

**Stable Isotopic Analysis of Equid (Horse) Teeth from Mongolia**

by

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This study examines the carbon and oxygen isotopic composition of bioapatite in equid tooth enamel as a potential record of environmental change in a north-central Mongolian sampling area between 51.4°N, 99.0°E and 44.6°N, 106.9°E (northwest to southeast). Mammal tooth enamel is useful as a palaeoclimate proxy because it is a durable material that directly reflects the isotopic composition of the body, and therefore organism diet and water intake. In addition, tooth enamel accumulates sequentially from crown to root over the period of months to years and often records seasonal variation. Thus, the inter- and intra-tooth variations in the stable carbon ( $\delta^{13}\text{C}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotopic composition of horse tooth enamel may provide a high-resolution record about climatic factors such as temperature and moisture availability as well as the composition and availability of forage during the tooth growth period. Sequential incremental samples from modern horses were analyzed to provide a record of the carbon and oxygen isotopic values preserved during tooth enamel formation and mineralization. To constrain the final composition of the bioapatite, modern enamel stable isotopic compositions were compared with the compositions of meteoric waters and plants from comparable localities. Modern teeth displayed a marked regional seasonal oscillation in  $\delta^{18}\text{O}$  and record a latitudinal shift observed in the  $\delta^{18}\text{O}$  of meteoric waters. In addition, bulk and sequentially sampled profiles from the modern teeth were used as a comparative set with samples from archaeological teeth (Bronze Age, ca. 1000 B.C.) and suggest that climatic patterns were roughly equivalent during both periods, with a similar plant communities, and similar summer precipitation/temperature patterns. However,

seasonality may have been more intense in Bronze Age ca. 1000 B.C., with similar summer highs but more severe winters. The difference could be due to This work contributes to ongoing research into the climatic history of Central Asia and to the application of equid tooth enamel as an environmental proxy in this region.

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## **PREFACE**

I am indebted to Dr. Rosenmeier for the opportunity to participate in this research, and for his guidance, patience and humor. Many thanks to Drs. Dan Bain and Charles Jones (University of Pittsburgh) and Dr. Francis Allard (Indiana University of Pennsylvania) for the time and assistance they have committed to this research. Marion Sikora at the University of Pittsburgh has provided endless direction with the lab procedures and constant aid. I am also grateful to Dr. Allard, and Drs. William Fitzhugh, and William Honeychurch (Smithsonian Institution) for their contributions to the archeological collections. This project was funded in part by a Brackenridge Undergraduate Fellowship Grant and the University of Pittsburgh's Honors College.

## 1.0 INTRODUCTION

Mongolia lies in a region whose ecology and economy are uniquely sensitive to climatic factors. Minimal annual precipitation and low relative-humidity determine the arrangement and species composition of the steppe and related biomes that cover much of the country. Traditional nomadic pastoralism is dependent on these precipitation-sensitive arid and semi-arid biomes of the Inner-Asia country.

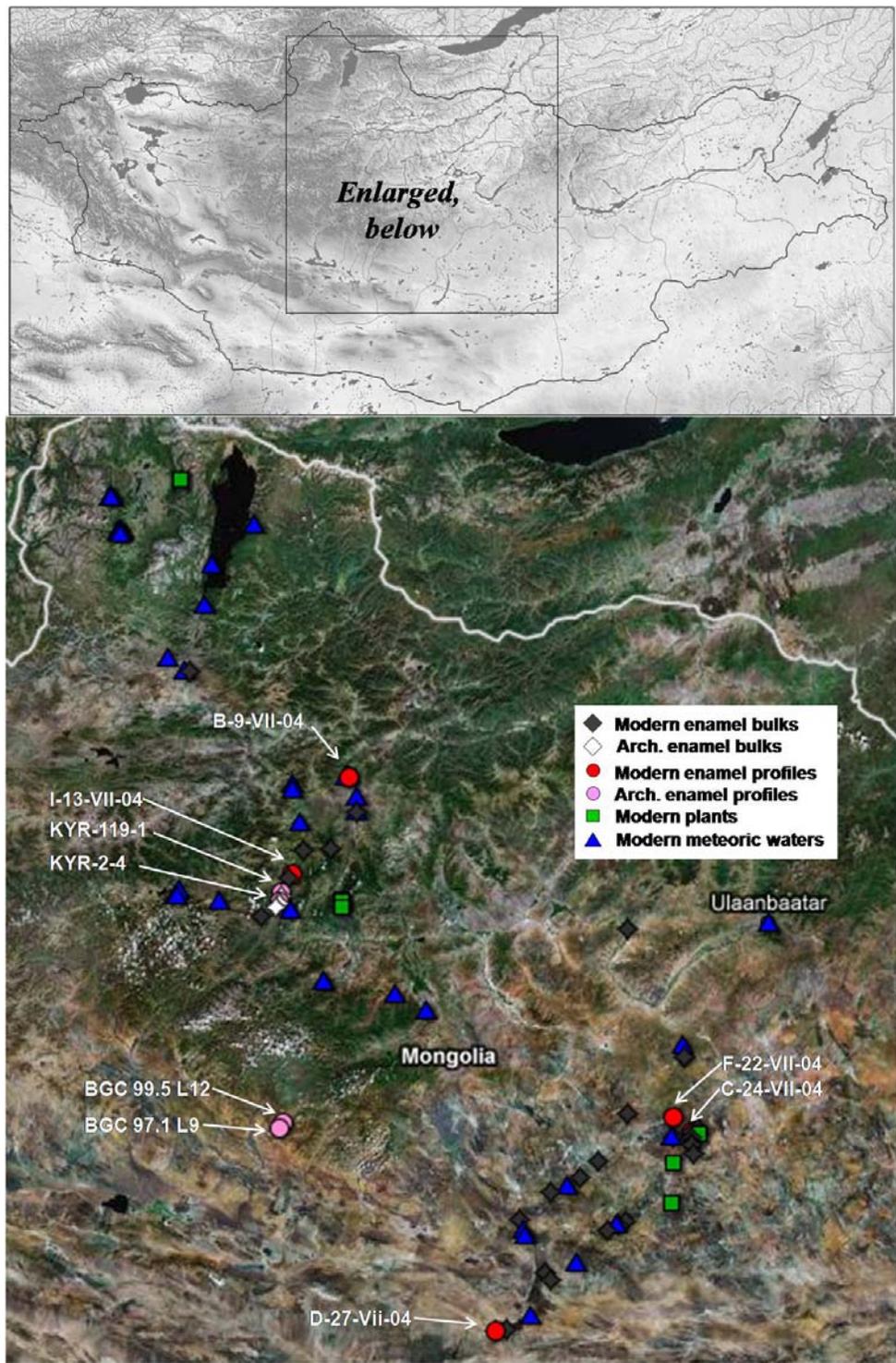
Nomadic pastoralism has been the most important economic activity in Mongolia for millennia. This study will focus on the regional Bronze Age, which ran from the mid-late second millennium B.C. until the mid-first millennium B.C.. During this time, the landscape was marked by an abundance of graves, stelae, and complex stone-built sites (Allard and Erdenebaatar 2005). There may have been agriculture during this time, but at least in the Khanuy Valley, a focus of this study, no above-ground remains of agriculture have been found. Archaeological investigations of Bronze Age sites in this region suggest that nomadic pastoralists erected the stone monuments and burials. Chronological examination of many of the sites indicates a time of social, economic, and cultural transition (Allard and Erdenebaatar 2005; Wright et al. 2007). A stable isotopic palaeoclimate exploration of this period will yield additional information on the climatic context in which these cultural changes, and the monument building developed, as well as allowing a comparison of the climatic and environmental context of Bronze-Age and modern nomadic pastoralism. Horse remains are present in many of the archaeological sites and thus

provide ample material for stable isotopic analysis in the framework of palaeodietary and palaeoecological reconstruction.

Because of its size, durability and diagenetic resistance, horse tooth enamel offers a high-resolution record of stable carbon and oxygen isotopes in vegetation and water ingested during tooth formation. In recent years, stable isotopes in mammal tooth enamel have been often studied as a proxy for palaeoclimate and palaeoseasonality (*e.g.* Hoppe *et al.* 2005, Nelson 2005; Wang *et al.* 2008) and to suggest the palaeodietary and palaeoenvironmental context for species evolution (*e.g.*, Sharp and Cerling 1998; Cerling *et al.* 1999; Passey *et al.* 2002) or archaeological sites (Lee-Thorp *et al.* 1989; Wiedemann *et al.* 1999). These stable isotopic analyses have been widely applied to the palaeoclimatic record in North America (Feranec and MacFadden 2000; Passey *et al.* 2002; Hoppe *et al.* 2005; Hoppe *et al.* 2006), Africa (Lee-Thorp *et al.* 1989; Kohn *et al.* 1998; Cerling *et al.* 1999) and parts of Asia (Wiedemann *et al.* 1999; Zazzo *et al.* 2002; Nelson 2005; Wang *et al.* 2008). However, few enamel-based palaeoclimatic studies exist in northern Central Asia. Cerling and Harris (1999) included specimens from five individual horses in northern Mongolia as part of a survey on isotopic patterns in mammal tooth enamel from a variety of climates around the world, but little else has been published on the isotopic. This current study in north-central Mongolia first establishes the relationships between modern teeth and modern environmental factors and then compares modern enamel oxygen and carbon isotopic values with those from Bronze-Age archeological samples in the same region. The record of isotopic variation preserved by the teeth contributes to a broader understanding of equid tooth enamel as an environmental proxy for this region and complements lower resolution but longer term lacustrine sediment records available from the region.

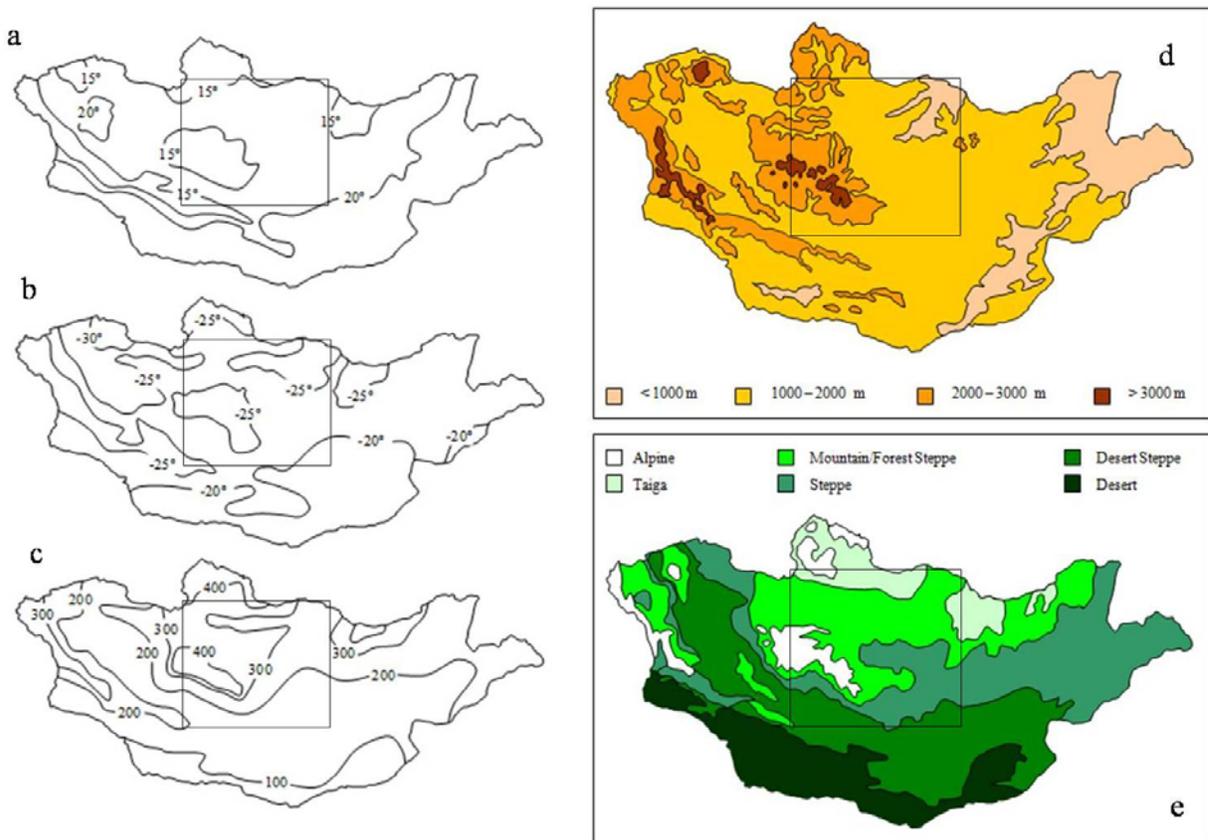
## 1.1 CLIMATE OF STUDY LOCATION

This study focuses on samples from two areas comprising two northeast-southwest transects in north-central Mongolia (between 44°N, 99°E and 49°N, 101°E ) (Fig. 1). The sampling area includes portions of the *aimags* (provinces) Hovsgöl, Bulgan, Arkhangai, Övörhangai, Töv, and Dundgovī. The sampling area is west of the Mongolian capital of Ulaanbaatar.



**Figure 1.** Study location. Digital Elevation model of Mongolia (above) and the central Mongolia sampling area (below) including bulk and profile enamel samples from archaeological and modern equid teeth, and the sample locations of modern meteoric waters and plants.

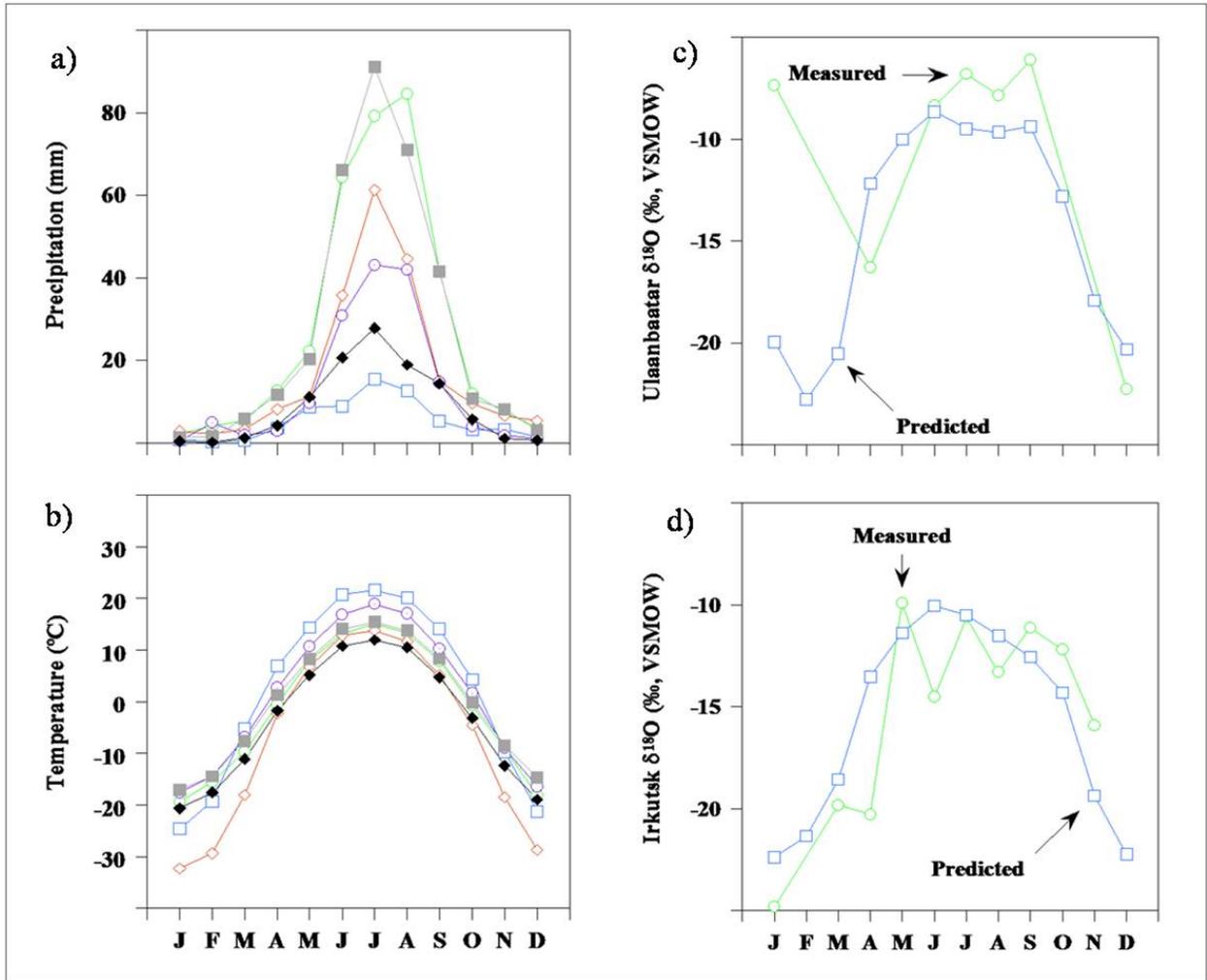
Mongolian climate displays a prominent latitudinal gradient, with more precipitation and cooler temperatures in the north, and warmer, more arid climates to the south (Fig. 2). The study area is characterized by a transition from desert steppe in the southern part through open grassland steppe and forest steppe to taiga in the northern reaches. Mongolian climate is highly seasonal with annual highs between 15-20°C and annual lows reaching -25 to -30°C. In the sampling region, 200-400 mm of precipitation falls annually, of which the majority occurs during the warmest months (Fig. 3 a & b).



**Figure 2.** Climatic variation in Mongolia: mean annual temperature highs (a) and lows (b), mean annual precipitation (c), elevation (d) and climate biomes (e). Sampling area from Fig. 1 marked in maps by boxes. Rainfall and temperature in Mongolia exhibit a prominent latitudinal gradient.

The mean monthly isotopic composition of precipitation at Ulaanbaatar and Irkutsk, two regional cities (Fig. 3, c and d), results from the seasonal oscillation of rainfall amount and

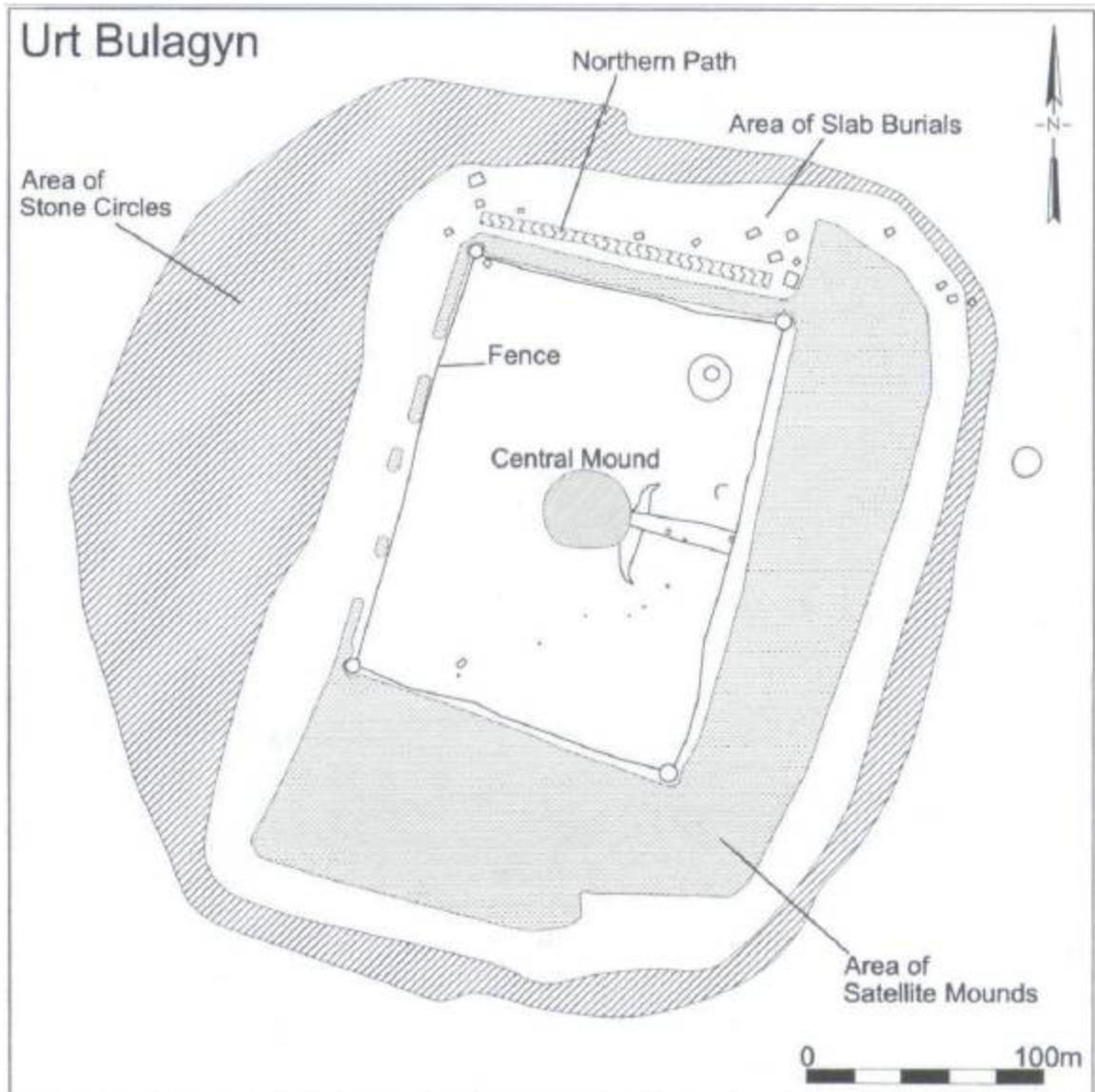
temperature (Fig. 3, a and b). For comparison, predicted values for isotopic composition of rainfall were calculated using the *Online Isotopes in Precipitation Calculator (OIPC)* (<http://www.waterisotopes.org>).



**Figure 3.** (a) Monthly variation in precipitation and (b) temperature in Ulaanbaatar (green), Hovd (blue), Tosontsengel (orange), Mandalgovi (purple), Bulgan, (gray), and Altay (black). (c, d) Variations in the mean monthly isotopic composition of precipitation in Ulaanbaatar and Irkutsk are represented by measured and calculated values (Measured values from International Atomic Energy Agency (IAEA) data. Predicted values calculated using the *Online Isotopes in Precipitation Calculator*).

## 1.2 ARCHAEOLOGICAL SITES IN MONGOLIA

One set of archaeological horse teeth used for comparison with modern teeth comes from the Khanuy Valley in Central Mongolia, near the central portion of the sampling region (Fig. 1). The valley contains thousands of Bronze Age archaeological sites, including many extensive, multi-faceted archaeological sites called *khirigsuurs* (large kurgans or burial mounds). The second largest *khirigsuur*, Urt Bulagyn, covers nearly 1600 square meters and consists of a 5-meter tall central mound with surrounding small satellite mounds, stone circles, and some slab burials (Fig. 4; see Allard and Erdenebaatar (2005) for a full description of the excavation). The site includes more than 1700 satellite mounds. Thus far, 45 satellite mounds have been excavated with horse remains (skulls, vertebrae, and occasionally leg bones) found in all. Preliminary radiocarbon dates from horse remains suggest that Urt Bulagyn may have been built over a short period of time (Allard pers. comm.). Overall, horse remains have been found at all 10 sites excavated in the valley.



**Figure 4.** Map of the *khirigsuur* Urt Bulagyn (burial mound and associated archaeological site) showing the extent of the site, which includes approximately 1700 satellite mounds. Each of the 45 satellite mounds excavated thus far contains horse remains, including teeth. (from Allard and Erdenebaatar 2005).

A second archaeological area further south of the Khanuy Valley provides a point of comparison to the central region. Baga Gazaryn Chuluu is a rocky area in the Gobi bordered by desert-steppe used throughout the Bronze and Early Iron Ages and the Xiongnu Period, the site is characterized by stone monuments and burials interspersed with areas containing materials

indicative of some agriculture (Wright *et al.* 2007). Materials found during the investigation of Baga Gazaryn Chuluu indicate continuity over several millennia, spanning times of evolving economies, social connections, and subsistence measures.

### **1.3 TOOTH ENAMEL AS A STABLE ISOTOPE RECORD**

#### **1.3.1 Background**

Mammal tooth enamel is useful as a proxy for palaeoclimate studies because it is a durable material whose composition is based on the isotopic composition of the body water, and thus the environment, during the mineralization period (Koch 1994). An animal's isotopic composition is based on that of the water and nourishment it consumes, which in turn relate to the environmental composition and climatic variables. Hypsodont (high-crowned) equid molars record sub-annual isotopic variations and may be preserved for millennia. They may be used to analyze diets, C3/C4 vegetation ratios, precipitation and evaporation, and other variables (Cerling and Harris 1999; Hoppe *et al.* 2004a; Wang *et al.* 2008)

Most organic animal parts decay and disappear with time after death leaving behind mineralized tissues such as bones and teeth. Studies using stable isotopes to focus on ancient periods must focus on these available materials. Even the organic fraction of these tissues, collagen, is susceptible to dissolution and degradation; little remains accurate enough for study after 10,000 years or so (Lee-Thorp *et al.* 1989). The inorganic portion, however, is a non-organic biogenically formed hydroxyapatite ( $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ ) with large crystals that is resistant to diagenesis (Koch *et al.* 1994; Sharp and Cerling 1998). Enamel, dentine, cementum and bone all

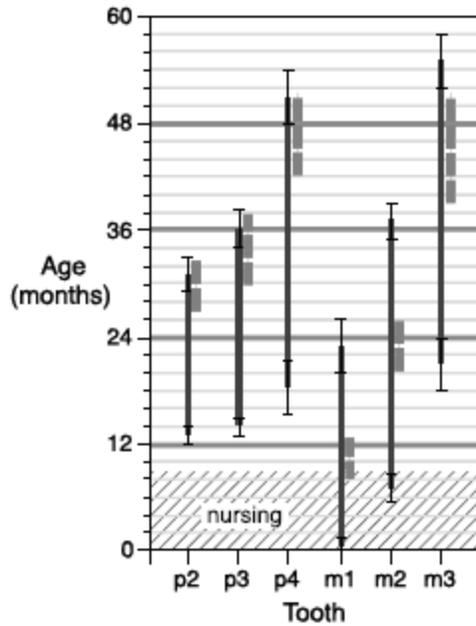
contain hydroxyapatite, but the last three also include up to 30% organic material. Enamel, in contrast, contains less than 3% organic matter (Hoppe *et al.* 2005). Enamel also exhibits the greatest degree of crystallization, which together with its low organic content makes it harder and more dense than other mineralized tissues. While the oxygen in apatite can be analyzed for its isotopic composition, in this study we analyze the carbonate ( $\text{CaCO}_3$ ) in horse teeth to obtain both  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ . The carbonate may be adsorbed into the matrix or substituted into the structure for the phosphate. The durability, resistance to post-mortem alteration, and presence of sufficient carbonate for sampling make enamel apatite a primary candidate for palaeoenvironmental studies (Wiedemann *et al.* 1999).

Because of variation in diet, behavior, and physiological processes, there is a species-specific relationship between tooth enamel  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ , diet and consumed water (Longinelli 1984; Lee-Thorp *et al.* 1989; Kohn 1996; Wang *et al.* 2008). Formation processes should therefore be considered by species, not generalized across all species. For each species, between diet and the averaged isotopic composition of the body, the fractionation varies based on various factors such as metabolism, available food and water, temperature and relative humidity. The enamel relates directly to the body composition during enamel formation. Oxygen isotopes in the enamel are related to the values of local precipitation while the carbon isotopic composition reflects dietary changes.

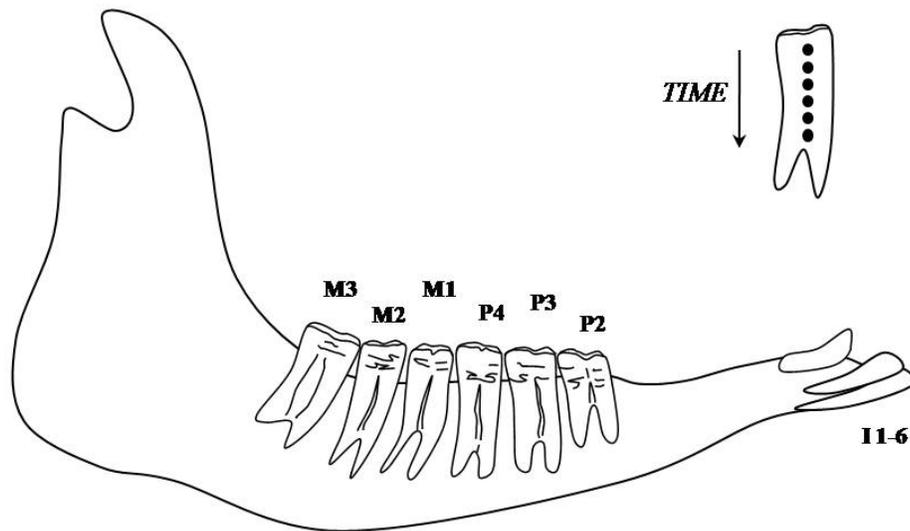
Horse teeth mineralize over a period of several months to years from the crown to the root. This incremental accumulation of horse tooth enamel preserves a temporal record that permits interpretation of seasonal changes, dietary behavior, or climatic changes (Sharp and Cerling 1998; Wiedemann *et al.* 1999; Feranec and MacFadden 2000; Wang *et al.* 2008). The size of the equid tooth allows successive samples of sub-seasonal resolution to be taken, even

with a hand-held drill. These serial micro-samples preserve isotopic variations (*e.g.* Sharp and Cerling 1998; Passey and Cerling 2002) that produce a profile of average body isotopic composition during formation.

Hoppe *et al.* (2004b) provide a thorough analysis of tooth formation and mineralization in horses. They found this complex multi-stage process, which involves an initial organic matrix and subsequent maturation phase, takes ~1.5 to ~2.8 years (Fig. 5). This process is longer than previously thought because the tooth continues to mineralize for ~6 months after eruption (Hoppe *et al.* 2004b). Thus, bulk samples and profiles should be at least 3-4 cm long to record full annual cycles. The complexity of the mineralization process in ungulates causes significant time-averaging, which attenuates the isotopic signal preserved in the enamel and may obscure more complex patterns in isotopic input (Passey and Cerling 2002). In addition, the M1 and M2 molars (see Fig. 6 for diagram of horse jaw) begin to form while the horse is still weaning, which may modify the isotopic composition in those teeth relative to those formed later in life (Hoppe *et al.* 2004b). The last teeth to form, the P4 premolar and the M3 molar, also mineralize the slowest, at a rate of ~3 cm/year (Hoppe *et al.* 2004b).



**Figure 5.** Timing of tooth mineralization (dark gray lines) and eruption (dashed light gray lines). Error bars account for timing variation in individuals and the population. The last molar in the jaw (M3) was the primary focus for sampling because forms after nursing and over a long period of time. (From Hoppe *et al.* 2004b).



**Figure 6.** Diagram of equid jaw showing the position of each tooth. The last molar in the jaw (M3) forms after weaning and covers a long period of time; this was the tooth sampled from each individual.

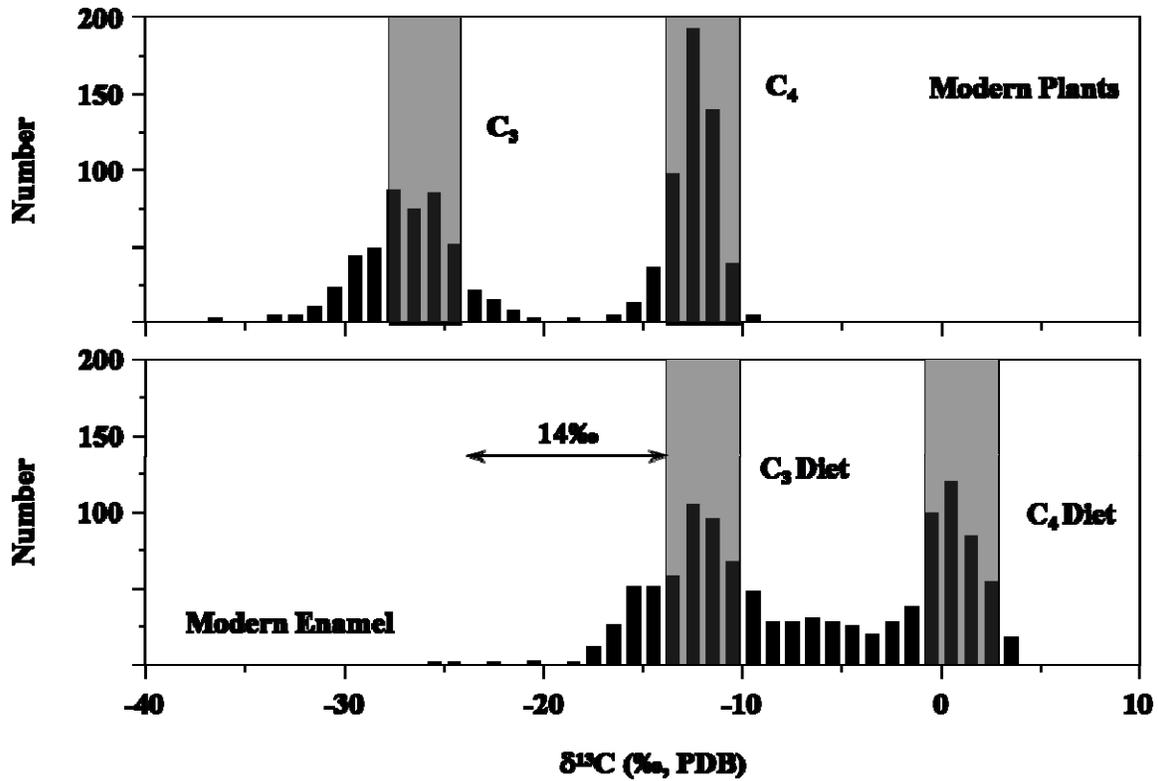
### 1.3.2 Horse enamel $\delta^{13}\text{C}$ as a palaeodietary indicator

Carbon isotopic fractionation occurs in C3 and C4 plants on the basis of their photosynthetic cycles. Most cool season grasses, trees, and herbs use the Calvin cycle (C3); while C4 plants, including warm season grasses and other plants adapted to warm climates, utilize the Hatch-Slack Cycle to complete photosynthetic processes (Ehleringer and Monson 1993). The composition of most C3 plants averages  $-13\pm 2\text{‰}$  while C4 plants have a distinct and more negative average of  $-27\pm 3\text{‰}$ ; plants using crassulacean acid metabolism (CAM) have an intermediate value (Ehleringer 1989; Hoppe *et al.* 2004a). Beyond photosynthetic routes, climate is a major influence on plant carbon isotopic composition. For example, water stress may induce a plant to close stomata, thereby restricting the isotopes available for photosynthesis because of reduced exchange with the atmosphere.

In Mongolia, defined seasonality and patterns of aridity in the climate contribute to the distribution and isotopic makeup of the plants. Mongolia's northern grasslands are dominated by C3 plants (Cerling and Harris 1999). The abundance of C4 grass species increases with decreasing geographical latitude and along regional north-south temperature and precipitation gradients (Pyankov *et al.* 2000). In general, C4 plants are confined to the southern portions of the country and account for only 3.5% of all of Mongolia's flora (Pyankov *et al.* 2000). In the north-central portion of Mongolia, the majority of vegetation uses a C3 metabolic pathway.

These differences in vegetation composition may be visible in horse tooth enamel because there is an established relationship between the isotopic composition of consumed plants and final enamel makeup. Average isotopic enrichment (positive fractionation) in large herbivores is 14.1‰ between diet and tooth enamel isotopic values (Cerling and Harris 1999). Their small sample set of Mongolia horses ( $n=5$ ) yielded a isotopic fractionation of  $+13.8\text{‰}\pm 1.9$ ,

while Hoppe *et al.* (2004a) found a higher value of  $+14.6\pm 0.5$ . Both values are statistically identical to the herbivore average, which will be considered herein when calculating an isotopic fractionation for this region. The distinct isotopic fractionation resulting from dissimilar photosynthetic processes is reflected in enamel (Fig. 7), generating a value of around  $-13\text{‰}$  for C3 feeders and  $+1\text{‰}$  for herbivores that feed primarily on C4 plants.



**Figure 7.** Fractionation of carbon between C3 and C4 plants (upper panel) and the distinct difference in enamel values (lower panel) (adapted from Kohn and Cerling, 2002).

### 1.3.3 Horse enamel $\delta^{18}\text{O}$ as a palaeoclimate indicator

Enamel is useful as a palaeoclimate indicator because its  $\delta^{18}\text{O}$  values reflect the oxygen isotopic composition of body water, and thereby contributing sources, during the period of mineralization. This relationship is dependant on a number of factors: (1) formation occurs at

constant temperature in homoeothermic animals; (2) body water is directly related to ingested meteoric waters for obligate drinkers; and (3) composition of these meteoric waters is regulated by climatic factors. The isotopic composition of the ingested food and drinking water and the physiological and metabolic processes of the body are the primary factors that determine the  $\delta^{18}\text{O}$  values of body water (Koch *et al.* 1994). Equid body water turnover time may be assumed rapid enough to record seasonal variations in ingested water (Sharp and Cerling 1998). Variations in metabolic functions may cause body water values to vary from those of drinking water by up to 3‰ in domestic horses (Hoppe and Amundson 2001). Times of water stress may also lead to enrichment in the water available from plants, which complicates interpretation of the relative abundances of C3/C4 plants (Wang *et al.* 2008). Body water values may also be more positive than meteoric precipitation because of evaporative fractionation in surface waters. While numerous factors affect the final stable isotopic composition of the enamel, the environmental signal is still visible in sequential samples.

Meteoric water sources are also affected by numerous factors. Beyond the effects of the timing and amount of precipitation; the relative amount of  $\delta^{18}\text{O}$  may also vary with continentality (decreases as precipitation reaches further inland, so-called “rainout”), and with temperature (decreases with decreasing temperature) (Dansgaard 1964; Rozanski *et al.* 1993).

#### **1.3.4 Stable isotopic variation of enamel in individuals and populations**

Throughout the year, factors such as precipitation amount, temperature, and plant community composition affect the environmental isotopic composition. Since bioapatite isotopic composition is dependent on environmental composition, intra-tooth values may vary a great deal. Intra-tooth  $\delta^{13}\text{C}$  variations may range from approximately 0.9‰ (Cerling and Harris 1999)

to more than 5‰ (Hoppe *et al.* 2005) depending on sample size and location, though a median value of ~2.8-2.9‰ is more common (Hoppe *et al.* 2004a; Wiedemann *et al.* 1999). Oxygen isotopic intra-tooth variation is often higher, around 6.5-9‰ (Hoppe *et al.* 2004a; Wiedemann *et al.* 1999). Enamel is a faithful recorder of diet and can be used to estimate the fraction of C3/C4 plants (Cerling and Harris 1999; Hoppe *et al.* 2004a). However, this is not a precise calculation. In one study, Hoppe *et al.* (2004a) estimated that enamel isotopic results underestimated the prevalence of C4 grasses in that environment by 10-20%, while another study found that disparity to be ~12% (Hoppe *et al.* 2005). These studies suggested that the underlying causes of these differences were higher levels of C3 browse consumption than assumed as well as variation in fractionation within the body. Individual species record the same environmental signal differently, as specific physiological, behavioral and dietary characteristics alter the enamel record (Kohn 1996; Wang *et al.* 2008). These factors make interpretation of the environmental signal more demanding.

In addition, Hoppe *et al.* (2004a) found that the difference in  $\delta^{18}\text{O}$  between two desert locations (one with ~100% C3 plants and cold season precipitation, and another with ~95% C4 and precipitation during the warm months) was less than the variation observed within populations. In a study on the Tibetan Plateau, Wang *et al.* 2008 found that oxygen isotopic variation was not linked to any single variable; multiple influential variables complicated interpretation. These ambiguities, taken together, introduce uncertainty into the use of enamel  $\delta^{18}\text{O}$  as a climatic proxy. Though there may be broad variations within single populations (*e.g.* Hoppe *et al.* 2005; Hoppe *et al.* 2004a) the assessment of many individuals from the same region and time period presents a broader picture of intra-population variation and may clarify and refine trends.

## **2.0 METHODS**

This study had three main parts. First of all, the environmental isotopic baseline is established by analysis of meteoric waters and plants from locations across the sampling region. Secondly, this baseline and the isotopic composition of modern teeth are compared to analyze their relationship. This relationship is examined because it often differs between each locality due to controlling factors. Once the relationship between the environmental and enamel isotopic composition is known, results from modern teeth are compared with those of archaeological teeth obtained from two Bronze-Age sites in the sampling area.

### **2.1 COLLECTION**

Tooth specimens and vegetation and water samples were collected along a latitudinal transect in north-central Mongolia. Elevation over this area ranges from about 1000 to 2400 meters above mean sea level (m.a.m.s.l). Modern teeth were collected in Mongolia over two summer field seasons in 2004 and 2006. Teeth from 90 modern individuals were collected during these field seasons. With assistance from collaborators Francis Allard, William Fitzhugh, and William Honeychurch, sample teeth from 35 Bronze-aged individuals were collected at archeological sites within the modern transect.

## 2.2 SAMPLING

Teeth were manually cleaned using mild soap and a brush prior to sampling. Sampled teeth were chosen on the basis of location and existing condition. M3 teeth were sampled to avoid the effects of weaning on stable isotopic composition and minimize discontinuities between teeth. Unworn juvenile teeth were excluded in an effort to avoid enamel that was not fully mineralized. Using a Dremel© variable speed hand drill with a diamond bit, the cementum and other contaminants were removed from the enamel, which was then sampled. Bulk samples (n=63 modern, 20 archeological) were taken along the growth axis. Profiles were obtained by drilling sequential samples perpendicular to the growth axis from the crown to the root of each tooth. Profile lengths ranged from 33-59 millimeters. Each sample was approximately 1 millimeter long with ~0.5 millimeter between sequential samples (see Fig. 8 for an example tooth).



**Figure 8.** Sequential sampling on tooth MN-EQ-B-9-VII-04 M3

### 2.3 TREATMENT AND ANALYSIS

Stable isotopes studies rely on the different isotopic weights to distinguish the relative abundance of each isotope in a mass spectrometer. Of the three naturally occurring oxygen isotopes,  $^{16}\text{O}$  is the most abundant and  $^{18}\text{O}$  makes up a small fraction (<1%). Carbon occurs naturally in three isotopic forms,  $^{12}\text{C}$ ,  $^{13}\text{C}$  and  $^{14}\text{C}$  (carbon-12 comprises ~99% of naturally occurring carbon, while 1% is carbon-13, and carbon-14 only occurs in trace amounts). Enamel samples reacted with acid yield carbon dioxide for analysis. The isotopic composition of this carbon dioxide may be deduced by the overall weight of the molecule (*e.g.* mass 46 is  $^{12}\text{C}^{16}\text{O}^{18}\text{O}$ ). The relative

abundance is expressed as the molar ratio of the less common (heavier) isotope to the more common (lighter) one in the sample compared to an international standard. This relative measure is conventional delta ( $\delta$ ) notation and written as  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  in parts per mil (‰).

Vegetation samples were freeze-dried and subsequently ground using a coffee grinder. Plant samples for stable carbon isotope analyses were measured with a Carlo Erba NA 1500 CNS elemental analyzer and VG PRISM II series mass spectrometer at the University of Florida. Tin capsules containing 400-500  $\mu\text{g}$  of sediment were combusted at 1040°C in an  $\text{O}_2$  atmosphere. Combustion gases were passed through a reduction column in a stream of helium gas and into a gas chromatograph where  $\text{CO}_2$  was separated. The gas stream then entered a VG PRISM II series mass spectrometer where the  $\text{CO}_2$  was concentrated in a cryogenic triple trap. Upon warming, the  $\text{CO}_2$  gas was analyzed in the mass spectrometer and compared to an internal gas standard. Plant sample  $\delta^{13}\text{C}$  values are expressed in conventional delta ( $\delta$ ) notation as the per mil (‰) deviation from the Vienna PeeDee Belemnite (VPDB). Precision for plant  $\delta^{13}\text{C}$  samples was  $\pm 0.08$  ‰.

Oxygen and carbon isotopic ratios were also measured on ground tooth enamel samples. Enamel samples were pretreated prior to analyses to a method developed with aid from Benjamin H. Passey (Department of Geology and Geophysics, University of Utah). Similarly to the shorter method described by Passey *et al.* (2002), samples were soaked for 15 minutes in 3%  $\text{H}_2\text{O}_2$  to remove organic matter, rinsed, soaked with buffered 1M acetic acid for 15 minutes to remove diagenetic calcium carbonate and adsorbed carbonate, and finally rinsed and dried. According to the work by Passey *et al.* (2002), there is no significant difference between this pretreatment method and a longer one in which samples are exposed to hydrogen peroxide and acetic acid for a 24 hour period.

Treated ground enamel samples weighing approximately 1000- $\mu\text{g}$  were reacted in 100% *o*-phosphoric acid at 90°C using a VG Instruments (IsoPrime, Ltd.) Multi-Prep™ inlet module and Gilson autosampler. Isotopic ratios of purified CO<sub>2</sub> gas were measured in-line (via dual inlet) with a VG Instruments IsoPrime™ stable isotope ratio mass spectrometer. Isotopic values are reported in conventional delta ( $\delta$ ) notation as the per mil (‰) deviation from Vienna Peedee Belemnite (VPDB). Precision for  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  samples were  $\pm 0.06$  and 0.08 ‰, respectively.

## 3.0 RESULTS

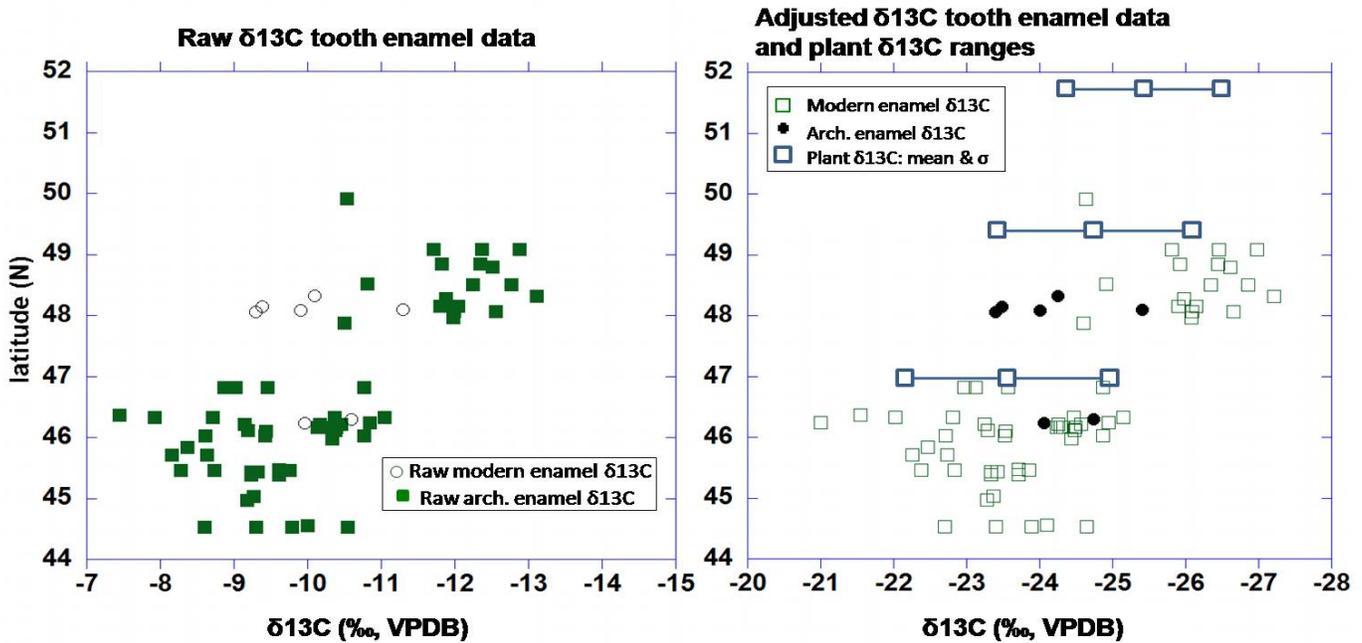
### 3.1 RELATIONSHIP WITH MODERN ENVIRONMENT

In order to accurately assess the palaeoenvironmental signal recorded by archeological teeth, the relationship needs to be established for the region. Samples from modern teeth were compared to water and plant samples from corresponding areas to determine the base environmental signal and the fractionation between the environmental composition and the enamel record.

#### 3.1.1 Local vegetation

Ethnographic interviews with herders at pasture sites identified plants typically consumed by horses (Allard, pers. comm.). In all, thirty plant samples were collected in three areas arranged north-south (between 51.4°N, 99.8°E and 45.6°N, 105.7°E), specifically ten samples from the northern Darkhat Valley, ten samples from the Khanuy Valley near the middle of the sampling area, and ten in the region of Baga Gazaryn Chuluu. One plant from the southern region had a value of  $-14.7\text{‰}$  which is within the range ( $-13\pm 2\text{‰}$ ) of C4 plants. Twenty-nine of the samples returned values between  $-23.4$  and  $-28.7\text{‰}$ , a range which encompasses the average value,  $-27\pm 3\text{‰}$ , calculated for C3 plants. Unless the survey missed a significant portion of C4 plants, the vast majority of plants consumed by horses in this region are C3 plants. C3 plants along each portion of the transect had different means. The Darkhat Valley mean was  $-27.2\pm 1.1\text{‰}$  ( $1\sigma$ ),

while the plants from Khanuy Valley centered on  $-26.5 \pm 1.3\text{‰}$ , and the southernmost nine plants averaged  $-25.3 \pm 1.4\text{‰}$ . While the Khanuy Valley average is not significantly different from either of the other plant averages, the  $\delta^{13}\text{C}$  average from north is significantly different from the south ( $p=0.014$ ). These means indicate a trend of increasing  $\delta^{13}\text{C}$  values with decreasing latitude, equivalent to  $\sim 0.4\text{‰}$  per degree of latitude (Fig. 9).



**Figure 9.** Left: Raw modern and archaeological bulk tooth enamel  $\delta^{13}\text{C}$  values plotted by latitude show decreasing  $\delta^{13}\text{C}$  values with increasing latitude. Right: Ingested  $\delta^{13}\text{C}$  values were calculated from bulk tooth enamel values (accounted for 14.1‰ fractionation between diet and enamel). For comparison, averages and means were calculated from the  $\delta^{13}\text{C}$  values of ten plant samples taken from each part of the sampling area.

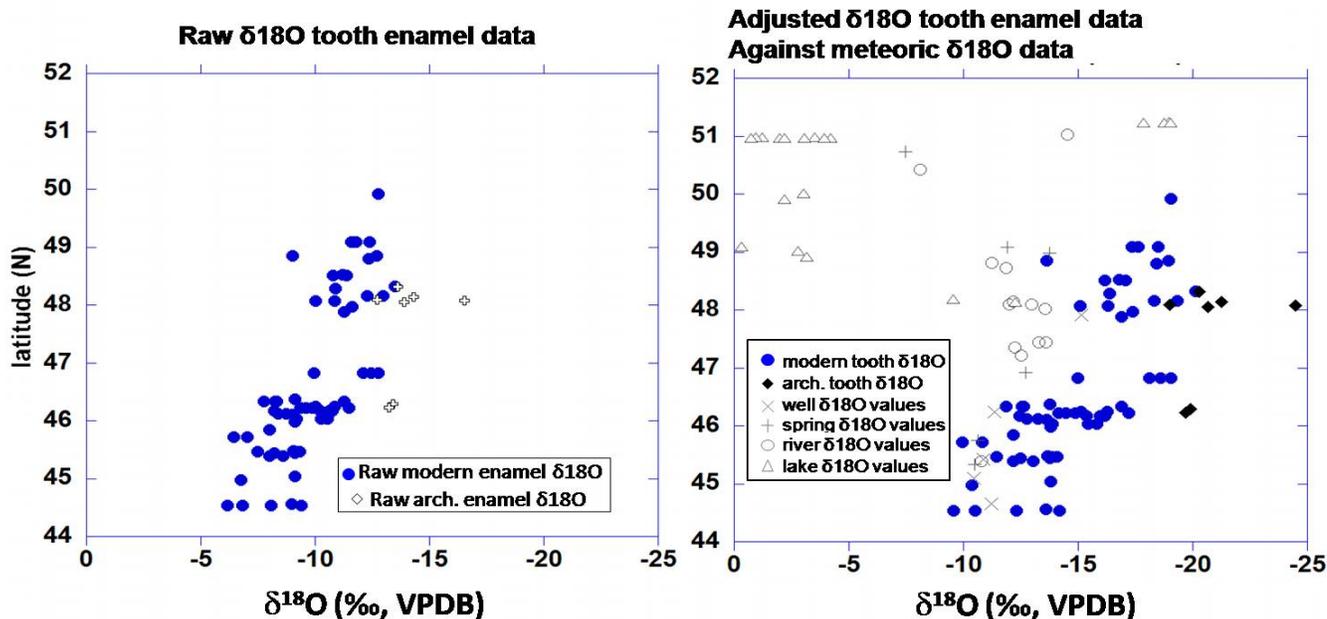
Since C4 plants account for only a small fraction of the region's plants, the variations in the enamel  $\delta^{13}\text{C}$  values, and thus the environmental signal, most likely reflect changing climatic variables. These variables, such as relative humidity or temperature, then affect metabolic processes such as plant respiration and carbon uptake. Plant water stress may also significantly impact the metabolic process. With increased aridity or other stress, plants must minimize water

loss, for example by closing stomata. This often results in reduced exchange with the atmosphere and increased use of the heavier isotope.

### 3.1.2 Local meteoric waters

Water samples were collected in north-central Mongolia (and 51.2°N, 99.0°E and 44.6°N, 106.9°E) from lakes, rivers, springs, wells, and rainfall during the early spring and summer seasons of the years 2003 and 2004. Sources included 21 lakes, three rainfall events, 13 rivers locations, six springs, and five wells between Oxygen isotopic values for surface waters (lakes, springs, rivers, and wells) analyzed by latitude (Fig. 10, right) demonstrate considerable variability (data available in Appendix A). The values for most of the lakes suggest evaporative enrichment (Fig. 10). Excluding the outlying lake values, meteoric waters sampled demonstrate a latitudinal influence, with increasing  $\delta^{18}\text{O}$  values observed with decreasing latitude. The average  $\delta^{18}\text{O}$  value of the rivers, springs, and wells is  $-11.9\text{‰}$ . In contrast, the mean value of all of the meteoric waters (i.e. including all of the lakes) is  $-9.3\text{‰}$ .

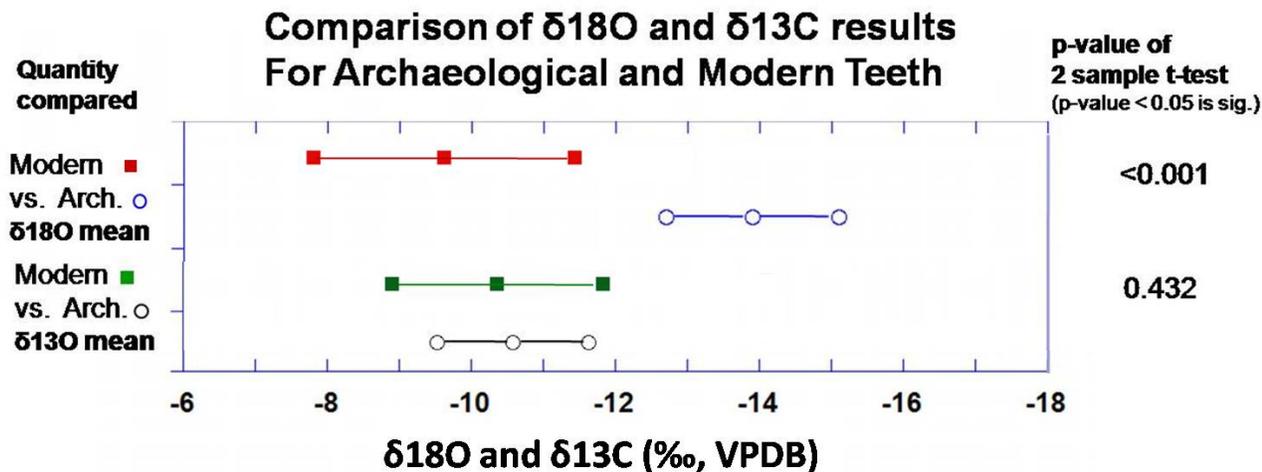
Ingested water values were calculated from the raw data using equations from Delgado Huertas *et al.* (1995) and Bryant *et al.* (1996). The resulting ingested water values are more negative than the meteoric waters (mean ingested value:  $-15.5\text{‰}$ ).



**Figure 10.** Left: Raw bulk modern and archaeological tooth enamel  $\delta^{18}\text{O}$  values plotted by latitude. Right: Ingested water  $\delta^{18}\text{O}$  values for modern and archaeological tooth enamel, plotted by latitude, were calculated from tooth enamel  $\delta^{18}\text{O}$  values using equations from Delgado Huertas *et al.* (1995) and Bryant *et al.* (1996). Modern meteoric water  $\delta^{18}\text{O}$  values shown for comparison.

### 3.1.3 Bulk tooth enamel $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values

Bulk  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values (Figures 9 and 10) plotted by latitude demonstrate trends similar to the ones shown by vegetation and meteoric waters. The average  $\delta^{13}\text{C}$  value for the collection of modern bulks is  $-10.2 \pm 1.5\text{‰}$ . While there is no significant difference between the  $\delta^{13}\text{C}$  bulk values averaged for the whole modern and archaeological groups, there is a difference in oxygen isotopic composition between the two groups (Fig. 11). A two-sample t-test shows the  $\delta^{18}\text{O}$  averages for modern ( $-9.9 \pm 1.8\text{‰}$ ) and archaeological enamel bulks ( $-14.0 \pm 1.2\text{‰}$ ) are significantly different ( $p < 0.005$ ).

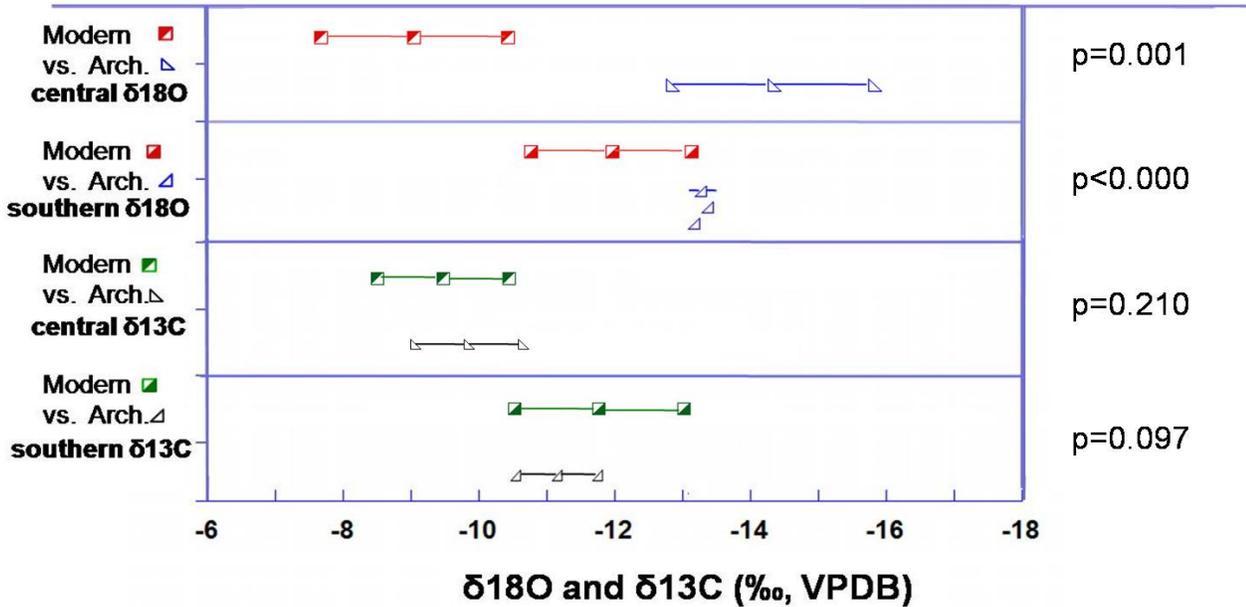


**Figure 11.** A comparison of the  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  means for all bulks and profiles illustrates the significant difference between the mean  $\delta^{18}\text{O}$  values, and the overlapping  $\delta^{13}\text{C}$  ranges.

Roughly dividing the bulk values at 47.5°N into latitudinal two groups permits analysis of central and southern groups (Fig. 12). Mean modern  $\delta^{18}\text{O}$  values are  $-11.6 \pm 1.1\text{‰}$  in the central area and  $-9.2 \pm 1.5\text{‰}$  in the south. The central and southern archaeological  $\delta^{18}\text{O}$  means are  $-14.2 \pm 1.4\text{‰}$  and  $-13.3 \pm 0.1\text{‰}$ , respectively. While there is no significant difference between the central and southern archaeological  $\delta^{18}\text{O}$  values (p-value=0.230), there is one for the modern  $\delta^{18}\text{O}$  values (p-value<0.001). The averaged modern  $\delta^{13}\text{C}$  values are  $-12.1 \pm 0.7\text{‰}$  and  $-9.5 \pm 0.9\text{‰}$  for the central and southern groups, respectively (p-value<0.001). In the central sample set, the fractionation between dietary isotopic composition and tooth enamel values is  $-14.4\text{‰}$ ; in the southern portion, that difference is  $-15.8\text{‰}$ . The difference between the archaeological  $\delta^{13}\text{C}$  central ( $-10.0 \pm 0.8\text{‰}$ ) and southern ( $-10.3 \pm 0.5\text{‰}$ ) means, is almost significant (p-value<0.068). Like the  $\delta^{13}\text{C}$  means averaging all samples, there is no significant difference between archaeological and modern means for either the central or southern group.

**Comparison of  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  results for  
Archaeological and Modern Teeth:  
central and southern portions of the sampling area**

**p-value of  
2 sample t-test  
(p-value<0.05 is sig.)**



**Figure 12.** A breakdown of  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  means for all bulks and profiles by portion of the sampling area. Again, the difference in  $\delta^{18}\text{O}$  values between archaeological and modern teeth is visible for both the central and southern areas; there are no further differences in  $\delta^{13}\text{C}$  between archaeological and modern teeth when divided by latitude. Each individual group, except  $\delta^{18}\text{O}$  in the archaeological enamel, shows a significant difference when divided by latitude.

Variation in  $\delta^{18}\text{O}$  values seen within equivalent latitudes is probably the result of variations in available drinking waters, though it could in part also be due to the effects of climate on plants. Since the modern equid enamel displays the prominent latitudinal controls representing similar trends in the environment, it is a useful as a palaeoclimate proxy for this region, and can be compared to archaeological teeth. There is only minimal variation (~3%) seen in the  $\delta^{13}\text{C}$  enamel values. Because the isotopic composition of C4 plants results in an enamel  $\delta^{13}\text{C}$  composition around +1‰, considerable changes in the relative abundance of C3/C4 plants would result in a greater variation in the enamel values than those exhibited by these

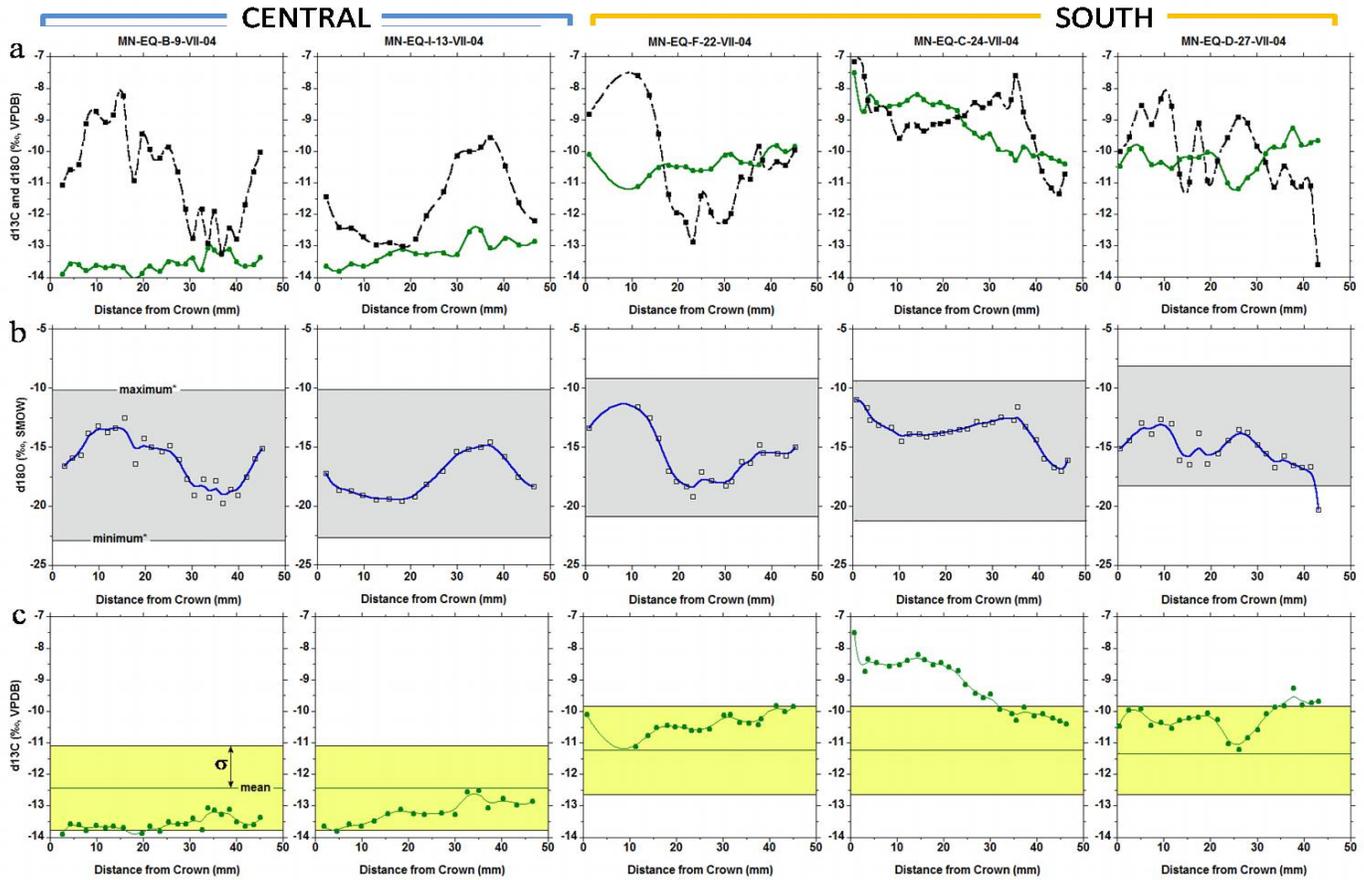
samples. Thus, the enamel  $\delta^{13}\text{C}$  results suggest that there has been little change in the C3/C4 composition between the modern period and the archaeological period sampled (Bronze Age ~1000 B.C.). Variations seen in the enamel in part may be due to minor changes in the relative abundance of C3/C4 plants consumed. However, such variations may also be the result of changing climate factors on the metabolic processes of the plants and the horses.

### 3.2 MODERN TOOTH PROFILES

Five sequentially sampled series were taken from modern horse teeth (1) B-09-VII-04 (49.08°N, 101.879°E), (2) I-13-VII-05 (48.32°N, 101.202°E), (3) F-22-VII-04 (46.33°N, 105.778°E), (4) C-24-VII-04 (46.21°N, 106.003°E), and (5) D-27-VII-04 (44.53°N, 103.651°E) (Fig. 13). Two profiles, B-09-VII-04 and I-13-VII-05 were from tooth sets found in the Khanuy Valley, in the central portion of the sampling area. Values from the F-22-VII-04, C-24-VII-04, and D-27-VII-04 profiles were averaged to obtain values for the southern region. C-24-VII-04 was found near the archaeological site from the south, Baga Gazaryn Chuluu.

Using equations from Delgado Huertas *et al.* (1995) and Bryant *et al.* (1996), ingested water values were calculated from tooth enamel  $\delta^{18}\text{O}$  values. Interpolated precipitation  $\delta^{18}\text{O}$  data points were derived from *OIPC* data (Bowen *et al.* 2005) using coordinates of the source location for each tooth. The maximum precipitation isotope values represents the mean for the months of June-August, when  $\delta^{18}\text{O}$  values peak, while the minimum was obtained by averaging the colder months of December-February (see center rows Figures 13 and 14 for comparison with ingested values from enamel). The  $\delta^{13}\text{C}$  enamel values are compared to an adjusted (by 14.1‰, as

previously established) average, flanked by the standard deviation, of the modern plant carbon isotopic values (row c in Figures 13 and 14).



**Figure 13.** (a) Intra-tooth  $\delta^{18}\text{O}$  (black dashed lines with open squares) and  $\delta^{13}\text{C}$  (green solid lines with filled circles) variations within modern horses collected from the sampling area. (b) Oxygen isotopic composition of ingested water (smoothed blue lines with black squares) compared to averaged warm and cold month precipitation  $\delta^{18}\text{O}$  values interpolated for the same locations. \*Maximum denotes the isotopic composition of precipitation averaged for three warmest months, while minimum the three coldest months. (c) Enamel carbon isotopic composition (green circles and smoothed lines) against average plant  $\delta^{13}\text{C}$  values and standard deviation (offset by established 14.1‰ fractionation between diet and enamel) for the corresponding location.

Profile lengths varied a great deal, from 33-59 millimeters. At the very least, each profile represents one annual cycle, as the growth rate of the M3 molar is approximately 30 millimeters

per year (Hoppe *et al.* 2004b). However, the longer profiles may be skewed by incorporating parts of more than one annual cycle. In order to judge the degree of irregularity, the bulk values were compared to a “cycle average” that incorporated ~30 millimeters of the profile, including one peak and one trough. A paired t-test showed that the difference between the bulk average value and the cycle average is insignificant for both carbon and oxygen data ( $p=0.632$  for  $\delta^{13}\text{C}$ ;  $0.594$  for  $\delta^{18}\text{O}$ ). Thus, the average cycle value from the profile was substituted for missing bulk values in order to calculate an overall average. In addition, several of the profiles have irregular patterns, with some data points interrupting peaks and troughs. To overcome this, mean maximums and minimums were calculated from five continuous points in peaks or troughs. Modern profile data is summarized in Table 1.

**Table 1.** Modern bulk and averaged  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values; italicized numbers are averages

	<b>Latitude (N)</b>	<b>Longitude (E)</b>	<b>Sample ID</b>	<b>Bulk value</b>	<b>Avg. min</b>	<b>Avg. max</b>
$\delta^{13}\text{C}$			<i>Central</i>	<i>-13.0</i>	<i>-13.2</i>	<i>-13.3</i>
	49.08	101.879100	EQ-B-9-VII-04	-12.9	-13.2	-13.7
	48.32	101.202350	EQ-I-13-VII-04	-13.1	-13.3	-12.8
			<i>South</i>	<i>-9.6</i>	<i>-9.6</i>	<i>-10.0</i>
	46.33	105.777650	EQ-F-22-VII-04	-10.4	-10.5	-10.4
	46.21	106.002633	EQ-C-24-VII-04	-9.1	-8.5	-9.5
	44.53	103.650733	EQ-D-27-VII-04	-9.3	-9.7	-10.2
		<i>Average <math>\delta^{13}\text{C}</math> values:</i>	<i>-11.3</i>	<i>-11.0</i>	<i>-11.3</i>	
$\delta^{18}\text{O}$			<i>Central</i>	<i>-13.0</i>	<i>-12.8</i>	<i>-9.8</i>
	49.08	101.879100	EQ-B-9-VII-04	-12.4	-12.7	-8.8
	48.32	101.202350	EQ-I-13-VII-04	-13.5	-12.9	-10.8
			<i>South</i>	<i>-8.5</i>	<i>-11.3</i>	<i>-8.8</i>
	46.33	105.777650	EQ-F-22-VII-04	-7.8	-12.1	-9.0
	46.21	106.002633	EQ-C-24-VII-04	-9.6	-10.7	-8.5
	44.53	103.650733	EQ-D-27-VII-04	-8.1	-11.0	-8.8
		<i>Average <math>\delta^{18}\text{O}</math> values:</i>	<i>-10.3</i>	<i>-11.9</i>	<i>-9.2</i>	

### 3.2.1 $\delta^{18}\text{O}$ in modern teeth

The modern  $\delta^{18}\text{O}$  isotopic profiles document the pronounced seasonal variation seen in Mongolian climate. Intra-tooth  $\delta^{18}\text{O}$  varies considerably, averaging  $-4.5\pm 1.3\text{‰}$  between maximum and minimum. However, compared to interpolated values, which have a mean range of  $-13.3\pm 1.2\text{‰}$ , the modern tooth profiles possess appreciably reduced amplitudes (Fig. 13, row b). The reduction in amplitude seen between precipitation and enamel values may be due to attenuation of the signal during formation. All of the profiles fall almost entirely within the range of modern precipitation. The mean value for the modern teeth is  $-10.3\pm 2.6\text{‰}$ . The averaged maxima for the five teeth range from  $-8.5$ – $-10.75\text{‰}$  and have a mean of  $-9.2\pm 0.9\text{‰}$ , while the minima range from  $-10.7$ – $-12.9\text{‰}$  with a mean of  $-11.9\pm 1.0\text{‰}$ . A t-test analyzing significance of the difference between the central and southern groups found no relationship between latitudinal group and  $\delta^{18}\text{O}$  values.

### 3.2.2 $\delta^{13}\text{C}$ in modern teeth

In comparison to the  $\delta^{18}\text{O}$  composition of the profiles, the  $\delta^{13}\text{C}$  enamel only has minimal variation through the annual cycles. The average range between minimum and maximum is  $1.5\pm 0.4\text{‰}$ , which is similar to the difference between the  $\delta^{13}\text{C}$  means of the northern group of vegetation and the southern group ( $-1.9\text{‰}$ ). The central profiles are slightly below the mean, while the southern profiles fall above the adjusted plant mean (Fig. 14, row c), suggesting a pattern of increasing carbon values with decreasing latitude. Four of the profiles—B-9-VII-04, 1-13-VII-04; F-22-VII-04 and D-27-VII-04—closely reflect the mean adjusted plant averages. The exception is part of the C-24-VII-04 profile, which strays from the mean. The mean modern

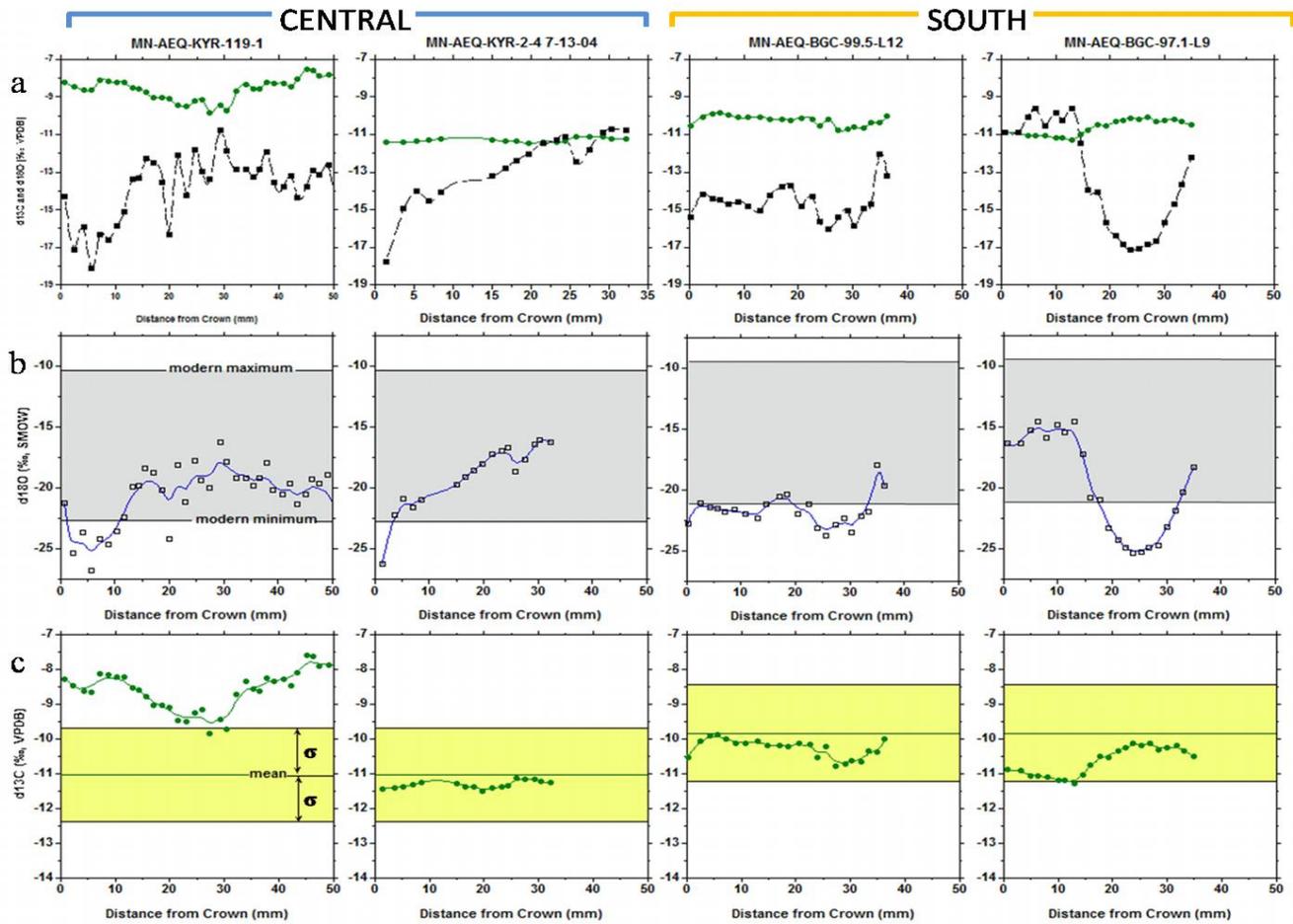
enamel  $\delta^{13}\text{C}$  value is  $-11.0 \pm 1.9\%$ . Because the “maxima” and “minima” were based on  $\delta^{18}\text{O}$  variation in the profiles, some of the  $\delta^{13}\text{C}$  maxima and minima have been reversed, causing more overlap in the ranges. The averaged maxima range from  $-9.5$ -  $-13.7\%$ , while the range of the minima is  $-8.5$ -  $-13.3\%$ .

### 3.3 ARCHAEOLOGICAL TOOTH PROFILES

Bulk samples were taken from 20 archeological teeth from several archeological locations in the sampling area. Profiles were taken from four archeological specimens: two each from Khanuy Valley and from Baga Gazaryn Chuluu (Fig. 14). Archeological profile data is summarized in Table 2.

**Table 2.** Archeological bulk and averaged  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values; italicized numbers are averages

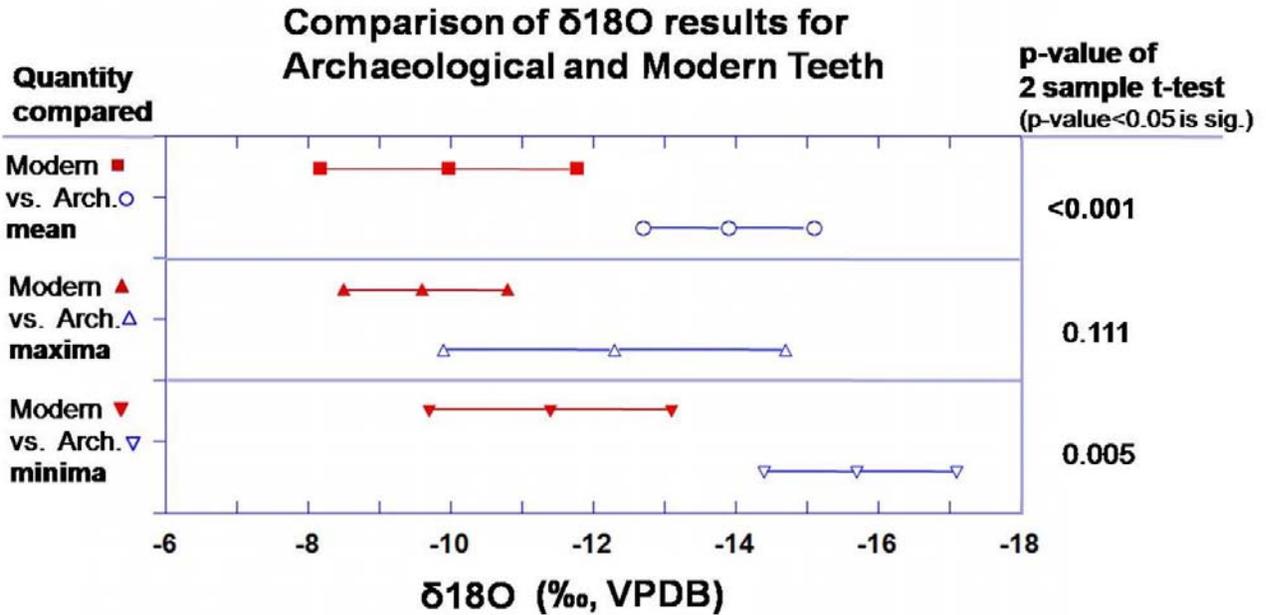
	<b>Latitude (N)</b>	<b>Longitude (E)</b>	<b>Sample ID</b>	<b>Bulk value</b>	<b>Avg. min</b>	<b>Avg. max</b>
$\delta^{13}\text{C}$			<i>Central</i>	<i>-10.0</i>	<i>-9.9</i>	<i>-10.4</i>
	48.32	101.202350	KYR-118-1	-9.4		
	48.32	101.202350	KYR-57-1	-9.3		
	48.32	101.202350	KYR-40-1	-9.9		
	48.32	101.202350	KYR-119-1	-10.2	-8.4	-9.5
	48.32	101.202350	KYR 2-4 7-13-04	-11.3	-11.4	-11.2
			<i>South</i>	<i>-10.0</i>	<i>-9.9</i>	<i>-10.4</i>
	46.23	106.036653	BGC 97.1 L9	-10.0	-10.2	-10.6
	46.23	106.036653	BGC 99.5 L12	-10.6	-10.2	-11.2
			<i>Average <math>\delta^{13}\text{C}</math> values:</i>	<i>-10.3</i>	<i>-10.2</i>	<i>-10.9</i>
$\delta^{18}\text{O}$			<i>Central</i>	<i>-14.2</i>	<i>-16.0</i>	<i>-11.8</i>
	48.32	101.202350	KYR-118-1	-14.3		
	48.32	101.202350	KYR-57-1	-13.9		
	48.32	101.202350	KYR-40-1	-16.5		
	48.32	101.202350	KYR-119-1	-13.6	-16.8	-12.2
	48.32	101.202350	KYR 2-4 7-13-04	-12.7	-15.1	-11.3
			<i>South</i>	<i>-13.3</i>	<i>-16.3</i>	<i>-12.1</i>
	46.23	106.036653	BGC 97.1 L9	-13.2	-15.6	-14.2
	46.23	106.036653	BGC 99.5 L12	-13.4	-16.9	-10.0
			<i>Average <math>\delta^{18}\text{O}</math> values:</i>	<i>-13.8</i>	<i>-16.1</i>	<i>-11.9</i>



**Figure 14.** As in the comparison for moderns: Archaeological intra-tooth  $\delta^{18}\text{O}$  (black dashed lines with squares) and  $\delta^{13}\text{C}$  (green solid lines with circles) profiles within modern horses collected in the sampling region (top row). Oxygen isotopic composition of ingested water (smoothed blue lines with black squares) compared to averaged modern warm and cold month modern precipitation  $\delta^{18}\text{O}$  values interpolated for the same locations (center row). Enamel carbon isotopic composition (green circles and smoothed lines) against average  $\delta^{13}\text{C}$  plant values (modern values shifted +1.4‰ based on the Seuss effect to estimate pre-industrial values) with standard deviation (plant values are also offset by established 14.1‰ fractionation between diet and enamel) for the corresponding location (bottom row).

### 3.3.1 $\delta^{18}\text{O}$ in archaeological teeth

Intra-tooth  $\delta^{18}\text{O}$  variation from the archaeological profiles is more complicated than the modern profiles, with less well-defined extremes because of more irregularity. However, it is clear that overall the isotopic values of the archaeological profiles are more negative. In contrast to the modern profiles, where the calculated ingested water values reflected the modern precipitation values, the archaeological profiles are much more negative (Fig. 14, row b). They fall near or straddle the averaged winter value for precipitation, and possess more negative troughs. The average value is  $-14.0\text{‰}$ , which is  $3.8\text{‰}$  more negative than the mean modern value. Minima range from  $-15.1$  to  $-16.9\text{‰}$ , approximately 2-6‰ lower than the observed modern minima. The mean archaeological averaged minimum is  $-16.1\pm 0.9\text{‰}$ . The archaeological maxima have a mean of  $-11.9\pm 1.8\text{‰}$  and range from  $-10.0$  to  $-14.2\text{‰}$ , overlapping the modern range. The maxima are not significantly different from the moderns ( $p=0.111$ ), whereas the minima are, with a p-value of 0.005. This difference is supported by the significant difference between the overall means. The average range between the maximum and minimum is  $-6.5\pm 1.6$ . This increased range implies a broader annual range, with roughly similar maxima, and lower troughs (minima) (see Figs. 14 & 15).



**Figure 15.** The difference between modern and archaeological  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  means (including all profiles and bulks) is further elucidated by separating the means of the peaks and troughs of the profiles. There is no significant difference between the modern and archaeological mean maxima, but there is a significant difference between the mean minima.

### 3.3.2 $\delta^{13}\text{C}$ in archaeological teeth

The mean  $\delta^{13}\text{C}$  for archaeological bulks is  $-9.9 \pm 0.9\text{‰}$ . Like the modern profiles, the archaeological  $\delta^{13}\text{C}$  profiles show minimal variation (Fig. 14). Three profiles, KYR-2-4, BGC 99.5 L12 and BGC 97.1 L9, are very similar to each other and the adjusted modern plant values (modern ranges shifted  $+1.4\text{‰}$  based on the Seuss effect to estimate pre-industrial values). In contrast, the carbon values from the KYR-119-1 profile demonstrate a positive shift, outside the range of the adjusted modern plant values. The average  $\delta^{13}\text{C}$  value falls at  $-9.9 \pm 0.9\text{‰}$ , with an average range of only  $-1.2 \pm 0.3\text{‰}$ . This average is higher than the modern mean ( $-10.96\text{‰}$ ), but

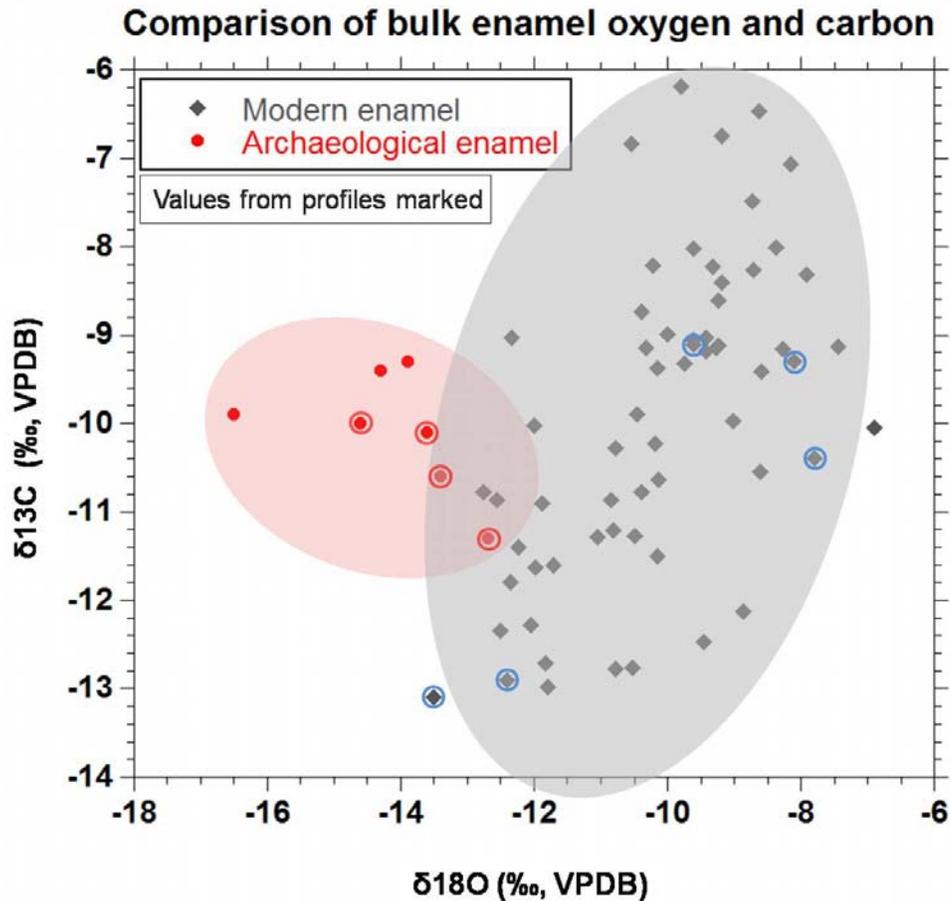
only by  $\sim 1\%$ . The archaeological average is more like the southern ( $-9.6\%$ ) mean than the central mean ( $-13.0\%$ ). The mean of the averaged maxima is  $-10.5 \pm 0.8\%$ , while that of the minima is  $-10.1 \pm 1.2\%$ . These means are not significantly different from those of the modern teeth (mean maximum:  $11.3\%$ ,  $p=0.543$ ; mean minimum:  $11.05\%$ ,  $p=0.351$ ). Excluding KYR-119-1 as an outlier, the remaining archaeological profiles are so similar that it appears the archeological teeth do not display any latitudinal trend of increasing values with decreasing latitude seen in the modern profiles.

## 4.0 DISCUSSION

Similar patterns are seen in the  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values of both modern and archaeological teeth: a defined  $\delta^{18}\text{O}$  oscillation that is within the range of meteoric waters, comparable average  $\delta^{18}\text{O}$  maxima, and minimal variation in the  $\delta^{13}\text{C}$  profiles. These similar patterns indicate that in general, modern day climate and Bronze Age climate were fairly similar. Statistical analyses of the  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values suggest there were very few differences between the archaeological and modern teeth. When the sampling area was divided into two groups by latitude, the mean modern  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values showed a significant latitudinal variation; however the archaeological  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values had no significant latitudinal pattern. The sample numbers for the groups divided by latitude were very small (2-3 for each area) and thus additional error may have been introduced by sample size. Based on a temperate climate analysis, Hoppe *et al.* (2005) suggests a data set of more than 9 was needed to estimate the mean  $\delta^{18}\text{O}$  value within 1‰ (CI=95%). At least 12 individuals would be needed to give an equivalent accuracy for the  $\delta^{13}\text{C}$  analysis.

Statistically significant differences appeared when considering the degree of seasonality presented by the  $\delta^{18}\text{O}$  in the teeth. There was a significant difference  $\delta^{18}\text{O}$  between the means obtained from bulks. Plotting  $\delta^{18}\text{O}$  against  $\delta^{13}\text{C}$  (Fig. 16) illustrates that while archaeological  $\delta^{13}\text{C}$  fell within the range of modern enamel values, the archaeological  $\delta^{18}\text{O}$  is much lower than

that of the modern teeth.



**Figure 16.** Oxygen and carbon isotopic values for modern and archaeological teeth illustrates trends in the results. The archaeological  $\delta^{13}\text{C}$  values fall within the range of modern values, but the archaeological  $\delta^{18}\text{O}$  values are much more negative than the modern set.

Mean maxima did not differ significantly, and thus the isotopic composition of the summer precipitation would also be similar. Instead, the main difference between modern and archaeological profiles derives from the mean minima. The archaeological minimum values are significantly less positive than the modern values ( $p=0.005$ ) by approximately 5‰. Because of the positive and direct relationship between horse enamel composition and ingested water, the higher temperature and increased precipitation of the summer, which influence meteoric waters, are represented by the enamel profile peaks. Similarly, declines in the relative  $\delta^{18}\text{O}$  during the

winter appear in the profiles as troughs. Thus, the lower troughs represent lower environmental  $\delta^{18}\text{O}$  values that could be caused by lower winter temperatures (Dansgaard 1964; Rozanski *et al.* 1993), a different amount of precipitation, or less evaporative enrichment, though this is not as much of a factor during the winter as the summer. These differences in winter weather patterns could be due to a change in the larger air mass trajectories.

Due to the complex matrix pattern of mineralization, samples are time-averages of the input signal (Hoppe *et al.* 2004b) which may attenuate the degree of seasonality. Furthermore, attenuation may complicate analysis of seasonal patterns; including masking a more complex annual climatic pattern (Passey and Cerling 2002), which may account for complicated profiles in the archaeological teeth. Large deviations from the norm in the summer periods may indicate a change in the source of the water for short periods of time (Sharp and Cerling 1998). Horses may be exposed to such changing conditions during seasonal migrations, or depending on the frequency and periodicity of precipitation. However, the changing values may need to last  $\geq 14$  days according to one estimate for the horse body water to adjust (Sharp and Cerling 1998). Alternatively, these profiles variations could be due to poor enamel preservation, or discrepancies in sampling or analysis.

A study of the prevalence of C4 species across Mongolia revealed that they account for only 3.5% of Mongolia's plant species (Pyankov *et al.* 2000). In fact, the values returned for 29 out of 30 (or ~97%) vegetation samples fall between -23 and -28 ‰. Only a small fraction of the horse's diet in this area includes C4 plants because the vast majority of the available vegetation is composed of C3 plants. Thus carbon isotopic variations in the teeth reflect the range of values present in the vegetation available for consumption, not a seasonal variation in the relative composition of C3/C4 plants. Sharp and Cerling (1998) explained a  $\delta^{13}\text{C}$  variation between 2 and

3‰ in their profiles by annual variations in the  $\delta^{13}\text{C}$  plant composition. This annual variation within C3 plants may be due to a variety of factors, such as water stress, heat, or other climatic influences on the isotopic composition of the plants. Positive shifts, such as those seen in the C-24-VII-04 and KYR-119-1 profiles may be due to the stress of high temperatures or aridity during the time of enamel formation. Instead of, or in addition to influences from environmental fluctuations, the relatively steady  $\delta^{13}\text{C}$  values could indicate a steady supplied diet (i.e. foddering).

Overall, the sequential sampling data suggest the presence of only one significant climatic difference between the averaged Bronze Age climate and present day conditions: that while mean summer temperatures or precipitation remained constant, the degree of oscillation increased because of a drop in the minima. Potentially, this was due to increased seasonality due to lower winter temperatures, but could also be influenced by differing storm or precipitation patterns. Furthermore, the low amplitude of  $\delta^{13}\text{C}$  values in both modern and archaeological teeth suggests minimal variation in the equid diet as well as minimal seasonal isotopic composition of plants.

## 5.0 CONCLUSION

The intent of this project was to analyze the connection of modern horse tooth enamel carbon and oxygen isotopic composition to modern environmental factors. This connection, once established, was used to examine palaeoclimatic factors through the isotopic composition of enamel from archaeological horse remains. It was found that horse teeth from Central Mongolia correlate well to modern environmental inputs, recording the distinct  $\delta^{18}\text{O}$  seasonality and the C3 species composition of the region. The  $\delta^{18}\text{O}$  composition of the archaeological teeth suggest there was potentially increased seasonality during the Bronze age, with a similar climate during warm months while decreased temperatures during the colder season made the precipitation  $\delta^{18}\text{O}$  composition more negative. However other variations in weather patterns may instead be the main factor in the dissimilarity of the modern and archaeological profiles.

In order to fully analyze the samples by latitude, more bulks and profiles from modern and archaeological teeth need to be run, particularly from the northern reaches of the transect (*e.g.* teeth from the Darkhat Valley). In addition, the sample size from the central and southern portions is small. While samples sizes may be limited by the quality and availability of specimens, more specimens are available than have been analyzed herein. Data from further tests could refine those averages and permit a more precise analysis of the modern-environmental correlation and the archaeological-modern differences.

## APPENDIX A

### DATA FOR WATERS AND VEGETATION

**Table 3.**  $\delta^{18}\text{O}$  water values

<i>Locale</i>	<i>Source</i>	<i>Latitude (N)</i>	<i>Longitude (E)</i>	<i>Elevation (m)</i>	<i>Delta O18</i>
<b>Asgat Nuur</b>	Lake	51.2459	099.0360	2213	-18.94
<b>Sant Nuur</b>	Lake	51.2382	099.0111	2360	-17.84
<b>Boorog Nuur</b>	Lake	51.2377	099.0362	2270	-18.75
<b>Sanjin Nuur</b>	Lake	51.2349	099.0222	2249	-19.01
<b>Dalbay Gol</b>	River	51.0245	100.7551	1665	-14.53
<b>Ar Holboo Nuur</b>	Lake	50.9836	099.1262	1732	-01.22
<b>Uvur Holboo Nuur</b>	Lake	50.9810	099.1303	1736	-00.92
<b>Adiya Nuur</b>	Lake	50.9806	099.1494	1692	-03.51
<b>Nogoon Nuur</b>	Lake	50.9707	099.1397	1719	-00.71
<b>Narmandah Nuur</b>	Lake	50.9688	099.1392	1714	-01.98
<b>Ongodoin Nuur</b>	Lake	50.9674	099.1421	1696	-02.20
<b>Buren Buht Nuur</b>	Lake	50.9661	099.1308	1723	-04.18
<b>Dagshid Nuur</b>	Lake	50.9661	099.1182	1747	-03.06
<b>Shalbag Nuur</b>	Lake	50.9648	099.1344	1726	-03.91
<b>Toilogt</b>	Spring	50.7269	100.2468	1642	-07.47
<b>Egiin Gol</b>	River	50.4182	100.1496	1648	-08.11
<b>Tagiin Nuur</b>	Lake	50.0121	099.7215	2038	-03.00
<b>Erkhel Nuur</b>	Lake	49.9125	099.9071	1535	-02.19
<b>Tsegeen Nuur</b>	Lake	49.0973	101.8607	1519	-00.30
<b>Ovoo</b>	Spring	49.0807	101.8324	1523	-11.91
<b>Doroo Tsagaan Nuur</b>	Lake	49.0221	101.2018	1727	-02.78
<b>Doroo</b>	Spring	48.9871	101.1983	1782	-13.74
<b>Sharga Nuur</b>	Lake	48.9228	101.9689	1336	-03.14
<b>Sharga</b>	Rainfall	48.9187	101.9707	1334	-06.31
<b>Khanuy Gol (North)</b>	River	48.8016	101.9695	1279	-11.24
<b>Zuslangiin Gol</b>	River	48.7204	101.2928	1527	-11.88
<b>Khorgo Nuur</b>	Lake	48.1858	099.8532	2154	-09.54
<b>Terkhiiin Tsagaan Nuur</b>	Lake	48.1425	099.7897	2065	-12.27
<b>Suman Gol</b>	River	48.1381	099.8295	2064	-12.20

<b>Chuluut Gol</b>	River	48.0940	100.3178	1833	-12.97
<b>Khanuy Gol (South)</b>	River	48.0891	101.0841	1664	-12.01
<b>Elstiin Gol</b>	River	48.0171	101.1821	1722	-13.57
<b>Ulaanbataar</b>	Well	47.9187	106.9222	1336	-15.12
<b>Tamir Gol</b>	River	47.4406	101.5773	1642	-13.62
<b>Tsenkher Gol</b>	River	47.4377	101.5752	1638	-13.28
<b>Tsagaan Sum Gol</b>	River	47.3430	102.4384	1470	-12.26
<b>Orkohn Gol</b>	River	47.2021	102.8019	1470	-12.52
<b>Zargol Hairhan</b>	Spring	46.9177	105.8762	1287	-12.69
<b>Dund Shandny</b>	Well	46.2451	106.0511	1516	-11.36
<b>Baga Gazaryn Chuluu (a)</b>	Rainfall	46.2162	105.9777	1582	-09.40
<b>Baga Gazaryn Chuluu (b)</b>	Rainfall	46.2162	105.9777	1582	-09.82
<b>Sangiin Dalai Nuur</b>	Lake	46.1583	105.7596	1383	-05.29
<b>Tsagaan Tolgoi</b>	Spring	45.7506	104.4967	1373	-10.61
<b>Mengit</b>	Well	45.4288	105.1016	1313	-10.87
<b>Ongiin Gol</b>	River	45.3845	103.9719	1279	-10.78
<b>Ongiin Khiid</b>	Spring	45.3397	104.0061	1285	-10.49
<b>Shorvog</b>	Well	45.1012	104.6124	1319	-10.46
<b>Mandal Ovoo</b>	Well	44.6537	104.0603	1066	-11.21

**Table 4.**  $\delta^{13}\text{C}$  values for vegetation

<i>Location</i>	<i>Sample ID</i>	<i>% C</i>	<i><math>\delta^{13}\text{C}</math> (‰, vs. VPDB)</i>
<b>Darkhat Valley</b>	SP-18-VI-06 A	43.42	-26.57
<b>Darkhat Valley</b>	SP-18-VI-06 B	36.31	-27.47
<b>Darkhat Valley</b>	SP-18-VI-06 G	40.23	-27.96
<b>Darkhat Valley</b>	SP-18-VI-06 L	41.70	-28.61
<b>Darkhat Valley</b>	SP-19-VI-06 D	37.31	-27.10
<b>Darkhat Valley</b>	SP-19-VI-06 F	40.39	-27.43
<b>Darkhat Valley</b>	SP-19-VI-06 K	41.28	-26.97
<b>Darkhat Valley</b>	SP-19-VI-06 M	42.17	-27.61
<b>Darkhat Valley</b>	SU-18-VI-06 C	40.71	-27.84
<b>Darkhat Valley</b>	SU-18-VI-06 G	40.85	-24.55
<b>Khanuy Valley</b>	Bromis-9-VI-06	39.84	-26.79
<b>Khanuy Valley</b>	Potentilla	40.75	-28.71
<b>Khanuy Valley</b>	Sedge9-9-VI-06	40.34	-27.99
<b>Khanuy Valley</b>	SP-10-VI-06 D	42.62	-27.24
<b>Khanuy Valley</b>	SP4-9-VI-06	44.28	-25.67
<b>Khanuy Valley</b>	SU1-8-VI-06	37.87	-25.21
<b>Khanuy Valley</b>	SU2-8-VI-06	38.42	-25.09
<b>Khanuy Valley</b>	SU4-8-VI-06	41.91	-24.58
<b>Khanuy Valley</b>	SU5-8-VI-06	38.07	-26.88
<b>Khanuy Valley</b>	Trifolium-9-VI-06	42.10	-27.16
<b>Erden Ger</b>	9-VII-06 A	42.37	-24.95
<b>Erden Ger</b>	9-VII-06 B	41.24	-24.45

<b>Erden Ger</b>	9-VII-06 C	43.25	-25.12
<b>Erden Ger</b>	9-VII-06 D	42.99	-24.13
<b>Erden Ger</b>	9-VII-06 F	34.73	-25.78
<b>Erden Ger</b>	9-VII-06 J	40.78	-14.76
<b>Erden Ger</b>	9-VII-06 s1	41.11	-23.44
<b>Erden Ger</b>	9-VII-06 s2	39.01	-27.81
<b>Baga Gazaryn Chulu</b>	8-VII-06 B	41.83	-27.15
<b>Gobi</b>	8-VII-06 B	41.74	-25.20

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