



Strengthening of the East Asian summer monsoon revealed by a shift in seasonal patterns in diet and climate after 2–3 Ma in northwest China

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ABSTRACT

To examine climate variability in northwest China in the late Cenozoic and to test hypotheses regarding the development of C_4 ecosystems and the dynamics of the Asian monsoons, the carbon and oxygen isotopic compositions of 32 bulk and 368 serial tooth enamel samples from herbivores in the Linxia Basin (Gansu Province), ranging in age from 25 Ma to the present, were determined. The results corroborate and improve the record previously obtained from the area, showing that all mammals in the Linxia Basin lived in habitats consisting primarily of C_3 vegetation prior to 2–3 Ma and that C_4 grasses did not become a significant component of local ecosystems until the Quaternary. The data also show that shifts in climate to drier and/or warmer conditions after about 14, 9.5, 7, and 2.5 Ma, as indicated by positive $\delta^{18}O$ excursions in the bulk enamel- $\delta^{18}O$ record, were accompanied by increased seasonality; whereas negative $\delta^{18}O$ shifts in the bulk data after about 11, 6, and 1.2 Ma, which indicate shifts to wetter and/or cooler climate, were associated with decreases in seasonality. Intra-tooth $\delta^{13}C$ and $\delta^{18}O$ profiles reveal significant changes in the seasonal patterns of diet and climate after ~2–3 Ma. Prior to ~2–3 Ma, there was little or no seasonal variation in herbivores' diets and all herbivores fed on C_3 vegetation year around. After that time, the data show a significant seasonal variation in the diets of horses and bovids, ranging from a pure C_3 to a mixed C_3/C_4 diet (with C_4 plants accounting for up to ~60% of the diet). An inverse relationship (or negative correlation) between $\delta^{13}C$ and $\delta^{18}O$ values within individual teeth – a pattern characteristic of the summer monsoon regime – is observed in younger (<2–3 Ma) horses and bovids but not in older fossils. These changes in intra-tooth isotopic patterns provide strong evidence for an enhanced monsoon climate since about 2–3 Ma.

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1. Introduction

Paleoclimatic and paleoecological records in western China are crucial for understanding the effects of the uplift of the Tibetan Plateau on regional climate and on the evolution of mammalian species. Stable carbon and oxygen isotopic analyses of fossil teeth and ancient soils collected along the margin of the Tibetan Plateau provide proxies for those records. Terrestrial plants can be grouped into two main categories based on their photosynthetic pathways: either C_3 or C_4 plants. C_3 plants, including trees, shrubs, forbs, and cool season grasses, use the Calvin Cycle photosynthetic pathway and have an average $\delta^{13}C$ value of -27‰ (with a range from -34 to -22‰). C_4 plants, including warm season grasses, a few shrubs, corn, sugar cane,

sorghum, and bamboo, use the Hatch-Slack photosynthetic pathway and have an average $\delta^{13}C$ value of -13‰ ($\delta^{13}C$ range = -17 to -9‰) (O'Leary, 1988; Farquhar et al., 1989; Cerling et al., 1997a,b). Studies of carbon isotopic compositions of fossil mammalian tooth enamel and paleosols from around the world suggest that prior to the late Miocene, terrestrial ecosystems consisted predominantly of C_3 plants. After the late Miocene (~7 to 5 Ma), C_4 grasses became a significant component of low- to mid-latitude and low-elevation regions (Quade et al., 1989; Cerling, 1992; Cerling et al., 1993, 1997a,b; MacFadden et al., 1994; MacFadden and Cerling, 1994; Wang et al., 1994). Oxygen isotopes in paleosols from Pakistan indicate that a dramatic increase in the oxygen isotope ratios of soil carbonates occurred with the late Miocene expansion of C_4 grasses, reflecting a change to a drier and/or warmer climate. It has been suggested that the Miocene global expansion of C_4 plants occurred in response to declining atmospheric CO_2 levels (Cerling et al., 1993, 1997a,b; Wang et al., 1994), and/or a strengthening of the Asian summer monsoon as a result of the uplift of the Tibetan Plateau (Quade et al., 1989). However, it has also been suggested that there was no globally simultaneous expansion of C_4

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plants (Morgan et al., 1994) and that no evidence exists for decreasing CO₂ levels during the late Miocene (Pagani et al., 1999; Retallack, 2001; Royer et al., 2001).

Because direct paleoenvironmental records, such as paleobotanical specimens, are rare from western China, the isotopic analyses of fossil mammalian herbivore tooth enamel and soil carbonates from the Linxia Basin in Gansu Province (with sequences of fossils and sediments that both pre- and post-date the late Miocene global expansion of C₄ grasses) are an important means of testing the global C₄ expansion hypothesis. These analyses also provide a way to explore the dietary evolution of extinct taxa and allow a greater understanding of ancient ecologies. Comparison of isotopic records of vegetation and climate changes based on these analyses with well-established records on the south side of the Himalayan–Tibetan Plateau and in other regions of the world will aid in understanding the effects of the Tibetan Plateau uplift on regional and global climate.

Wang and Deng (2005) presented a late Cenozoic record of vegetation and environmental change from the Linxia Basin, based on the stable isotope analyses of bulk enamel samples, paleosols and modern soils. Their data suggest that C₄ grasses did not expand into the Linxia Basin during the period of “global C₄ expansion” but rather in the Quaternary. They hypothesized that the East Asian summer monsoon (EASM), currently controlling the climatic conditions in the area, was probably not strong enough to affect this part of China throughout much of the Neogene, and delayed C₄ expansion into the area until the EASM was further strengthened after 2–3 Ma. Based on fossil enamel and paleosol carbonate isotope data from several localities on the Chinese Loess Plateau, Passey et al. (2009) recently offered a contrasting hypothesis suggesting a stronger EASM during the late Miocene and Pliocene than today.

In this study, we analyzed the carbon and oxygen isotopic compositions of 368 serial enamel samples from 23 fossil and modern herbivore teeth from the Linxia Basin. We also analyzed 32 bulk samples that complement the 126 bulk enamel analyses previously reported by Wang and Deng (2005). In addition, the carbon isotope compositions of 54 modern plants from the Linxia Basin were determined for comparison with estimated C₃/C₄ compositions of ancient ecosystems.

The new isotope data and previously published data were utilized to examine the climate variability in NW China during the late Cenozoic and to test hypotheses regarding the development of C₄ ecosystems and the dynamics of the Asian monsoons.

2. Study area

The Linxia Basin (103°E, 35°N) is located in Gansu Province, China, about 100 km south of the provincial capital, Lanzhou, on the northeastern margin of the Tibetan Plateau (Fig. 1). It is currently considered a temperate steppe biome, with an elevation of ~1917 m (Wang and Deng, 2005), a mean annual temperature of 7 °C, and an annual rainfall of 515 mm. The present-day climate is strongly influenced by the East Asian monsoon system (An et al., 2000).

The Linxia Basin is a flexural basin bounded by the Lajishan fault to the west, the North Qinling fault to the south, and the Maxian mountains to the north; but to the east, the boundary is poorly defined. It is believed that deposition in the Linxia Basin began at ~29 Ma and continued nearly uninterrupted until the Holocene (Fang et al., 1997, 2003) and that the Tibetan Plateau grew in a step-wise fashion towards the northeast through time (Fang et al., 1997, 2003; Tapponnier et al., 2001). Evidence suggests that the deformation front of the Tibetan Plateau had propagated into this region by 6 Ma (Fang et al., 1997, 2003).

The Cenozoic sedimentary sequence in the Linxia Basin is well-exposed, with an overall thickness of more than 500 meters, and spans almost continuously from the late Oligocene to the Holocene (Fig. 2). Additionally, the strata contain abundant, diverse, and well-preserved mammalian fossils, making the Linxia Basin an ideal place to study paleoecology and paleoclimatology using stable carbon and oxygen isotope analyses of mammalian tooth enamel (Deng et al., 2004a, b; Wang and Deng, 2005). The chronology of the Cenozoic deposits has been determined by magnetostratigraphic and biostratigraphic techniques (Fang et al., 1997, 2003; Deng et al., 2004a, b). The deposits consist primarily of fluvial and lacustrine sediments, with Pleistocene eolian deposits covering most of the region (Fang et al., 2003; Deng et al., 2004a,



Fig. 1. Location of the Linxia Basin and selected International Atomic Energy Agency (IAEA) stations within and outside of the summer monsoon region in China. The dotted line is a rough estimation of the modern northern extent of the Asian summer monsoon based on the $\delta^{18}\text{O}$ records of precipitation from the IAEA stations.

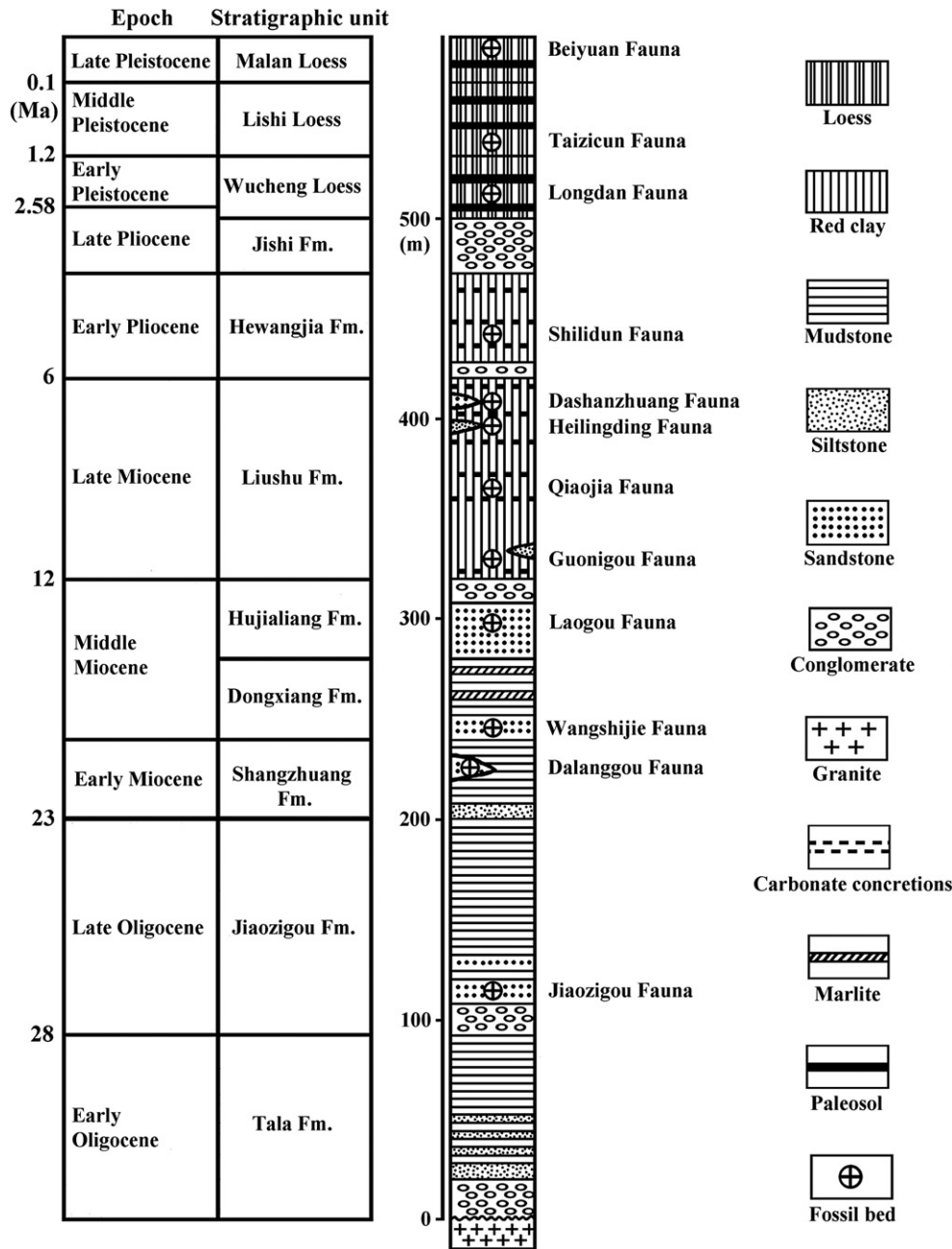


Fig. 2. Late Cenozoic sedimentary sequence of the Linxia Basin. Modified from Fang et al., 1997, 2003 and Deng et al., 2004b.

b; Wang and Deng, 2005). The stratigraphic sequence has been divided into eleven formations based on lithofacies and paleontology (Fig. 2).

3. Materials and methods

Well-preserved fossil tooth samples were selected for carbon and oxygen isotopic analyses from the Hezheng Museum of Natural History in Hezheng County, Gansu Province, or from the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP) in Beijing, China. All fossils were originally collected from the Linxia Basin by IVPP. The teeth were chosen from several groups of late Cenozoic mammals, including horses (Family Equidae), rhinoceroses, bovids, deer, giraffes, pigs, and elephants. Most samples collected were third molars and premolars to reduce the effects of pre-weaning on the $\delta^{18}\text{O}$ of the tooth enamel (e.g., Fricke and O'Neil, 1996; Bryant et al., 1996).

Multiple specimens from each stratum were collected when possible so that variations in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values within populations, between different taxa of the same age, and between similar taxa of different ages could be determined. Most of the bulk enamel isotope data were reported previously in Wang and Deng (2005). Based on the isotopic results of the bulk samples, we selected 23 tooth samples that spanned the time intervals where significant changes in vegetation and climate occurred for serial isotopic analyses in order to reconstruct seasonal patterns in diet and climate or changes in behaviors of individuals (e.g., Koch et al., 1995; Fricke and O'Neil, 1996; Sharp and Cerling, 1998). We also analyzed 32 bulk enamel samples to fill in some of the gaps in the previously published bulk enamel isotope record from the area (Wang and Deng, 2005). In addition, 54 plants were collected throughout the Linxia Basin for carbon isotope analyses in order to estimate the C_3/C_4 composition of the modern ecosystem and then compare it with

ancient ecosystems inferred from the $\delta^{13}\text{C}$ of fossil teeth. The oxygen isotopic composition of tooth enamel phosphate ($\delta^{18}\text{O}\text{-PO}_4^{3-}$) was also determined for 10 rhino samples from various strata in order to test for the preservation of the primary isotopic compositions of the tooth carbonate samples by comparison with the phosphate isotopic data (e.g., [Iacumin et al., 1996](#)). The results of this study are reported in standard notation as $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in reference to the international carbonate standard V-PDB.

Bulk enamel samples were obtained by either drilling along the entire length of a tooth using a slow-speed rotary drill or by cutting off a section of each tooth, lengthwise from the crown to the root, and manually separating the enamel from the dentine using a rotary tool. Then, the samples were ground into a fine powder using a mortar and pestle. Serial samples were drilled, using a slow-speed rotary tool, perpendicular to the growth axis of each tooth from crown to root, with the youngest samples being near the root and the oldest samples being near the crown.

Powdered tooth enamel samples were pretreated in 5% reagent grade sodium hypochlorite for approximately 20 to 24 h at room temperature to remove organic material, rinsed with deionized water, and then treated in 1 M acetic acid overnight to remove non-structural carbonate from the enamel. The samples were then rinsed with deionized water and freeze-dried. The enamel samples were then converted to CO_2 by reaction with 100% phosphoric acid for 3 to 18 h at 72 °C (bulk samples) or for approximately 72 h at 25 °C (serial samples; modified from [McCrea, 1950](#)). Then, the carbon and oxygen isotope ratios of the resulting CO_2 were analyzed using a Thermo-Finnigan Gas Bench II interfaced with a Delta Plus XP stable isotope ratio mass spectrometer (IRMS) at the Florida State University (FSU). Some of the bulk enamel samples were analyzed on a VG Prism in the Stable Isotope Lab at the University of Florida. The $\delta^{13}\text{C}$ values of the plant samples were measured using a Carlo Erba elemental analyzer (EA) connected to the IRMS at FSU. The analytical precision (based on replicate analyses of lab standards processed with each batch of samples) is $\pm 0.1\%$ (1σ) or better for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$.

To prepare the tooth enamel phosphate samples, tri-silver phosphate was precipitated from the enamel samples following the [Dettman et al. \(2001\)](#) procedure, which is a modification of the approach by [O'Neil et al. \(1994\)](#). First, samples were dissolved in 2 M HF in an ultrasonic bath overnight, which simultaneously precipitated CaF_2 . The solutions were then decanted and were brought to a nearly neutral pH with the addition of 20% NH_3OH . Next, 2 M AgNO_3 was added to each sample, causing rapid precipitation of Ag_3PO_4 . Finally, the samples were centrifuged, decanted, rinsed with deionized water, and freeze-dried. The O isotopic composition of Ag_3PO_4 was analyzed using a Finnigan Thermal Conversion Elemental Analyzer (TC/EA) connected to the IRMS at FSU. All samples and standards were run in triplicate to ensure that the TC/EA produced no memory effect. The analytical precision is $\pm 0.3\%$ (1σ) or better for $\delta^{18}\text{O}$ (based on replicate analyses of lab standards processed with each batch of samples).

4. Results and discussion

4.1. Assessment of fossil tooth preservation

Tooth enamel is resistant to diagenetic alteration and, therefore, is well-suited to preserve the original isotopic composition of an animal. This is a result of a large crystal size, larger than in other biogenic materials, such as bone or dentine, and a low ($\sim 1\%$) porosity that allows very little influx of diagenetic fluids ([Ayliffe et al., 1994](#); [Wang and Cerling, 1994](#)). Tooth enamel has an inorganic component ($>96\%$ by weight) mainly in the form of hydroxyapatite crystals ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), which contain $\sim 1\text{--}3\%$ structural carbonate (CO_3^{2-}) substituting for phosphate (PO_4^{3-}) and hydroxyl (OH^-) ions ([Wang and Cerling, 1994](#)). It has been demonstrated that an $\sim 8\text{--}9\%$ fractionation occurs

between coexisting phosphate and structural carbonate in enamel hydroxyapatite of extant mammals ([Bryant et al., 1996](#); [Iacumin et al., 1996](#)). Because carbonates in fossil tooth enamel are more susceptible to dissolution and recrystallization processes during diagenesis than are phosphates, it is generally believed that phosphates are more likely to retain original oxygen isotope compositions ([Kolodny and Luz, 1991](#); [Ayliffe et al., 1994](#)). Even so, evidence suggests that microbial activity can modify $\delta^{18}\text{O}(\text{PO}_4^{3-})$ values of biogenic apatite during early diagenesis ([Ayliffe et al., 1994](#); [Kolodny et al., 1996](#); [Sharp et al., 2000](#)). Therefore, comparison of $\delta^{18}\text{O}(\text{CO}_3^{2-})$ with $\delta^{18}\text{O}(\text{PO}_4^{3-})$ values of fossil tooth enamel can be useful in evaluating the preservation of the original oxygen isotopic compositions of the structural carbonate and phosphate components of enamel hydroxyapatite ([Iacumin et al., 1996](#); [Fricke et al., 1998](#); [Wang et al., 2008a](#)).

Selected fossil enamel samples from the Linxia Basin were analyzed for both $\delta^{18}\text{O}(\text{CO}_3^{2-})$ and $\delta^{18}\text{O}(\text{PO}_4^{3-})$ values ([Fig. 3](#)). The $\delta^{18}\text{O}_{\text{c-p}}$ values are $8.3 \pm 0.8\%$ (all mean reported $\pm 1\sigma$), which is consistent with predicted values for the formation of coexisting structural carbonates and phosphates from the same body water in modern biogenic apatite ([Longinelli and Nuti, 1973](#); [Bryant et al., 1996](#); [Iacumin et al., 1996](#)). This suggests that there was little or no alteration of the oxygen isotopic compositions of either the structural carbonate or phosphate components of these samples ([Fig. 3](#)).

4.2. Late Cenozoic climate, diet and vegetation changes in the Linxia Basin

The $\delta^{18}\text{O}$ of tooth enamel from large-bodied mammalian herbivores largely reflects the $\delta^{18}\text{O}$ of meteoric water that is ingested by the herbivores, through drinking and plant consumption ([Longinelli, 1984](#); [Luz et al., 1984](#); [Ayliffe and Chivas, 1990](#)). In turn, the $\delta^{18}\text{O}$ of meteoric water is controlled by climate ([Dansgaard, 1964](#); [Rozanski et al., 1992](#)). As a result, oxygen isotopic analysis of mammalian tooth enamel can be a valuable tool for reconstruction of regional paleoclimates. A significant shift over time in the $\delta^{18}\text{O}$ values of tooth enamel of a given taxon, from a given region, indicates a change in regional climate (e.g., [Ayliffe and Chivas, 1990](#); [Quade et al., 1992](#); [Ayliffe et al., 1994](#); [Bryant et al., 1994](#); [Kohn and Cerling, 2002](#)). At any given time interval, a large range of $\delta^{18}\text{O}$ values for individuals of a given taxon may reflect differences in dietary behavior or local and seasonal variability in precipitation ([MacFadden,](#)

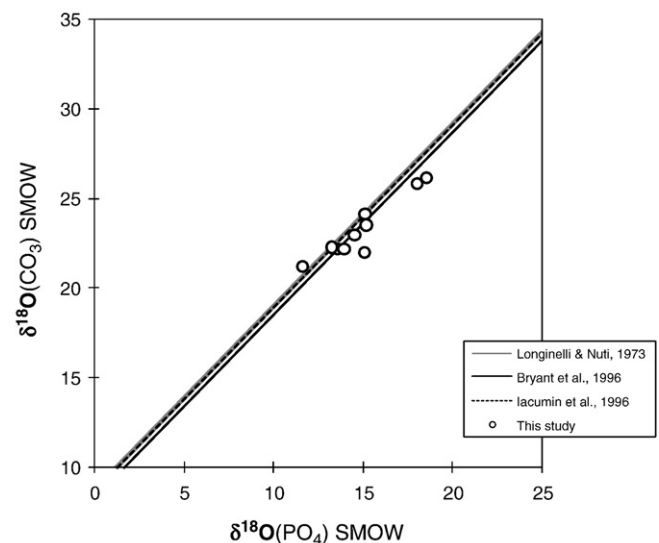


Fig. 3. $\Delta^{18}\text{O}_{\text{c-p}}$ ($= \delta^{18}\text{O}[\text{CO}_3] - \delta^{18}\text{O}[\text{PO}_4]$) values of fossil rhino enamel from this study vs calculated linear regressions from $\Delta^{18}\text{O}_{\text{c-p}}$ values of tooth enamel and bone from extant mammals ([Bryant et al., 1996](#); [Iacumin et al., 1996](#)) and the shells of extant marine organisms ([Longinelli and Nuti, 1973](#)). The $\Delta^{18}\text{O}_{\text{c-p}}$ values of the fossil rhino samples plotted on or near the equilibrium lines from previous studies, suggesting that there was little or no alteration of the oxygen isotopic compositions of either the structural carbonate or phosphate components of the fossil rhino samples.

1998; Kohn and Cerling, 2002). Therefore, mean $\delta^{18}\text{O}$ values can be used rather than individual data to reconstruct long-term trends in regional paleoclimates, as mean values attenuate $\delta^{18}\text{O}$ variability caused by behavioral differences and/or local and seasonal weather changes (Kohn and Cerling, 2002; Wang and Deng, 2005). If, in fact, the mean $\delta^{18}\text{O}$ values of tooth enamel reflect regional climate, the $\delta^{18}\text{O}$ values of tooth enamel from different large-bodied mammalian taxa living in the same region should display similar shifts in their respective $\delta^{18}\text{O}$ records.

Comparing the $\delta^{18}\text{O}$ values of horses and rhinos that lived concurrently in the Linxia Basin throughout the last 25 million years (Fig. 4), it is demonstrated that the $\delta^{18}\text{O}$ fluctuations throughout the late Cenozoic are nearly identical for both taxa, strongly supporting that the oxygen isotopic composition of mammalian herbivore tooth enamel largely reflects regional climate. As shown in Fig. 4 and Table 1, the $\delta^{18}\text{O}$ values of horse tooth enamel show significant shifts to more negative values after ~11 and ~6 Ma, indicating changes in regional climate toward either cooler temperatures or less arid conditions, or both. A large negative shift in the $\delta^{18}\text{O}$ values of horse enamel also occurs after ~7.5 Ma, but more data are needed to determine statistical significance. Likewise, significant shifts to more negative values occur in the $\delta^{18}\text{O}$ values of rhino enamel after ~11 and ~9 Ma. Based on deep-sea $\delta^{18}\text{O}$ and Mg/Ca records, a general cooling trend with two main cooling phases occurred over the last

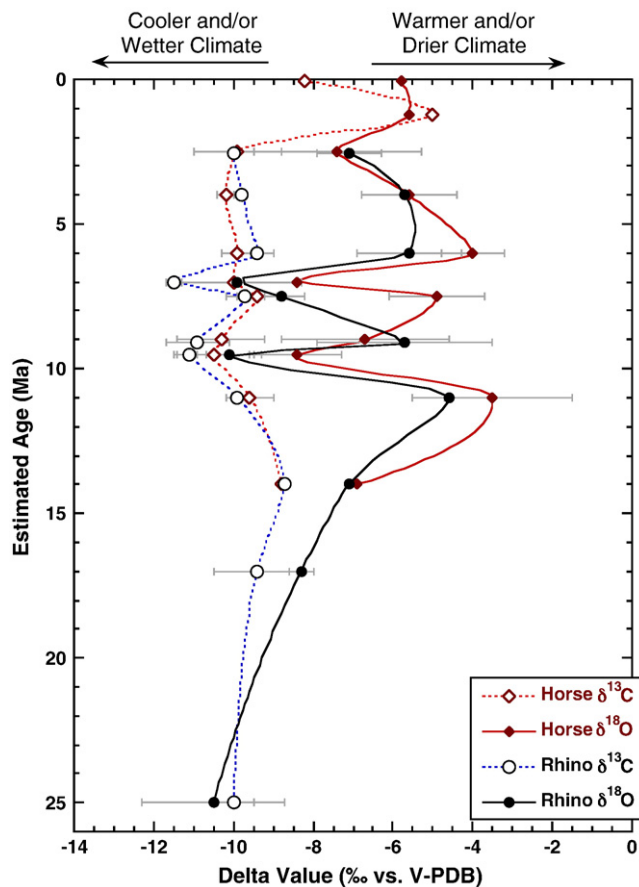


Fig. 4. Carbon and oxygen isotopic compositions of horses and rhinos lived concurrently in the Linxia Basin throughout the last 25 million years. The curves represent calculated mean δ values of tooth enamel from multiple horse or rhino individuals at given ages and include data from this study, as well as those from Wang and Deng (2005), which provides improved time resolution as well as a more robust data set compared to the previous study. Negative shifts in $\delta^{18}\text{O}$ values indicate changes to cooler and/or wetter climates and positive shifts indicate changes to warmer and/or drier climates. $\delta^{13}\text{C}$ values indicate diet composition (i.e., C_3 or C_4 plants) and shift to more positive values with water stress and more negative values in forested or closed-canopy environments. Error bars on $\delta^{18}\text{O}$ curves represent 1 standard deviation from the mean.

Table 1

Statistical significance of differences in mean oxygen isotopic compositions of various taxa at different ages as determined by two-tailed *t*-tests.

Sample	Mean difference (‰)	df	<i>t</i>	<i>p</i>	Significant difference at 95% CI?
Horse $\delta^{18}\text{O}$ (11.0 Ma) vs horse $\delta^{18}\text{O}$ (9.5 Ma)	4.9	13	4.97	0.0003	Yes
Horse $\delta^{18}\text{O}$ (9.5 Ma) vs horse $\delta^{18}\text{O}$ (9 Ma)	1.7	7	-1.63	0.1468	No
Horse $\delta^{18}\text{O}$ (9.5 Ma) vs horse $\delta^{18}\text{O}$ (7.5 Ma)	3.5	14	-5.69	0.0001	Yes
Horse $\delta^{18}\text{O}$ (9 Ma) vs horse $\delta^{18}\text{O}$ (7.5 Ma)	1.8	11	-1.90	0.0839	No
Horse $\delta^{18}\text{O}$ (6 Ma) vs horse $\delta^{18}\text{O}$ (4 Ma)	1.6	14	3.16	0.0069	Yes
Horse $\delta^{18}\text{O}$ (6 Ma) vs horse $\delta^{18}\text{O}$ (2.5 Ma)	3.3	19	4.38	0.0003	Yes
Horse $\delta^{18}\text{O}$ (4 Ma) vs horse $\delta^{18}\text{O}$ (2.5 Ma)	1.7	15	1.70	0.1105	No
Horse $\delta^{18}\text{O}$ (2.5 Ma) vs horse $\delta^{18}\text{O}$ (<2.5 Ma)	1.6	11	-1.01	0.3356	No
Rhino $\delta^{18}\text{O}$ (25 Ma) vs rhino $\delta^{18}\text{O}$ (17 Ma)	1.6	10	-1.29	0.2261	No
Rhino $\delta^{18}\text{O}$ (17 Ma) vs rhino $\delta^{18}\text{O}$ (14 Ma)	0.7	6	1.21	0.2705	No
Rhino $\delta^{18}\text{O}$ (14 Ma) vs rhino $\delta^{18}\text{O}$ (11.5 Ma)	4.4	8	-10.51	<0.0001	Yes
Rhino $\delta^{18}\text{O}$ (11.0 Ma) vs rhino $\delta^{18}\text{O}$ (9.5 Ma)	5.2	8	9.97	<0.0001	Yes
Rhino $\delta^{18}\text{O}$ (9.5 Ma) vs rhino $\delta^{18}\text{O}$ (9 Ma)	2.8	6	-2.90	0.0274	Yes
Rhino $\delta^{18}\text{O}$ (9 Ma) vs rhino $\delta^{18}\text{O}$ (7.5 Ma)	1.9	7	2.74	0.0291	Yes
Rhino $\delta^{18}\text{O}$ (7.5 Ma) vs rhino $\delta^{18}\text{O}$ (7 Ma)	1.0	6	1.40	0.2118	No
Rhino $\delta^{18}\text{O}$ (7 Ma) vs rhino $\delta^{18}\text{O}$ (6 Ma)	4.3	6	-3.96	0.0075	Yes
Bovid $\delta^{18}\text{O}$ (7 Ma) vs bovid $\delta^{18}\text{O}$ (4 Ma)	1.1	5	-0.59	0.5824	No
Bovid $\delta^{18}\text{O}$ (4 Ma) vs bovid $\delta^{18}\text{O}$ (2.5 Ma)	1.0	7	-1.20	0.2677	No
Bovid $\delta^{18}\text{O}$ (2.5 Ma) vs bovid $\delta^{18}\text{O}$ (0 Ma)	1.3	4	1.85	0.1373	No
Deer $\delta^{18}\text{O}$ (7 Ma) vs deer $\delta^{18}\text{O}$ (4 Ma)	6.4	2	-5.01	0.0376	Yes

25 million years (Shackleton and Kennett, 1975; Shackleton et al., 1995; Lear et al., 2000; Zachos et al., 2001). The first significant cooling event related to the rapid expansion of the east Antarctic ice sheet occurred during the middle Miocene from ~15 to 10 Ma (Shackleton and Kennett, 1975; Zachos et al., 2001). The negative shift in horse and rhino $\delta^{18}\text{O}$ after 11 Ma indicates a shift to cooler temperatures or moister conditions and is roughly consistent with cooling due to the establishment of a major ice sheet by 10 Ma. The second cooling phase occurred during the Plio-Pleistocene with a small-scale expansion of the west Antarctic ice sheet followed by the onset of the Northern Hemisphere Glaciation (Shackleton and Kennett, 1975; Lear et al., 2000; Zachos et al., 2001). The horse and rhino $\delta^{18}\text{O}$ values show a negative shift from 6 to 2.5 Ma, which is, again, in general agreement with the deep-sea records.

Significant positive shifts in $\delta^{18}\text{O}$ values after ~14 Ma, ~9.5 Ma and ~7 Ma in horses and rhinos (Fig. 4, Table 1), indicate shifts to drier and/or warmer conditions. $\delta^{18}\text{O}$ values of deer tooth enamel from the Linxia Basin also suggest a significant positive shift in $\delta^{18}\text{O}$ values after ~7 Ma (Table 1). The deep-sea $\delta^{18}\text{O}$ and Mg/Ca records show a general warming trend from ~26 to 15 Ma (interrupted by an abrupt return to a cooler climatic state at ~23 Ma during the "Early Miocene Mi-1 glaciation"), with a climatic optimum occurring at 17 to 15 Ma (Zachos et al., 2001). Although the low time resolution of the enamel $\delta^{18}\text{O}$ record does not permit detailed comparison with marine record, rhino

$\delta^{18}\text{O}$ values show a warming trend from the late Oligocene to the mid-Miocene (Fig. 4) that broadly agrees with deep-sea records. Dettman et al. (2003) suggested, based on $\delta^{18}\text{O}$ values of fluvial and lacustrine carbonates, that the Linxia Basin shifted to more arid and/or warmer conditions at 12 Ma. The positive shift observed in both horse and rhino $\delta^{18}\text{O}$ values from ~14 to 11 Ma also indicates a shift to drier conditions and/or increased temperatures. The positive shift in $\delta^{18}\text{O}$ values of horses, rhinos and deer from the Linxia Basin after ~7 Ma is similar in timing to a positive $\delta^{18}\text{O}$ shift observed in fossils and paleosols from Pakistan and Nepal (Quade et al., 1989; Quade et al., 1992; Quade et al., 1995). This suggests a shift toward a drier and/or warmer climate on both the north and south sides of the Tibetan Plateau during the late Miocene.

The $\delta^{18}\text{O}$ values of fossil tooth enamel from different taxonomic groups from the Linxia Basin, including bovids, horses, rhinos, deer, giraffes, pigs, and elephants, all fall within or close to (<2‰ difference) the ranges of horse and rhino $\delta^{18}\text{O}$ values at any given age, with the exception of a single deer sample at 4 Ma, which is >4‰ more positive than any horse or rhino individual at that age (Fig. 5). This difference could be the result of either diagenetic alteration or differences in dietary or drinking behavior between the deer at that age and all other taxa. A difference in dietary behavior is likely, as the deer $\delta^{18}\text{O}$ values are also more positive than those of all other taxa at 14 and 7 Ma. Deer ingest a large amount of water from leaves, and leaf water is strongly affected by relative humidity (Cormie et al., 1994; Koch, 1998). As a result, leaf $\delta^{18}\text{O}$ values will increase with increases in aridity. Overall, the oxygen isotopic compositions of all mammalian taxa collected from the Linxia Basin (Table 1) are consistent with the positive and negative trends in horse and rhino mean $\delta^{18}\text{O}$ values shown in Fig. 4, reflecting changes in regional climate.

Carbon isotope compositions of mammalian herbivore tooth enamel serve as proxies for the diets of animals. Because tooth enamel carbonate is ^{13}C -enriched by ~14‰ relative to diet, tooth enamel $\delta^{13}\text{C}$ values that are <−9‰ usually indicate a pure C_3 diet and $\delta^{13}\text{C}$ values that are >−2‰ indicate a pure C_4 diet (Lee-Thorp and van der Merwe, 1987; Koch, 1998; Cerling et al., 1997a; Cerling and Harris, 1999; Kohn and Cerling, 2002; Passey et al., 2002; Wang and Deng, 2005). If plants

become water-stressed, $\delta^{13}\text{C}$ values increase (Farquhar et al., 1989), and thus the conservative “cut-off” enamel- $\delta^{13}\text{C}$ value for a pure C_3 diet in water-stressed environments should be −8‰ for modern herbivores and −7‰ for fossil herbivores due to changes in the $\delta^{13}\text{C}$ value of atmospheric CO_2 (Cerling et al., 1997a; Wang et al., 2008b). In closed environments, such as forests, $\delta^{13}\text{C}$ values decrease, due to the influence of soil respiration (Schleser and Jayasekera, 1985; Sternberg et al., 1989; van der Merwe and Medina, 1989). Therefore, temporal shifts in the $\delta^{13}\text{C}$ values for horses or rhinos from the Linxia Basin (Fig. 4) may indicate either a shift in diet, habitat, or regional climatic conditions.

The mean $\delta^{13}\text{C}$ values for horses and rhinos at nearly all time intervals are very similar and range from −8.2 to −12.4‰ (Figs. 4 and 5), which suggests that both taxa had pure C_3 diets throughout most of the late Cenozoic and confirms the initial finding of Wang and Deng (2005). Notably, at 1.2 Ma, the horse $\delta^{13}\text{C}$ values increase to ~−5‰, indicating a change to a mixed C_3/C_4 diet after 2.5 Ma. The horses analyzed in this study include *Anchitherium sp.*, *Hipparion sp.*, and *Equus sp.* Anchitheres had low-crowned teeth and are presumably browsers feeding on soft vegetation like leaves, which is consistent with a pure C_3 diet inferred from the carbon isotope data. *Hipparion* and *Equus* in the Linxia Basin had high-crowned teeth, suggesting adaptation to grazing on grasses. However, unlike their counterparts in the Indian subcontinent, Africa and the Americas, these horses all had pure C_3 diets and were presumably grazing on C_3 grasses throughout the late Miocene and Pliocene; C_4 grasses became a part of their diets only after ~2.5 Ma (Fig. 8A,B). Because all $\delta^{13}\text{C}$ values from different taxonomic groups from the Linxia Basin are greater than −13‰ (Fig. 5), a relatively open environment, such as a mixed woodland/steppe biome is indicated at all time intervals. Most positive and negative shifts in mean $\delta^{13}\text{C}$ values occur at the same time and in the same direction as the shifts in $\delta^{18}\text{O}$ values (Fig. 4), indicating that the variation in $\delta^{13}\text{C}$ values is probably a result of fluctuations in water availability. This in turn suggests that the variation in $\delta^{18}\text{O}$ values most likely reflect changes in humidity or aridity, as opposed to changes in temperature. There is an exception from ~14 to 11 Ma for both horses and rhinos, where $\delta^{13}\text{C}$ values decreased as $\delta^{18}\text{O}$ values increased. This suggests that the plants were not water-stressed at this time interval and,

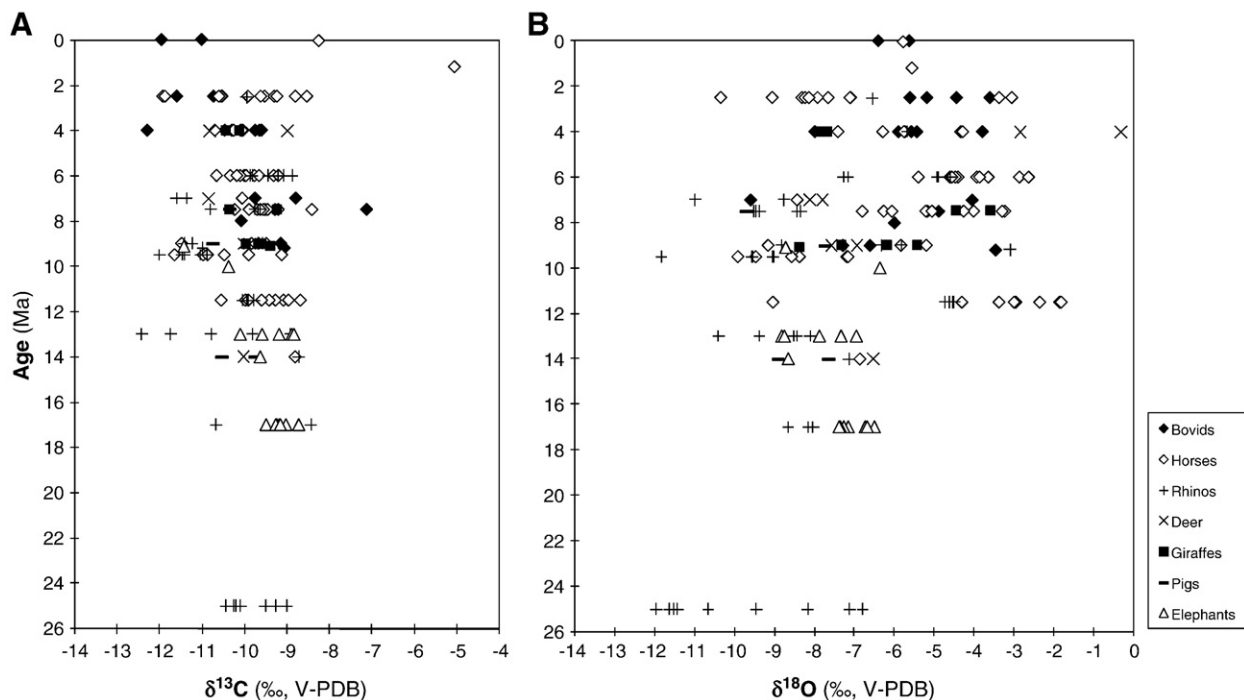


Fig. 5. Individual (A) $\delta^{13}\text{C}$ and (B) $\delta^{18}\text{O}$ values of fossil tooth enamel from different taxonomic groups from the Linxia Basin vs age (including data from this study as well as data from Wang and Deng, 2005).

therefore, the positive shift in $\delta^{18}\text{O}$ values may be due to warmer temperatures rather than increased aridity at ~11 Ma compared to ~14 Ma.

Individual $\delta^{13}\text{C}$ values of fossil tooth enamel from other taxonomic groups from the Linxia Basin including bovid, deer, giraffe, pig, and elephant fall within or near the ranges of horse and rhino $\delta^{13}\text{C}$ values at any given age (Fig. 5), also indicating pure C_3 diets for those animals. A single bovid sample at 7.5 Ma had a $\delta^{13}\text{C}$ value of -7.2% , which suggests that this individual may have consumed a small amount of C_4 plants. This would be consistent with a late Miocene C_4 expansion, but because there is no indication of mixed C_3/C_4 diets for any other individual (including horses) at 7.5 Ma, it is likely that very little C_4 vegetation was present in the Linxia Basin at that time. Alternatively, this $\delta^{13}\text{C}$ value could indicate that this individual was feeding on plants experiencing water stress or had migrated from another region outside the Linxia Basin (Cerling et al., 1997a; Wang et al., 2008b; Passey et al., 2009; Zhang et al., 2009). In general, the carbon isotopic compositions of all mammalian taxa collected from the Linxia Basin are consistent with the horse and rhino $\delta^{13}\text{C}$ trends shown in Fig. 4.

4.3. Changes in seasonality and monsoon strength

Because enamel mineralizes incrementally along the growth axis of a tooth, intra-tooth variation in the carbon and oxygen isotopic compositions of enamel reflects seasonal fluctuations in diet and in regional climate experienced by an animal (Koch et al., 1989; Bryant et al., 1994, 1996; Fricke and O'Neil, 1996; Sharp and Cerling, 1998; Feranec and MacFadden, 2000). Seasonal patterns in diet and climate reconstructed from serial carbon and oxygen isotopic analyses of individual fossil teeth may offer clues to the behaviors of various fossil taxa and may provide insight into the timing of the development of the Asian monsoon in this region.

The uplift of the Tibetan Plateau during the Cenozoic Era is considered to be the driving force in the development of the Asian monsoons (Hahn and Manabe, 1975; Ye, 1981; Murakami, 1987; Kutzbach et al., 1993; Yanai and Li, 1994; An et al., 2001; Kitoh, 2004), and the timing history of this development is important to the understanding of mammalian evolution in China. The Asian monsoon is caused by the strongly contrasting heat capacities of the ocean and continent. The high elevation difference between the Tibetan Plateau and the ocean results in strong temperature contrasts of air masses, creating a pressure gradient that drives warm, moist winds from the ocean to over the land in the boreal summer (Summer Monsoon). As a result, large amounts of precipitation fall over the Asian continent during the summer monsoon season. In the winter, cold and dry winds move from Siberia southward across East Asia toward the ocean (Winter Monsoon), resulting in relatively dry winters. This leads to distinct seasonal differences in precipitation on the Asian continent (Webster, 1987; Clark et al., 2000).

The oxygen isotope ratios of meteoric water vary from region to region as a result of evaporative and condensative processes. Evaporation favors water molecules containing the light isotope ^{16}O , and condensation favors water molecules with the heavy oxygen isotope ^{18}O (Epstein and Mayeda, 1953; Craig, 1961). In temperate zones that lie outside of monsoon regions and have relatively small amounts of annual precipitation, as well as large fluctuations in monthly temperatures, temporal variation in $\delta^{18}\text{O}$ values largely reflects changes in ambient temperature. In this type of environment, $\delta^{18}\text{O}$ values increase during summer months and decrease during winter months. In monsoonal regions, where there is seasonal fluctuation in temperature and precipitation, temporal variation in $\delta^{18}\text{O}$ values mainly reflects changes in rainfall amounts, the so-called "Amount Effect" (Dansgaard, 1964). Because precipitation preferentially removes ^{18}O from the vapor, the more it rains, the more ^{18}O -depleted the atmospheric water vapor becomes, and the precipitation that forms subsequently, likewise,

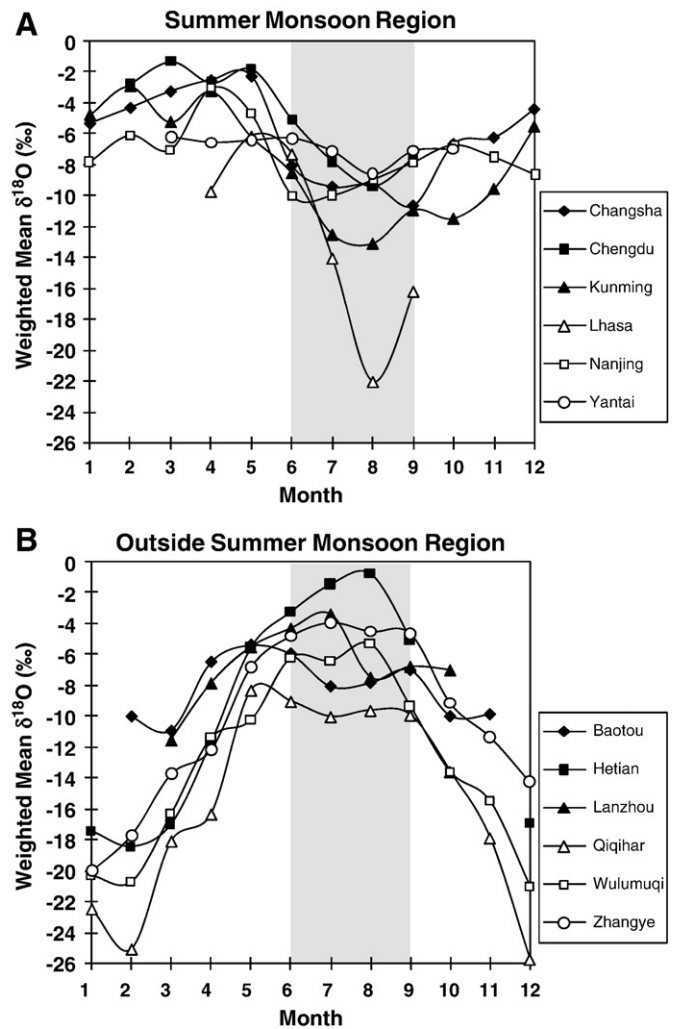


Fig. 6. Weighted monthly mean $\delta^{18}\text{O}$ values of precipitation at selected International Atomic Energy Agency (IAEA) stations (A) within the summer monsoon region and (B) outside of the summer monsoon region in China.

becomes more ^{18}O -depleted. In a monsoonal environment where most of the annual precipitation falls in the summer, $\delta^{18}\text{O}$ values of precipitation decrease during summer months, despite higher ambient temperatures, and increase during winter months (Dansgaard, 1964; Gonfiantini, 1985; Rozanski et al., 1993; Araguas-Araguas et al., 1998).

Weighted monthly mean $\delta^{18}\text{O}$ values of precipitation at selected International Atomic Energy Agency (IAEA) stations (Fig. 1) both within and outside of the summer monsoon region in China are shown in Fig. 6. At the six localities within the summer monsoon region, the weighted monthly mean $\delta^{18}\text{O}$ values of precipitation are lowest during the summer months (June–August), with up to a 16‰ difference between highest and lowest monthly $\delta^{18}\text{O}$ values (Fig. 6A). At the six localities outside the summer monsoon region, the mean $\delta^{18}\text{O}$ values of precipitation are highest during the summer months (June–August) and are lowest during the winter months (December–February), with up to an 18‰ difference between summer and winter $\delta^{18}\text{O}$ values (Fig. 6B). These data are consistent with expected oxygen isotope compositions of precipitation in temperate regions outside monsoonal regions and those of precipitation within monsoonal regions.

Because the $\delta^{18}\text{O}$ of tooth enamel is a proxy for regional climate, serial isotopic analyses of fossil tooth enamel should show the same patterns observed in the IAEA precipitation data. That is, in regions that are not strongly influenced by monsoons, low amounts of precipitation and warmer temperatures should result in increased

$\delta^{18}\text{O}$ values during the summer months, and within monsoon regions, high amounts of summer precipitation should result in decreased $\delta^{18}\text{O}$ values during the warmer months. Therefore, depending on the type of climate experienced by an animal, either peaks or troughs in the serial data could reflect the summer months. As a result, it is necessary to determine whether the highest or the lowest $\delta^{18}\text{O}$ values within the serial isotope records of given individuals occurred during the summer months in order to differentiate between mammals that lived within and those that dwelled outside of monsoon regions. It may be possible to distinguish between summer and winter $\delta^{18}\text{O}$ values by looking at concurrent carbon isotope records (Fig. 7). Because C_3 plants are dominant year-round in regions with little summer precipitation, little or no change in $\delta^{13}\text{C}$ values occur throughout the year. In this case, we may assume that the peaks, or increased $\delta^{18}\text{O}$ values occur during the summer due to little precipitation and warmer temperatures and that this isotopic pattern reflects regions that are not strongly affected by the summer monsoon. On the other hand, because C_4 grasses are able to grow in regions with summer precipitation and warm temperatures, an increase in $\delta^{13}\text{C}$ values occurs in enamel formed during summer months when C_4 grasses are present. Here, we may assume that the troughs or decreased $\delta^{18}\text{O}$ values occur during the summer months as a result of high amounts of precipitation and that this pattern is reflective of a summer monsoon region (Fig. 7). Thus, an inverse relationship exists between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values in a summer monsoon region where C_4 grasses are available for consumption by herbivores. However, due to complicated animal behaviors, this expected intra-tooth isotope pattern may not always emerge. For example, for animals that are selective in their diets and eat only C_3 plants regardless of the availability of C_4 food resource, there will be no inverse relationship between intra-tooth $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values.

In order to investigate changes in seasonality in the Linxia Basin from the Miocene to the present and to determine the timing of the strengthening of the summer monsoon in northwestern China, serial tooth enamel samples from 12 fossil horses dated ~11, 9.5, 7, 6, 4, 2.5, 1.2, and 0.05 Ma were analyzed (Fig. 8), as were those from two 17 Ma and two 13 Ma elephants, two 4 Ma giraffes (Fig. 9), and three 2.5 Ma

and two modern bovids (Fig. 10). The data show that, in general, positive $\delta^{18}\text{O}$ shifts in the horse and rhino bulk data, indicating shifts to either drier and/or warmer conditions after ~14, 9.5, 7, and 2.5 Ma, were accompanied by increased seasonality, as inferred from the relatively greater $\delta^{18}\text{O}$ ranges in the serial data (Fig. 11). Likewise, negative $\delta^{18}\text{O}$ shifts in the bulk data after ~11, 6 and 1.2 Ma were associated with decreases in seasonality, or relatively smaller $\delta^{18}\text{O}$ ranges in the serial data (Fig. 11).

The oxygen isotopic compositions of water sources have a great influence on seasonal $\delta^{18}\text{O}$ signals in mammalian tooth enamel. It has been shown that precipitation displays much larger seasonal $\delta^{18}\text{O}$ variability than groundwater, as the oxygen isotopic composition of groundwater reflects a weighted mean annual $\delta^{18}\text{O}$ of precipitation in the catchment area modified by evaporation. Therefore, the $\delta^{18}\text{O}$ values of lakes and larger rivers that consist predominantly of groundwater reflect average annual isotope compositions, and ephemeral streams and small ponds that consist mainly of local rainwater and have short water residence times have $\delta^{18}\text{O}$ values that more accurately reflect the oxygen isotope composition of seasonal precipitation (Koch et al., 1989; Clark and Fritz, 1997). Thus, tooth enamel from animals that drank from large bodies of water would be expected to show dampened seasonal signals compared to that from animals that drank from smaller and more temporary ponds or pools. Furthermore, consumption of plant material affects the oxygen isotope compositions of animals. Plants consist mainly of water (>80%) and leaf water is generally ^{18}O -enriched relative to local meteoric water. This is due to the preferential loss of isotopically light water molecules during evapotranspiration. The extent of ^{18}O -enrichment is dependent on relative humidity (Dongmann et al., 1974; Epstein et al., 1977; Yakir, 1992). Consequently, leaf water tends to have more enriched $\delta^{18}\text{O}$ values and displays a larger range of $\delta^{18}\text{O}$ variation in open habitats compared to more closed forested habitats in a given geographic area. Thus, it would be expected that individuals living in more open habitats or those migrating between habitats would display greater $\delta^{18}\text{O}$ variability in their enamel than would non-migratory forest-dwellers. Therefore, mammalian individuals with relatively small $\delta^{18}\text{O}$ ranges may have received most of their water intake from longstanding lakes, springs, or rivers, which tend to dampen

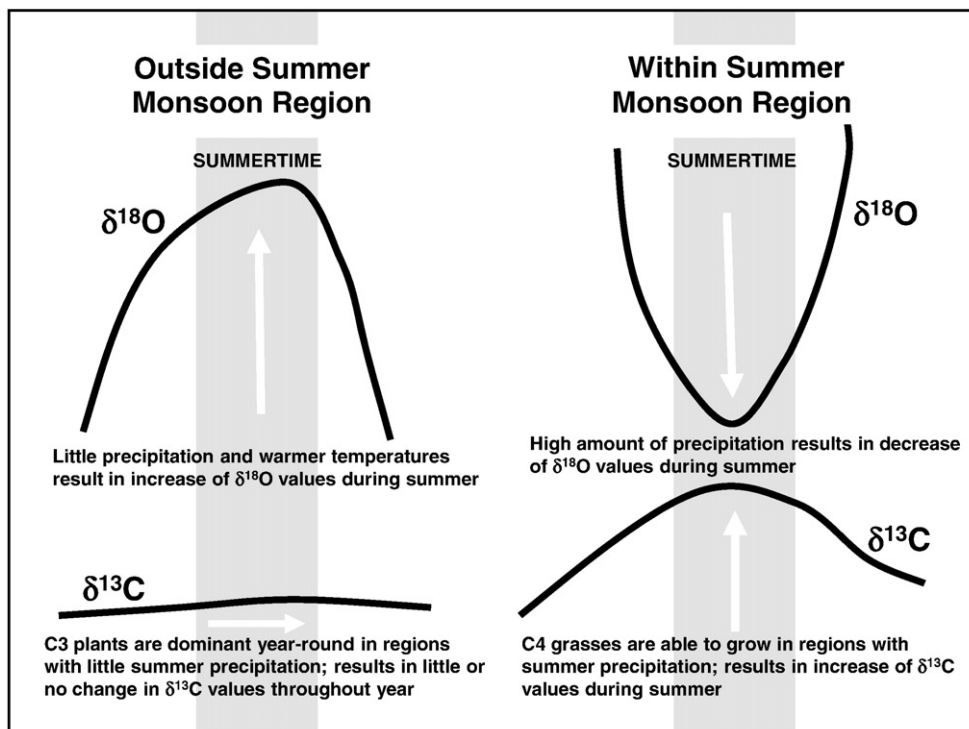


Fig. 7. Expected patterns in serial $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ records for tooth enamel samples from localities within and outside of the summer monsoon region in China.

seasonal $\delta^{18}\text{O}$ signals, or they may have lived in or migrated between more forested habitats. This is consistent with a cooler and/or wetter environment as inferred from the bulk oxygen isotope data (Fig. 4). Conversely, individuals with relatively larger $\delta^{18}\text{O}$ ranges may have drunk from more transient water sources or lived in a more open environment, which would be consistent with higher aridity and/or warmer temperatures.

The serial isotopic data also show a marked change in the seasonal $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ patterns after ~2–3 Ma. Before ~2.5 Ma, there was little or no intra-tooth $\delta^{13}\text{C}$ variations, indicating that all herbivores had pure C_3 diets year around. After that time, the data show a significant seasonal variation in horses' diets, ranging from a C_3 to a mixed C_3/C_4 diet with C_4 grasses accounting for up to ~60% of the diet. This confirms the conclusion based on the bulk enamel isotope data as discussed in the previous section and suggests that C_4 grasses may have not spread into the basin until after 2.5 Ma, much later than the proposed global C_4 expansion during the late Miocene. This may

indicate a strengthening of the Asian summer monsoon after 2.5 Ma, as C_4 plants are favored in warm rainy summers. Most importantly, decreases in the $\delta^{18}\text{O}$ values within individual teeth of the horses from 1.2 and 0.05 Ma occurred simultaneously with increases in the $\delta^{13}\text{C}$ values (Fig. 8B). That is, there is an inverse relationship or negative correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values within individual teeth of the horses from 1.2 and 0.05 Ma ($r = -0.434$ for By-05; $r = -0.465$ for Tz-02) (Fig. 8B). As mentioned previously, C_4 grasses are summer grasses that require summer precipitation and an animal that consumes C_4 vegetation will have increased $\delta^{13}\text{C}$ values in its enamel formed during summer months. Increased summer precipitation (i.e., summer monsoon) will result in a decrease in $\delta^{18}\text{O}$ values during summer months. That is, in the Asian monsoon region where C_4 grasses are available for consumption, one would expect that higher $\delta^{13}\text{C}$ values correlate with lower $\delta^{18}\text{O}$ values (representing summer months) within an individual tooth (Fig. 7). Thus, the inverse (or negative) correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values observed in teeth

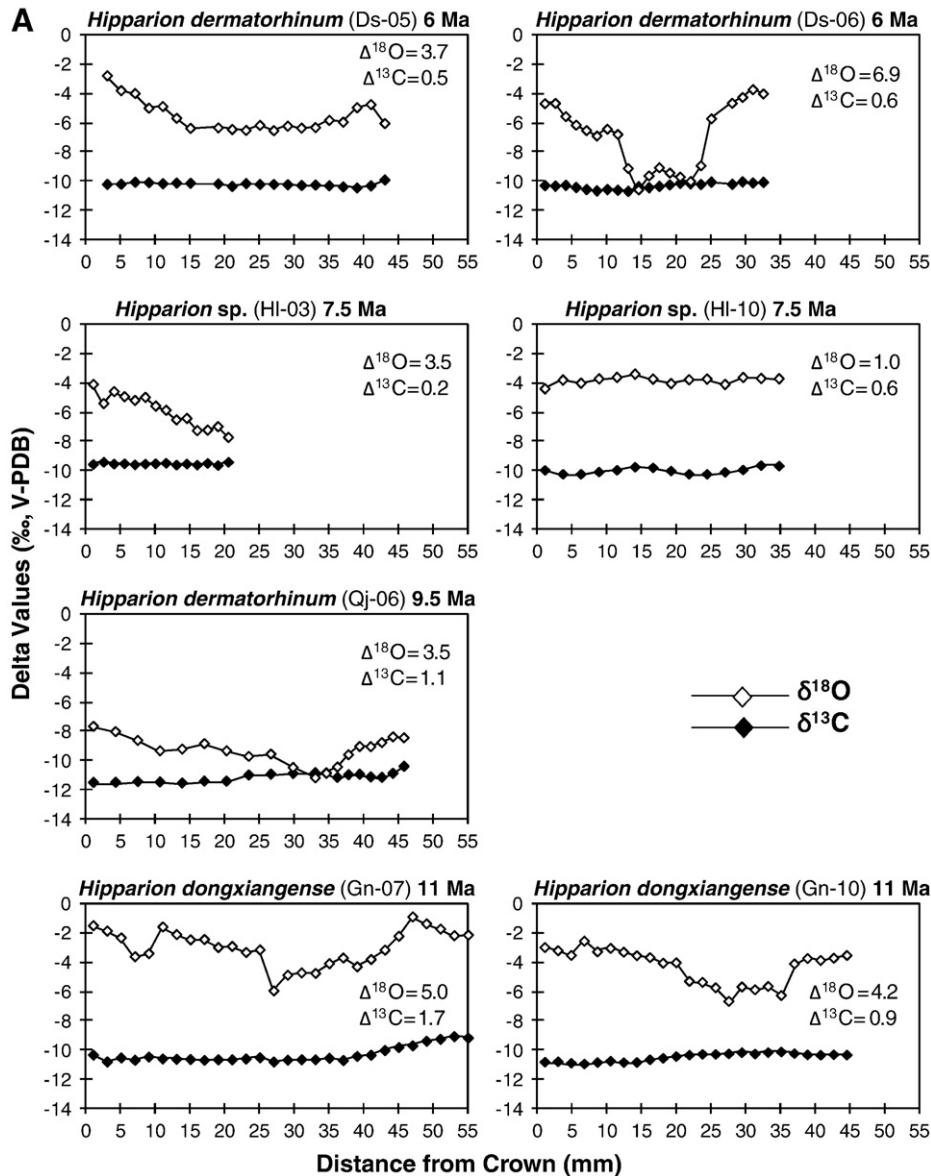


Fig. 8. A. Serial carbon and oxygen isotope compositions of horse tooth enamel from 11 to 6 Ma. Open diamonds represent oxygen isotope compositions and closed diamonds represent carbon isotope compositions. The data labels in parentheses are the sample numbers of the horse individuals. Serial samples decrease in biological age with distance from the crown. $\Delta^{18}\text{O}$ and $\Delta^{13}\text{C}$ = the ranges of the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ seasonal cycles. B. Serial carbon and oxygen isotope compositions of horse tooth enamel from 4 to 0.05 Ma. Open diamonds represent oxygen isotope compositions and closed diamonds represent carbon isotope compositions. The data labels in parentheses are the sample numbers of the horse individuals. Serial samples decrease in biological age with distance from the crown. $\Delta^{18}\text{O}$ and $\Delta^{13}\text{C}$ = the ranges of the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ seasonal cycles. Shaded areas represent inferred summer months.

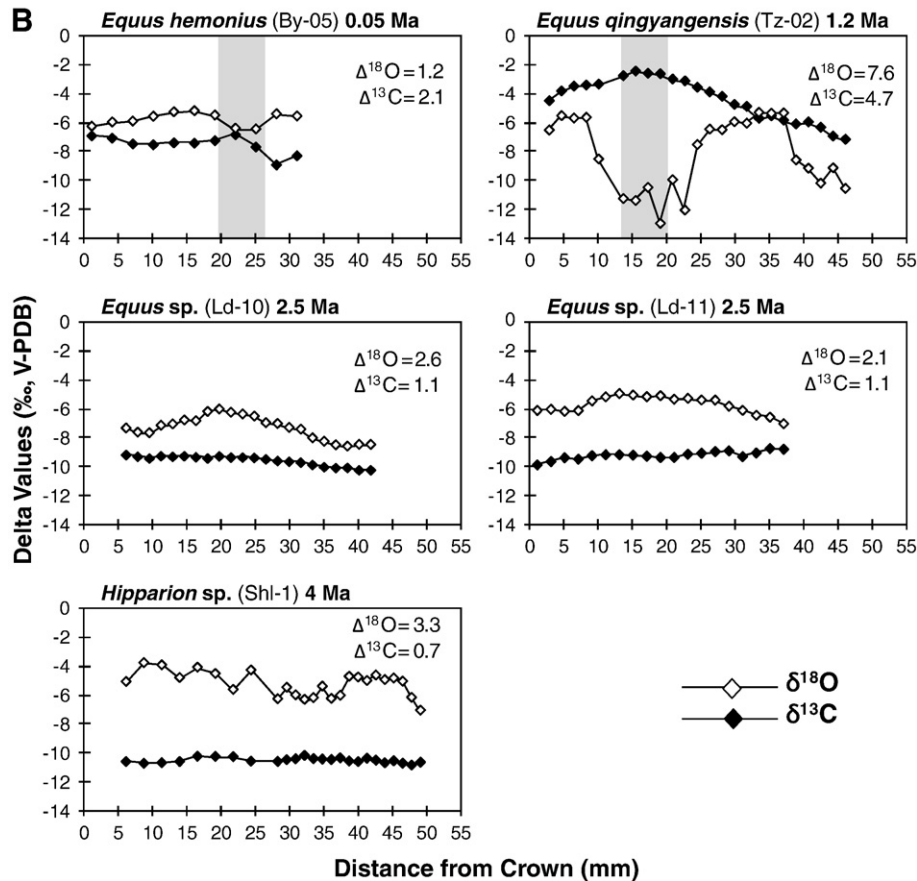


Fig. 8 (continued).

of the 1.2 Ma and 0.05 Ma horses (Fig. 8B) strongly supports a strong monsoonal environment in the Linxia Basin after, but not prior to ~2.5 Ma. Serial analyses of bovid teeth from ~2.5 Ma and later also showed an inverse correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values for all individuals ($r = -0.190$ to -0.975) (Fig. 10), providing further support for an enhanced monsoon climate since ~2–3 Ma.

In addition, a significant increase in seasonality after 2.5 Ma, as indicated by increased intra-tooth $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variations in both horses and bovids (Figs. 8 and 10), is consistent with a strengthening of the summer monsoon in the region after ~2–3 Ma. The $\delta^{18}\text{O}$ range of *Equus hemionus* at 0.05 Ma was not as large as that of *Equus qingyangensis* at 1.2 Ma (Fig. 8B), which could be due to having an incomplete seasonal record for *E. hemionus*, as the tooth was very worn. This could also be the result of a temporary decrease in seasonality during the years recorded by the 0.05 Ma individual. It has been shown that the Asian monsoon has distinct interannual variations and that a year of heavy rainfall is often followed by a year of diminished rainfall (Clark et al., 2000). For example, extensive snow cover over Eurasia following a strong monsoon season can slow down the summer heating of the landmass, which is necessary for the formation of large-scale monsoon flow in the following year (Shukla, 1987; Barnett et al., 1989). The $\delta^{18}\text{O}$ range of the modern cow was, likewise, not as large as that of the modern goat (Fig. 10). Again, the records of these two individuals may represent two different time spans and the difference in seasonality between the two animals may be due to interannual variation in monsoon strength. It is also possible that the O isotopic difference between the modern cow and goat is due to different drinking and dietary behaviors of these two domestic animals. Most fossil horses showed weak or no cyclicity in the serial oxygen isotope data (that is, no clear sinusoidal pattern), which suggests that the horses may have been migratory, and therefore, had mixed seasonal signals from drinking at

multiple locations, or drinking from a large water body such as a lake or a river that had smaller seasonal $\delta^{18}\text{O}$ variation than precipitation.

The $\delta^{13}\text{C}$ ranges of all horses from 11 to 2.5 Ma and the elephants *Gomphotherium* sp. at 17 Ma and *Platybelodon grangeri* at 13 Ma are $\leq 1.7\%$ (Figs. 9 and 10), indicating no significant seasonal variation in feeding behavior or composition of diet. *Palaeotragus microdon* (giraffe) at 4 Ma has a relatively larger $\delta^{13}\text{C}$ range of 2.3‰ (Fig. 9), but all $\delta^{13}\text{C}$ values are $< -8\%$ and there is no inverse relationship between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values. This suggests that the greater variation in the serial $\delta^{13}\text{C}$ values of *P. microdon* is likely due to seasonal changes in relative humidity rather than seasonal variation in the proportion of C_3 and C_4 plants consumed by the animal. Three bovid individuals from 2.5 Ma and two modern bovids have $\delta^{13}\text{C}$ ranges of 0.6, 2.2, 1.5, 2.0, and 1.6‰, with almost all $\delta^{13}\text{C}$ values $< -10\%$ (Fig. 10). Although all five individuals appear to have been pure C_3 feeders based on their bulk $\delta^{13}\text{C}$ values, there is a negative correlation ($r = -0.190$ to -0.975) between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values for all individuals. This suggests that these animals may have incorporated a small amount of C_4 grasses into their diets (with up to 14% C_4 , assuming that the end-member enamel- $\delta^{13}\text{C}$ values for pure C_3 and C_4 diet are -12% and $+2\%$, respectively) during the summer monsoon season (Fig. 10). This is quite possible, as the modern flora in the Linxia Basin includes C_4 grasses, which account for ~13% of all species collected from the basin (Table 2). Carbon isotope analysis of modern soil carbonates has shown that C_4 plants are unevenly distributed over the modern landscape and make up about 10 to 40% of the local biomass in the Linxia Basin (Wang and Deng, 2005). Thus, both the modern cow and goat could have ingested some C_4 vegetation during summer months. Also, because the modern-day environment of the Linxia basin is strongly influenced by the East Asian summer monsoon, increased serial $\delta^{13}\text{C}$ values that occur in tandem with decreased serial $\delta^{18}\text{O}$ values of tooth enamel from

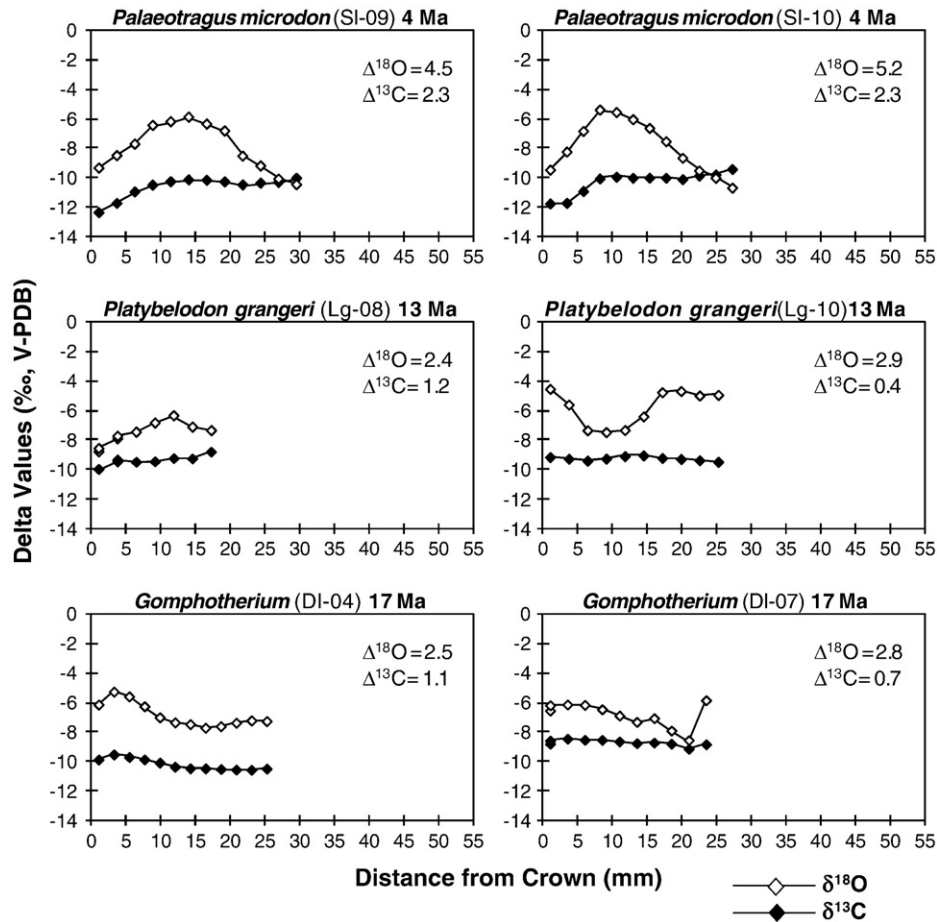


Fig. 9. Serial carbon and oxygen isotope compositions of elephant (*Gomphotherium* and *Platybelodon*) and giraffe (*Palaeotragus*) tooth enamel from 17 to 4 Ma. Open diamonds represent oxygen isotope compositions and closed diamonds represent carbon isotope compositions. The data labels in parentheses are the sample numbers of the individuals. Serial samples decrease in biological age with distance from the crown. $\Delta^{18}\text{O}$ and $\Delta^{13}\text{C}$ = the ranges of the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ seasonal cycles.

modern animals would require the incorporation of some C_4 vegetation during the summer. These data are also consistent with a stronger summer monsoon since $\sim 2\text{--}3$ Ma.

5. Conclusions

Carbon and oxygen isotopic analyses of tooth enamel indicate that significant changes occurred in the climates and diets of mammalian taxa from the Linxia Basin over the last 25 million years. Positive and negative shifts in the mean bulk $\delta^{18}\text{O}$ values of enamel from both horses and rhinos were roughly consistent with deep-sea records that indicated a general warming trend from ~ 26 to 15 Ma and two major cooling phases during the Neogene, respectively. A positive shift in both horse and rhino mean bulk $\delta^{18}\text{O}$ data was also consistent with a previous study that indicated the Linxia Basin shifted to warmer and/or more arid conditions at 12 Ma, based on oxygen isotope compositions of fluvial and lacustrine carbonates. A positive shift at ~ 7 Ma in the bulk enamel- $\delta^{18}\text{O}$ record from the Linxia Basin was similar in timing to a positive $\delta^{18}\text{O}$ shift observed in fossils and paleosols from Pakistan and Nepal, suggesting a shift toward a drier and/or warmer climate on both the north and south sides of the Tibetan Plateau during the late Miocene. Additionally, individual bulk $\delta^{18}\text{O}$ values of fossil tooth enamel from bovids, deer, giraffes, pigs, and elephants were consistent with the positive and negative trends in horse and rhino mean $\delta^{18}\text{O}$ values.

The enamel- $\delta^{13}\text{C}$ values indicate that various herbivores in the Linxia Basin, including horses, rhinos, bovids, deer, giraffes, pigs, and elephants, were all feeding predominantly on C_3 plants from 25 to

~ 2.5 Ma and that C_4 grasses did not become an important dietary component until after $\sim 2\text{--}3$ Ma. The data also indicate an open environment, such as a woody grassland or mixed woodland/steppe biome in the Linxia Basin from ~ 25 to 0.05 Ma. Serial oxygen isotopic analyses showed that, in general, positive $\delta^{18}\text{O}$ shifts in the horse and rhino bulk data, indicating shifts to either drier and/or warmer conditions after about 14, 9.5, 7, and 2.5 Ma, were accompanied by increased seasonality, as inferred from the relatively greater $\delta^{18}\text{O}$ ranges in the serial data. Likewise, shifts to wetter and/or cooler climate after about 11, 6.0, and 1.2 Ma, as indicated by negative $\delta^{18}\text{O}$ shifts in the bulk data, were associated with decreases in seasonality, or relatively smaller $\delta^{18}\text{O}$ ranges in the serial data. The serial carbon isotope results showed little or no seasonal variations in the $\delta^{13}\text{C}$ of diets before $\sim 2\text{--}3$ Ma. An increase in the serial $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ ranges of both horses and bovids after 2.5 Ma is consistent with a strengthening of the summer monsoon in the region after $\sim 2\text{--}3$ Ma. An inverse-correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values within individual teeth – a pattern characteristic of the summer monsoon regime – was observed in horses from 1.2 and 0.05 Ma as well as bovids of 2.5 Ma or younger ages but not in older fossils, providing further support for a strengthened monsoon circulation since about 2–3 Ma.

Acknowledgements

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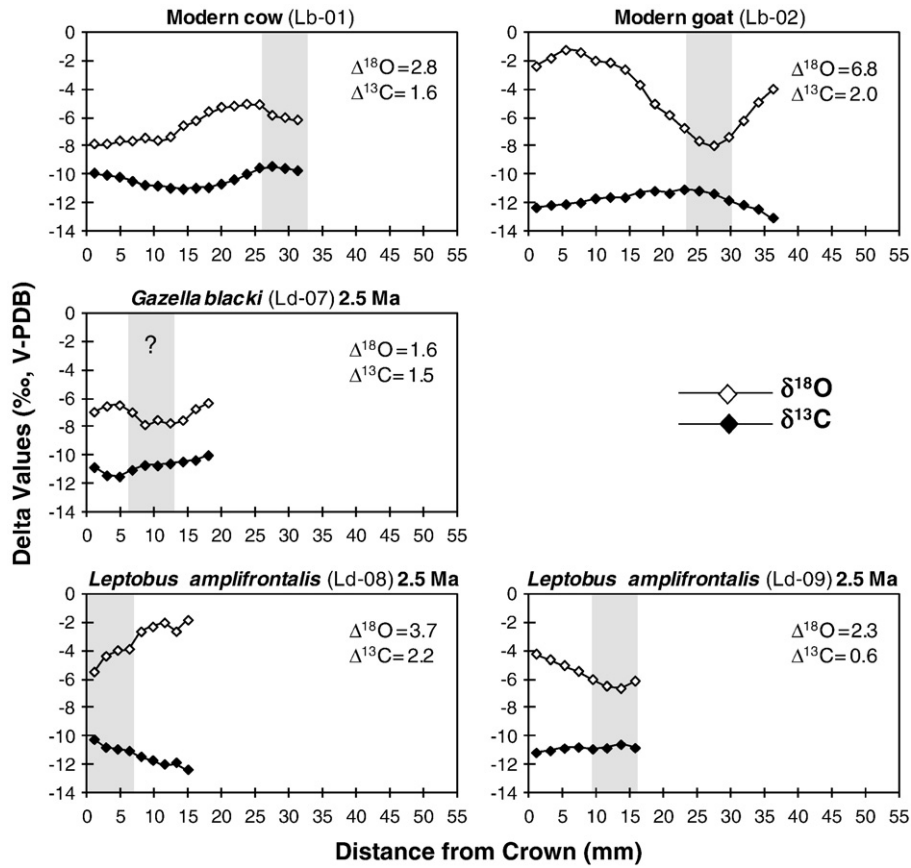


Fig. 10. Serial carbon and oxygen isotope compositions of bovid tooth enamel from 2.5 Ma to the present. Open diamonds represent oxygen isotope compositions and closed diamonds represent carbon isotope compositions. The data labels in parentheses are the sample numbers of the bovid individuals. Serial samples decrease in biological age with distance from the crown. $\Delta^{18}\text{O}$ and $\Delta^{13}\text{C}$ = the ranges of the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ seasonal cycles. Shaded areas represent inferred summer months.

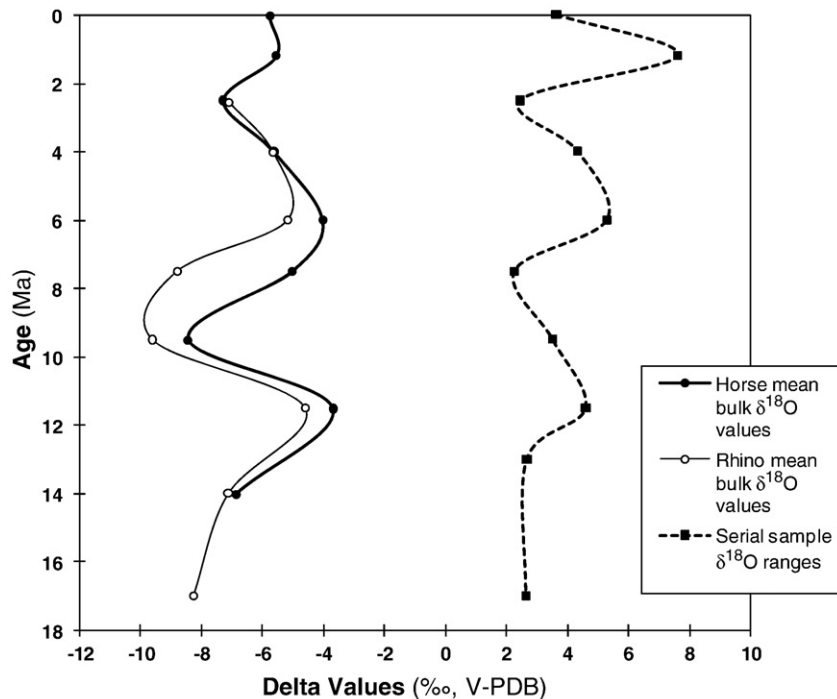


Fig. 11. Horse and rhino mean bulk $\delta^{18}\text{O}$ values vs $\delta^{18}\text{O}$ ranges of all serial-sampled mammals of various ages.

Table 2
Carbon isotope compositions of plants from the Linxia Basin.

Lab no.	$\delta^{13}\text{C}$ value	C3 or C4	Lab no.	$\delta^{13}\text{C}$ value	C3 or C4	Lab no.	$\delta^{13}\text{C}$ value	C3 or C4
Lx-01	-28.1	C3	Lx-19	-12.4	C4	Lx-37	-27.4	C3
Lx-02	-26.5	C3	Lx-20	-27.6	C3	Lx-38	-27.3	C3
Lx-03	-25.4	C3	Lx-21	-25.8	C3	Lx-39	-24.4	C3
Lx-04	-25.9	C3	Lx-22	-26.0	C3	Lx-40	-27.2	C3
Lx-05	-27.1	C3	Lx-23	-25.0	C3	Lx-41	-26.4	C3
Lx-06	-28.6	C3	Lx-24	-28.7	C3	Lx-42	-26.7	C3
Lx-07	-28.8	C3	Lx-25	-28.5	C3	Lx-43	-24.6	C3
Lx-08	-26.8	C3	Lx-26	-25.1	C3	Lx-44	-27.7	C3
Lx-09	-25.0	C3	Lx-27	-13.3	C4	Lx-45	-27.2	C3
Lx-10	-13.0	C4	Lx-28	-25.8	C3	Lx-46	-26.7	C3
Lx-11	-28.4	C3	Lx-29	-26.5	C3	Lx-47	-28.5	C3
Lx-12	-27.5	C3	Lx-30	-26.5	C3	Lx-48	-27.3	C3
Lx-13	-27.5	C3	Lx-31	-25.5	C3	Lx-49	-27.2	C3
Lx-14	-25.9	C3	Lx-32	-25.4	C3	Lx-50	-26.8	C3
Lx-15	-13.5	C4	Lx-33	-25.1	C3	Lx-51	-26.7	C3
Lx-16	-27.5	C3	Lx-34	-25.5	C3	Lx-52	-25.2	C3
Lx-17	-27.1	C3	Lx-35	-13.9	C4	Lx-53	-12.7	C4
Lx-18	-24.6	C3	Lx-36	-27.6	C3	Lx-54	-13.0	C4

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