalcolithic, and that it was the incorporation of these secondary products that enabled populations to become the larger, more complex political systems of the Bronze Age [6–8]. While many of Sherratt’s ideas have been validated by subsequent research, testing of this hypothesis through zooarchaeological and lipid analysis of ceramic vessels has shown that dairying practices appear to have begun earlier. Evidence of dairy fats in ceramics are present shortly after, if not concurrently, with the first domestication of ruminant animals (sheep, goat, cattle) [8–12]. This incorporation of dairying practices into dietary systems resulted in a multitude of observable changes in the archaeological record. For example, optimization of dairy production, i.e. increasing milk production through retaining
milk-producing females to old age and killing males at a young age results in alterations in the ratio of females to males in herd composition, and animal age at death profiles [6,12].

Evidence for past dairying can be identified through the presence of dairy processing and storage materials, as well as through slaughter patterns of dairy animals for optimal milk exploitation [13]. For example, protein and lipid (fatty acid) evidence has shown the presence of milk (among other dietary materials) in ceramics in early Anatolia at (ca. 6500 BC) [10,14]. The movement of dairy practices northwards into Europe has been demonstrated through additional lipid evidence for ruminant dairy products from Neolithic sites in modern day Poland, where dairy fats were present in sieve-like ceramics with holes, suggestive of cheese making vessels [11,12]. The use of dairy in Europe is well-documented and has been extensively published [11,15–18], and while a few recent studies have touched on on the eastern spread of dairying into Asia, much more work is needed for a fully nuanced understanding of this dispersal. So far, a few published studies demonstrate the presence of dairy animals and practices in the central and eastern steppe through archaeological, lipid, and protein research [19–21] (Figure 1). Zooarchaeological evidence that suggests mortality profiles of zooarchaeological assemblages associated with dairy pastoralism have been found in southern Russia and Kazakhstan [19,22] indicating an eastward migration of herding practices. aDNA research documents the migrations of Bronze Age western steppe populations eastward, reaching as far as the Altai Mountains [23–25]; however, these studies suffer from a lack of contemporaneous samples feast of the Altai, and these migrations may have travelled further east into Mongolia.

Figure 1: Examples of lipid and proteomic evidence for early dairying on the Eurasian Steppe (Craig et al. 2011; Evershed et al. 2008; Salque et al. 2013; Xie et al. 2016; Yang et al. 2014; Outram et al., 2009).
1.2 The antiquity of dairying in Mongolia

In contemporary Mongolia, dairy pastoralism is practiced by approximately 40% of the population living outside of the urban capital of Ulaanbaatar [28]. Depending on the environment and climate, seven different animal species are commonly milked in Mongolia: sheep, goat, cow, yak, reindeer, camel and horse (Figure 2). From the milk of these species many different dairy products are produced including fresh and fermented milk, yogurt, butter, and a diversity of cheeses and dried consumables [29]. In addition to caloric intake, milk consists of between 80-90% water [30], providing a source of vital hydration on the arid steppe. Dairying is regarded as a long-standing subsistence practice, and is believed to have extended far into the past, however, it remains unclear when or where the first animals were used for dairying on the Eastern Steppe.

Mongolia’s history is marked by imperial periods, such as the Mongol (1200 - 1400 CE) and Xiongnu Empires (ca. 200 BCE – 200 CE), and preceding prehistoric populations of the Early Iron Age (800 - 300 BCE), the Bronze Age (3000 - 800 BCE), and Eneolithic and Neolithic periods (prior to 3000 BCE). The Mongol and Xiongnu Empires have been colloquially understood as polities of hostile, horseback raiders that lacked the complexity of other well-known global imperial structures [31,32]. Nonetheless, as demonstrated in this thesis, both empires relied on diverse, multi-resource dietary economies including animals (milk, meat, transport, leather), crops, and imported foods [33–35]. Prior to the Xiongnu period, in the Early Iron Age, Bronze Age, and Neolithic periods, little is known about subsistence strategies due to a lack of historical evidence and the sparsity of excavated occupation sites [36,37].
There is ample historic evidence for dairy consumption from Mongol period texts, where specific mentions of ruminant and horse milk consumption are common. One such example is the “Secret Histories of the Mongols”, translated into English by DeRachewiltz [38]. This written work is the first book known to have been composed by Mongols in the Mongolian language, and it details the life and rise of Chinggis Khan. Born as Temujin, a young boy from a rural pastoral family, Chinggis Khan grew up to unite disparate tribes from across the region into the Mongol Empire. “The Secret Histories of the Mongols” [38] repeatedly details the social importance of fermented horse milk and the prevalence of other ruminant dairy products in Mongol dietary traditions. In other documents, foreign visitors to the Mongol capital of Kharkorin noted the presence of a silver airag fountain, commissioned by one of the Khans, and meant to dispense fermented horse milk and wine to members of the Mongol elite [39]. Artistic and written depictions of this infamous fountain are common, through no physical remains of the fountain have been recovered.

During the Xiongnu period, there is additional historic evidence for ruminant and equine dairy consumption, although these texts were not written by Xiongnu scribes, but rather by members of Han Chinese groups who were frequently in contact with the Xiongnu. These texts (ca. 100 BCE - 200 CE) specifically mention the consumption of dairy products, but also the drinking of lao, which translates to “horse alcohol”, at important Xiongnu social and political events [40]. Archaeologically, Xiongnu period ceramics indicative of cheese-making straining vessels suggest the manufacture of dairy products, and zooarchaeological evidence of pastoral kill-off patterns suggests dairy-focused subsistence practices were common in their multi-resource economies [33].

Without the guidance of historic documents for the time periods prior to the Xiongnu period in Mongolia, we have only archaeological evidence to consider. During the Bronze Age, there are few instances of occupation sites with recovered material objects in Mongolia [36,37]. Rather, the archaeological landscape is dominated by stone mounds, which often contain human burials [41]. Faunal remains of dairy animals are occasionally also recovered from satellite mounds placed around the primary human inhumations, yet these remains are often very fragmentary or charred, making it difficult to determine whether the animals were domesticated or wild [37,42]. While few occupation sites have been recovered and published to date, this trend is changing as more intensive survey techniques have been used to locate areas of prehistoric human domestic activity [37].
Prior to the Early Bronze Age in Mongolia very little is known about settlement structure or subsistence patterns largely due to the lack of the excavated sites prior to the Bronze Age. It has been suggested that early domesticated cattle could have been present in south-eastern Neolithic Mongolia [43], but these claims have not been substantiated with documented zooarchaeological methodologies and the faunal remains have been lost [44]. It is widely assumed that most Neolithic populations practiced hunting, gathering, and fishing, but this is difficult to substantiate due to the dearth of archaeological materials and skeletal evidence [43–45].

1.3 Grain cultivation in Central and Eastern Asia

Mongolia’s economy today is heavily focused on animal products, but crop cultivation, in the form of grains, potatoes, and other vegetables, provides an important part of modern Mongolian diets. Only a small percentage of the total landmass is arable (between 1 and 9.5% per aimag), and the arid climate as well as short growing seasons makes crop cultivation challenging [46]. Due to the small amount of land available for crop agriculture, many cereal grains and grain products are imported into Mongolia from nearby Central Asian countries of Russia and Kazakhstan [47].

Mongolia’s historic imperial systems have long been viewed as populations of horseback raiders, dependent on dairy pastoralism, who primarily extorted outside resources through military intimidation and extortion from neighbouring populations. However, others have suggested these empires were far more intricate, with complex economic systems including grain agriculture, craft specialization, and extensive trade networks [33,48]. When these systems were first put into place is unknown, and an assumption of a specialized reliance on pastoral products has pervaded archaeological approaches to Mongolian societies and empires, often leading to them having a unique position in global comparisons of imperial economies. Cereal grain cultivation was undertaken during the Middle and Late Bronze Age in Central Asian regions surrounding Mongolia, but is not known to have been grown within its modern borders. Excavations from second and first millennium sites in southern Siberia, Kazakhstan, and the Xinjiang region of northwest China contain archaeobotanical evidence for millet (broomcorn and foxtail), as well as other grains (Figure 3) [49–53].
During Mongolia’s imperial periods there is ample historic and archaeobotanical evidence for the presence of locally cultivated grains and internationally traded crops [13,34,54]. For example, unprocessed millet grains have been recovered from within the burials of Xiongnu rulers and other elites [55,56], suggesting that they were likely grown locally rather than traded over long distances [54]. Previously published stable isotope analysis and recovered archaeological material evidence, in the form of plowshares [57], suggest active, local cultivation and consumption which is further supported by historical recounts of administrative roles assigned to monitor millet production and distribution [40]. There remains a lack of direct archaeobotanical evidence for C$_4$ grain cultivation and consumption before the Iron Age, meaning that the time period when this grain was first included in eastern steppe diets remains a mystery. Through the application of stable isotope analysis to 155 individuals from prehistory to the Mongol period, we hope to add to the understanding of the range C$_3$ and C$_4$ grain consumption of each period, and identify differences between prehistoric and imperial subsistence economies. These data will add to the growing corpus of evidence detailing the importance of pastoral resource systems in early populations, as well as the complexities of preindustrial Mongolian empires.
1.4 Methods used in this study

1.4.1 The history and methodologies used in ancient protein research

The presence of proteins in archaeological materials has been studied since the 1950s, and the methods with which proteinaceous materials have been identified have evolved over the past 70 years. Initially, protein analysis was used to identify the presence of amino acids in fossils [58] and to determine the relative age of archaeological and paleontological materials containing amino acids, through a method known as amino acid racemization [59,60]. Other early protein studies delved into the likelihood of proteins remaining present in dinosaur bones [61], spurring the realization that while still affected by diagenesis, proteins could persist longer than DNA in the archaeological record. Immunoassays allowed for the identification of proteins by 1974, albeit only those that were specifically targeted for, and Edman sequencing, developed in the early 1980’s, worked well with undamaged modern samples, but is not a reliable method for use with ancient degraded proteins [62].

The incorporation of mass spectrometry into protein studies has opened new avenues in the identification ancient peptide sequences, and has been heavily applied to zooarchaeological and palaeontological materials to investigate evolutionary and taxonomic questions, which is especially important when aDNA analysis is no longer possible [63–68]. One key development in the history of this field has been the use of Matrix Assisted Laser Desorption Ionization mass spectrometry (MALDI-MS) on collagen peptides extracted from palaeoanthropological and palaeofaunal skeletal remains, enabling the taxa-specific identification of bone collagen through a peptide mass fingerprinting approach [63,64,69]. The subsequent development and application of tandem mass spectrometry (MS/MS), and the development of ‘shotgun proteomics’ allowed researchers to identify the specific amino acid sequences of digested peptides, where an entire proteome could be recovered [70,71]. More recently, this method has been applied to archaeological materials beyond bone, such as dental calculus [72–74], ceramic residues and matrices [14,75], preserved organic materials [20,76], cultural heritage items [77], and mummified tissues [78].

Over the past 10 years, ancient protein analysis methods have become more widely applied, and recently researchers have explored some of the optimal strategies for identifying
endogenous proteins and generating replicable, shared data [79]. A number of methods have been developed enabling the extraction and identification of ancient proteins [74,80–83] (For a more detailed description protein extraction methods used in these studies see: Supplementary Information in Manuscript 1). Broadly, all these ‘bottom-up’ methods involve protein extraction, denaturation, digestion and purification before mass spectrometry analysis. Purified peptides are ionized and injected into an MS/MS via liquid chromatography (LC). During the MS/MS analysis, the mass over charge (m/z) of each peptide is measured; occurring rapidly as the sample’s peptides are ionized and sent into the machine. The most frequently identified precursor ions (charged peptides) are then sent on for fragmentation and each of the resulting fragments is measured again by a mass analyser (Figure 4). Once the m/z of each of the precursor ions (MS1), and each of their daughter fragments (MS2) are known, the amino acid sequence of a peptide can be inferred by analysing mass shifts corresponding to amino acid losses in the daughter ions (Figure 5) [70]; For a more detailed description MS/MS methods and settings used in these studies see Supplementary Information in Manuscripts 1 and 2).

Figure 4: Diagram of a Thermo Scientific Q-Exactive HF tandem mass spectrometer (From Thermo Scientific at: https://www.thermofisher.com/order/catalog/product/IQLAAEGAAPFALGMBFZ)
1.4.2 Identifying milk proteins in ancient dental calculus

During the past five years, ancient protein analysis using tandem mass spectrometry has been applied to human dental calculus in order to identify dietary [73,74,84,85] and human oral microbiome proteins [72,86]. Excitingly, this form of protein analysis has offered an indirect form of zooarchaeology by uncovering ancient human-animal interactions through the presence of animal dietary proteins and this has been especially important in cases where associated faunal remains are absent (Manuscript A; Manuscript B). As the studies included in this dissertation are specifically focused on dietary information from dental calculus, other methods focused on microbial or pathogenic protein identification will not be discussed here (See: [72,85–87]). Importantly, milk has a high protein content when compared to other beverages (between 1-14%), yet this varies widely by species (Table 6). It also has a distinctive protein contribution. The majority of proteins found in milk are caseins (alpha-S1-, alpha-S2-, beta-, and kappa-), followed by whey proteins (beta-lactoglobulin [BLG], alpha-lactalbumin, serum albumin, immunoglobulins) and others (lysozyme, lactoferrin, lactoperoxidase).

Studies into dietary proteins from ancient dental calculus have often identified dairy proteins, with examples of ruminant milk recovered from multiple archaeological contexts [70,81,82]. Ruminant milk protein sequences are largely conserved across the infraorder Pecora (even-toed hoofed mammals with ruminant digestion), with a few important differences between specific genera and species. While the peptides from largely conserved regions of a protein can determine that the identified milk protein was from an animal from the overall Pecora infraorder, for example, other peptides contain sequence differences that can identify the protein as deriving specifically from Ovis (sheep) or Capra (goat).
Interestingly, beta-lactoglobulin (BLG) is not produced by humans, and offers a way to specifically look at a protein only found in animal milks (Figure 7).

<table>
<thead>
<tr>
<th>Species</th>
<th>Fat</th>
<th>Protein</th>
<th>Lactose</th>
<th>Ash</th>
<th>Total Solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ass (donkey)</td>
<td>1.3</td>
<td>6.9</td>
<td>4</td>
<td>1.3</td>
<td>25.2</td>
</tr>
<tr>
<td>Bison</td>
<td>1.7</td>
<td>4.8</td>
<td>5.7</td>
<td>0.96</td>
<td>13.2</td>
</tr>
<tr>
<td>Camel</td>
<td>4.9</td>
<td>3.7</td>
<td>5.1</td>
<td>0.7</td>
<td>14.4</td>
</tr>
<tr>
<td>Cow (Holstein)</td>
<td>3.5</td>
<td>3.1</td>
<td>4.9</td>
<td>0.7</td>
<td>12.2</td>
</tr>
<tr>
<td>Cow (Zebu)</td>
<td>4.9</td>
<td>3.9</td>
<td>5.1</td>
<td>0.8</td>
<td>14.7</td>
</tr>
<tr>
<td>Goat</td>
<td>3.5</td>
<td>3.1</td>
<td>4.6</td>
<td>0.79</td>
<td>12</td>
</tr>
<tr>
<td>Human</td>
<td>4.5</td>
<td>1.1</td>
<td>6.8</td>
<td>0.02</td>
<td>12.6</td>
</tr>
<tr>
<td>Horse</td>
<td>4.6</td>
<td>2.7</td>
<td>6.1</td>
<td>0.51</td>
<td>11</td>
</tr>
<tr>
<td>Reindeer</td>
<td>22.5</td>
<td>10.3</td>
<td>2.5</td>
<td>1.4</td>
<td>36.7</td>
</tr>
<tr>
<td>Sheep</td>
<td>5.3</td>
<td>5.5</td>
<td>4.6</td>
<td>0.9</td>
<td>16.3</td>
</tr>
</tbody>
</table>

Figure 6: Solid content variation between commonly milked animal species [30]

Figure 7. Differences in amino acid sequences of two commonly recovered peptides from beta-lactoglobulin, the most frequently recovered dairy protein. Each letter represents a specific amino acid, and those in red are those which indicate species specificity.
Due to the deep evolutionary divergence between ruminants and equines, there are proteins which are present in horse milk but not in the milk of other ruminants (Figure 8). For example, horses produce BLG II, which remains genetically present in ruminants, but is no longer expressed into proteins, and lysozyme C, a specific isozyme identifiable to horses [30,88]. Camels lack the ability to express proteins (BLG) that ruminants and horses produce, yet they synthesize others that have been specifically identified in camel milk (peptidoglycan recognition protein 1) [89]. Horse and camel milk contain high percentages of lactose and have low casein content in comparison to other dairy species, causing these milks to be difficult to process into other products such as cheeses or yogurts (Table 6). These same factors present both horse and camel milk as ideal materials for fermentation into alcoholic beverages. To date, in protein extraction from human dental calculus beta-lactoglobulin (BLG) is the most abundantly recovered milk protein, with alpha-S1-casein second, and an aggregate of other proteins following at far lower frequencies [73,74,84]. This could be due to the types of dairy preferred in the past (liquid whey rather than curd), but it seems more likely that this is due to the structural stability of BLG [90], allowing it to persist through time, or the ability of specific peptides to be recovered through current methods.

1.4.3 History and application of stable carbon and nitrogen isotope analysis

The application of stable isotope analysis to dietary studies extends back to the early 1960’s, when the difference in $^{13}$C/$^{12}$C ratio between C$_3$ and C$_4$ photosynthetic pathways was discovered. The fact that these values don't overlap indicates that this analysis can be used to
differentiate C$_3$ and C$_4$ plants from each other [91]. This idea was then applied to animals that consumed C$_3$ and C$_4$ plants, revealing that the $^{13}\text{C}/^{12}\text{C}$ ratio of the types of plants eaten would be measurable in the bones on the consumer [92]. Shortly after this discovery, it was demonstrated that $^{15}\text{N}/^{14}\text{N}$ stable isotope ratios of consumers vary depending on the trophic level of their food source(s) with a 3‰ addition per level, for example, an herbivore will have a $\delta^{15}\text{N}$ ratio that is 3‰ higher than that of the plants they consume [93]. While these methods were first applied to modern plants and animals, it can also be used on archaeological skeletal remains that contained adequate amounts of collagen.

$\delta^{13}\text{C}$ variability in terrestrial ecosystems is primarily driven by two dominant photosynthetic pathways, C$_3$ (wheat, barley, most wild plants) and C$_4$ (millet, maize, most of the remaining wild plants), which differ in their net discrimination against $^{13}\text{C}$ during photosynthesis [94]. These biological and ecological distinctions are passed into the tissues of consumers depending on the proportion of plants, and consumers of different plants that were consumed. Significantly, $\delta^{13}\text{C}$ analysis of human bone collagen primarily reflects the isotopic values of the protein input to the diet, with a minor contribution from lipids and carbohydrates [95]. This means that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bone collagen will be heavily influenced by protein-rich foods [95,96]. The rate of replacement of bone cells varies by skeletal element, and provide information over a specific time period, generally the last 15-20 years of the individual’s life [97]. In contrast to bone collagen, $\delta^{13}\text{C}$ measurements of tooth enamel bioapatite reflect the carbon in the ‘whole-diet’ (proteins, carbohydrates, lipids, etc.) during the period of life of the enamel formation, and this varies depending on the species and which tooth is sampled [98].

In bone collagen, stable nitrogen isotope ratios ($\delta^{15}\text{N}$) provide additional dietary insights. The trophic effect is seemingly a result of the loss of $^{15}\text{N}$-depleted products during excretion, though it should be noted that diet-tissue distinctions are highly variable between animals [99,100]. Freshwater fish typically have higher $\delta^{15}\text{N}$ values than terrestrial fauna due to the fact that aquatic systems often have longer food-chains, although $\delta^{13}\text{C}$ values of terrestrial and freshwater sources often overlap [96]. The $\delta^{15}\text{N}$ of plants, as well as their consumers, also has the potential to be influenced by environmental conditions, including aridity, salinity, and soil fertility [101–103] (for a more detailed background and methods used in this thesis, see the Supplementary Text 1 of Manuscript 3).
These methodologies have also been widely used in the Holocene to identify dietary patterns and economies. For example, carbon values have been used to identify early North American maize consumers (C₄ pathway), distinguishing them from consumers of a hunter-gatherer diet (primarily C₃ pathways) [104–106]. Differences in the carbon values of certain members of populations has also been used to respond to questions of social inequities visible through differences in dietary intake, for example, those in elite burials likely subsisted on more nutritious meats over the less nutrient-dense maize that was widely available [107]. Studies, such as that from the midwestern individuals from the modern day United States, detailed a continued reliance on maize products, but could also indicate that diets also contained fish due to the change in trophic level indicated by the δ¹⁵N ratios [108].

Recently, stable isotope analysis of archaeological human and associated faunal remains has emerged as an increasingly powerful methodology for tracking paleodietary and subsistence changes in Central and East Asia. Through large-scale studies of individuals over time, we can see broad changes in dietary intake, especially changes in C₃ and C₄ grain consumption. Studies using archaeobotanical evidence combined with stable carbon isotope ratios of western, northern, and central Eurasian Steppe zones human [109–111] and animal herd populations [112] detail the widespread use of wheat, barley, and millet across most of the steppe, and a lack of evidence in Mongolia until the Iron Age [111,113].
2. Aims of the thesis

The combination of proteomic and stable isotope biomolecular analyses incorporated into existing archaeological and historic evidence offers insights into individual and population-level trends in dietary intake and economic diversity. Here, we illuminate the evolution of subsistence strategies through environmental, social, and political transformations over 5000 years of Mongolia’s past through the following three aimed studies.

We aim to determine whether Late Bronze Age (c. 1500 - 800 B.C.E.) individuals in northern Mongolia were consuming dairy products, and if the dairy taxa identified in this approach align with associated zooarchaeological evidence. We expect that dairy pastoralism was common by the Late Bronze Age, and would consist of primarily sheep and goat dairy resources. We also include an ancient DNA analysis to determine whether northern Mongolian dairying populations had local or western steppe genetic ancestry.

To determine the development and diversity of Mongolia’s dairy traditions, through the identification of milk proteins recovered from ancient human dental calculus. Through the extraction and analysis of species-specific peptides we demonstrate ancient and historic human-animal interactions. These data will be coordinated with established archaeological and historic evidence to contextualize early dairying practices. Based on current archaeological knowledge, we hypothesize ruminant dairy to be present in the dental calculus beginning in the Late Bronze Age, and that horse milk proteins will first be identified during the Xiongnu period (ca. 200 BCE – 200 CE).

We will assess the importance of C₄ grain consumption and cultivation through stable carbon and nitrogen isotope analysis from bone collagen, and carbon isotope analysis from dental enamel bioapatite. Resulting data from the Xiongnu, Mongol (c. 1200 -1400 CE), and pre-imperial populations will be combined with archaeological, botanical, and historical evidence to explore and compare the dietary complexities of each period. We hypothesize that millet, or other C₄ grain consumption, would become visible through stable carbon isotope analysis and archaeobotanical evidence recovered from Xiongnu period.
3. Manuscript A

“Bronze Age population dynamics and the rise of dairy pastoralism on the eastern Eurasian steppe”


Published in *PNAS*, November 6, 2018

This study focused on the proteomic analysis of dental calculus in combination with ancient DNA analysis. This project aimed to test for the presence of dairy consumption in north central Mongolia during the Late Bronze Age while also conducting genome analysis to determine whether the population was purely local or shared ancestry with western steppe populations. The following specific analyses were performed:

- Protein extraction and analysis of nine distinct individuals from sites across the Khovsgol aimag of northern Mongolia spanning between 1380 - 975 BCE.
- Ancient DNA analysis of 20 individuals from the same aimag and time period
- Comparison of protein data to faunal remains common in Mongolia’s Late Bronze Age
- Comparison of genomic data to known western steppe and local ancient northern Eurasian populations to determine ancestry

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Jeong, Shevan Wilkin, William T.T. Taylor, Richard W. Hagan, Sabri Bromage, Soninkhisig Tsolomon, Christian Trachsel, Jonas Grossmann, Cheryl A. Makarewicz, John Krigbaum, Ashley Scott, Ganmaa Davaasambuu, Joshua Wright, Nicole Boivin, Martine Robbeets, Johannes Krause, Bruno Frohlich, Jessica Hendy, and Christina Warinner analyzed data; and Choongwon Jeong, Shevan Wilkin, Jessica Hendy and Christina Warinner wrote the paper. In total, Shevan Wilkin contributed to 50% of the project.
Bronze Age population dynamics and the rise of dairy pastoralism on the eastern Eurasian steppe

Choongwon Jeong,a,b,1,2 Shevan Wilkin,c,1 Tsend Amgalantugsd, Abigail S. Bouwman,e William Timothy Treaylor, Richard W. Hagan,f Sabri Bromage,f Soninkhishig Tsolmon,g Christian Trachsel,h Jonas Grossmann,h Judith Littleton,k Cheryl A. Makarewicz,l John Krigbaum,l Marta Burri,l Ashley Scott,l Gannaa Davasaambu,l Joshua Wright,l Franziska Irmer,l Erdene Myagmar,m Nicole Boivinc and Martine Robbeetsb, Frank J. Rühli,e Johannes Krausea, Bruno Frohlichh,o Jessica Hendyq, and Christina Warinnera,2

*Department of Archaeogenetics, Max Planck Institute for the Science of Human History, 07745 Jena, Germany; †The EurasiaAngle Project, Max Planck Institute for the Science of Human History, 07745 Jena, Germany; ‡Department of Archaeology, Max Planck Institute for the Science of Human History, 07745 Jena, Germany; §Institute of Archaeology, Mongolian Academy of Sciences, 14200 Ulaanbaatar, Mongolia; ¶Institute of Evolutionary Medicine, University of Zürich, 8057 Zürich, Switzerland; #Department of Nutrition, Harvard T. H. Chan School of Public Health, Boston, MA 02115; $Nutrition and Biotechnology Department, Mongolian University of Science and Technology, 14191 Ulaanbaatar, Mongolia; ‖Functional Genomics Centre Zürich, University of Zürich/Edigeräumäische Technische Hochscule Zürich, 8057 Zürich, Switzerland; ‡Department of Anthropology, University of Auckland, 1010 Auckland, New Zealand; ¶Institute of Prehistoric and Protohistoric Archaeology, Christian Albrechts University, 21118 Kiel, Germany; †Department of Anthropology, University of Florida, Gainesville, FL 32611; ‡Department of Archaeology, University of Aberdeen, AB24 3FX Aberdeen, United Kingdom; ‖Department of Anthropology and Archaeology, National University of Mongolia, 14200 Ulaanbaatar, Mongolia; †Department of Anthropology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560; and ¶Department of Anthropology, University of Oklahoma, Norman, OK 73019.

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Recent paleogenomic studies have shown that migrations of Western steppe herders (WSH) beginning in the Eneolithic (ca. 3300–2700 BCE) profoundly transformed the genes and cultures of Europe and central Asia. Compared with Europe, however, the eastern extent of this WSH expansion is not well defined. Here we present genomic and proteomic data from 22 directly dated Late Bronze Age burials putatively associated with early pastoralism in northern Mongolia (ca. 1380–975 BCE). Genome-wide analysis reveals that they are largely descended from a population represented by Early Bronze Age hunter-gatherers in the Baikal region, with only a limited contribution (~7%) of WSH ancestry. At the same time, however, mass spectrometry analysis of dental calculus provides direct protein evidence of bovine, sheep, and goat milk consumption in seven of nine individuals. No individuals showed molecular evidence of lactase persistence, and only one individual exhibited evidence of >10% WSH ancestry, despite the presence of WSH populations in the nearby Altai-Sayan region for more than a millennium. Unlike the spread of Neolithic farming in Europe and the expansion of Bronze Age pastoralism on the Western steppe, our results indicate that ruminant dairy pastoralism was adopted on the Eastern steppe by local hunter-gatherers through a process of cultural transmission and minimal genetic exchange with outside groups.

Significance

Since the Bronze Age, pastoralism has been a dominant subsistence mode on the Western steppe, but the origins of this tradition on the Eastern steppe are poorly understood. Here we investigate a putative early pastoralist population in northern Mongolia and find that dairy production was established on the Eastern steppe by 1300 BCE. Milk proteins preserved in dental calculus indicate an early focus on Western domesticated ruminants rather than local species, but genetic ancestry analysis indicates minimal admixture with Western steppe herders, suggesting that dairy pastoralism was introduced through adoption by local hunter-gatherers rather than population replacement.


The authors declare no conflict of interest.

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Data deposition: The sequences reported in this paper have been deposited in the NCBI Sequence Read Archive (SRA) (bioproject accession no. PRJNA429901). The protein spectra have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository (accession no. PXD008217).

See Commentary on page 12083.

1C.J. and S.W. contributed equally to this work.
2To whom correspondence may be addressed. Email: jeong@shh.mpg.de or warinner@shh.mpg.de.

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Botai, in central Kazakhstan, evidence for Eneolithic dairying has been reported through the presence of ruminant and equine dairy lipids in ceramic residues as early as 3500 BCE (8, 9). In the Altai and Tarim basin, where WSH populations have left strong genetic footprints (1, 3, 10, 11), archaeological evidence supports the presence of dairy products by the early second Millennium BCE and later (8, 12, 13). In the Eastern steppe, however, no direct observations of dairy consumption have been made for a comparable time period, despite the fact that skeletal remains of domestic livestock (such as sheep, goats, cattle, and horses) have been found at Mongolian ritual sites and in midden contexts as early as the 14th century BCE (14–17). In the absence of direct evidence for Bronze Age milk production or consumption on the Eastern steppe, it remains unclear whether these animals are merely ritual in nature or signify a major shift in dietary ecology toward dairy pastoralism, and whether their appearance is connected to possible WSH migrations onto the Eastern steppe.

To understand the population history and context of dairy pastoralism in the eastern Eurasian steppe, we applied genomic and proteomic analyses to individuals buried in Late Bronze Age (LBA) burial mounds associated with the Deer Stone-Khirigsuur Complex (DSKC) in northern Mongolia (SI Appendix, Figs. S1–S3 and Table S1). To date, DSKC sites contain the clearest and most direct evidence for animal pastoralism in the Eastern steppe before ca. 1200 BCE (18). Focusing on six distinct burial clusters in Arbulag soum, Khövsgöl aimag, Mongolia (Fig. 1 and SI Appendix, Figs. S1–S3), we produced genome-wide sequencing data targeting ~1.2M single nucleotide polymorphisms (SNPs) for 22 DSKC-associated individuals directly dated to ca. 1380–975 calibrated BCE (SI Appendix, Fig. S4 and Table S2), as well as sequenced whole genomes for two individuals (>3x coverage). Nine of the individuals in this group yielded sufficient dental calculus for proteomic analysis, and we tested these deposits for the presence of milk proteins using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Overall, our results find that DSKC subsistence strategy included dairying of Western domesticated ruminants, but that there was minimal gene flow between analyzed DSKC populations and WSH groups during the LBA. Thus, in contrast to patterns observed in western Europe where, for example, the arrival of WSH is associated with population replacement and continental-level genetic turnover (5), contact between WSH and Eastern steppe populations is characterized by transcultural transmission of dairy pastoralism in the near absence of demic diffusion.

Results

Ancient DNA Sequencing and Quality Assessment. We built and sequenced uracil-DNA-glycosylase-half (19), double-indexed Illumina libraries for genomic DNA extracted from teeth or femora from DSKC-associated burials in Khövsgöl, Mongolia. Twenty of 22 libraries exhibited good human DNA preservation, with a mean host endogenous content of 14.9% (range 0.2–70.0%); two libraries yielded very little human DNA (<0.05%) and were excluded from further analysis (SI Appendix, Table S2). Libraries were then enriched for 1.2 million variable sites in the human genome (1240K) using in-solution hybridization (2, 3). All individuals (12 males, 8 females) showed characteristic patterns of chemical modifications typical of ancient DNA (SI Appendix, Fig. S5), and 18 individuals yielded both low estimates of modern DNA contamination (≤1% mitochondrial and nuclear contamination) and sufficient genome coverage for subsequent analysis (0.11× to 4.87× mean coverage for target sites (SI Appendix, Table S3). No close relative pairs were identified among the ancient individuals (SI Appendix, Fig. S6). Two individuals with high endogenous content on screening (ARS008, 70.0%; ARS026, 47.6%) were deeply sequenced to obtain whole genomes (~3.3x coverage) (SI Appendix, Table S3). We intersected our ancient data with a published world-wide set of ancient and contemporary individuals (Dataset S1) whose genotypes are determined for 593,124 autosomal SNPs on the Affymetrix HumanOrigins 1 array (20).

Fig. 1. Map of the Eurasian steppes. (A) Distribution of the Western (brown) and Eastern (green) steppes and the locations of ancient (red) and modern (black) populations discussed in the text. Population codes are provided in the Dataset S1. A box indicates the location of the LBA burial mounds surveyed in the Arbulag soum of Khövsgöl aimag. (B) Enhanced view of LBA burial mounds (white circles) and burial clusters selected for excavation (boxes a–f) with the number of analyzed individuals in parentheses (SI Appendix, Table S1). (C) Photograph of burial 2009-52 containing the remains of ARS026, a genetic outlier with Western steppe ancestry.
Characterization of the Genetic Profile of the Khövsgöl Gene Pool. To characterize the genetic profile of DSKC-associated LBA Khövsgöl individuals (Khövsgöl), we performed principal component analysis (PCA) of Eurasian populations ([SI Appendix], Fig. S7). PC1 separates eastern and western Eurasian populations, with central and north Eurasian populations falling in an intermediate position ([SI Appendix], Fig. S7). PC2 separates eastern Eurasian populations along a north–south cline, with northern Siberian Nganasans and the Ami and Atayal from Taiwan forming the northern and southern end points, respectively. Most LBA Khövsgöl are projected on top of modern Tuvinians or Altaians, who reside in neighboring regions. In comparison with other ancient individuals, they are also close to but slightly displaced from temporally earlier Neolithic and Early Bronze Age (EBA) populations from the Shamanka II cemetry (Shamanka_EN and Shamanka_EBA, respectively) from the Lake Baikal region ([SI Appendix], Fig. S7) (4, 21). However, when Native Americans are added to PC calculation, we observe that LBA Khövsgöl are displaced from modern neighbors toward Native Americans along PC2, occupying a space not overlapping with any contemporary population (Fig. 2A and [SI Appendix], Fig. S8). Such an upward shift on PC2 is also observed in the ancient Baikal populations from the Neolithic to EBA and in the Bronze Age individuals from the Altai associated with Okunevo and Karasuk cultures (1). These observations are consistent with LBA Khövsgöl and other ancient Siberians sharing more ancestry with Native American-related gene pools than modern populations in the region do.

Notably, two individuals fall on the PC space markedly separated from the others: ARS017 is placed close to ancient and modern northeast Asians, such as early Neolithic individuals from the Devil’s Gate archaeological site (22) and present-day Nivhs from the Russian far east, while ARS026 falls midway between the main cluster and western Eurasians (Fig. 2A). Genetic clustering with ADMIXTURE (23) further supports these patterns (Fig. 2B and [SI Appendix], Fig. S9). We quantified the genetic heterogeneity between Khövsgöl by calculating F_{sym} symmetry statistics (24) in the form of F_{sym}(chimpanzee, outgroup; Khövsgöl, Khövsgöl) for all pairs against 18 outgroups representative of world-wide ancestries ([SI Appendix], Fig. S10). As expected, the two outliers did not form a clade with the rest of individuals and therefore we treated each individual separately in subsequent analyses. For the remaining 16 individuals, 14 were merged into a single main cluster based on their minimal genetic heterogeneity. The other two individuals (ARS009 and ARS015) were excluded from this cluster because they broke symmetry with four and two individuals (maximum

![Fig. 2.](https://www.pnas.org/cgi/doi/10.1073/pnas.1813608115)
| = 3.9 and 4.7 SE), respectively, and were also slightly displaced from the others in our PCA (Fig. 2A).

Next, we quantified the genetic affinity between our Khövsgöl clusters and world-wide populations by calculating outgroup- statistics with Central African Mbuti as an outgroup (25). For the main cluster, top signals were observed with earlier ancient populations from the Baikal region, such as the early Neolithic and EBA individuals from the Shamanka II cemetery (4), followed by present-day Siberian and northeast Asian populations, such as Negidals from the Amur River basin and Nganasans from the Taimyr peninsula (Fig. 3A and SI Appendix, Figs. S11 A and B). As expected based on their nonoverlapping positions on PCA, however, Khövsgöl do not form a cluster with these high-affinity groups, as shown by symmetry tests in the form of (Mbuti, X; Siberian, Khövsgöl). Interestingly, Upper Paleolithic Siberians from nearby Afontova Gora and Mal’ta archaeological sites (AG3 and MA-1, respectively) (25, 26) have the highest extra affinity with the main cluster compared with other groups, including the eastern outlier ARS017, the early Neolithic Shamanka_EN, and present-day Nganasans and Tuvinians ( Z > 6.7 SE for AG3) (red shades in Fig. 3B and SI Appendix, Fig. S11 C and D). This extra affinity with so-called “Ancient North Eurasian” (ANE) ancestry (27) may explain their attraction toward Native Americans in PCA, because Native Americans are known to have high proportion of ANE ancestry (20, 25). Main-cluster Khövsgöl individuals mostly belong to Siberian mitochondrial (A, B, C, D, and G) and Y (all Q1a but one N1c1a) haplogroups (SI Appendix, Table S4).

**Source of ANE Ancestry in the LBA Khövsgöl Population.** Previous studies show a close genetic relationship between WSH populations and ANE ancestry, as Yamaya and Afanasievo are modeled as a roughly equal mixture of early Holocene Iranian/Caucasus ancestry (IRC) and Mesolithic Eastern European hunter-gatherers, the latter of which derive a large fraction of their ancestry from ANE (20, 28). It is therefore important to pinpoint the source of ANE-related ancestry in the Khövsgöl gene pool: that is, whether it derives from a pre-Bronze Age ANE population (such as the one represented by AG3) or from a Bronze Age WSH population that has both ANE and IRC ancestry. To test these competing hypotheses, we systematically compared various admixture models of the main cluster using the qpAdm program (20). Ancient Baikal populations were chosen as a proxy based on both their spatiotemporal and genetic similarities with the Khövsgöl main cluster (Figs. 2 and 3). When the early Neolithic Shamanka_EN is used as a proxy, we find that Baikal+ANE provides a better fit to the main cluster than Baikal+WSH, although no two-way admixture model provides a sufficient fit ( P ≥ 0.05) (SI Appendix, Table S5). Adding a WSH population as the third source results in a sufficient three-way mixture model of Baikal+ANE+WSH with a small WSH contribution to the main cluster (e.g., P = 0.180 for Shamanka_EN+AG3+Sintashta with 3.7 ± 2.0% contribution from Sintashta) (Fig. 4 and SI Appendix, Table S6).

Using the temporally intermediate EBA population Shamanka_EBA, we can narrow down the time for the introduction
of WSH ancestry into the main cluster. Shamanka_EBA is modeled well as a two-way mixture of Shamanka_EN and ANE (P = 0.158 for Shamanka_EN+AG3) (Fig. 4) but not as a mixture of Shamanka_EN and WSH (P \leq 2.91 \times 10^{-7}) (SI Appendix, Table S5), suggesting no detectable WSH contribution through the early Bronze Age. Similar results are obtained for other Late Neolithic and EBA populations from the Baikal region (SI Appendix, Table S5). In contrast, the Khövsgöl main cluster is modeled well by Shamanka_EBA+WSH but not by Shamanka_EBA+ANE (P \geq 0.073 and P \leq 0.038, respectively) (SI Appendix, Table S5). A three-way model of Shamanka_EN+ANE+WSH confirms this by providing the ANE contribution around zero (SI Appendix, Table S6). The amount of WSH contribution remains small (e.g., 6.4 \pm 1.0% from Sintashta) (Fig. 4 and SI Appendix, Table S5). Assuming that the early Neolithic populations of the Khövsgöl region resembled those of the nearby Baikal region, we conclude that the Khövsgöl main cluster obtained \sim 11\% of their ancestry from an ANE source during the Neolithic period and a much smaller contribution of WSH ancestry (4–7\%) beginning in the early Bronze Age.

Admixture Testing of Genetic Outliers. Using the same approach, we obtained reasonable admixture models for the two outliers, ARS017 and ARS026. The eastern outlier ARS017, a female, shows an extra affinity with early Neolithic individuals from the Russian far east (Devil’s Gate) (22) and in general with contemporary East Asians (e.g., Han Chinese) compared with the Khövsgöl main cluster (Fig. 3B and SI Appendix, Fig. S12). ARS017 is also similar to Shamanka_EN in showing no significant difference in qpAdm (SI Appendix, Fig. S12 and Table S7). Using contemporary East Asian proxies, ARS017 is modeled as a mixture of predominantly Ulchi and a minor component (6.1–9.4\%) that fits most ancient western Eurasian groups (P = 0.064–0.863) (SI Appendix, Table S7). This minor Western component may result from ANE ancestry; however, given the minimal western Eurasian contribution, we do not have sufficient power to accurately characterize this individual’s western Eurasian ancestry.

The Western outlier ARS026, a male dating to the end of the radiocarbon series, has the highest outgroup-f1 with the main LBA Khövsgöl cluster, with extra affinity toward Middle Bronze Age (MBA) individuals from the Sintashta culture (Fig. 3B and SI Appendix, Fig. S13) (1). DNA recovered from this individual exhibited expected aDNA damage patterns (SI Appendix, Fig. S5) but was otherwise excellently preserved with >47\% endogenous content and very low estimated contamination (1\% mitochondrial; 0.01\% nuclear). ARS026 is well modeled as a two-way mixture of Shamanka_EBA and Sintashta (P = 0.307; 48.6 \pm 2.0\% from Sintashta) (SI Appendix, Table S7). Similar to ARS026, contemporaneous LBA Karasuk individuals from the Altai (1400–900 BCE) (1, 29) also exhibit a strong extra genetic affinity with individuals associated with the earlier Sintashta and Andronovo cultures (SI Appendix, Fig. S14). Although two-way admixture models do not fit (P \leq 0.045) (SI Appendix, Table S8), the Karasuk can be modeled as a three-way mixture of Shamanka_EBA/Khövsgöl and AG3 and Sintashta, suggesting an eastern Eurasian source with slightly higher ANE ancestry than those used in our modeling (P \geq 0.186) (SI Appendix, Table S8). Like ARS026, admixture coefficients for the Karasuk suggest that MBA/LBA groups like the Sintashta or Subruyaya are a more likely source of their WSH ancestry than the EBA groups, like the Yamnaya or Afanasievo. Notably, Karasuk individuals are extremely heterogeneous in their genetic composition, with the genetically easternmost Eurasian individual nearly overlapping with the EBA Baikal groups (Fig. 2A and SI Appendix, Figs. S7 and S8). Earlier groups, such as the Afanasievo, Sintashta, and Andronovo, are mostly derived from WSH ancestries, and this may suggest that admixture in the Altai-Sayan region only began during the LBA following a long separation since the Eneolithic. Although ARS026 exhibits substantial WSH ancestry, strontium isotopic values obtained from his M3 enamel resemble local fauna and fall within the range of the main Khövsgöl cluster (SI Appendix, Fig. S15 and Table S9); however, because the enamel this individual also exhibited elevated manganese levels, postmortem trace element alteration from soil could not be excluded.

Dairy Subsistence and Lactase Persistence. Contemporary Mongolia has a dairy- and meat-based subsistence economy, and to more precisely understand the role of dairy products in the diets of present-day mobile pastoralists in Khövsgöl aimag, we conducted a detailed nutritional investigation of summer and winter diets. We find that dairy-based foods contribute a mean of 35\% total dietary energy, 36–40\% total carbohydrate, 24–31\% total protein, and 39–40\% total fat to rural summer diets in Khövsgöl aimag, with liquid milk and dairy product consumption of 216–283 and 172–198 g/d, respectively (SI Appendix, Table S10 and Dataset S2).

Despite the importance of dairying today, its origins in Mongolia are poorly understood. Given the limited WSH ancestry of the main Khövsgöl cluster, we sought to determine if dairy pastoralism was practiced by this putatively pastoralist LBA population by testing for the presence of milk proteins (30) in the dental calculus of these individuals. We extracted proteins from 12 dental calculus samples representing 9 individuals (SI Appendix, Table S11) and analyzed tryptic peptides using LC-MS/MS (31). Observed modifications included deamidation (N, Q) and oxidation (P, M) (SI Appendix, Table S12). All protein identifications were supported by a minimum of two peptides across the dataset, and only peptides with an E value \leq 0.001 were assigned; the estimated peptide false-discovery rate (FDR) across the full dataset was 1.0\%, and protein FDR was 4.6\%. Milk proteins were detected in seven of the nine individuals analyzed (SI Appendix, Table S13 and Dataset S3), confirming that dairy foods were consumed as early as 1456 BCE (1606–1298 BCE, 95\% probability of the earliest directly dated individual) (SI Appendix, Fig. S4 and Table S2). Specifically, we detected the milk whey protein β-lactoglobulin (Fig. 5A and B) and the curd protein α-S1-casein, with peptides matching specifically to sheep (Ovis), goat (Capra), Caprinae, Bovinae, and a subset of Bovidae (Ovis or Bovinae) (Fig. 5C, SI Appendix, Table S13, and Dataset S3). These peptides exhibited asparagine and glutamine deamidation, as expected for ancient proteins (32), and the frequency and distribution of recovered β-lactoglobulin (Fig. 5B) and α-S1-casein peptides closely matched that empirically observed for modern bovine milk (33), thereby providing additional protein identification support through appropriate proteotypic behavior.

Given the evidence for dairy consumption by the LBA Khövsgöl population, we sought to determine if the dairy-adaptive -13910*T (rs4988235) lactase persistence (LP) allele found today in Western steppe (34) and European (35) populations was present among LBA Khövsgöl dairy herders, and we examined this position in our SNP-enriched dataset. The -13910*T LP allele was not found in the LBA Khövsgöl (SI Appendix, Fig. S17 and Table S14), and additionally all observed flanking sequences in the lactase transcriptional enhancer region contained only ancestral alleles.

Discussion

In this study, we find a clear genetic separation between WSH populations and LBA Mongolians more than a millennium after the arrival of WSH at the furthest edges of the Western steppe and the earliest appearance of the WSH Afanasievo cultural elements of the Altai-Sayan mountain range. This genetic separation between Western and Eastern steppe populations appears to be maintained with very limited gene flow until the
The 3,000-y legacy of dairy pastoralism in Mongolia poses challenging questions to grand narratives of human adaptation and natural selection (36). For example, despite evidence of being under strong natural selection (36), LP was not detected among LBA Khövsgöl individuals, and all identified milk proteins originated from ruminants, specifically the Western dairy domesticates sheep, goat, and Bovinae. These findings suggest that neighboring WSH populations directly or indirectly introduced dairy pastoralism to local indigenous populations through a process of cultural exchange. Further research on other regional cultures in Mongolia, such as Chemurchek, Hemsteg, and Ulaanzuukh, is needed to determine if this pattern of cultural adoption observed among DSKC sites is broadly shared across other Bronze Age cultures throughout the Eastern steppe.

Bronze Age trade and cultural exchange are difficult to observe on the Eastern steppe, where mobile lifestyles and ephemeral habitation sites combine to make household archaeology highly challenging. Burial mounds are typically the most conspicuous features on the landscape, and thus much of Mongolian archaeology is dominated by mortuary archaeology. However, unlike WSH, whose kurgans typically contain a range of grave goods, many LBA mortuary traditions on the Eastern steppe did not include grave goods of any kind other than ritually deposited animal bones from horse, deer, and bovids. Given that Mongolian archaeological collections are typically dominated by human remains with limited occupational materials, the ability to reconstruct technological exchange, human–animal interaction, and secondary product utilization through the analysis of proteins preserved in dental calculus represents an important advance.

Materials and Methods

Experimental Design. Based on an 850-km² archaeological survey of DSKC-associated burial mounds in Arbūlgū soum, Khövsgöl, Mongolia, we selected 22 burial mounds from 6 distinct burial mound groupings (A–F) for excavation and analysis (Fig. 1 and SI Appendix, sections 1 and 2 and Table S1). Bone and tooth samples from 22 individuals (11 femora, 11 teeth) were analyzed for ancient DNA, and 12 dental calculus samples from 9 individuals were analyzed for ancient proteins (SI Appendix, Table S2). Twenty-one individuals were successfully direct radiocarbon dated to ca. 1380–975 BCE (SI Appendix, section 3 and Table S2).
Ancient DNA Extraction, Library Construction, and Sequencing. DNA extraction and library construction was performed in a dedicated clean room facility at the Max Planck Institute for the Science of Human History in Jena, Germany, following previously published protocols (38), including partial uracil-DNA-glycosylase treatment (19). Following screening, 20 samples with ≥0.1% endogenous content were enriched for 1.2 million informative nuclear SNPs (1240K) by in-solution hybridization (2, 3). Additionally, preenrichment libraries for two well-preserved samples (AR5008 and AR5026) were deep-sequenced to generate 3.3× genomes. All sequencing was performed using single-end 75-bp (for screening and enriched libraries) or paired-end 50-bp (for whole-genome sequencing of two preenrichment libraries) sequences on an Illumina HiSeq 4000 platform following the manufacturer’s protocols (SI Appendix, section 4).

DNA Sequence Data Filtering and Quality Assessment. DNA sequences were processed using the EAGER v1.92.50 pipeline (38). Adapter-trimmed reads ≥30 bp were aligned to the human reference genome using BWA aln/samse v0.7.12 (40) with the nondefault parameter “n 0.01,” and PCR duplicates were removed using dedup v0.12.2 (39). The first and last three bases of each read were masked using the trimbam function in bamUtils v1.0.13 (41). For each target SNP, a single high-quality base (Phred-scaled quality score ≥30) from a high-quality read (Phred-scaled mapping quality score ≥30) was randomly chosen from the 3-bp masked BAM file to produce a pseudodiploid genotype for downstream population genetic analysis. DNA damage was assessed using mapDamage v2.0.6 (42), and mitochondrial DNA contamination was estimated using Schmutzi (43). For males, nuclear contamination was estimated using ANGSD v0.910 (44) (SI Appendix, section 4).

Uniparental Haplotype and Kinship Analysis. Mitochondrial haplotypes were determined by generating a consensus sequence using the log2fasta program in Schmutzi (43), followed by haplogroup assignment both by HaploGrep2 (45) and HaploFind (46). The Y haplogroup was determined using the yHaplo program (47). Genetic relatedness was estimated by calculating pairwise mismatch rate of pseudodiploid genotypes (48) (SI Appendix, section 4).

Population Genetic Analysis. Khövsgöl SNP data were merged with published ancient genome-wide data for the 1240K panel (1, 3, 4, 20–22, 25–28, 49–59) (Dataset S1). A comparative dataset of present-day individuals was compiled from published datasets either genotyped on the Affymetrix Axiom Human Origins 1 array (HumanOrigins) or sequenced to high-coverage in the Simons Genome Diversity Project (20, 60–62) (SI Appendix, section 4). Intersecting with SNPs present in the HumanOrigins array, we obtain data for 593,124 autosomal SNPs across worldwide populations. Population structure was investigated by PCA as implemented in the smartpca v13050 in the University of Georgia Center for Applied Isotope Studies (sections 4 and 5). The f2 and f3 statistics were calculated using the qp3Pop (v400) and qpDstat (v711) programs in the qp3Pop program in Schmutzi (43), followed by haplogroup assignment both by HaploGrep2 (45) and HaploFind (46). The Y haplogroup was determined using the yHaplo program (47). Genetic relatedness was estimated by calculating pairwise mismatch rate of pseudodiploid genotypes (48) (SI Appendix, section 4).

Strontium Isotope Analysis. Strontium isotopes (87Sr/86Sr) measured from human and faunal tooth enamel (n = 16) and bone (n = 5) were analyzed at the University of Georgia Center for Applied Isotope Studies (n = 17) and the University of Florida Department of Geological Sciences (n = 4) using a thermo-ionization mass spectrometer (SI Appendix, section 6).

Dietary Analysis in Contemporary Khövsgöl, Mongolia. Up to 6 d of weighed diet records were collected from 40 subjects (n = 231 total person-days) randomly sampled from the rural soum of Khangai and the provincial cen-
ter of Mörn in June 2012 and January 2013 by trained medical students from the Mongolian National University of Medical Sciences and Ach Medical Institute. Nutrient content determination was performed using a purpose-built food composition table (64), which we appended with unpublished food composition data from the Mongolian University of Science and Technology and the Mongolian Public Health Institute, as well as published data from the United States and Germany (65, 66) (SI Appendix, section 7). Contemporary dietary data were collected under Harvard Institutional Review Board Protocol #21002.

Protein Extraction, Digestion, and LC-MS/MS. Ancient protein analysis was performed in a dedicated clean room facility at the Max Planck Institute for the Science of Human History following recommended guidelines (32). Dental calculus was decalcified in 0.5 M EDTA, and proteins were extracted and trypsin-digested using a modified low-volume Filter-Aided Sample Preparation protocol (67). The resulting peptides were analyzed by LC/MS/MS using the yHaplo program (47). Genetic relatedness was estimated by calculating pairwise mismatch rate of pseudodiploid genotypes (48) (SI Appendix, section 4).
4. Manuscript B

“Dairy pastoralism sustained eastern Eurasian steppe populations for 5000 years”


This study was published in *Nature Ecology and Evolution* in March 2020

The second project expands upon the resulting data from the first by analysing the dental calculus from 32 individuals across Mongolia spanning between the Early Bronze Age and the Mongol Empire (ca. 3000 BC - 1500 AD), and has the following specific outcomes:

- To locate the earliest dairy consuming culture group in Mongolia
- To classify the range of dairy species exploited in early and imperial Mongolia
- To identify the earliest use of horse milk on the eastern Eurasian steppe

Author contributions: Shevan Wilkin, William Taylor, Christina Warinner, Nicole Boivin, and Jessica Hendy designed the research plan; Choongwon Jeong, Shevan Wilkin, Richard W. Hagan, Christian Trachsel, Jonas Grossmann, Abigail Ramsøe, Erdene Myagmar, Jessica Hendy, and Christina Warinner performed research; Richard W. Hagan, Christian Trachsel, Jonas Grossmann, and Ashely Scott contributed analytic tools; Nicole Boivin contributed personnel and material resources; Shevan Wilkin, William T.T. Taylor, Richard W. Hagan, Christian Trachsel, Jonas Grossmann, Jessica Hendy, and Christina Warinner analyzed data; and Shevan Wilkin and Jessica Hendy wrote the paper. In total, Shevan Wilkin contributed to 80% of this research.
Dairy pastoralism sustained eastern Eurasian steppe populations for 5,000 years


Dairy pastoralism is integral to contemporary and past lifeways on the eastern Eurasian steppe, facilitating survival in agriculturally challenging environments. While previous research has indicated that ruminant dairy pastoralism was practiced in the region by circa 1300 bc, the origin, extent and diversity of this custom remain poorly understood. Here, we analyse ancient proteins from human dental calculus recovered from geographically diverse locations across Mongolia and spanning 5,000 years. We present the earliest evidence for dairy consumption on the eastern Eurasian steppe by circa 3000 bc and the later emergence of horse milking at circa 1200 bc, concurrent with the first evidence for horse riding. We argue that ruminant dairying contributed to the demographic success of Bronze Age Mongolian populations and that the origins of traditional horse dairy products in eastern Eurasia are closely tied to the regional emergence of mounted herding societies during the late second millennium bc.

In contemporary central and eastern Eurasia, mobile dairy-based pastoralism is a key subsistence practice for many people. Much of the eastern Eurasian Steppe is covered by dryland grasses which, while challenging for grain agriculture, can sustain large meat and dairy-producing herds. Across the steppe, dairy is a staple food and the product of rich culinary traditions. In the Mongolian countryside, fresh, fermented, processed and distilled dairy products provide a major source of hydration and up to 50% of summer calorific intake. Moreover, milk provides a protein- and fat-rich dietary component, while the processing of milk into dairy products enables the creation of a storable and transportable food source. In Mongolia today, dairy livestock, including sheep, goat, horse, yak, reindeer and camel, are exploited for milk, meat, traction and transport across diverse environmental niches.

The adoption of dairy into adult human diets was a major transition in prehistoric subsistence. In western Eurasian contexts, biomolecular approaches have been extensively applied to investigate the archaeological antiquity of a dairy diet. Following their initial domestication in Southwest Asia, cattle (Bos taurus), sheep (Ovis aries) and goats (Capra hircus) spread eastwards across the Eurasian steppe into Central Asia. Biomolecular evidence for dairy lipids has been identified in ceramics from Neolithic Anatolia and eastern Europe, as well as Copper and Bronze Age Kazakhstan, indicating the potential spread of dairying out of Southwest Asia. As far east as the Tarim Basin of the Xinjiang region in northwestern China in the Middle Bronze Age, milk proteins have been identified in a woven basket and pieces of well-preserved kefir cheese (Fig. 1). In Bronze Age Mongolia, a recent study of human dental calculus from individuals across multiple sites in the northern Khövsgöl aimag identified milk proteins from sheep, goat and cattle. Ancient DNA analysis of the same population found that almost all individuals were of predominately local ancestry and only a single individual had over 10% of western steppe herder ancestry. This suggests that by the late second millennium bc, dairy pastoralism had been fully adopted by, or originated with, local northern populations, leaving an open question of when and how dairy subsistence arrived in this region.

Direct evidence into the timing and nature of pastoral economies in Mongolia from other datasets is exceedingly rare. On the eastern Steppe, the ephemeral nature of pastoral campsites and severe wind deflation in most contexts makes detecting occupational sites with direct information on subsistence economies challenging. As a result, archaeologists have often been forced to form conclusions about local subsistence from materials found in ritual
human burials under stone monuments that dominate the Bronze Age archaeological landscape and occasionally include satellite animal burials. Specific features of burial mounds (stone type, shape and ringed fences) can be used to identify interred individuals into different culture groups as mound construction styles changed alongside evolving cultural traditions in Bronze Age Mongolia\cite{15,16}.

Prior to the Bronze Age, before the presence of constructed stone burial mounds, there are very few uncovered occupation or ritual sites, and pre-Bronze Age subsistence strategies are not well understood. However, it is assumed that Neolithic subsistence strategies included hunting, gathering and fishing, although the possibility of pastoralism should not be completely discounted\cite{14}. Human burials associated with Afanasievo and Chemurchek culture groups (circa 3000–2500 BC) contain faunal remains of ovicaprid, bovine, equid and dog remains\cite{15,17,18}; yet it is unclear whether the remains of each were of a domesticated variety or their wild relatives, such as Ovis ammon and Capra ibex in the case of ovicaprids\cite{19}. Later Bronze Age (1500–800 BC) campsites containing ruminant and equine remains suggest the dietary consumption of horses and cattle, as well as sheep and/or goats as part of a fully pastoral economy\cite{21}. Satellite burials containing multiple animals surrounding ritual human internments attest to pastoral culling and herd management patterns of sheep, goat, cattle and horses during this period\cite{22,23}. By 1200 BC, remains of domesticated horses became almost ubiquitous at ritual burials sites in Northern Mongolia, with some of the crania showing evidence for equine dentistry and horse bridling and riding\cite{24,25}.

In later time periods, written records from neighbouring regions, such as China and the Middle East, as well as within Mongolia, document the importance of ruminant and equine dairy in day-to-day subsistence, particularly the consumption of fermented horse milk as early as the Xiongnu Empire (circa 200 BC to AD 100)\cite{26} along with camel milk by the Mongol period (circa AD 1206–1398)\cite{27,28}. Even though many archaeologists and historians assume milk had been included in ancient steppe diets, little direct evidence has been available about where and when specific animal species were first exploited for dairy on the steppe east of the Altai Mountains.

The analysis of ancient proteins extracted from ancient human dental calculus (calciﬁed dental plaque or tooth tartar) has been established as an approach for detecting milk consumption in past individuals\cite{29,30}. Differences in amino acid sequences between taxa enable the detection of the livestock species or ‘zooarchaeology by proxy’—a method to detect past animal use by the analysis...
of human remains alone. Here, we apply the proteomic analysis of ancient dental calculus to 32 individuals spanning from the late Neolithic through the Middle Ages to characterize the antiquity and species diversity of ruminant and equine dairying in Mongolia. We report the earliest direct evidence for dairy consumption in East Asia (east of the Altai Mountains), finding that ruminant dairy consumption was a feature of ancient diets in Mongolia from its initial pastoral occupation, circa 3000 BC and occurred in association with archaeological sites linked to western steppe cultures. We show that ruminant dairying became widespread by the Middle Bronze Age (1800–1200 BC) and from 1200 BC we observe the onset of horse milk consumption, perhaps the progenitor of the alcoholic drink airag, in tandem with the first evidence for mounted horseback riding and highly mobile economies—a tradition that remained important through the great nomadic empires and into the modern era.

**Results**

Milk proteins were identified in 72% of individuals analysed (23 of 32 individuals, Table 1), indicating the widespread consumption of dairy foods across multiple time periods in prehistoric and historic Mongolia. Specifically, we detected evidence of the milk proteins β-lactoglobulin (BLG I and II), α-S1-casein, kappa-casein, α-lactalbumin and β-casein, lysozyme C and peptidoglycan recognition protein 1. BLG was the most frequently detected milk protein, a pattern consistent with previous observations from ancient dental calculus. We observe evidence of milk consumption from diverse taxa across multiple environmental zones within Mongolia (Fig. 2). Of the seven dairy livestock species used in contemporary Mongolia, we identified milk peptides from goat, sheep, cattle, horse and camel but did not detect peptides that could be assigned specifically to reindeer or yak milk.

We find evidence of milk proteins in the earliest directly dated individual in our sample set, AT-26 (3316–2918 cal BC; 2σ range) at the Afanasievo burials of Shatar Chuluu, the earliest known mounded burial features associated with pastoral economies in the territory of Mongolia. Specifically, we observe peptides deriving from a taxonomically ambiguous region of the milk whey protein BLG, where the species can be assigned as a bovid in Bovinae subfamily (cow, yak, bison and water buffalo) or Ovis genus (sheep), making a more specific taxonomic assignment for this individual's milk consumption more challenging. In the two individuals associated with Chermurcheek culture at the sites of Khundii Gobi (2886–2577 cal BC; 2σ range) and Yagshiin Khuduu (2567–2468 cal BC; 2σ range), we detect milk peptides matching to the subfamily Caprinae (sheep or goat) and the genus Ovis (sheep) from BLG and also α-S1-casein. At Khundii Gobi, these identifications are specific to sheep (with six BLG peptides identified) among others that are specific only to the subfamily Caprinae and the infraorder Pecora (all even-toed ruminant mammals). These results align with recent archaeofaunal data from the early second millennia BC, which suggest a significant role for sheep in the prehistoric economy of pastoral occupants of the Mongolian Altai.

In the Middle Bronze Age (circa 1800–1200 BC) the consumption of ruminant dairy milk can be seen in four of seven individuals analysed in the central eastern site of Ulaanzuukh and in seven of nine individuals in the previously published Khövsgöl sites in northern central Mongolia. Ulaanzuukh individuals date to slightly earlier than the Bronze Age burial sites analysed in Jeong et al., where evidence of ruminant milk consumption was found in individuals associated with slope burials and khirigsuur ritual monuments. At Ulaanzuukh, BLG is the most frequently recovered protein across all samples, with peptides from α-S1-casein and kappa-casein (proteins that are associated more with milk curds than milk whey) identified to a lesser extent. The dental calculus from the three Ulaanzuukh individuals who did not show evidence of dairy consumption showed a poor level of protein preservation, with a general absence of typical salivary and bacterial proteins reported previously in dental calculus studies, which may suggest that an absence of evidence for milk consumption in these individuals could be due to overall poor biomolecular preservation.

At Bronze Age sites dating to after 1200 BC, we found evidence for dairy consumption in all four individuals tested. We identified ruminant milk peptides from the same two proteins (BLG and α-S1-casein) identified in the previous time period, as well as from two additional proteins: α-lactalbumin and β-casein. In addition to ruminant milk proteins, we also detected the first palaeoproteomic evidence of horse (Equus) milk, including horse-specific peptides from BLG I and II (horse BLG is derived from two paralogous genes) in two of four individuals. An individual from the site of Shunklai Uul (circa 1000 BC), in central Mongolia, had 126 peptide spectral matches (PSMs) from ruminant BLG, another 50 from horse BLG I and II (Fig. 3) and an additional nine from α-S1-casein, β-casein and α-lactalbumin.

Of the three Early Iron Age individuals (800–400 BC), two from the northwestern Mongolia site of Chandman Mountain and another from Dartsag (north-central Mongolia), all showed moderate to high preservation and contained an abundance of human and oral microbiome proteins. Two of these contained no robustly identified milk peptides; in contrast, one individual from Chandman Mountain contained an abundance of PSMs to milk proteins. During this period, BLG peptides specific to both sheep and goat were detected, along with others that can be assigned to the higher taxonomic orders of Caprinae and Bovidae. Also, we find peptides derived from casein and α-lactalbumin specific to caprines, and those from both BLG I and II specific to equine milk.

In the Late Iron Age, during the tenure of the Xiongnu Empire (circa 200 BC to AD 300), calculus from each of the three individuals studied contained evidence for dairy consumption. One individual had PSMs specific to only horse BLG, while the second had Bos-specific BLG and caprine α-S1-casein peptides. The third individual had ruminant casein and whey proteins (BLG and α-S1-casein) as well as lysozyme C peptides specific to Equus. In the post-Xiongnu period, a single individual archaeologically classified as Turkic era did not show any evidence of milk consumption.

Nine of the eleven Mongol Empire individuals showed evidence for the consumption of dairy, with many individuals showing evidence for the consumption of milk from multiple species. For example, one individual showed evidence for the consumption of ruminant, equine and camel milk, whilst a further five individuals showed evidence for the consumption of both ruminant and horse dairy products (Fig. 3). During this period, we observed the first evidence for the consumption of camel milk through the detection of peptidoglycan recognition protein 1 (UniProtKB: Q9GK12), an immune protein that has been isolated from modern camel milk.

Deamidation of glutamine and asparagine has been proposed as a marker of taphonomic degradation in ancient proteins. We applied an analysis of bulk deamidation using a previously published approach to five individuals from this study to examine potential patterns of archaeological degradation in milk, which may suggest that the milk proteins in our samples are ancient, as they appear to be of similar deamidation levels as the human oral proteins in the calculus. All deamidation is reported in Supplementary Table 2. We observed that the older samples generally showed higher levels of deamidation. The milk-origin peptides retrieved from the early Bronze Age individuals showed an average of 23.9% glutamine deamidation, the Late Bronze Age 13.5% and the Mongol period 3.3%. The same pattern was observed in the deamidation of asparagine, with an average of 52.6% deamidation of milk-origin peptides in the Early Bronze Age and 32.9% in the Late Bronze Age. However, the milk peptides recovered from the Mongol period individual do not fit this pattern in terms of asparagine deamidation, with 48.3% deamidation (Supplementary Table 2).
Earliest evidence for dairying is associated with western steppe herder archaeological cultures. Our results demonstrate the oldest known evidence of dairy consumption in Mongolia and the eastern Eurasian steppe (circa 3000–2500 bc), in the form of Early Bronze Age Afanasievo- and Chemurchek-associated individuals in both central and western Mongolia (Table 1). Previous ancient DNA analysis of one of the individuals at Shatar Chuluu showing ruminant dairy proteins (AT-26) was shown to have a non-local mitochondrial haplogroup consistent with western steppe herder populations37, supporting the interpretation of individuals associated with the Afanasievo culture as migrants from eastern Europe.

### Table 1 | Presence of dairy proteins by individual and archaeological site

<table>
<thead>
<tr>
<th>Archaeological culture</th>
<th>Archaeological site</th>
<th>Individual ID</th>
<th>Calibrated radiocarbon date</th>
<th>Milk species/taxonomic group identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Bronze</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afanasievo</td>
<td>Shatar Chuluu</td>
<td>AT-26</td>
<td>3316–2918 bc</td>
<td>Bovinae/Ovis*</td>
</tr>
<tr>
<td>Chemurchek</td>
<td>Khundii Gobi</td>
<td>AT-628</td>
<td>3310–2919 bc</td>
<td>Ovis, Bovinae/Ovis</td>
</tr>
<tr>
<td>Chemurchek/ Afanasievo</td>
<td>Khurai Gobi</td>
<td>AT-635</td>
<td>2618–2487 bc</td>
<td>None detected</td>
</tr>
<tr>
<td>Chemurchek</td>
<td>Yagshin Huduu</td>
<td>AT-590b</td>
<td>2567–2468 bc</td>
<td>Ovis, Bovinae/Ovis*, Caprinae</td>
</tr>
<tr>
<td>Middle Bronze</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulaanzuukh</td>
<td>Ulaanzuukh</td>
<td>AT-823</td>
<td>1391–1209 bc</td>
<td>None detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT-921</td>
<td>1412–1266 bc</td>
<td>Capra, Ovis, Bovidae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT-923</td>
<td>No date</td>
<td>Bovinae/Ovis*</td>
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<td></td>
<td></td>
<td>AT-824</td>
<td>1402–1279 bc</td>
<td>None detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT-769A</td>
<td>1608–1416 bc</td>
<td>Bovidae, Bovinae/Ovis*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT-769B</td>
<td>1509–1439 bc</td>
<td>None detected</td>
</tr>
<tr>
<td>Late Bronze</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baitag</td>
<td>Uliastai River, Central Terrace</td>
<td>AT-676</td>
<td>1277–1057 bc</td>
<td>Equus, Capra, Ovis, Bovinae</td>
</tr>
<tr>
<td>Deer Stone/ Khirigsuur</td>
<td>Berkh Uul</td>
<td>AT-905</td>
<td>1371–1121 bc</td>
<td>Ovis, Capra, Bovinae/Ovis*</td>
</tr>
<tr>
<td>Slab Burial</td>
<td>Shunkhlaa Uul</td>
<td>AT-233</td>
<td>1072–903 bc</td>
<td>Equus, Capra, Ovis, Bos</td>
</tr>
<tr>
<td>Undefined</td>
<td>Khoit Tsenkher</td>
<td>AT-398</td>
<td>1056–904 bc</td>
<td>Capra, Bovinae/Ovis*</td>
</tr>
<tr>
<td>Early Iron</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chandmani</td>
<td>Chandman Uul</td>
<td>AT-56</td>
<td>971–843 bc</td>
<td>Equus, Capra, Ovis, Bovinae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT-121</td>
<td>358–195 bc</td>
<td>None detected</td>
</tr>
<tr>
<td>Slab Grave</td>
<td>Dartsagt</td>
<td>AT-766</td>
<td>750–407 bc</td>
<td>None detected</td>
</tr>
<tr>
<td>Late Iron</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xiongnu</td>
<td>Tamiryn Ulaan Khoshuu</td>
<td>AT-728</td>
<td>No date</td>
<td>Equus</td>
</tr>
<tr>
<td>Xiongnu</td>
<td>Duulaga Uul</td>
<td>AT-605</td>
<td>43 bc to AD 51</td>
<td>Bos, Bovinae/Ovis*, Caprinae</td>
</tr>
<tr>
<td>Iron Age</td>
<td>Ulaanzuukh</td>
<td>AT-885</td>
<td>cal 96 bc to AD 61</td>
<td>Caprinae, Bovinae/Ovis*</td>
</tr>
<tr>
<td>Early Medieval</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkic</td>
<td>Uliastain dood denj</td>
<td>AT-675</td>
<td>cal AD 650–765</td>
<td>None detected</td>
</tr>
<tr>
<td>Late Medieval (Mongol period)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Sharga Uul</td>
<td>AT-701</td>
<td>No date</td>
<td>Equus</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Del Khad</td>
<td>AT-775</td>
<td>No date</td>
<td>Equus, Ovis, Camelus</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Zaraa Tolgoi</td>
<td>AT-271</td>
<td>No date</td>
<td>Equus, Bovinae/Ovis*</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Banzart Khairkhan</td>
<td>AT-846</td>
<td>No date</td>
<td>Equus, Bovinae/Ovis*</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Tahlityn Khotgot</td>
<td>AT-360</td>
<td>No date</td>
<td>Equus</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Burgaldain Khundii</td>
<td>AT-650</td>
<td>No date</td>
<td>Equus, Caprinae,</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Ganzagad</td>
<td>AT-835</td>
<td>No date</td>
<td>Equus, Capra, Bovinae/Ovis*</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Uguumur Tsuvaraa Uul</td>
<td>AT-549</td>
<td>No date</td>
<td>Capra, Ovis, Bovinae</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Khoit Tsenkher, Tarvagatain Am</td>
<td>AT-354</td>
<td>AD 1158-1252</td>
<td>Equus, Caprinae, Bovidae</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Kharkhorin</td>
<td>AT-512</td>
<td>No date</td>
<td>None detected</td>
</tr>
<tr>
<td>Mongol Empire</td>
<td>Mori Baridag</td>
<td>AT-800</td>
<td>No date</td>
<td>None detected</td>
</tr>
</tbody>
</table>

Radiocarbon dates are calibrated to a 2σ probability (Supplementary Table 6). Bovinae/Ovis* identifications refer to the detection of a polymorphic amino acid position where in the identified peptide TPEVD(D/N/K)EAELEK, D is specific to Bovinae, N is specific to Ovis and K is specific to Capra, Because asparagine (N) deamidates to aspartic acid (D), the presence of a D at this position cannot be unambiguously assigned to Bovinae or Ovis. References and details of archaeological site excavations can be found in the Supplementary Table 1. All identified dairy peptides per individual are detailed in Supplementary Dataset 2.
via the Russian Altai38–40 and a probable vector for the initial introduction of ruminant domestic animals into Mongolia41. The identification of ruminant milk proteins (Ovis and Bovinae/Ovis) supports the domestic nature of fauna found in these burials and indicates that dairy pastoralism formed an important element of the subsistence base of these late fourth millennium bc transcontinental migrants. Most significantly, they suggest that human migrations associated with the expansion of the Afanasievo culture present a viable candidate for the initial introduction of dairy and domestic livestock into eastern Eurasia. In this study, the earliest individual in this dataset showed evidence of dairy consumption. To track the earliest instances of dairy consumption in the eastern Steppe, it would be necessary to analyse individuals from earlier time periods. This would be particularly informative to untangle the presence or absence of dairying before Yamnaya–Afanasievo migrations.

The antiquity of eastern steppe horse milk consumption. Temporal patterning in our protein results suggest that horse milk consumption played a key role in the emergence and proliferation of mobile pastoralism in Mongolia (Fig. 4). Today, horses play a vital role in traditional Central Asian pastoral lifeways, improving herd management as well as providing a primary source of meat and milk. Horseback herders can manage larger herd sizes. Horses can break through snow and ice to access the sustenance underneath, exposing grass oases for other animals in the herd42–43. We observe direct evidence of horse milk consumption on the eastern steppe, in the form of equine (Equus)-specific peptides from the milk whey proteins BLG (I and II) and lysozyme C in individuals associated with the Baitag and Slab Grave cultures in western Mongolia dated to the late second millennium bc (Table 1). In addition to the appearance of horses in dietary assemblages41, this time period is linked with the proliferation of horses in ritual sites, the first direct archaeological evidence for horse bridling and riding44, the first evidence for horse breeding and management45,46, innovations in horse healthcare47, an expanded use of dry intermontane grasslands41–45 and the emergence of mobile, horse-facilitated pastoralism in eastern Eurasia. Our findings suggest that the incorporation of horses into dairy herds may have been closely linked to this multifaceted economic transformation in the use of horses48.

Following the Bronze Age, direct proteomic evidence for horse milk consumption continues through the imperial Xiongnu and Mongol periods (Table 1), in agreement with extensive textual and zooarchaeological evidence underscoring their significance to historic economies. During the Iron Age, we identify horse milk proteins at Tamiryin Ulaan Khoshuu, a site within the heartland of the Xiongnu Empire49–51. Historical Chinese documents record that an assortment of dairy products were consumed during the Xiongnu period (circa 200 bc to ad 100), including dried curds from ruminant
milk (aaruul) but lao (horse alcohol) was the most consistently referenced dairy product and it held a prominent place in the cultural practices and identities of the steppe peoples. By the Mongol period, horse milk consumption appears in among >80% (9 of 11) of tested individuals, a finding that matches historical accounts for widespread consumption of fermented mare’s milk, known as airag (Mongolian) or koumiss/kymyz (Turkic languages).

Horse milk differs from ruminant milks in important ways, in particular, it contains less curd protein (caseins) and much more lactose. While the lower casein content makes it undesirable for producing most dried curd products (like aaruul, a staple of the traditional Mongolian diet), its high lactose content makes it highly suitable for making alcoholic beverages with ethanol contents as high as 10–12% (ref. 33). Communal airag-drinking was and continues to be an important social activity and it has been frequently referenced in historical texts. The cultural significance of airag continued throughout the Mongol period, with social gatherings at the time referred to as ‘going to drink airag with people’, and social rankings reinforced by how close one sat in relation to the pitchers of airag. Favoured associates were charged with serving the airag and with choosing the order in which airag was served at feasts. At the Mongol capital of Kharkorum, a silver fountain was said to flow with fermented mare’s milk, dispensing airag.

Identification of other milks and fermentation agents. In addition to cattle, sheep, goats and horses, domesticated Bactrian camels (Camelus bactrianus), yaks (Bos grunniens) and reindeer (Rangifer tarandus) are also milked in contemporary Mongolia; however, very little is known about the dairying history of these three species. Here we report protein evidence of camel milk consumption (together with horse and sheep milk) in a Mongol period individual buried in the Gobi Desert (Table 1), a habitat of Bactrian camels. Although not unique to milk in other mammal species, the camel protein peptidoglycan recognition protein 1 (PRP1), is an important component of camel milk whey. Mongol period historical accounts, such as the twelfth-century Secret History of the Mongols, and historic accounts from foreign travellers into the empire, such as Marco Polo, include stories of camels used for subsistence (milk, meat and blood) and transport of people and gers (round tents). While probably not the earliest instance of camel milk-drinking, the PRP1 protein data presented here provide biomolecular evidence for its consumption. Camel dairy use is not well understood and this finding provides an insight into exploitation of this historic traction animal for milk.

We did not detect any milk peptides specifically identified as yak or reindeer in this study. While yak is a commonly herded species in Mongolia today, their past use as a dairying animal is not well understood. Yak proteins are difficult to specifically identify due to their sequence similarity to other cattle species. Yak BLG, for example, differs from that of cattle at only a single amino acid across the entire BLG protein. While it is possible that some of the peptides assigned in this study to Bos or the higher taxonomies of Pecora,
Bovidae and Bovinae could have originated from domestic yak, we only observed cattle-specific variants when sufficient protein coverage enabled it. The absence of reindeer-specific milk consumption may be expected based on historical accounts on the use of this animal for dairying in this region. Contemporary reindeer herders in Mongolia do milk their animals but these families migrated from Siberia into northern Mongolia only in the last century.

In the present study, we did not identify any proteins specific to any bacterial taxa or other fermentation agents used in the processing of milk into other dairy products. Although processing agents were not found in the samples analysed in this study, it does not mean these were not used in ancient and historic Mongolia and future studies should continue to look for their presence alongside milk peptides.

These results provide the earliest evidence for dairy pastoralism in Mongolia’s first herding societies, pushing back previous estimates of dairying in the Eastern steppe by more than 1,700 years and tracing pastoralist connections between the western and eastern steppes to the Early Bronze Age. These results show that within 5,000 years after the earliest evidence for dairying in the Near East, this practice and its associated animals had spread more than 7,000,000-km eastward to become a successful mode of subsistence on the Mongolian steppe. While the routes of this movement remain to be fully understood, our data suggest that dairy pastoralism, and in particular the emergence of horse riding and horse milking circa 1200 bc, provided the economic, political and social support necessary for the success of subsequent nomadic empires on the vast grasslands of Eurasia.

Methods
Sample collection. Dental calculus samples were collected from the Department of Anthropology and Archaeology at the National University of Mongolia (NUM) from 32 previously excavated individuals (Table 1, listed by NUM accession number; see Supplementary Table 1 for site details). Individuals were selected from archaeological sites assigned to time periods between the Neolithic and the Mongol period. Dental calculus was removed from the tooth using sterilized dental scalars and stored in Eppendorf tubes until extraction. Nitrile gloves were used during sample collection to avoid contamination from skin proteins. Samples were
exported to the Max Planck Institute for the Science of Human History under permission from the Ministry of Culture, Education, Science and Sports (export no. 10/413 (7b/52) was received on 2 February 2017, no. A010258, MN DE 7 643). Protein extractions were conducted in a dedicated laboratory for the determination of ancient proteins at the Max Planck Institute for the Science of Human History, Jena, using a filter-aided sample preparation protocol previously published in Jeong et al. Following protein extraction, digested peptides were stored at −80°C before being analysed by liquid chromatography tandem mass spectrometry (LC–MS/MS) at the Functional Genomics Center Zürich, ETH/University of Zürich.

LC–MS/MS analysis. Mass spectrometry analysis was performed on a Q Exactive HF mass spectrometer (Thermo Scientific) equipped with a Digital PicoView source (New Objective) and coupled to an m-Class UPLC (Waters). Solvent composition at the two channels was 0.1% formic acid for channel A and 0.1% formic acid, 99.9% acetonitrile for channel B. Column temperature was 50 °C. For each sample 4 µl of peptides were loaded on a commercial ACQUITY UPLC M-Class Symmetry C18 Trap Column (100 Å, 5 µm, 180 × 2 mm, Waters) followed by ACQUITY UPLC M-Class HSS T3 Column (100 Å, 1.8 µm, 75 µm × 250 mm, Waters). The peptides were eluted at a flow rate of 300 nl/min by a gradient from 40% B to 100% B in 120 min. Column was cleaned after the run by increasing to 98% B and holding 98% B for 5 min before re-establishing loading condition. Samples were acquired in a randomized order. The mass spectrometer was operated in data-dependent mode (DDA), acquiring a full-scan MS spectra (350–1,500 m/z) at a resolution of 120,000 at 200 m/z after accumulation to a target value of 5,000,000, and a maximum injection time of 50 ms followed by higher energy collision dissociation fragmentation on the 12 most-intense ions per cycle. Higher-energy collision dissociation spectra were acquired at a resolution of 30,000 using a normalized collision energy of 28 and a maximum injection time of 50 ms. The automatic gain control was set to 100,000 ions. Charge state screening was enabled. Singly, unsingly and charge states higher than eight were rejected. Only precursors with intensity above 90,000 were selected for MS/MS. Precursor masses previously selected for MS/MS measurement were excluded from further selection for 30 s and the exclusion window was set to 10 ppm. The samples were acquired using internal lock mass calibration on m/z 371.0122 and 445.1200.

Data analysis. To account for as much variation of milk-associated proteins as possible during MS/MS ion searches, a supplementary database of unreviewed milk protein sequences was curated from UniProtKB. As an additional source of dairy protein sequences, genomic data covering the two BLG genes in ancient horses generated by Gaunitz and colleagues were translated into amino acid sequences, aligned with their respective modern sequences, and any putatively divergent proteins were concatenated to the supplementary database. In total, 244 additional accessions from UniProtKB and four putatively divergent BLG accessions from ancient horse genomes were added (Supplementary Dataset 1). Peak lists were generated from raw files by selecting the top 100 peaks using MzConvert from the ProteoWizard software package v.3.0.11781 (ref. 1). Each sample was searched using Mascot (v2.6.2) against Swiss-Prot in combination with the curated milk protein database (Supplementary Dataset 1). Results were exported from Mascot as csv files and further processed through an internally created tool, MS-MARGE (ref. 2) to estimate the validity of peptide identifications and summarize the findings. MS-MARGE is an R script that relies on an Rmarkdown file to generate the following: an R markdown report summarizing the search (an example of the Supplementary Information file), a csv file containing confidently identified PSMs and a FASTA file of confidently identified peptide sequences. As input, MS-MARGE accepts a csv file exported from a Mascot MS/MS ion search against an amino acid database containing decoyed sequences with the Group Protein Families option turned off. Additionally, two parameters can be provided: an expected value (e-value) cutoff and a minimum number of peptides to support an identification (default 0.01 and 2, respectively). To estimate false discovery rate (FDR) at the PSM and protein level, MS-MARGE counts the number of decoy hits after filtering for e-value and minimum peptide support, and divides this value by the number of target hits minus the number of decoys. The resulting value is multiplied by 100 to provide the estimate of FDR as a percentage. We aimed for a protein FDR of under 5% and a peptide FDR of under 2% (Supplementary Dataset 2). A minimum of two individual PSMs were required for specific protein identifications and only peptides with an e-value below 0.01 were accepted. After filtering criteria were applied, we observed a range of variation in the numbers of proteins identified, with samples ranging from 2 to 209 confidently identified protein families.

Demarcation levels in five samples (AT-628, AT-590b, AT-26, AT-233 and AT-835) were calculated to assess authenticity using a previously published approach (Extended Data Fig. 1 and Supplementary Table 2). These samples were specifically chosen to verify the antiquity of the oldest samples in this study, as well as a more recent sample from the Mongol period for comparison of deamination patterns. The raw MS/MS files for each were run through MaxQuant v1.6.2.6a against the previously described milk database and against the human proteome. Settings including a semitryptic search strategy allowing for a maximum of two missed cleavages were used, and the score cutoff for modified and unmodified peptides was set to 60 with no correction for FDR. Carbamidomethyl (C) was added as a fixed modification, while variable modifications included: oxidation (M), acetyl (protein N-term), deamidation (NQ), Gln-pyro-Glu (N-term E), Glu-pyro-Glu (N-term E), phospho (ST) and hydroxyproline. The deamination levels of all milk proteins were averaged per sample. All identified peptides, including their posttranslational modifications, were reported in Supplementary Dataset 2.

Radiocarbon dating. A total of 26 bones and teeth from 24 individuals were radiocarbon dated at two research facilities, the Oxford Radiocarbon Accelerator Unit (ORAU, laboratory code OxA) and the Groningen Radiocarbon Laboratory (laboratory code GrM). The ORAU followed routine pretreatment and measurement protocols for bone and dentine. In brief, 200–600 mg of bone or dentine was drilled using a hand-held dentist drill and collagen was extracted through a series of chemical steps that involved immersion in HCl, removal of humic acids using NaOH and removal of adsorbed CO2 via a final HCl wash. Only four of the samples prepared at the ORAU (OxA-36230, OxA-36231, OxA-36232 and OxA-36233) underwent ultrafiltration using Vivaprot ultraturfers, due to initial indications of poor collagen preservation. Extracted collagen was frozen overnight and lyophilized. Between 2 and 5 mg of collagen was combusted in an elemental analyser (EA) and its C and N stable isotope ratios were measured at an isotope ratio mass spectrometer (IRMS) instrument linked to the EA, before excess CO2 was collected, graphitized and measured at a high Voltage Engineering Europa (HVVE) accelerator, alongside blanks and standards. These were used for contamination calculation and final correction of the data. For these samples, collagen yields ranged greatly from 0.8% to 17.8%, C/N ratios of the extracted collagen fall within expected ranges (3.2–3.4), with the exception of OxA-36233 (CN = 3.6), and percentage C in the combusted collagen was 37–46%. The measurements are reported in radiocarbon years before present (BP), where BP = AD − year. Samples from Oxford were calibrated using OxCal v4.3.2 (ref. 1) and an IntCal13 atmospheric curve (ref. 1).

For radiocarbon dates processed at the Groningen Radiocarbon Laboratory, samples were decalcified over at least a 24-h period using mild acid (HCl, 2–4% w/v; room temperature) at the Center for Stable Isotope Research at the University of Groningen. For each sample still not fully decalcified, the solution was refreshed, removing and storing soft portions separately in demineralized water until further preparation. Soft and pliable fragments were rinsed thoroughly with demineralized water. Extracts were then exposed to NaOH (1%, −30 min) to eliminate humic acids, rinsed to neutrality and treated once more with acid (HCl, 4% w/v, 15 min). The raw collagen fraction was denatured to gelatin in acidified demineralized water (pH 3) at 80 °C for 18 h. Before drying, the dissolved gelatin was filtered through a 50-µm mesh to eliminate any remaining foreign particulates and the crystalline collagen scraped from the glass. Approximately 4-mg aliquots of the reduced carbon fraction were then weighed into tin capsules for combustion in an EA (IsotopeCube NCS, Elemental). The EA was coupled to an IRMS (Isoprime 100), allowing the δ13C value of the sample to be measured, as well as a fully automated cryogenic system to trap CO2 liberated on combustion. After run completion, the individual reaction vessels were transferred to a graphitization manifold, where a stoichiometric excess of H2 gas (1.25) was added and the CO2 gas reduced to graphite over an Fe(s) catalyst. The graphite samples were then pressed and the radioisotopic ratio determined on a MICA/ADAS accelerator mass spectrometer. Samples from Groningen were calibrated using OxCal v4.3.2 (ref. 1) and an IntCal13 atmospheric curve (ref. 1).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Raw and processed MS/MS data from blanks, instrument washes and samples are available to download via the PRIDE partner repository under accession codes PXD014730 and PXD014730. The authors declare that all other data supporting the findings of this study are available within the paper and its supplementary information files. We have commissioned a Mongolian translation of this manuscript, available at https://natureecoevocommunity.nature.com/manage/posts/59870-mongolian-translation-of-dairy-pastoralism-sustained-eastern-steppe-populations-for-5000-years/edit.

Code availability. MS-MARGE, an R script used to estimate the validity of peptide identifications and summarize the findings is available for use via Bitbucket: https://bitbucket.org/whagan/ms-marge/src/master/. The custom dairy database used to analyse the data in this study is available to download via the York Research Database at https://doi.org/10.15122/0589742b-287a-4576-40a0-30df53d9f52c.

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Author contributions

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | Milk and bulk deamidation and peptide counts.
5. Manuscript C

“Economic Diversification Supported the Growth of Nomadic Mongolian Empires”


This project was published in *Scientific Reports* in March 2020

Project 3 explores the first and continued use of C₄ plants (millet) in Mongolia through stable isotope analysis of human bone collagen and dental bioapatite spanning between the Neolithic and Mongol periods (4500 BCE – 1400 CE).

- Measure range of carbon and nitrogen stable isotope values for human bone collagen and the range of carbon stable isotope values for human tooth enamel in each time period in Mongolia
- Identify when C₄ plants became a primary part of eastern steppe diets
- Investigate how dietary economies changed during times of empire and whether characterization as specialised pastoralists is appropriate

Author contributions: **Shevan Wilkin**, William Taylor, Nicole Boivin, and Patrick Roberts designed the research plan; **Shevan Wilkin**, Alicia Ventresca Miller, Bryan K. Miller, Richard W. Hagan, Ricardo Fernandes, Madeleine Bleasdale, Erdene Myagmar, and Patrick Roberts performed research; Nicole Boivin contributed personnel and material resources; **Shevan Wilkin**, Alicia Ventresca Miller, Bryan K. Miller, William Taylor, Ricardo Fernandes, Richard W. Hagan, analysed data; and **Shevan Wilkin**, Alicia Ventresca Miller, Bryan K. Miller, and Patrick Roberts wrote the paper. In total, **Shevan Wilkin** contributed to 80% of this research.
Economic Diversification Supported the Growth of Mongolia’s Nomadic Empires

Shevan Wilkin1*, Alicia Ventresca Miller1,2, Bryan K. Miller1, Robert N. Spengler III1,2, William T. Taylor1,3, Ricardo Fernandes1,4,5, Richard W. Hagan6, Madeleine Bleasdale6,7, Jana Zech3, S. Ulziibayar7, Erdene Myagmar8, Nicole Boivin1,9,10,11 & Patrick Roberts1,9*

Populations in Mongolia from the late second millennium B.C.E. through the Mongol Empire are traditionally assumed, by archaeologists and historians, to have maintained a highly specialized horse-facilitated form of mobile pastoralism. Until recently, a dearth of direct evidence for prehistoric human diet and subsistence economies in Mongolia has rendered systematic testing of this view impossible. Here, we present stable carbon and nitrogen isotope measurements of human bone collagen, and stable carbon isotope analysis of human enamel bioapatite, from 137 well-dated ancient Mongolian individuals spanning the period c. 4400 B.C.E. to 1300 C.E. Our results demonstrate an increase in consumption of C₄ plants beginning at c. 800 B.C.E., almost certainly indicative of millet consumption, an interpretation supported by archaeological evidence. The escalating scale of millet consumption on the eastern Eurasian steppe over time, and an expansion of isotopic niche widths, indicate that historic Mongolian empires were supported by a diversification of economic strategies rather than uniform, specialized pastoralism.

Mongolian empires, such as the Xiongnu and Mongols, are some of the most renowned imperial entities in public and academic thought. This is, in part, due to their historical portrayal as highly mobile, predatory horseback polities with a specialized dairy and meat-based economy1–4, an image that is perpetuated in cinema, novels, and documentaries alike. While such stereotypes likely arose from the hyperbolized accounts of neighboring adversaries, starting with the Han, who fought against the Xiongnu5, they have persisted and now pervade academic evaluations of the economic basis of these ancient peoples. The modern economic focus on pastoralism in rural areas of Mongolia today is frequently viewed as a relic of the past and has been drawn upon to interpret the often-fragmentary archaeological record of this region6–7, although ethnoarchaeological approaches often ignore the role of urban markets and motorized transport in modern mobile pastoralism. The view of uniformly specialized pastoral economies has also furthered the scholarly fascination with historical Mongolian populations, resurrecting the long-standing question of whether an empire can meet the costs and challenges of long-term political and economic organization in the absence of grain surpluses6–7.

Empires are, however, inherently complex and, by definition, extend their control over multiple societies, cultures, and economies, as well as heterogeneous landscapes8–10. Crop surplus has traditionally been viewed as an essential component of stable political entities and complex imperial food production and procurement systems are often over-simplified by historians and archaeologists, leading to their characterization as single-resource systems (such as maize for the pre-Columbian empires of South America). Refined analyses generally reveal diverse and dynamic economies supporting imperial expansions, which draw together a variety of food sources11.

1Max Planck Institute for the Science of Human History, Department of Archaeology, Jena, Germany. 2University of Michigan, Department of Anthropology, Ann Arbor, Michigan, USA. 3University of Colorado, Department of Anthropology, Museum of Natural History, Boulder, CO, USA. 4School of Archaeology, University of Oxford, Oxford, UK. 5Faculty of Arts, Masaryk University, Brno, Czech Republic. 6Max Planck Institute for the Science of Human History, Department of Archaeo genetics, Jena, Germany. 7Institute of Archaeology and Ethnology, Mongolian Academy of Sciences, Jukovin orgon chuluu 77, Ulaanbaatar, Mongolia. 8National University of Mongolia, Ulaanbaatar, Mongolia. 9School of Social Science, The University of Queensland, Brisbane, Australia. 10Department of Anthropology and Archaeology, University of Calgary, Calgary, Alberta, Canada. 11Department of Anthropology, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA. *email: wilkin@shh.mpg.de; roberts@shh.mpg.de
As a consequence, it is perhaps unsurprising that archaeological, archaeobotanical, and historical records are beginning to strongly hint at the possibility that historical Mongolian empires were not solely reliant on dairy pastoralism, but also featured agriculture, as well as craft specialization, and participation in trade systems that spanned thousands of kilometers.

Of particular interest in this context has been the growing archaeobotanical evidence from across Central Asia that demonstrates an influx of millet, both broomcorn (Panicum miliaceum L.) and foxtail (Setaria italica L.), and other domesticated grains in the surrounding steppeland lands of Siberia, Kazakhstan, and northwestern China during the second and first millennia B.C.E. There are some archaeobotanical data suggesting the use of crops in Mongolia starting around c. 100 B.C.E. – 200 CE, though these have been dismissed as reflective of trade rather than local production. Overall, due to issues of wind deflation and a lack of sampling during excavation, archaeobotanical evidence from Mongolia is severely lacking. Moreover, where present, it is difficult to determine the degree to which an archaeobotanical assemblage represents overall dietary reliance. To date, there have been no systematic, direct analyses of the consumption of domesticated crops among peoples in this region over the past three millennia, leaving the economic basis for some of the world’s most famous empires unresolved.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Date Range</th>
<th>Mean δ13C (%) (VPDB; SD)</th>
<th>δ13C (VPDB) Range</th>
<th>Mean δ15N (%) (AIR; SD)</th>
<th>δ15N (%) (AIR) Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early (n = 14)</td>
<td>4400–800 B.C.E.</td>
<td>−17.3 ± 0.8</td>
<td>−18.5 – −16.2</td>
<td>+12.8 ± 1.0</td>
<td>+11.0 – +14.6</td>
</tr>
<tr>
<td>Early Iron (n = 7)</td>
<td>800–200 B.C.E.</td>
<td>−16.0 ± 0.8</td>
<td>−16.8 – −14.8</td>
<td>+13.6 ± 1.1</td>
<td>+12.2 – +14.8</td>
</tr>
<tr>
<td>Xiongnu (n = 47)</td>
<td>200 B.C.E.–250 C.E.</td>
<td>−16.0 ± 1.3</td>
<td>−18.5 – −13.1</td>
<td>+13.2 ± 1.3</td>
<td>+7.9 – +15.5</td>
</tr>
<tr>
<td>Mongol (n = 38)</td>
<td>1200–1375 C.E.</td>
<td>−16.5 ± 1.7</td>
<td>−20.4 – −12.4</td>
<td>+12.8 ± 1.7</td>
<td>+6.9 – +16.2</td>
</tr>
<tr>
<td>Faunal* (n = 53)</td>
<td>2000 B.C.E. – 200 C.E.</td>
<td>−18.4 ± 1.8</td>
<td>−21.8 – −13.16</td>
<td>+8.1 ± 2.4</td>
<td>−3.5 – +12.6</td>
</tr>
</tbody>
</table>

Table 1. Average bone collagen values for individuals in this study by time period, individual values presented in Supplementary Table 1 (results include additional individuals from previously published articles).

Results

Preservation of samples. We analyzed 80 bone collagen and 108 dental enamel samples from 137 individuals from 60 archaeological sites (Tables 1–4; Fig. 1). Samples were separated into four chronological periods based on relative and absolute dating (Early [Neolithic - Bronze Age], Early Iron, Xiongnu, and Mongol; see Supplementary Table S4 for AMS dates). As there is only a single individual from the Neolithic period (c. 4400–3000 B.C.E.), this sample was combined with Bronze Age individuals dating to prior to 800 B.C.E. (n = 23; collagen n = 14, enamel n = 16) to create a single period labelled as ‘Early’. The Iron Age samples were split into two chronological periods, corresponding to the pre-imperial Early Iron Age (c. 800–200 B.C.E.) and the Xiongnu (c. 200 B.C.E. – 250 C.E.). The Early Iron Age samples include 16 individuals (collagen n = 7, enamel n = 16) from one site. From the subsequent Xiongnu, we analyzed 59 individuals (collagen n = 23, enamel n = 54) from 28 sites. Individuals from the later Mongol Empire (c. 1200–1375 C.E.) are grouped together and consist of 28 individuals (collagen n = 28, enamel n = 21) from 19 sites.

All of the human bone collagen samples included in this study had atomic C:N ratios between 3.1 and 3.5 and were thus within the accepted range for good collagen preservation (Supplementary Table S1). The collagen yields of these samples ranged between 6 and 30%, with none falling below 1%, a further check of data quality. Furthermore, the majority of collagen samples have greater than 11% N and greater than 30% C, within acceptable ranges. Each bone collagen sample was run in duplicate, and averages are presented in Supplementary Table S1 along with their standard deviation.

Bone collagen carbon and nitrogen stable isotope results. δ13C and δ15N results from human bone collagen are grouped into four chronological periods, as detailed above, for comparative analysis. The pre-Bronze and Bronze Age average values are the closest to those of the average faunal values, although the faunal values
Table 2. Average tooth enamel bioapatite values by time period.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Date Range</th>
<th>$\delta^{13}$C (%) (VPDB) range</th>
<th>$\delta^{15}$N (%) (VPDB) range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early (n = 17)</td>
<td>4400–800 BC</td>
<td>$-12.9 \pm 0.8$</td>
<td>$-14.3 - -11.9$</td>
</tr>
<tr>
<td>Early Iron (n = 14)</td>
<td>800–200 BC</td>
<td>$-11.0 \pm 2.1$</td>
<td>$-13.3 - -5.7$</td>
</tr>
<tr>
<td>Xiongnu (n = 56)</td>
<td>200 BC–250 AD</td>
<td>$-11.2 \pm 2.3$</td>
<td>$-14.9 - -3.1$</td>
</tr>
<tr>
<td>Mongol (n = 21)</td>
<td>1200–1375 AD</td>
<td>$-11.3 \pm 1.9$</td>
<td>$-15.1 - -6.8$</td>
</tr>
</tbody>
</table>

have a higher standard deviation. The widest range of carbon and nitrogen isotope values were found in the Xiongnu and Mongol Period populations. For all of the individual $\delta^{13}$C and $\delta^{15}$N values from each time period see Supplementary Table 1.

Dental enamel carbon stable isotope results. The data from $\delta^{13}$C values of human enamel bioapatite are divided into the same chronological periods as the bone collagen data. The Xiongnu population had the largest range of stable carbon isotope values, followed by the Mongol period and Early Iron Age (Table 2). The pre-Bronze/Bronze period had the lowest range of stable carbon isotope values when compared to the later populations. For all of the individual $\delta^{13}$C and $\delta^{18}$O values from each time period see Supplementary Table 2 (Samples with both collagen and enamel Supplementary Table 3).

Environmental differences. As stable carbon and nitrogen isotope values may vary in different environments (i.e. temperature and aridity), to adequately assess human $\delta^{13}$C and $\delta^{15}$N values from normal steppe (>200 mL of annual precipitation) and dry (<200 mL of annual precipitation) regions, we also determined the average values for each environmental type (Table 3). In these tables we have separated the previously published faunal stable carbon and nitrogen isotope values into the "steppe" or "dry" regions according to modern annual rainfall[27,28].

Statistical tests. Boxplots of our results can be found in Fig. 2A–C, and statistical comparisons between the time periods can be found in Supplementary Table 5 ($\delta^{13}$C bone collagen), Supplementary Table 6 ($\delta^{13}$C enamel bioapatite), and Supplementary Table 7 ($\delta^{15}$N bone collagen). For the bone collagen data, both the Xiongnu and the Mongol average $\delta^{13}$C values were significantly higher than those of Early individuals (p < 0.05). The same trend was seen for tooth enamel $\delta^{13}$C, with Early Iron, Xiongnu, and Mongol samples having $\delta^{13}$C significantly higher than that of the Early group (for the overall p < 0.05, and the specific pairwise comparisons are available in Supplementary Table 6). There was no significant difference between average dental enamel values for the Early Iron, Xiongnu, and Mongol periods $\delta^{13}$C (p > 0.05). Bronze Age $\delta^{15}$N values were also significantly higher (p < 0.05) than those of the Early Iron, Xiongnu, and Mongol periods (Supplementary Table 7).

Isotopic temporal trends in Mongolia and environmental impacts. Higher $\delta^{13}$C values in individuals from the Early Iron Age, Xiongnu, and Mongol periods could be the product of the increased direct consumption of C4 crops or wild plants or animals consuming C4 plants. It should also be understood that both Mongolians and foreign travellers would have been moving within and outside of the imperial borders, and dietary intake likely varied greatly in different regions. In areas with environmentally-linked variation in wild C4 and C3 plant distributions, such as Mongolia, it is important to rule out a climatically driven change (see Supporting Information Text 1). Modern plant samples from Mongolia have yielded $\delta^{13}$C values ranging from −28.3 to −23.4% for C4 photosynthetic pathways and an average $\delta^{13}$C of −14.7% for plants following the C3 photosynthetic pathway[48]. Notably, wild C4 plants make up a much smaller proportion of Mongolian and other Central Asian environments than C3 plants[45,81]. Overall, contemporary studies suggest that leaf $\delta^{13}$C values decrease with increasing mean annual precipitation[82, both as a product of reduced C4 plants in wetter landscapes and aridity-driven changes in $\delta^{13}$C among C3 plants (see Supplementary Text S1). While C4 plants make up a relatively limited portion of the biotic community today, we established local isotopic baselines for Mongolia in the past using archaeological fauna in order to determine if shifts in $\delta^{13}$C values through time are the product of environmental variations or social and economic choices. Isotopic studies of modern and archaeological herb animals have shown differences in $\delta^{13}$C values between more and less arid regions[81,85,86, and that there is variation in the availability of C4 and C3 plants across the country[87,88]. While there were no fauna associated with the human remains collected for this study, we were able to use previously published faunal stable isotope data from the Minusinsk Basin of Siberia (just north of Mongolia) (MNSK, AD, AM; n = 21)[89,90, the Gobi (BGC; n = 14)[91,92, Gobi-Alta (SBR; n = 5)[93, and north central Mongolia (EG; n = 13)[94, areas to show that regional herbivores generally consumed C4 plants, with some having higher stable carbon isotope values, indicative of C4 plant consumption, in the hyper-arid desert regions[95,96. Statistical tests further support this assessment, with humans having higher $\delta^{13}$C values than the available fauna in all periods, with the greatest difference occurring in the Xiongnu and Mongol periods (p < 0.005) (Figs. 2A and 3A). For terrestrial faunal remains (Fig. 3A), there is a significant correlation between $\delta^{15}$N and $\delta^{13}$C bone collagen values ($R^2 = 0.64$, p-value < 0.001) which is a product of higher levels of aridity leading to a higher availability of C4 plants in the natural vegetation cover. However, no such correlation is observed in humans (Fig. 3A,B), either between $\delta^{15}$N and $\delta^{13}$C bone collagen values ($R^2 = 0.01$, p-value = 0.15) or between $\delta^{15}$N bone collagen and $\delta^{13}$C enamel values ($R^2 = 0.05$, p-value = 0.13). Given this, alongside the consistent elevation of human $\delta^{13}$C values over the available fauna $\delta^{13}$C values, this indicates that higher $\delta^{13}$C values in human bone collagen and enamel is a product of direct consumption of non-wild C4 plants.
Mean bone collagen δ¹³C values for faunal remains from steppe regions are typically C3 (−19.3 ± 1.3‰), and the stable carbon isotopic offset between bone collagen of herbivores and carnivores is c. 1‰⁴⁹. Thus, human bone collagen steppe samples dating to the Early period (prior to 800 B.C.E.) do not show δ¹³C values indicative of a millet dietary contribution (−18.3 ± 0.1‰). This same offset applies to human bone collagen samples from dry regions from all periods since these are elevated by up to 1‰ (Early −17.2 ± 0.7‰; Early Iron = −16.2 ± 0.9‰; Xiongnu − 16.1 ± 1.1‰; Mongol − 16.1 ± 1.4‰) when compared to the bone collagen mean for faunal samples from dry regions (−17.2 ± 1.5‰). However, for later time periods in steppe regions average human bone collagen δ¹³C values are elevated by c. 3‰ (Early Iron −16.3 ± 0.7‰; Xiongnu − 16.1 ± 1.2‰; Mongol − 16.8 ± 2.2‰) when compared to faunal values. Thus, the higher (c. 2‰) human bone collagen δ¹³C values observed for later periods in steppe regions when compared to the early period is indicative of a temporal increase in millet-based

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**Figure 1.** Maps of sites used in this study. These maps were created for this study and were produced using QGIS 3.0⁹⁹ https://qgis.org/en/site and using the Natural Early Data maps from https://www.naturaeartdta.com/downloads/ by Shevan Wilkin and Michelle O’Reilly (Graphic Designer for the Max Planck Institute for the Science of Human History, Jena, Germany).
Our results clearly demonstrate an increase of C4 plant caloric consumption. To further confirm that the increased δ13C values in human bone collagen and tooth enamel through time is a product of the consumption of crops rather than changing availabilities of baseline C3/C4 plant ratios or the availability of samples in different local environments, we developed a Bayesian model to produce a C4 dietscape, representing estimates of spatial distribution of C4 plants based on per capita caloric consumption (See SI for detailed discussion). Stable carbon isotope data of dental enamel was used, and individuals were separated into two periods, Early (Neolithic - Bronze Age) or Late (Xiongnu, and Mongol). The results for the two models show that during the Early period C4 caloric contributions were very low across Mongolia, likely including consumption of local plants and livestock consuming natural vegetation, with mean estimates varying between <2.5 and 5.0% of calories (interpolation 1-sigma uncertainty up to 0.5% calories) (Fig. 5A,B). During the later periods, the variability in millet-based food consumption increases considerably as shown by the range in the mean estimate (between 3 and 26% of per capita millet calories) and in the 1-sigma interpolation uncertainty for each location (between c. 3 and 6% of per capita millet calories) (Fig. 5C,D). The C4 plant dietscape for the late period also shows that millet consumption is concentrated in central northern Mongolia (reaching the highest mean value [26% per capita millet calories]), an area where environmental increase of carbon values would not be expected naturally (Figs. 4 and 5).

### Discussion

#### Isotopic indicators of diet through time in mongolia.

Our results clearly demonstrate an increase of human consumption of C4 plants during the imperial periods in ancient and historic Mongolia (Figs. 2, 3, and 5). While high δ15N values in human bone collagen relative to the faunal data (Fig. 2A and 3A) supports evidence for human reliance on dairy and meat products throughout the periods under study, the change in C4 plant...
consumption represents the major dietary shift within this timeframe. The significant decrease in δ15N values in the later periods, in comparison to the Bronze Age, further supports this point, potentially indicating reduced consumption of meat and milk and increased consumption of grains. Moreover, comparisons with faunal datasets and environmental background data allows us to confidently state that this shift is a consequence of increasing rates of consumption of C4 resources. Stable carbon isotope values from individuals before the Early Iron Age show little indication of C4 plant consumption beyond the local natural baseline identified through the fauna from similar environments. Individuals from the cemetery of Chandman Mountain (c. 900–400 B.C.E.)

Figure 2. Boxplots showing the range of carbon values for all individuals from each period. Outliers are shown as individual data points. (A) Comparison of the bone collagen carbon values for humans and fauna. Faunal data derives from previously published data,[20][29][30][44] and all human data is from this study. (B) Difference in human enamel values between Early, Early Iron, Xiongnu, and Mongol periods. The Early Iron, Xiongnu, and Mongol period average values are significantly higher than the Early period average. (C) Boxplots showing the range of oxygen values from enamel samples. There are no significant differences between any of the time periods.

Figure 3. Carbon and nitrogen values from bone collagen with ellipses showing ranges at 95% confidence. (A) Individuals included in this study as well as humans and faunal values from previously published data.[31] (B) Humans included in this study showing the variation between those in the “Dry” and “Steppe” zones. “Dry” sites have less than 250 mm of annual precipitation, “Steppe” sites have over 250 mm of precipitation per year. (C) δ15N from bone collagen versus δ13C values from dental enamel demonstrating the shift from primarily C3 reliant diets in the Early period to a wider range of carbon and nitrogen values, indicating an increase in the diversity of diets in the later three periods. (D) δ18O from dental enamel versus δ15N from bone collagen showing the values in “Dry” and “Steppe” areas.
analyzed in this study show the first visible evidence of C\textsubscript{4} plant consumption as part of a mixed agro-pastoral diet. However, this particular site in northwest Mongolia is more a part of the Minusinsk Basin region of southern Siberia, an area where millet consumption was common by the Late Bronze Age, than the rest of Mongolia. Previously published contemporaneous human and faunal isotope analyses within this region are indicative of animals consuming primarily C\textsubscript{3} plants\textsuperscript{20}, with the human population postulated as having a diet additionally composed of C\textsubscript{4} plants in concert with meat and milk.

In the rest of Mongolia during the tenure of the Xiongnu and Mongol empires human stable carbon values became more varied with increasing numbers of individuals displaying bone collagen and tooth enamel $\delta^{13}\text{C}$ values suggestive of moderate to high C\textsubscript{4} plant consumption, with the number of individuals with such values reaching their peak during these imperial periods. We also observe the largest range and diversity of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values during the imperial periods. This is likely due to diverse subsistence strategies being pursued across each empire, reflecting different environmental zones and levels of imperial support. This is result of the extensive range of each empire, and includes the knowledge that not everyone that died in Mongolia would have been “Mongolian”, but these individuals likely lived and died within the empires. Since the majority of the individuals analyzed were excavated from elite imperial tombs, and human remains representing other sectors of society are lacking at present, attributing all outliers to non-local outsiders would be to dismiss the agency of Mongolian populations and provide something of a ‘colonial’ narrative.

Figure 4. Sites in and around Mongolia with archaeological or archaeobotanical evidence for C\textsubscript{3} (wheat and barley) and C\textsubscript{4} (broomcorn and foxtail millet) grain cultivation during the Iron Age. This map was newly created for this study and produced using QGIS 3.0\textsuperscript{89} https://qgis.org/en/site and using the Natural Early Data maps from https://www.naturalearthdata.com/downloads/ by Shevan Wilkin, Bryan K. Miller, and Michelle O’Reilly (Graphic Designer for the Max Planck Institute for the Science of Human History, Jena, Germany).

Individual bone collagen and tooth enamel $\delta^{13}\text{C}$ values for the Xiongnu and Mongol empires range between those indicative of a pure C\textsubscript{3} diet to those that suggest heavy C\textsubscript{4} plant consumption. Interestingly, during this period, a few individuals had $\delta^{13}\text{C}$ values lower than those of the Early period which, along with lower $\delta^{15}\text{N}$ values, indicates a staple intake of C\textsubscript{3} plants, likely crops such as wheat and barley. Historical and archaeobotanical sources suggest that cereal crops were commonly cultivated or obtained through trade during the Mongol period\textsuperscript{13,51–56}. In addition to grains, carbonized fruit and nut remains have been recovered from sediments at the Mongol capital of Karakorum (also used during the Mongol rule in the Yuan Dynasty) showing the diversity of imported plants through the presence of rice (\textit{Oryza sativa} L.), over a dozen cultivated fruits, including grapes (\textit{Vitis vinifera} L.), figs (\textit{Ficus carica} L.), and jujube (\textit{Ziziphus jujube} Mill.), as well as vegetable and oil-seed crops. There are also remains of spices – notably a few, such as black pepper (\textit{Piper nigrum} L.) and caraway (\textit{Carum carvi} I.), that were imported along the trade routes with South Asia, and would have involved transport across distances of up to 2000 kilometers\textsuperscript{12}.

The resulting bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values have been plotted to show this increase of dietary diversity over time (Fig. 2A). From our data, alongside the growing corpus of biomolecular, archaeological, and historical data, it is evident that the Xiongnu and Mongol Empires had complex imperial structures that facilitated increasingly diverse subsistence economies. The combination of crop cultivation in tandem with dairy pastoralism would have allowed these empires to sustain a diverse economic surplus that defended against livestock depletion from harsh winters, crop loss, or volatile political episodes. Diverse dietary values likely also reflect an increasingly cosmopolitan society in which dietary heterogeneity within populations increased with growing migration, trade and interaction, and the emergence of increasingly elaborated elite statuses. The diversity could also reflect temporal political shifts within the time-span covered by our sampling groups, with trade routes to Karakorum decreasing in volume during the Mongol Period after the switch of the capital in 1260 and ending with the end of the Yuan Dynasty in 1368\textsuperscript{12,57}, for example.
Mongolian empires in context. Historical and ethnographic research indicates the importance of pastoralism on the historic and proto-historic eastern Eurasian Steppe. Recent proteomic research has demonstrated the clear importance of dairy-based pastoralism to Mongolian dietary practices from at least c. 1500 B.C.E. 58. Nevertheless, our data clearly highlight that pastoral lifestyles did not preclude the inclusion, and later intensification, of crop use. Millet’s suitability to arid environments combined with its short growing period is compatible with the often peripatetic, mobile lifestyles of pastoralists22,26. Indeed, during the Xiongnu and Mongol empires, we see clear evidence for human dietary reliance on millet in a significant proportion of individuals. Although some scholars contend that all grains were either extorted or imported from China and other exterior polities6, we argue that our data, alongside existing archaeobotanical and archaeological findings25,59,60, provide clear evidence for imperial reliance on locally grown crops in the Xiongnu and Mongol heartlands, as well as the coordination of diverse economic connections and exchanges12. These discoveries bolster the notion of an economically diverse population across much of Mongolian history14,61,62.

Agricultural tools for plowing, hoeing, and grinding have been uncovered from permanent Xiongnu settlements in Mongolia, implying local plant cultivation and processing63, and charred remains of millet, barley, and wheat grains have been recovered through flotation at pit-house villages at Boroo64 and ephemeral campsites61. Studies in the Egiin Gol valley and at the large site complex of Ivolga have illustrated the presence of long-season cereal crops (wheat and barley) in the Iron Age, which represent more labor investment in farming practices than millets61,63. At Ivolga, this occurs alongside evidence of ploughshares at permanent settlements63, as well as written accounts of crops suitable for the northern steppe being managed by imperial Xiongnu administrators, such as the ‘Lord of Millet Distribution’, referred to in the 1st century C.E. Chinese accounts54. Millet grains, still articulated in their chaff, have been found within the graves of Xiongnu rulers at Gol Mod and Noyon Uul27 as well as of local elites throughout the steppe60. There are also uncharred grains found within Xiongnu pit-house villages, all of which were unprocessed (i.e. with palea and lemma) and thus most likely not transported long distances60 instead representing local production and consumption (Fig. 4).

Scholars working in Mongolia have extensively discussed the formation of hierarchical political systems and greater concentrations of population densities in the absence of farming, often describing imperial systems in Central Eurasia as unique due to their economic basis65–69. In other parts of Asia, farming is linked to demographic expansion and the congregation of greater population densities70. Notably, millet farming is linked to urbanization71,72 and imperial formation73 in East Asia. Boserupian economics suggest that increased investment in farming, along with a diversified economy and higher levels of cultural exchange, often lead to a demographic transition74–76. The data presented in this paper suggests that, while Mongolian empires have often been seen as outliers in global comparisons of imperial structures, they were in fact, like many others around the world, highly reliant on economic diversification, local adaptations to a diversity of environments, and the creation of reliable and stable subsistence resources and economic surpluses8–10.
Mongolian empires have traditionally conjured up exotic ideas of mobile pastoral specialists who roamed the Asian steppes attacking more sedentary communities. While prominent in the public sphere, such preconceptions have also directed the type of questions academics have asked. For example, comparative analysis of Mongolian empires with others around the world has been limited, with ‘Steppe Empires’ often portrayed as deficient or somehow doomed to failure in the absence of reliable crop-based surplus. As in other parts of Central Asia, where occupation sites have been hard to come by, simplistic projections of ethnographic and ethnohistoric datasets into the past have been common in Mongolian archaeology. We hope to have demonstrated how multidisciplinary approaches, built on datasets from different parts of Mongolian imperial networks, can begin to provide novel insights into their economic systems and, perhaps most importantly, their geographic and temporal variability. While there is no doubt that the Xiongnu and Mongol empires were unique, they were also built upon many of the same tenets of economic diversity, stability, and reliability that have characterized imperial structures throughout prehistory and history, demonstrating the importance of a core set of underlying variables in both enabling and driving the formation of empires.

**Methods and Materials**

**Sites and materials analyzed.** All bone and tooth samples included in this study were collected from the National University of Mongolia’s Department of Archaeology during the winter of 2016. Bone collagen was analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes, and the carbonate of dental enamel bioapatite was measured for carbon stable isotopes ($\delta^{13}C$), with some individuals analyzed for both bone collagen and bioapatite (see Supplementary Table 3). Time periods for samples ranged from the mid-fifth millennium B.C.E. to the Mongol Empire, as dated by AMS radiocarbon methods where possible (see below).

Samples of bones and teeth were collected from archaeological sites across the country of Mongolia, through varying environmental and topographical zones. Where possible, we collected a tooth and long bone fragment from individuals from each time period. While we aimed to assemble an equal number of samples from all time periods, the collection was dominated by individuals from the Iron Age periods, resulting in fewer individuals prior to the Iron Age. Bone collagen was preferably extracted from rib bones, but occasionally other bone fragments were employed (clavicle, femur, crania). $\delta^{13}C$ and $\delta^{15}N$ stable isotope measurements of human bone collagen inform primarily on protein source, and the bones sampled (i.e. ribs) represent a period of diet of approximately the last 20 years of life.

By contrast, tooth enamel $\delta^{13}C$ values are indicative of the whole dietary carbon (carbon mix of protein, lipids, and carbohydrates) consumed during enamel formation. First molars mineralize before an individual is 3 years old, second molars are fully formed around age 8, and third molars, if present, are completely mineralized and temporal variability. While there is no doubt that the Xiongnu and Mongol empires were unique, they were also built upon many of the same tenets of economic diversity, stability, and reliability that have characterized imperial structures throughout prehistory and history, demonstrating the importance of a core set of underlying variables in both enabling and driving the formation of empires. First molars were chosen only when both the M2 and M3 were unavailable.

**Stable isotope analysis methods.** Bone collagen. We selected ribs for bone collagen analysis as representative of the last c. 20 years of life. Collagen was extracted from each rib sample following standard procedures. Approximately 1 gram of pre-cleaned bone was demineralized in 10 ml aliquots of 0.5 M HCl at 4 °C, with changes of acid until CO2 stopped evolving. The residue was then rinsed three times in deionized water before being gelatinized in pH 3 HCl at 75 °C for 48 hours. The resulting solution was filtered, with the supernatant then being lyophilized over a period of 24 hours.

After calculating the collagen yield, all purified collagen samples (~1 mg) were located in tin capsules to be analyzed in duplicate at the Department of Archaeology, Max Planck Institute for the Science of Human History by the elemental analyzer/continuous flow isotope ratio mass spectrometry (EA-IRMS) using a ThermoFisher Elemental Analyzer coupled to a ThermoFisher Delta V Advantage Mass Spectrometer via a ConFloIV system. $\delta^{13}C$ and $\delta^{15}N$ values were compared and calibrated against International Standards (USGS40 ($\delta^{13}C_{\text{Craw}} = −26.4 ± 0.1‰, \delta^{13}C_{\text{true}} = −26.4 ± 0.0‰, \delta^{15}N_{\text{raw}} = −4.4 ± 0.1‰, \delta^{15}N_{\text{true}} = −4.5 ± 0.2‰), IAEA N2 ($\delta^{15}N_{\text{raw}} = +20.2 ± 0.1‰, \delta^{15}N_{\text{true}} = +20.3 ± 0.2‰), IAEA C6 ($\delta^{13}C_{\text{raw}} = −10.9 ± 0.1‰, \delta^{13}C_{\text{true}} = −10.8 ± 0.0‰)). Replicate analysis of an in-house fish gelatin standard suggests that machine measurement error is c. 0.1‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

Tooth enamel. Teeth or tooth fragments were cleaned using air-abrasion to remove any adhering external material. 8 mg of enamel powder was obtained using gentle abrasion with a diamond-tipped drill along the full length of the buccal surface or fragment in order to maximize the period of formation represented by the resulting isotopic analysis for bulk samples. Enamel powder was pre-treated using a protocol to remove any organic or secondary carbonate contaminants. This consisted of a series of washes in 1.5% sodium hypochlorite for 60 minutes, followed by three rinses in purified H2O and centrifuging, before 0.1 M acetic acid was added for 10 minutes, followed by another three rinses in purified H2O (as per).

Following reaction with 100% phosphoric acid, gases evolved from the samples were analyzed to stable carbon and oxygen isotopic composition using a Thermo Gas Bench II connected to a Thermo Delta V Advantage Mass Spectrometer at the Max Planck Institute for the Science of Human History, Jena (MPI-SHH). Carbon and oxygen isotope values were compared against an International Atomic Energy Agency (NBS 19) and in-house standard (MERCK). Replicate analysis of internal bovid enamel standards suggests that machine measurement error is c. ± 0.2‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{18}O$. Using a Thermo Delta V Advantage Mass Spectrometer at MPI-SHH, gases produced from a reaction with 100% phosphoric acid were analyzed for stable carbon and oxygen isotopic composition. We compared the resulting values against International Standards (IAEA-603 ($\delta^{13}C = 2.5; \delta^{18}O = −2.4$); IAEA-CO-8 ($\delta^{13}C = −5.8; \delta^{18}O = −22.7$); USGS44 ($\delta^{13}C = −42.2$)); as well as an in-house standard.
standard of (MERCK ($\delta^{13}C = -41.3; \delta^{18}O = -14.4$)). The data from these standards suggest that the machine-measurement error is $c. \pm 0.1\%$ for $\delta^{13}C$ and $\pm 0.2\%$ for $\delta^{18}O$. We increased the precision of our analyzed samples by measuring repeats of extracts using a tooth enamel bovid standard ($n = 20, \pm 0.2\%$ for $\delta^{13}C$ and $\pm 0.3\%$).

**Statistical tests.** To determine whether the differences in human $\delta^{13}C$ between each period were significant, we performed a Wilcoxon rank sum test, with multiple test correction using the Benjamini-Hochberg procedure. All tests were performed using the free R statistical software.

**Bayesian dietary modelling.** Caloric estimates of millet intakes were obtained using the Bayesian mixing model FRUITS having as input data individual tooth enamel $\delta^{13}C$ values and local food isotopic values adjusted for spatial variations due to varying environmental conditions. To achieve the latter, we grouped site locations into the categories of “steppe” and “dry” depending on modern day annual precipitation. Steppe sites have a range from 250–350 mm in precipitation per annum and arid sites have below 250 mm of yearly rainfall. It was assumed that the enamel $\delta^{13}C$ signal is defined by the dietary carbon mix. To extrapolate the spatial distribution of per capita millet caloric intakes (dietscape) a Bayesian additive mixed model with error-in variables available as an online app via the Pandora & IsoMemo initiatives was employed. Dietscapes were generated for two main periods corresponding to a temporal divide defined by the intensification of millet consumption as observed from the interpretation of raw isotopic data, into Early (Bronze Age) and Late (combining the Early Iron Age; Xiongnu; Mongol periods). Modelling at a higher chronological resolution was not possible given a lack of data for shorter time periods. Further details on dietscape modelling are available in Supplementary Text S3.

**Radiocarbon and archaeologically classified dates.** AMS radiocarbon dates were conducted at the Oxford Radiocarbon Accelerator Unit (ORAU), Oxford, England, UK ($n = 14$; bone collagen and dentine) and at the University of Groningen, Faculty of Science and Engineering, Groningen, The Netherlands ($n = 25$; bone collagen and dentine). All pre-Xiongnu samples and 30% of the Xiongnu samples were radiocarbon dated to solidify the dating of individuals from early time periods. Most Xiongnu and Mongol samples were separated into periods based on archaeological materials and burial styles as assessed by excavators and curators from the National University of Mongolia. See Supplementary Text S2 for additional details.

**Data availability and ethical approval statement.** All of the data included in the study have been made available in Tables 1 and 2, the Supplementary Information. Samples analyzed for this study (AT- denoted codes) are currently curated at the Max Planck Institute for the Science of Human History, Jena, Germany. Samples were exported to the Max Planck Institute for the Science of Human History under permission from the Ministry of Culture, Education, Science and Sports (Export number 10/413 (7b/52) which was received on 2nd February, 2017 # A0109258, MN DE 7 643)

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Author contributions
S.W., P.R. and N.B. designed the experiments. S.W., M.B., S.U. and E.M. collected the samples. S.W. and J.Z. completed laboratory analyses. S.W., A.V.M., R.E., R.H., J.Z. and P.R. analyzed the data. S.W., P.R., A.V.M., B.K.M., R.F., WT, R.N.S., S.U. and E.M. contextualised the data and wrote the article with input from all authors.

Competing interests
The authors declare no competing interests.

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6. Discussion

Together, these three, independent studies illustrate the evolution of subsistence patterns over 6000 years in ancient and imperial Mongolia. This research clearly shows the presence of ruminant dairy consumption from the Early Bronze Age (ca. 3000 BCE) (Manuscript B), which extends the date of the earliest dairying on the eastern steppe over 1500 years earlier than previously established (Manuscript A). Importantly, the earliest evidence for dairy in this study was identified from individuals in Afanasievo burial sites, supporting our hypothesis that dairy was integrated into Mongolia through Early Bronze Age migrations of western steppe herding groups crossing the Altai. As detailed in Manuscripts A and B, our studies demonstrated that while the first dairying practices were introduced by western steppe groups, by c. 1300 BCE, dairying had been adopted by local northern populations with very little western steppe ancestry.

The identification of horse milk proteins in the Late Bronze Age (c. 1200 BCE), concurrent with equine skeletal evidence for horseback riding, is especially informative as both traditions have persisted for millennia and are still commonly practiced today (Manuscript B). That Bronze Age Mongolian horses are a distinctly different equine species that those from the Eneolithic Botai sites in northern Kazakhstan [114], a site with lipid evidence suggestive of horse dairying [27]. Our data may represent either an independent emergence of horse dairying, or possibly, the first evidence for horse milk consumption. Whichever scenario these data represent, we show that in the Late Bronze Age there was a dramatic shift in how people on the eastern steppe used horses in their subsistence and economic strategies (Manuscript B).

Our third study illuminated dietary differences between the pre-imperial populations and those of the later Xiongnu and Mongol Empires. The individuals in the “early” groups (Eneolithic and Bronze Age) did not display carbon values indicative of moderate- to high-level C₄ consumption, while the imperial individuals had carbon values suggestive of low- to high-input C₄ grain consumption - likely as part of a diversified dietary economy. A significant decrease in δ¹⁵N ratios from the “early” to “late” periods further supports a decrease in the dependence on meat and dairy once the consumption of cultivated grains became more common (Manuscript C).
6.1 Dairying in ancient Mongolia

Recent studies combining ancient genomics with archaeological data discuss the migrations of the dairy-focused pastoral Yamnaya populations north of the Caspian Sea at 3000 BCE across the central steppe [23,25,115]. Our data support the introduction of dairy pastoralism into Mongolia by western steppe herders moving across Eurasia and over the Altai Mountains. The earliest individual in our sample set, from an archaeologically-identified Afanasievo site in central Mongolia, is from a clear western steppe mitochondrial haplogroup and contains peptides from a sheep/cattle milk proteins [116]. This early evidence of western steppe style burial mounds in the central Mongolian Khangai Mountains concurrently with Yamnaya populations in the far western steppe [23] indicates that the Afanasievo populations spread further into East Asia than originally thought (Figure 9).

While the first evidence of dairy consumption in Mongolia is found at Afanasievo and Chemurchek Early Bronze Age burial sites (c. 3000 - 2500 BCE), Late Bronze Age dairy reliant sites in northern Mongolia have a very different demographic profile. In the first study of this thesis, ancient DNA analysis revealed that while 9 of the 11 calculus samples contained dairy proteins, the associated individuals were of a local ancestral background, rather than from the western steppe [74]. This indicates that although early dairying was likely introduced from the migrating western steppe herders (Manuscript B), the practice was thoroughly adopted by local Mongolian populations over the subsequent 1500 years.

Evidence for the consumption of horse dairy beginning around 1200 BCE is especially important, as this coincides chronologically with the earliest morphological evidence for horse riding in Mongolia [117–119]. The incorporation of horse riding and milking into pastoralist systems resulted in dramatic demographic changes and shifts in occupation site location strategies (Figure 9). Horses offer faster ways to travel, and with the addition of horses into the pastoral economy, herding larger numbers of animals becomes possible [120]. Furthermore, horses can use their hooves to break through the snow cover allowing themselves and other taxa in the herd to access the plant sustenance below, which is especially important over the frigid Mongolian winters. In the Late Bronze Age, our protein data combined with morphological skeletal indications of horse riding [119–121], and evidence of occupation sites in previously unoccupied intermontane areas of the steppe [118] suggest a dramatic shift in the
economic role of horses in Mongolia, as well as changes in the efficiency of pastoral resource systems.

Figure 9. Four panel map of individuals analysed in this study, showing archaeological culture group affiliations, the presence or absence of dairy consumption and taxa consumed. Solid filled circles indicate individuals with a positive identification of dairy consumption. Circles with a line-through indicate no dairy consumption detected. Individual AT-923, associated with Ulaanzuukh, is not directly radiocarbon dated and is not included in this figure. Taxonomic icons only indicate the most specific taxa identified in a phylogenetic branch. The full list of dairy species identified for each individual is listed in Table 1 of Manuscript B.

6.2 The diversification of diets in the imperial periods

Our protein study demonstrates that dairy pastoralism continued as a part of subsistence strategies through the Xiongnu and Mongol periods. However, our study into stable carbon isotope analysis of human remains across these time periods establishes a significant increase in millet consumption by the Xiongnu period, demonstrating that the imperial economy was diverse, including both dairy and cultivated grains. As archaeological and botanical studies have identified cereal grains as well as tools for farming and processing we can see that the use of locally grown grains was an important part of eastern steppe diets by the Iron Age.
Through the combination of stable carbon isotope data with established archaeological and historical evidence, we can see the presence of local millet cultivation in concert with other grains acquired through long distance trade. The significant increase in dietary diversity during the imperial periods (Figure 10) demonstrates the innovative ways each empire increased surpluses to combat food shortages, and confirms that these empires relied on complex subsistence systems. The focused, and possibly high-investment cultivation of millet within the empire secure the Xiongnu and Mongol Empires as complex political systems relying on multi-resource agro-pastoral economies. These new data, combined with the established historic and botanical evidence, have overturned the long-standing, popular assumptions of Mongolian empires as primarily dairy pastoralists that derived grains from neighbouring regions.

![Graph showing ∆13C values for different time periods](image)

Figure 10. Boxplot showing the range of ∆13C values for all dental enamel samples in each time period. Difference in human enamel values between Bronze, Early Iron, Xiongnu, and Mongol periods. The Early Iron, Xiongnu, and Mongol period average values, noted with * are significantly higher than the Bronze Age average.

6.3 Future directions

Archaeological studies, including lipid and protein analyses, have spatially and temporally bookended the origin and extent of the spread of ruminant dairying across the Eurasian steppe [10,11,74]. While it is evident that western steppe populations with pastoral dairy herds migrated eastward from Neolithic Anatolia across the steppe into
Mongolia, the pathways via which these pastoral populations traveled remains unclear. Also unclear is the directionality of the movement of horse dairying, as lipid analysis of ceramics from the Eneolithic Botai culture (c. 3800 - 3000 BCE) is suggestive of equine milk [27], yet the horses from Botai site are from a different species than those of Bronze Age Mongolia [114]. In tracing the movement, or possibility of multiple independent origins of horse dairying future work could focus on the analysis of calculus and ceramic materials from across Eneolithic and Bronze Age Kazakhstan and central Russia for the presence of ancient horse milk proteins. In order to properly assess ancient samples for proteins from the modern and ancient equine species, we have translated published data from ancient horse genetics studies into their associated protein sequences [114, Manuscript B]. As this work is ongoing, it is vital that we expand reference protein sequence databases with possible protein sequence variations from modern and ancient animal populations, in collaboration with animal geneticists. Data produced from these upcoming analyses will further illuminate the pathways and directionality of the spread of dairy animals and practices across the steppe (Figure 11).

Figure 11: Map of Eurasia demonstrating the studies which bookend the evidence for dairying identified through biomolecular analyses, including lipid and protein studies, and the open questions regarding directionality and the pathways of the transmission of dairy practices.

In order to illuminate these migratory pathways, it is necessary to conduct protein studies of human dental calculus from sites between southwestern Asia and the eastern steppe spanning
between the Neolithic and Bronze Age. Prior to the advent of horse riding, early pastoralists travelled on foot and primarily lived along mountainous regions with transhumant herding patterns. Based on these observations, we have identified specific regions with similar environments, as well as other sites that contain other evidence of dairy animals and practices that present ideal populations for further study [19,22,27,114].

To expand our understanding of grain consumption in ancient and historic Mongolian diets, it would be extremely helpful to create additional datasets from faunal remains. As fauna primarily eat wild grasses and occasionally cultivated fodder, carbon values from their skeletal and dental remains offer insights into local isotopic baselines [112,122,123]. Data from fauna would be especially telling as wild plant ground cover changes dramatically across Mongolia’s varied environmental zones, and factors such as aridity and temperature can affect $\delta^{13}C$ values. Furthermore, studies in migration and transhumant patterns through oxygen and strontium isotope analysis of animal and human dental enamel can also be especially telling in regards to animal herd management and the ranges of seasonal movements [124,125]. New studies using these methods across large temporal periods, such as those used in Manuscripts B and C, can uncover changes in migratory movements over time and through political and environmental changes.

6.4 Conclusion

The combination of proteomics, stable isotope analysis, archaeological, botanical, and historical evidence has allowed us to clearly demonstrate a dramatic shift from an almost purely pastoral early subsistence strategy to diversified dietary economies during the Xiongnu and Mongol Empires. We show that early Mongolian (c. 4000 - 800 BCE) subsistence strategies clearly included milk and meat from numerous animal species, but was also likely supplemented with hunting, gathering, and fishing. A slight, but evident increase in human stable carbon isotope values at c. 800 BCE demonstrates the low-level incorporation of millet into a heavily pastoral diet that included both ruminant and equine dairy. It was not until the subsequent Xiongnu and Mongol Empires (c. 200 BCE - 300 CE) that we see an extremely significant ($p < 0.00027$) diversification in the range of carbon values demonstrating the importance of high-input C$_4$ grain consumption. However, even as the Xiongnu and Mongol Empire dietary economies dramatically shifted to include locally grown and internationally traded grains, fruits, and vegetables, dairy remained economically and culturally important.
Our multidisciplinary approach has overturned previous assumptions regarding the arrival of ruminant dairying on the eastern steppe, the origins of Mongolian horse dairying, and the extreme dietary diversity of Xiongnu and Mongol empires. These datasets in these papers provide a template for future work uncovering the pathways of the dairying populations followed across the continent from Anatolia to Mongolia, revealing the extent of early horse dairying in Central Asia, and adding further resolution to our understanding of cultivated crop consumption. Furthermore, our work on understanding changes to (low- to high-investment cultivation) and the resilience of (ubiquity of dairy pastoralism through time) specific subsistence strategies in antiquity can help inform modern populations of optimal strategies for sustainable approaches to future subsistence.
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8. Summary

This dissertation explores the dietary histories of ancient Mongolia through the study of stable isotope and protein analyses, with a specific focus on the origins of dairy pastoralism and cereal grain cultivation on the eastern Eurasian steppe. In contemporary Mongolia, dairy pastoralism is a frequently practiced subsistence strategy in rural areas, and milk from as many as seven species is processed into numerous different dairy products. While dairy pastoralism and milk consumption are common in Mongolia’s history, the antiquity of dairying has been unknown. The situation is similar with grain cultivation which makes up a small but important component of Mongolia’s economy today, facilitated by the use of modern irrigation methods. It remains unclear, however, when grain cultivation began on the eastern steppe, and which crops were utilized. This dissertation, through three separate studies using proteomics and stable isotope analysis, aims to discover when dairy and millet were first incorporated into early Mongolian diets.

In order to better understand the antiquity of dairying on the eastern steppe, shotgun proteomics was applied to ancient human dental calculus. Through this analysis I identified the presence of ruminant (sheep or cow) milk proteins in human dental calculus at c. 3000 BCE from an Afanasievo-associated site in Central Mongolia. Evidence for ruminant milk consumption was also identified in the Bronze Age, Xiongnu, and Mongol Empire individuals included in this study, demonstrating the extensive and continuous history of dairy use in eastern steppe diets. Furthermore, I detected horse milk proteins in dental calculus dating to c. 1200 BCE, coinciding with archaeological evidence for horseback riding, indicating a dramatic shift in the economic roles of horses in the Late Bronze age. The incorporation of ridden horses into herds allowed more animals to be herded and opened the intermontane regions of the steppe for occupation and grazing. These changes may have led to an increase if population size and inter-population interactions, both of which likely impacted the formation of the subsequent Xiongnu Empire.

To refine the existing knowledge surrounding the origins of grain cultivation on the eastern steppe, analysis of stable carbon isotope ratios from human bone collagen and dental bioapatite was conducted. The results of these studies indicate the beginning of C₄ grain consumption in the Early Iron Age in north-western Mongolia, and a significant diversification of dietary intake, including grains, in the imperial regimes of Mongolia’s Iron Age and Medieval periods.
These data, combined with historical documents and well-established archaeological and archaeobotanical evidence, show the extensive diversity and range of historic Mongolian diets.

This thesis identifies changes and uniformities in dietary trends in populations from over 5000 years in Mongolia. My protein data show the earliest known dairy consumption on the eastern steppe beginning at 3000 BCE, and the drinking of horse milk from 1200 BCE, both practices that continues to the modern day. I also show the slow incorporation of grain consumption into pastoral systems, through local millet cultivation, in the Early Iron Age, which increased alongside the creation of a grain-supplemented economic surplus (local and imported) during Mongolia’s imperial periods. Through these studies, we can see the early and continued use of dairy in the pre-imperial periods, as well as throughout the Xiongnu and Mongol Empires, and a significant increase in the cultivation of millet through imperial-supported agriculture.
9. Zusammenfassung (Summary in German)

Diese Doktorarbeit bietet eine neue Perspektive zur Geschichte der Ernährung in der Mongolei durch die Analyse von Proteinen und stabilen Isotopen. Der Fokus besteht hierbei vor allem auf die Herkunft der Milchproduktion und der Kultivierung von Getreiden in der Ost-Eurasischen Steppe.


Um die Geschichte der Milchproduktion in der ostasiatischen Steppe besser zu verstehen, wurde das Konzept der Shotgun-Proteomik auf menschliche Zahnsteinproben aus archäologischen Kontexten angewendet. Durch diese Untersuchungen habe ich festgestellt, dass Milchproteine von Kühen und/oder Schafen in menschlichem Zahnstein niedergelegt wurden, der in einer auf 3000 v. Chr. datierten Fundstelle gefunden wurde, die mit der Afanasievo-Kultur im Zentrum der Mongolei verbunden wird. Beweise für den Verzehr von Milch wurden auch in Individuen gefunden, die auf die Bronzezeit datiert wurden und auch auf die Zeiten des Xiongnu und mongolischen Reiches, was auf eine umfangreiche und kontinuierliche geschichtliche Bedeutung der Milchproduktion für die Ernährung in der Steppe hinweist.


Durch diese Untersuchungen habe ich die frühen Anfänge und kontinuierliche Bedeutung der Milchproduktion aufgezeigt und den deutlichen Anstieg des Getreideanbaus als ein wichtiger Teil der Wirtschaft des mongolischen Reiches.
10. Statement of Honour (Eigenständigkeitserklärung)

I declare that I am familiar with Course of Examination for Doctoral Candidates in the Faculty of Biology and Pharmacy. I have written this dissertation myself, without using any assistance or sections of texts from third parties, or from other of my own works, without identifying them through citations or inclusions as co-authors. No one has been enlisted as a doctoral consultant, and no third parties have received any direct or indirect payment for work connected to this dissertation. This dissertation, beyond the included manuscripts that have been submitted for publication, has not be previously submitted for any other scientific examination, and none of the work presented here has been submitted to any other postsecondary school.

Jena, the 27.08.2019

Shevan Wilkin

Shevan Wilkin
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12. Author Contributions

Manuscript A.

“Bronze Age population dynamics and the rise of dairy pastoralism on the eastern Eurasian steppe”

**Author contributions:** Choongwon Jeong, Frank J. Rühli, Bruno Frohlich, Jessica Hendy and Christina Warinner designed the research plan. Choongwon Jeong, *Shevan Wilkin*, Tsend Amgalantugs, Abigail S. Bouwman, Richard W. Hagan, Sabri Bromage, Soninkhishig Tsolomon, Christian Trachsel, Judith Littleton, John Krigbaum, Marta Burri, Ganmaa Davaasambuu, Franziska Irmer, Erdene Myagmar, Bruno Frohlich, Jessica Hendy, and Christina Warinner performed research; Richard W. Hagan, Christian Trachsel, Jonas Grossmann, and Ashely Scott contributed new reagents/analytic tools; Nicole Boivin, Martine Robbeets, and Johannes Krause contributed personnel and material resources; Choongwon Jeong, *Shevan Wilkin*, William T.T. Taylor, Richard W. Hagan, Sabri Bromage, Soninkhisig Tsolomon, Christian Trachsel, Jonas Grossmann, Cheryl A. Makarewicz, John Krigbaum, Ashley Scott, Ganmaa Davaasambuu, Joshua Wright, Nicole Boivin, Martine Robbeets, Johannes Krause, Bruno Frohlich, Jessica Hendy, and Christina Warinner analyzed data; and Choongwon Jeong, *Shevan Wilkin*, Jessica Hendy and Christina Warinner wrote the paper. In total, Shevan Wilkin contributed to 50% of the project.

Manuscript B

“Dairy pastoralism sustained eastern Eurasian steppe populations for 5000 years”

**Author contributions:** *Shevan Wilkin*, William Taylor, Christina Warinner, Nicole Boivin, and Jessica Hendy designed the research plan; Choongwon Jeong, *Shevan Wilkin*, Richard W. Hagan, Christian Trachsel, Jonas Grossmann, Abigail Ramsøe, Erdene Myagmar, Jessica Hendy, and Christina Warinner performed research; Richard W. Hagan, Christian Trachsel, Jonas Grossmann, and Ashely Scott contributed analytic tools; Nicole Boivin contributed personnel and material resources; *Shevan Wilkin*, William T.T. Taylor, Richard W. Hagan, Christian Trachsel, Jonas Grossmann, Jessica Hendy, and Christina Warinner analyzed data;
and Shevan Wilkin and Jessica Hendy wrote the paper. In total, Shevan Wilkin contributed to 80% of this research.

Manuscript C

“Economic Diversification Supported the Growth of Nomadic Mongolian Empires”

Author contributions: Shevan Wilkin, William Taylor, Nicole Boivin, and Patrick Roberts designed the research plan; Shevan Wilkin, Alicia Ventresca Miller. Bryan K. Miller, Richard W. Hagan, Ricardo Fernandes, Madeleine Bleasdale, Erdene Myagmar, and Patrick Roberts performed research; Nicole Boivin contributed personnel and material resources; Shevan Wilkin, Alicia Ventresca Miller, Bryan K. Miller, William Taylor, Ricardo Fernandes, Richard W. Hagan, analysed data; and Shevan Wilkin, Alicia Ventresca Miller, Bryan K. Miller, and Patrick Roberts wrote the paper. In total, Shevan Wilkin contributed to 80% of this research.
13. Curriculum Vitae

Shevan Wilkin
Melanchtonstraße 10 • 07743 – Jena, Germany
Phone: +49 (0)3641 686-733 • E-Mail: shevanwilkin@me.com

Education


Publications


William Taylor, Shevan Wilkin, Joshua Wright, Michael Dee, Erdene Myagmar, Accepted
William Timothy Treal Taylor, Julia Clark, Tumurbaatar Tuvshinjargal, Jessica Thompson Jobe, Svetlana Shnaider, Frederik Seersholm, Isaac Hart, Nicholas Case, Jamsranjav Bayarsaikhan, Ulrike Thuering, Jessica Hendy, Bryan K. Miller, Alicia Ventresca Miller, Shevan Wilkin, Andrea Picin, Nils Vanwezer, Franziska Irmer, Samantha Brown, Aida Abydkanova, Daniel Schultz, Victora Pham, Michael Bunce, Katerina Douka. Emily Lena Jones, Nicole Boivin. Horses and Herding Transitions in the Bronze Age Eurasian Steppes. (Current Anthropology) Accepted


Presented talks

Food Security in Flu, Archaeological methods for economic sustainability, MPI-SHH, Department of Archaeology, Jena, Germany – “Dairy pastoralism and millet agriculture on the ancient Eurasian Steppe” May 2019

UKAS Conference 2019, University of Manchester, UK – “Pastoralism and agriculture on the ancient Eastern Eurasian Steppe through multi-proxy archaeological data sets” April 2019

Invited talk, Botai Culture and other Neolithic Monuments of Central Asia, Al-Farabi University, Department of Archaeology, Almaty, Kazakhstan – “Using proteomics to trace dairying in ancient Eurasia” January 2019

Invited talk, Vilnius University, Department of History – “Exploring Equine Dairying through shotgun proteomics of human dental calculus” December 2018

Invited lecture, National Museum of Ethnology Osaka, Japan – “Processing and Consumption of Dairy Products in Ancient Eurasia: The Forefront of Milk Studies by Biomolecular Archaeology and the Connection with Northeast Asia” October 2018

Departmental talk, MPI-SHH, Department of Archaeology – “Ancient Mongolia through proteomics, isotopes, and mummies: An update of PhD progress” September 2018

ISBA8, Jena, Germany – “Ancient Dairying in Mongolia through LC-MS/MS bottom-up proteomics”
EAA Conference 2018, Barcelona, Spain – “Understanding ancient Mongolian dairying through shotgun proteomics of dental calculus” September 2018

Ancient Proteins @20, Copenhagen, Denmark – “The antiquity of dairying on the Eastern Eurasian Steppe” August 2018

Scientific Advisory Board Lightning Talk, MPI-SHH, Jena, Germany – “Early dairy on the Eastern Eurasian Steppe through proteomics” May 2018

Ancient Protein Workshop at the Functional Genomics Center Zurich, Switzerland – “Using LC-MS/MS methods for paleoproteomics” January 2018

Departmental update, MPI-SHH Department of Archaeology, Jena, Germany – “Update on preliminary protein results” September 2017

UKAS conference 2017, University College London, UK – “Pastoralism and dairying in ancient Mongolia: Insights from proteins in dental calculus” April 2017

Eurasia Triangle invited talk, MPI-SHH Department of Linguistics and Cultural Evolution, Jena, Germany – “Early Pastoralism on the Eastern Steppe” February 2017

Departmental PhD project overview, MPI-SHH, Department of Archaeology, Jena, Germany – “Early pastoralism on the Mongolian steppe” February 2017

Jena, the 27.08.2109

Shevan Wilkin

Shevan Wilkin