Article

Chinese Science Bulletin

Geology

November 2013 Vol.58 No.31: 3788–3795
doi: 10.1007/s11434-013-5894-z

Diets, eco-environments and seasonal variations recorded in the oxygen and carbon isotopic compositions of mammal tooth enamel from the Shunshanji site, Sihong County, Jiangsu Province, China

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Received November 29, 2012; accepted April 15, 2013; published online May 30, 2013

The carbon and oxygen isotopic compositions of the tooth enamel of mammals, including deer, wild pigs, buffaloes and domesticated pigs from the Shunshanji site, Sihong County, Jiangsu Province, China, were analyzed to reconstruct the mammals' eco-environments and diets, and to evaluate seasonal variations in the study area. Carbon isotopic compositions of buck samples revealed that the deer ate completely C_3 plants and the environments they inhabited were relatively open and that wild pigs ate primarily C_3 plants. Oxygen isotopic compositions indicated that the body sources of these two mammals were different, i.e. the deer and pigs lived in different niches within a relatively similar ecosystem. Modern domesticated pigs were isotopically more positive than the ancient wild pigs in carbon $\delta^{13}C$ values, suggesting the former ingested more C_3 plants relative to the latter. Although the $\delta^{18}O$ data showed modern domesticated and ancient pigs had similar oxygen isotope compositions, their water sources were different. The carbon and oxygen isotopic patterns of premolar microsamples of ancient and modern buffaloes indicated that the plants ingested by the ancient buffalo varied with seasonal shifts, but plants ingested by the modern buffalo were relatively constant. The eco-environment of the modern buffalo was more open, warmer and drier than eco-environment of the ancient buffalo, which may be the result of the deforestation and other human activities. Ancient and modern seasonal changes were clearly recorded in the isotopic patterns and the seasonal variation amplitudes of the ancient and modern eco-environments were similar.

Shunshanji site, enamel, stable carbon isotope, stable oxygen isotope, palaeodiets, eco-environment, seasonal variations

Citation: Tian X S, Zhu C, Shui T, et al. Diets, eco-environments and seasonal variations recorded in the oxygen and carbon isotopic compositions of mammal tooth enamel from the Shunshanji site, Sihong County, Jiangsu Province, China. Chin Sci Bull, 2013, 58: 3788–3795, doi: 10.1007/s11434-013-5894-z

For the past decades, analysis of stable carbon and oxygen isotopes from mammalian bones and teeth has been used extensively for the reconstruction of the palaeoclimate, palaeoenvironment and palaeodiets from various species [1]. Carbon and oxygen isotopic compositions incorporated in tooth enamel can reveal the diets of ancient carnivorous and omnivorous animals. For herbivores, those data allow inferences regarding their diet types, such as C_3 plants or C_4 plants, and in turn, enable temperature and precipitation estimates of their habitat eco-environments [2]. Some studies have suggested that oxygen and carbon isotopic compositions in wild and domesticated mammal enamels may be closely correlated with diet sources and ambient environments [3,4]. Unlike bone, tooth enamel, which is not replaced once it is formed, and therefore is more resistant to diagenetic alteration because of its lower organic matter content and higher degree of crystallinity [5]. Thus *in vivo* biogenic isotope signals may remain unchanged for long periods. For example, stable carbon isotopes in tooth enamel were used to reconstruct the palaeodiet of Miocene hippopotamids unearthed in Chad [6]. Another study quantitatively reconstructed a Cretaceous palaeoenvironment using oxygen

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isotopes from tyrannosaurid tooth enamel [7]. In addition, enamel grows serially perpendicular to the tooth length axis; hence carbon and oxygen isotopes in enamel can reflect environmental factors, such as temperature and precipitation, during the time they are incorporated [8]. Unlike entire incisor samples yielding average isotopic values during the time span of tooth enamel formation, serial microsamples of tooth enamel provide finer temporal information in isotopic compositions, usually on the order of weeks, months and seasons [9]. Some studies have shown that carbon and oxygen isotopic compositions in modern bovine and horse tooth microsamples vary with seasons [10,11]. Based on such results, carbon and oxygen isotopic compositions in ancient mammal tooth enamel have been used to probe seasonal variations of the past [12,13]. In China, preliminary work was carried out to explore the relation between oxygen and carbon isotopic compositions and environmental factors [14–16]. Research was also carried out in northern China and Tibet to relate oxygen and carbon isotopic compositions in tooth enamel of wild herbivorous mammals in relation to vegetational ecotypes and environments [17,18]. Other researchers have reconstructed regional environmental evolution [19–21]. However, in China few studies so far have utilized isotopic compositions in serial microsamples of tooth enamel to investigate seasonal change.

In this contribution, the carbon and oxygen isotopic compositions of buck tooth enamel of mammals, including ancient deer, wild pigs and domesticated pigs, were analyzed to reconstruct the eco-environments and diets of mammals. Moreover, the carbon and oxygen isotopic compositions of serial tooth enamel samples from ancient and modern buffaloes were used to reveal seasonal variations in the study area.

1 Background

1.1 Stable carbon isotopes in tooth enamel

Terrestrial plants are classified into three types in relation to their photosynthetic pathways of fixing CO_2 from atmosphere: the C_3 pathway, the C_4 pathway and the CAM pathway [22]. C_3 plants, including most trees, herbs, shrubs, and cool-climate grasses, use the C_3 photosynthetic pathway to fix CO_2, forming a three-carbon sugar, whereas C_4 plants, including sedges and temperate and tropical grasses, use the C_4 photosynthetic pathway to fix CO_2, resulting in a four-carbon acid. CAM plants mainly include desert-adapted succulents, such as cacti, which have different photosynthetic pathways during the day and at night. The CAM plants were discarded in this study because the study region is not in the desert. C_3 plants and C_4 plants exhibit non-overlapping isotope compositions because they fractionate carbon isotopes to different degrees. C_3 plants show a mean δ^{13}C value of –27‰, with a range between –21‰, and –35‰. Plants growing in open environments or in water-stressed ecosystems have more positive values relative to those from closed forests because of the canopy effect. In contrast, C_4 plants, which grow in open environments, exhibit a mean δ^{13}C value of –12.5‰ with a narrow range of from –10‰ to –15‰ [23]. Furthermore, the δ^{13}C value of ingested food is enriched in the formed tooth enamel, with an enrichment of about 14‰ for herbivores and 9.5‰ for carnivores. Therefore, tooth enamel δ^{13}C values of herbivores that have fed on C_3 plants should range from –20‰ to –7‰, and those of herbivores that have fed on C_4 plants should range from –4‰ to 1‰ [24].

1.2 Stable oxygen isotopes in tooth enamel

The oxygen isotopic compositions of enamel phosphates are controlled by ingested water and may be affected by various factors, such as aridity, latitude, altitude, precipitation, relative humidity, temperature and metabolism [25]. For large sized mammals, body temperatures are kept constant and the oxygen isotopic compositions of their enamel are not affected by metabolic processes, but rather by meteoric water. Hence, the oxygen isotopic compositions of mammalian enamel, especially for herbivores, may record isotopic compositions of meteoric water and thus reflect the climatic and eco-environmental characteristics of the period during which they lived [26]. It is known that δ^{18}O values of meteoric water change spatially and temporally. In general, δ^{18}O values of meteoric water in closed, cool and humid environments are more negative than those in open, dry and warm habitats. Drought-tolerant mammals exhibit enriched δ^{18}O values relative to those frequently drinking water. Grazers living in grasslands have higher δ^{18}O values than browsers living in forests [27].

1.3 Tooth enamel maturation and time resolution

Tooth formation begins at the crown and the tooth grows progressively downwards toward the cervical junction. The organic components in the initially mineral-poor and protein-rich tooth matrix are gradually replaced with inorganic crystalline calcium hydroxyapatite [28]. During maturation, tooth enamel will incorporate carbon and oxygen. Consequently, both carbon and oxygen compositions incorporated at different times may be isotopically different, and sequential sample intervals from the crown to the cervical junction potentially may demarcate isotopic variability, reflecting seasonal changes in carbon and oxygen compositions of diet and ingested water. The tooth enamel from high-crowned mammals, such as sheep, cattle and deer, are often used to study palaeoenvironments. Because premolar enamel forms after the high-crowned mammals are weaned, δ^{18}O and δ^{13}C values of the high-crowned mammal premolar enamel are not affected by the milk of the mother [3,29]. As a result, variations in δ^{18}O and δ^{13}C values reflect the local environmental changes during the course of enamel formation

and, in turn, reflect variations in conditions, such as local precipitation, temperature and humidity. Thus, such variations record local seasonal changes.

2 Materials and methods

The mounded Shunshanji site is located in Sihong County, Suqian City, Jiangsu Province, China, with an area of 15000 m^2, at 33°34′58″N and 118°10′8″E. The landscape immediately surrounding the site is dominated by plains, while a series of mounds of about 50 m above sea level are scattered across the plains. The climate is a transitional zone of Northern subtropical to Northern temperate, typical of the East Asian monsoon region. Rainfall and temperature average about 890 mm and 14.6°C per year.

Scanning electron microscope (SEM) images of tooth enamel were used to judge the degree of digenetic alteration. A small piece of enamel from each tooth sample was rinsed with distilled water and then was oven-dried. Gold was sputtered onto the enamel under vacuum until it coated the enamel surface, which is used to improve image contrast. SEM images were made with a S-3400N II scanning electron microscope at the Modern Analyses Center of Nanjing University.

We determined the carbon and oxygen stable isotope values of tooth enamel from pigs, buffaloes and deer to evaluate the palaeoenvironment of the study site. Carbon and oxygen isotopic compositions of a buck enamel from animals are used to obtain the time-averaged information. Because the premolars are the last to develop and because the teeth of ancient and extant buffaloes are available in the study area, isotopic compositions of premolar microsamples from an ancient and modern buffaloes were determined to better understand the environmental and seasonal variations. The modern buffalo was raised for plowing fields, and mainly fed on plants growing in the wild such that the effect of human fodder on the isotopic compositions was minor. In this study 10 microsamples were cut serially from tip to the base of two buffalo premolars (Figure 1), which were about 55 mm long and record cyclic information for just over one year. Thus, every 5-mm represented 1.5 months' growth in tooth length for this animal [30].

The enamel samples were pretreated and conducted according to the standard methods [31]. The enamel was cut from the teeth with a dental drill. Powders of less than 75 μm were obtained from finely ground enamel. First, organic components were removed by soaking powdered samples in 2%–3% NaOCl overnight. Next, exogenous carbonates were removed after the samples were treated with 0.2 mol/L acetic acid for 20 h. The samples were rinsed with distilled water and then freeze-dried. The dry powder was reacted with 100% H_3PO_4 at 60°C for 5 h under vacuum to release CO_2 from the structural CO_3 in the apatite. The stable isotope ratios of carbon and oxygen from the resultant CO_2 were determined on a Delta plus Advantage isotope ratio mass spectrometer at the Key Laboratory of Lake Sediments and Environments, CAS. Replicate analyses show analytical precision of about 0.01‰ for carbon isotopes and 0.2‰ for oxygen isotopes. The isotopic ratios of carbon and oxygen are expressed as $\delta^{13}C$ values and $\delta^{18}O$ values ($\delta=(R_{sample}-R_{standard})/R_{standard}\times1000$, where $R=^{13}C/^{12}C$ or $^{18}O/^{16}O$).

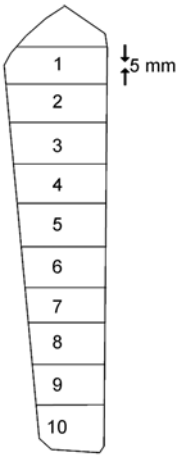

Figure 1 Sequential sampling of tooth enamel.

Student's *t*-test (software SPSS 16.0) is used to judge whether the differences between two groups of data were significant with respect to mean isotopic values. If results show that *P*-value is less than the hypothesized value of 0.05, then the differences between two groups are significant in mean values or *vice versa* [32].

3 Results and discussion

3.1 Preservation of primary isotopic compositions of enamel

During the process of tooth fossilization a range of factors, such as digenetic processes, the destruction of crystalline structure, recrystallization, and microbially induced corrosion can alter the primary isotopic compositions of tooth enamel [31]. The SEM images from tooth enamel are shown in Figure 2. The surface microstructures of ancient mammalian tooth enamel, similar to those of extant examples, were compact, with no cracks on the surfaces, indicative of no modification of the hydroxyapatite crystal structure or no intrusion of exogenous chemical compounds. Therefore, the enamel in the fossil samples was well preserved [33], and *in vivo* isotopic signals have been maintained because the fossils are still quite young and therefore have undergone little to no diagenesis.

3.2 Carbon isotopes and diets

Isotopic measurements are shown in Table 1 and Figure 3. Mean $\delta^{13}C$ and $\delta^{18}O$ values and their standard deviations are

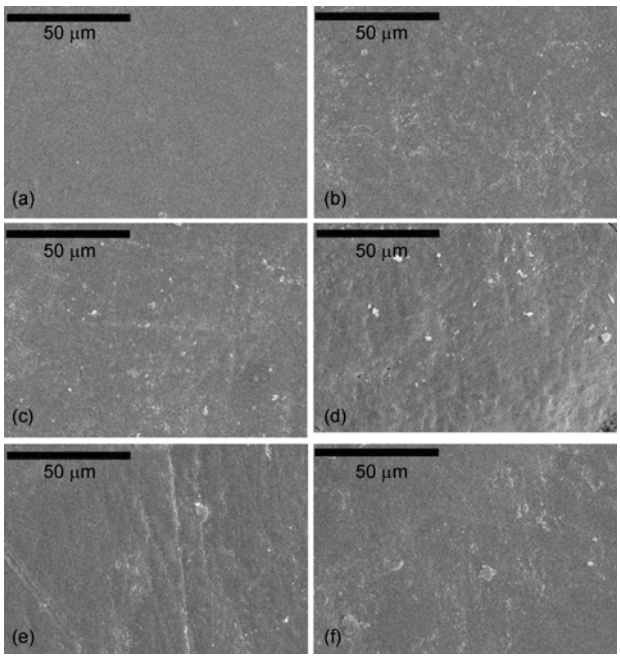

Figure 2 Scanning electron microscope images of mammal enamel from the Shunshanji site. (a) Modern pig; (b) ancient wild pig; (c) modern buffalo; (d) ancient buffalo; (e) and (f) ancient deer.

presented in Figure 4. Moreover, δ^{13}C values of plants consumed by complete wild mammals can be calculated using the δ^{13}C values of these mammal tooth enamel and thus the types of these plants can be inferred [34]. Enamel δ^{13}C values of deer averaged 13.9±0.4‰ (n=4) and the enamel δ^{13}C values of wild pig averaged −12.5±0.8‰ (n=15). Carbon isotopes showed that enamel δ^{13}C values of deer and wild pig range of from −20‰ to −7‰. Assuming that herbivore enamel δ^{13}C values more negative than −7‰ are indicative of 100% C_3 feeding, the deer in the study area consumed only C_3 plants. Deer are classified as browsing herbivores, whereas wild pigs occasionally eat small animals and are classified as omnivores [35]. A Student's t-test (P=0.003, n_1=4, n_2=15) shows a significant difference between wild pigs and deer in their mean δ^{13}C values, indicating significant differences in their diets. Yet the wild pigs also ingested mainly C_3 plants; therefore, the deer and wild pigs lived in a relatively similar ecosystem. Plants in closed forests have lower δ^{13}C values (as low as −35‰) relative to trees in open habitats because of the canopy effect. Moreover, plants in water-stressed ecosystems in arid environments are less depleted in ^{13}C and have more positive values (as high as −21‰) than the mean C_3 δ^{13}C value of −27‰ [36]. In

Table 1 δ^{13}C and δ^{18}O values of mammal enamel samples from the Shunshanji site[a)]

Sample number	Specimen	δ^{13}C (‰)	δ^{18}O (‰)	Sample number	Specimen		δ^{13}C (‰)	δ^{18}O (‰)
K125792	M_2 (deer)	−13.3	−5.9	K126220	M_2 (domestic pig)		−7.2	−7.2
K125793	M_2 (deer)	−14.2	−5.7	K126241	M_3 (domestic pig)		−6.8	−7.3
K126210	M_3 (deer)	−14.3	−4.5	K126221		P1-1	−7.7	−8.9
K126211	M_2 (deer)	−13.9	−6.2	K126222		P1-2	−11.2	−7.8
K126202	M_1 (wild pig)	−12.2	−7.9	K126223		P1-3	−10.4	−8.4
K126203	M_2 (wild pig)	−12.8	−7.2	K126224		P1-4	−10.4	−8.6
K126204	M_2 (wild pig)	−11.7	−7.7	K126225	Ancient buffalo	P1-5	−10.3	−8.7
K126205	M_3 (wild pig)	−10.5	−7.7	K126226		P1-6	−9.4	−9.8
K126206	M_3 (wild pig)	−13.6	−5.9	K126227		P1-7	−7.7	−10.2
K126207	M_2 (wild pig)	−13.8	−6.0	K126228		P1-8	−6.1	−11.0
K126208	M_3 (wild pig)	−12.5	−6.2	K126229		P1-9	−5.9	−10.5
K126209	M_2 (wild pig)	−12.3	−7.7	K126230		P1-10	−6.3	−10.5
K125794	M_2 (wild pig)	−12.2	−8.0	K126231		P1-1	−3.7	−8.2
K125795	M_2 (wild pig)	−13.01	−6.9	K126232		P1-2	−4.7	−4.9
K126212	M_1 (wild pig)	−12.6	−5.5	K126233		P1-3	−4.2	−5.4
K126213	M_2 (wild pig)	−12.9	−6.9	K126234		P1-4	−3.1	−5.7
K126214	M_2 (wild pig)	−12.9	−5.2	K126235	Modern buffalo	P1-5	−2.5	−5.7
K126215	M_2 (wild pig)	−12.3	−8.5	K126236		P1-6	−2.5	−5.8
K126216	M_3 (wild pig)	−12.6	−8.3	K126237		P1-7	−2.6	−5.7
K126217	M_2 (domestic pig)	−4.8	−6.6	K126238		P1-8	−2.7	−6.3
K126218	M_3 (domestic pig)	−6.0	−6.5	K126239		P1-9	−3.3	−6.9
K126219	M_2 (domestic pig)	−6.1	−7.1	K126240		P1-10	−3.6	−8.3

a) M_1, the first molar; M_2, the second molar; M_3, the third molar; P, premolar.

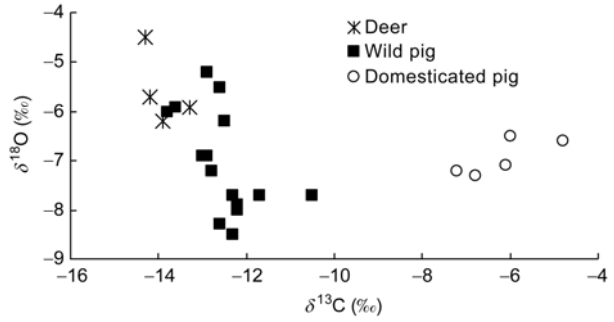

Figure 3 δ^{13}C and δ^{18}O values of mammal tooth enamel.

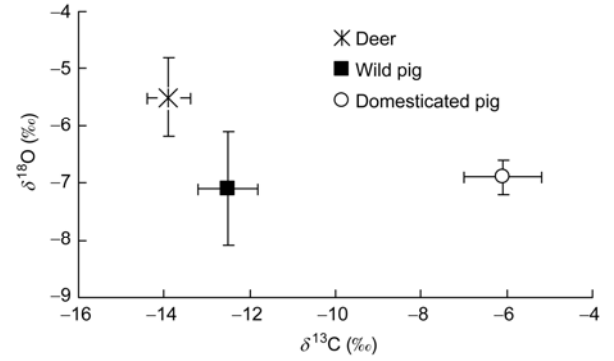

Figure 4 Mean values and standard deviations for δ^{13}C and δ^{18}O values of mammal tooth enamel.

light of a measured enrichment of 14‰ δ^{13}C in tooth enamel and their mean δ^{13}C value of about −27‰, the environments inhabited by deer were relatively open [37].

Modern domesticated pig enamel δ^{13}C values displayed an average of −6.2±0.9‰ (n=5) with a range of 2.4‰. Domesticated pigs are resumed as carnivores; hence, their diets had a mean δ^{13}C value of about −15.7‰, a value between 100% C_3 plants and 100% C_4 plants. An independent-samples t-test showed that the mean enamel δ^{13}C values between modern pigs and ancient pigs were significantly different (Student's t-test, P<0.001, n_1=5, n_2=15), with the former more positive than the latter. This difference may be caused by one of two reasons. Either the fodder of domesticated pigs may comprise cultivated crops of C_4 plants with elevated δ^{13}C values, or the samples were derived from individual rural households rather than large farms, and therefore may have included human refuse and animal remains, resulting in elevated enamel δ^{13}C values [38].

3.3 Oxygen isotopes and water sources

Oxygen isotopes from mammals have diverse sources and directions. Mammals with different habits in the same eco-environment will have different oxygen isotope compositions. Oxygen isotope compositions are generally sensitive to different water sources, different plant tissues consumed by the mammals, different living areas, and the amount and nature of secretion and sweating [39]. The mean deer δ^{18}O value was −5.5±0.7‰ (n=4). The enamel δ^{13}C values of wild pigs averaged −7.0±1.0‰ (n=15). Mean enamel δ^{18}O values between ancient wild pigs and deer were significantly different (Student's t-test, P=0.02, n_1=4, n_2=15). Three possible factors may cause such a difference. The first is that their water sources could have been different. Compared with local water, leaf water is significantly enriched in ^{18}O because of preferential loss of ^{16}O via evapotranspiration [40]. Another possibility is that δ^{18}O values in different plant tissues were different; therefore, wild pigs and deer may have eaten different plants or different plant tissues. The third explanation is that these mammals inhabited different niches, which also could have caused differences in their δ^{18}O values due to the effects of respiration, secretion and sweating. Thus, despite their being in the same ecosystem, the deer and wild pigs lived in different niches.

The enamel δ^{18}O values of modern domesticated pigs averaged −6.9±0.40‰ (n=5), which was not significantly different from the mean value of the ancient pigs (Student's t-test, P=0.2, n_1=5, n_2=15). Nonetheless, this result does not necessarily signify that they shared the same water sources. As discussed above, the diets that supplied water from food of the ancient pigs differed greatly from modern domesticated pigs. For ancient pigs the ^{18}O mainly came from C_3 plants, and for the modern domesticated pigs, the sources may include C_3 plants, C_4 plants, human refuse, or animal remains [41]. In addition, their drinking water sources also differed because the oxygen from modern and ancient natural water sources, such as ponds and rivers, should be isotopically distinct. If the modern domesticated pigs ingested some household water, the oxygen isotopic signature would be affected by human activities. To understand how the factors mentioned above affected the enamel δ^{18}O values of domesticated and wild pigs more detailed analyses will be carried out.

3.4 Environmental and seasonal change

Buffaloes are fully herbivorous, so variations of their tooth δ^{18}O values and δ^{13}C values reflect changes in the isotopic compositions of the plants consumed and, in turn, shifts in the eco-environment [42]. Serial oxygen and carbon isotope measurements across enamel surfaces revealed a cycle of about one year of growth. The mean δ^{18}O values for ancient and modern buffaloes premolar microsamples were −9.4±1.0‰ (n=10) and −6.2±1.1‰ (n=10), respectively (Table 1, Figure 5). The ancient buffalo samples yielded more negative δ^{18}O values than the modern buffalo samples. Thus, we infer that the eco-environment of the ancient buffaloes was more moist and cold than in modern times. In the study area, numerous ape fossils have been unearthed and a large number of fish, crocodile, turtle, rodents, rhinos and other fossils also have been found. Furthermore, stone tools and pottery

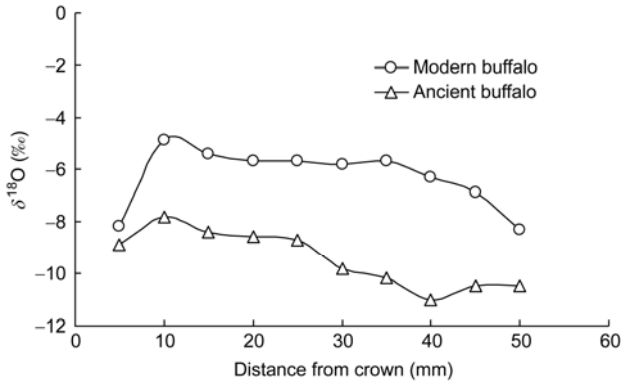

Figure 5 δ^{18}O value profiles of buffalo premolars from the Shunshanji site.

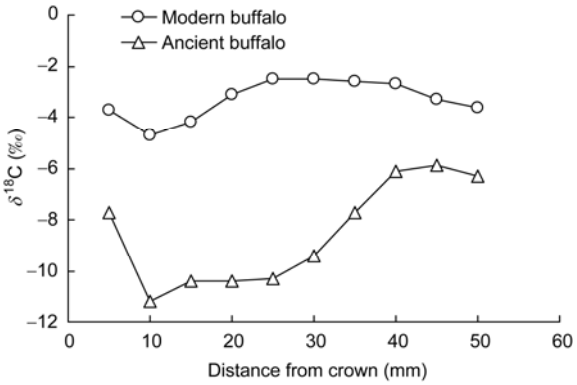

Figure 6 δ^{13}C value profiles of buffalo premolars from the Shunshanji site.

with graceful lines and polished surfaces have also been discovered from the site. These finds signify that the climate was humid and suitable for animal and plant growth and human living at that time. A moat around the site also was found, measuring up to 1000 m long, 1.5–3 m deep, and 6–24 m wide. This also shows that the climate was moist in the past. Both of the two series of buffalo enamel δ^{18}O values show similar seasonal variations. High values of the serial δ^{18}O indicated dry and warm seasons, and typically were followed by low δ^{18}O values, which suggested wet and cold seasons [43].The ranges of intra-tooth enamel δ^{18}O values for the ancient and modern samples were 3.2‰ and 3.4‰ respectively, and were very similar, signifying that seasonal variation amplitudes of modern and ancient samples were very close.

The mean δ^{13}C values for ancient and modern buffalo premolar microsamples were −8.5±2.0‰ (n=10) and −3.2± 0.7‰ (n=10), respectively. The difference between their mean enamel δ^{13}C values was significant (Student's t-test, P<0.01, n_1=10, n_2=10), implying that modern buffaloes diets include more C_4 plants than those of ancient buffaloes. Thus, similar to the δ^{18}O data, the δ^{13}C values also supported the idea that it was warmer and drier in modern times than in the past. The serial δ^{13}C values of the ancient buffaloes decreased from the base to the crown, implying that they ate different plants with seasonal changes. The ranges of intra-tooth enamel δ^{13}C values for the ancient and modern samples were 5.3‰ and 2.2‰, respectively (Table 1, Figure 6). Fluctuations of modern buffalo δ^{13}C values indicated that they consumed plants without seasonal shifts, which may be caused by the effects of precipitation and temperature on the plants consumed [44]. In contrast, the ancient buffaloes ingested C_4 plants occasionally. In contrast to ancient times, C_4 plants today may be more readily available to buffaloes in the local community, and therefore environmental change is a plausible explanation for the isotopic signatures. It is generally accepted that seasonal variations in the eco-environment will be obvious if the ranges of intra-tooth enamel δ^{18}O and δ^{13}C values are up to 2‰–3‰ [45]. Serial δ^{13}C and δ^{18}O values clearly showed both modern and ancient seasonal variations. Nonetheless, these conclusions are preliminary because of a small number of tooth samples; therefore, additional samples and analysis will be needed to confirm these inferred patterns.

4 Conclusions

Results from this study show that enamel δ^{13}C values of deer and wild pigs fell into the range of −20‰ to −7‰, indicating that deer diets consisted entirely of C_3 plants and wild pigs primarily ingested C_3 plants. The plants consumed by deer showed a mean δ^{13}C value of about −27‰, suggesting the environments that the deer inhabited were relatively open. The deer and pigs lived in a similar ecosystem. There were differences between the wild pigs and deer in their diets and water sources, suggesting the two species of mammals lived in different niches.

Enamel δ^{13}C values of modern pigs were more positive than those of ancient wild pigs. A hypothesis to explain this difference is that the fodder for domesticated pigs may comprise cultivated crops of C_4 plants with elevated δ^{13}C values along with human refuse and animal remains, resulting in elevated enamel δ^{13}C values for the domesticated pigs. Although the enamel δ^{18}O values of modern domesticated and ancient wild pigs were not significantly different, they clearly differed in natural water sources from their foodstuffs and from their drinking water.

Enamel δ^{13}C values showed that modern buffaloes included more C_4 plants than the ancient samples. Serial δ^{13}C values showed that ancient buffalo ingested different plants with seasonal changes, yet modern buffalo have diets without seasonal shifts. The isotopic patterns showed that the eco-environment of the modern period was colder and moister than the ancient setting of the study site, which may be the result of deforestation and other human activities. Ancient and modern seasonal changes were clearly recorded, and the seasonal variation amplitudes of modern and ancient samples were similar.

It is relevant to reconstruct the paleodiets and eco-envi-

ronments of human inhabitants based on analyses of carbon and oxygen isotope compositions. However, because the sample data set was limited, more research is needed to confirm the conclusions mentioned above.

This work was supported by the National Natural Science Foundation of China (40971115 and 411711163), the University Doctoral Foundation (20090091110036), the Test Foundation of Modern Analyses Center of Nanjing University (0209001309), the Foundation of the Ministry of Science and Technology of the People's Republic of China (2010BAK67B02) and the China Postdoctoral Science Foundation (2010048113). Two anonymous reviewers are thanked for their reviews of this manuscript.

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