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A preliminary application of dental cementum incremental analysis to determine the season-of-death of equids from the Xujiayao site, China

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Abstract Seasonality study of Paleolithic archaeological faunal assemblages is important for reconstructing modes of animal death and ancient hominin strategies for prey acquisition. The dental cementum of animal teeth records the season of an individual animal in which it died, providing the necessary evidence for determining any patterns in season of death. This article presents the procedure and preliminary results of the application of cementum incremental analysis to equid teeth, which comprise the dominant component of the fauna from the Xujiayao Site—an early Late Pleistocene archaeological site in China. Results show multi-seasonal use in both the upper and lower layers of this site. Furthermore, attention is given to the method's future application in seasonality studies at Paleolithic archaeological sites in China.

Keywords Cementum Analysis, Seasonality, Paleolithic, Xujiayao site, Zooarchaeology

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1. Introduction

Seasonality studies of Paleolithic archaeological sites are important for reconstructing critical aspects of ancient hominin behaviors, such as foraging habits, hunting strategies, land use, and residential mobility (Klein, 1978, 2009; Monks, 1981; Speth, 1987; Stiner, 1994). Previous studies have established that the analysis of incremental growth marks in peripheral cementum of various ungulate mammal teeth is a valid technique for obtaining estimates of season of death, because tooth cementum has been recognized to grow according to seasonal rhythm (Burke and Castanet, 1995; Kay, 1974; Miller, 1974; Pike-Tay, 1995; Ransom, 1966; Stallibrass, 1982; Stutz, 2002; Stutz and Lieberman, 2007). Thus, it has been used to analyze animal fossils in a wide variety of archaeological sites (Burke and Castanet, 1995; Jones, 2012; Kay, 1974; Lubinski and O'brien, 2001; Ma et al., 2012; O'Brien, 1994; Pike-Tay, 1995; Spiess, 1976; Stutz, 2002; Stutz and Lieberman, 2007).

Although equids including the two species, *Equus przewalskii* and *Equus hemionus* discussed in this paper are abundant in many Chinese archaeological sites, limited research has been done on the strategy for economic exploitation of these animals. Specifically, there has been little previous study on the seasonal use of equid resources because the fundamental information on equid season of death has not been available. Among the methods suggested to address the shifts in seasonal activity pattern in the context of Pleistocene cultural and biological evolution, cementum incremental analysis stands out because this technique could yield highly de-

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tailed seasonal results across populations, species and time periods (Stutz, 2002). Cementum analysis has not been used in China because the required procedures are complex and tooth specimens from known Paleolithic sites are often recovered in poor condition after taphonomic changes, making it challenging to obtain accurate results of age and season of death (Greenfield et al., 2015; Li and Tong, 2008). Since there have been some successful cases of application of cementum analysis in studies of equid teeth (Burke and Castanet, 1995; Greenfield et al., 2015; Mitchell et al., 2003; Sahara, 2014; Smuts, 1974), it is reasonable to use this technique to explore seasonality in Chinese archaeological sites.

In this article, we describe the necessary procedures, and present the preliminary results of the application of cementum incremental analysis to equid teeth from Xujiayao, an early Late Pleistocene archaeological site in China. The purpose of this research is to test the viability of cementum increment analysis in determining season-at-death of archaic equid teeth in east Asia. Then, we use the results to study the seasonality of equid acquisition through time at the Xujiayao site. Furthermore, since this is the first attempt to use this technique in the study of China's Pleistocene, the potential value of cementum incremental analysis to the seasonality study of Paleolithic fauna in China is assessed.

2. Materials and methods

2.1 Materials

Xujiayao site (40°06′028″N; 113°58′414″E; c. 970 m above sea level) is an early Late Pleistocene open-air site with abundant artifacts, *Homo* and animal remains, as well as the first identified horse-kill site in northern China. This site was discovered in 1974, and has since been excavated on more than five occasions. It consists of two localities—Locality 74113 and Locality 74093. Most of the specimens were uncovered from Locality 74093, which is situated in lacustrine deposits and at a depth of 8–12 m below the present-day surface and forms the foundation of this study. According to the erosion surface and the difference between clay layers, the cultural sequence can be divided into two layers: an upper layer and a lower layer (Chen et al., 1982; Jia and Wei, 1976; Jia et al., 1979; Norton and Gao, 2008).

Thirty samples of fossil equid mandibular cheek (including those of *Equus przewalskii* and *Equus hemionus*) were selected randomly from the 94 mandibular M1s or M2s, that were retrieved relatively intact from the Xujiayao site. In this study, all *E. przewalskii* and *E. hemionus* teeth were integrated into one equid group because their morphology and ecological niche are similar (Feh et al., 2001). A. Burke's experimental results, which were established through the observation of 29 modern horses' teeth sections (Burke and Castanet, 1995), were selected as the controlled sample to contrast with the study the equids from Xujiayao.

2.2 Thin-section preparation

Before doing the cementum experiment, every important feature of these teeth was measured according to conventional measurement rules (Driesch, 1976), and pictures of the teeth were taken. The main procedure for making the sections is as follows:

Occlusal surfaces (well above the gumline) and root tips were sectioned off using a thick wire saw (EXAKT E300CP) in order to reduce the size of specimens. Then, the teeth were ultrasonically cleaned in water for one hour. After that, we put the teeth onto a DPX mounting media box which was filled with liquid DPX mounting media. The box was transferred to a vacuum container for approximately two hours or even longer at 0.1–0.2 bars until the samples were totally penetrated. Then, the box was transferred to a light curing instrument (EXAKT 530) and exposed in blue followed by yellow light for six hours each. Next, solidified teeth were carefully oriented and sectioned longitudinally (labio-lingually) by the thick wire saw (EXAKT E300CP) to approximately 150–200 μm. The thin sections were then ground and polished to the thickness of 40–80 μm using an EXAKT 400CS grinder-polisher. Finally, the sections were ultrasonically cleaned with water and mounted onto cover slips using DPX mounting media (Burke and Castanet, 1995; Greenfield et al., 2015; Hu et al., 2012).

2.3 Determination of season-of-death

We can estimate the age of death by adding the number of seasonal bands to the age at which the cementum begins to grow, and we can infer the season when the animal dies by observing the outermost band (Lieberman, 1994). A polarizing microscope was used to observe the sections because the cementum bands are relatively clearly visible under polarized light (Figure 1). We utilized the microscope to enlarge the sample from 50 to 100 times so that we could count the cementum bands and observe the outer bands. There were two steps in documenting the cementum. First, evaluate the preservation via comparison with modern horse's structure. Second, observe the outer increment band to explore the season-of-death distribution. The contrasting cementum growth of different seasons is as follows (Table 1).

3. Results

It could be seen that the visual aspect of the growth bands in the fossil teeth correspond to the modern samples (Figure 2), as well as the orientation of collagen fibers (Figure 3), which have shown that the growth structures observed in mineralized tissues of modern equid correspond to homologous structures observed in Asian fossil equid.

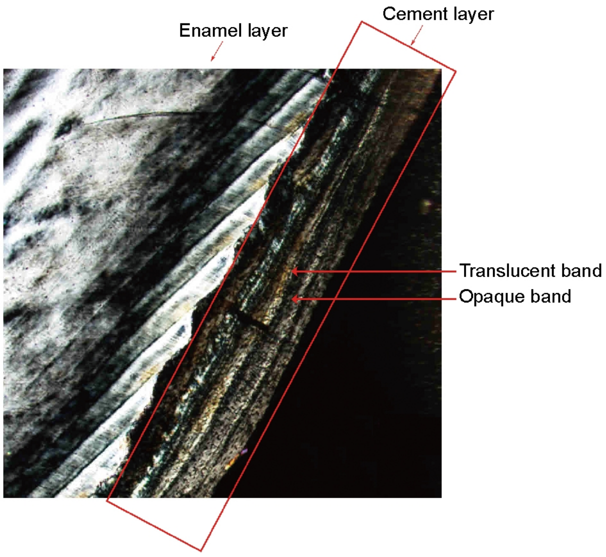

Figure 1 Translucent and opaque bands of equid cementum under a polarizing microscope.

The statistics recorded in the experimental process of cementum analysis as well as under the crown height model are presented above (Table 2). Crown height measurement is widely used to estimate the age of various hypsodont herbivores, and a significant correlation exists in equids between the height of the crown and the age of the individual. We relied on Philippe Fernandez's (Fernandez and Legendre, 2003) computing model which highlights the variations of every tooth and has different parameters for each tooth. We compare the results on age at death with the results on crown height measurements to examine the condition of preservation of the samples. Statistical tests, including the One-Sample Kolmogorov-Smirnov Test, a paired test, and Fisher's Exact test have been used to compare them and are run in SPSS Statistics 23, with results considered significant if $P \leq 0.05$.

Sample One defined by us consists of the age estimation by the crown height method, and the mean value of age estimation by cementum analysis composes Sample Two. It should be noted that we had excluded six specimens before we did the test because the growth bands of four teeth (No. C-4, No. C-5, No. C-24, No. C-28) were preserved poorly. The other two specimens (No. C-17, No. C-19) were eliminated as the ages from cementum analysis deviated from those of crown height to a very great degree (the deviations were almost more than 50%).

One-Sample Kolmogorov-Smirnov Test shows that the two significance levels are higher than 0.05, so the test distributions are both normal. From the paired test result, correlation between the paired samples is 0.642, which means that the paired samples are strongly correlated. Two-tailed probability is 0.052>0.05, which means the difference between the two samples is not marked. In conclusion, the ages which were inferred by cementum increment analysis are not significantly different from those calculated by the tooth height model, which means the equine cementum preserved well, and cementum analysis is relatively accurate at least in this experiment.

Combining the observations of the final growth marks, we obtain the seasonality results (Figure 4). The seasonal data obtained through this study show multi-seasonal use in both the upper and lower layers, although predominantly in the cold season. When we compared the seasonal data from different layers via Fisher's Exact test, the seasonal results have no significant difference (P=0.64).

Table 1 The cementum growth in different seasons[a)]

Season	Month	Cementum growth	Under microscope
Cold season	November	LAG forming intermittently	Opaque
	December–February	LAG formed	
	March–April	Intermittent growth zone forming	
Warm season	May–October	Growth band formed	Translucent

a) LAG=Lines of arrested growth. Burke and Castanet (1995).

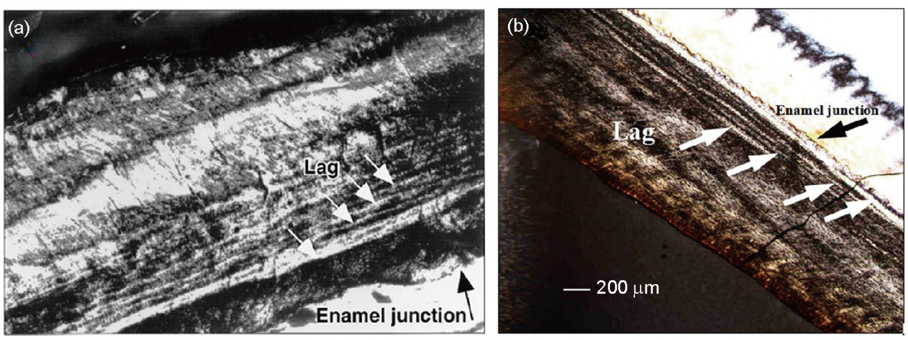

Figure 2 Growth marks in dental cementum of equids. (a) *Equus caballus* (Burke and Castanet, 1995); (b) *Equus hemionus* from Xujiayao site–Specimen No.C-2.

Table 2 The results of the cementum incremental analysis and the tooth-height technique[a)]

Specimen number	Age by tooth height model (years)	Number of cementum bands	Age range by cementum analysis (years)	Mean age (years)	Season of death	Location of specimen	Species
C-1	12.41	6	6.67–8	7.34	Cold	L	*E. przewalskii*
C-2	8.97	8	8.67–10	9.34	No info	L	*E. hemionus*
C-3	11.8	9	9.67–11	10.34	No info	L	*E. przewalskii*
C-4	7.78	Not clear	No info	No Info	No info	L	*E. przewalskii*
C-5	9.63	Not clear	No info	No Info	No info	L	*E. przewalskii*
C-6	9.75	7	7.67–9	8.34	Cold	L	*E. przewalskii*
C-7	3.07	4	4.67–6	5.34	No info	L	*E. przewalskii*
C-8	11.94	10	10.67–12	11.34	Cold	L	*E. przewalskii*
C-9	10.64	10	10.67–12	11.34	Warm	L	*E. przewalskii*
C-10	5.67	7	7.67–9	8.34	Cold	L	*E. przewalskii*
C-11	7.74	6	6.67–8	7.34	Cold	L	*E. hemionus*
C-12	9.16	More than 8	8.67–	8.67	Cold	L	*E. przewalskii*
C-13	10.05	9	9.67–11	10.34	Cold	L	*E. hemionus*
C-14	10.54	7	7.67–9	8.34	Warm	L	*E. hemionus*
C-15	10.03	5	5.67–7	6.34	Cold	L	*E. przewalskii*
C-16	10.68	6	6.67–8	7.34	Cold	U	*E. hemionus*
C-17	12.19	5	5.67–7	6.43	No info	U	*E. przewalskii*
C-18	11.45	11	11.67–13	12.34	Cold	U	*E. przewalskii*
C-19	8.54	3	3.67–5	4.34	Warm	U	*E. hemionus*
C-20	6.83	More than 5	5.67–	5.67	Warm	U	*E. przewalskii*
C-21	11.49	8	8.67–10	9.67	Warm	U	*E. hemionus*
C-22	12.41	8	8.67–10	9.34	Cold	U	*E. przewalskii*
C-23	8.53	6	6.67–8	7.34	Warm	U	*E. przewalskii*
C-24	14.35	More than 4	4.67–	4.67	Cold	U	*E. przewalskii*
C-25	5.21	5	5.67–7	6.34	Warm	U	*E. hemionus*
C-26	8.1	6–8	6.67–10	8.34	Cold	U	?
C-27	9.23	7	7.67–9	8.34	Cold	U	*E. przewalskii*
C-28	5.64	Not clear	No info	No info	None	U	*E. przewalskii*
C-29	7.94	7	7.67–9	8.34	Cold	U	*E. przewalskii*
C-30	8.97	7	7.67–9	8.34	Cold	U	*E. przewalskii*

a) As cementum bands form after the eruption of teeth (Burke and Castanet, 1995), the duration from birth to the eruption should be considered when it comes to the calculation of age. As m1/m2 erupts after 6 to 24 months (Fernandez and Legendre, 2003), 0.67 to 2 years should be added to the age calculated by counting the number of cementum bands. L=lower layer, U=upper layer. "?", this tooth sample cannot be identified

4. Discussion and conclusions

According to the seasonal results, the distributions of season-at-death in both upper and lower layers are similar, showing that equids were hunted throughout the year but predominantly in the cold season. In the deposits at Schöningen 13II-4, equids died in both the cold season and wet season (Rivals et al., 2015). During the Pleniglacial period, equids died in both cold and warm season sites as well (Burke and Castanet, 1995). The seasonality results for equid hunting at Xujiayao may indicate extended, multi-seasonal use of the Xujiayao site in northern China.

Cold season dominance of equid hunting at Xujiayao may reveal that early hominins probably faced levels of seasonal food stress comparable to the levels faced by contemporary Hadza and San hunter-gatherers in Africa. During the late dry season and early rainy season, these modern hunter-gatherers underwent significant loss of body weight. The food pressure leads the San to concentrate on high protein feedback animals (Speth, 1987). Hadza always obtain more ungulates in the dry season than in the wet season, because their blind hunting near water holes brings more prey animals in the dry season and because tracking animals is easier then (Bunn, 1993, 2001; O'Brien, 1994). Moreover, there will likely be more carcasses for scavenging during the more stressful dry season (Blumenschine et al., 1987).

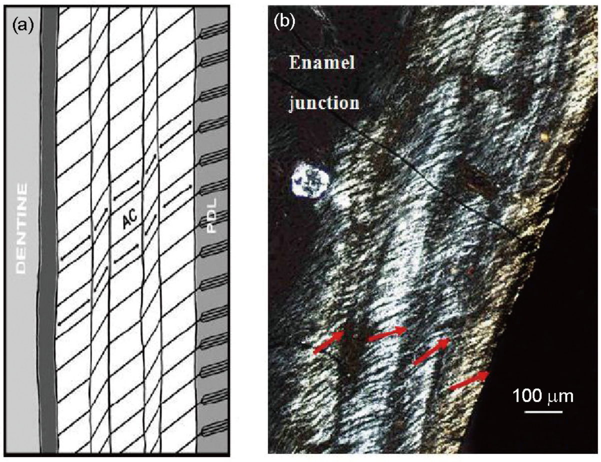

Figure 3 The angle of collagen fibers of cementuma (a). C=Cementum; PDL=Periodontal Ligament (Stutz, 2002). (b) Xujiayao site–Specimen No.C-25. Arrows point to the angle of collage fiber mineralization in response to seasonal changes in food toughness.

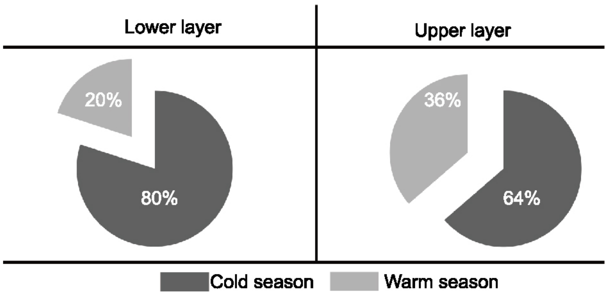

Figure 4 Results of season of death.

Though the sample size in this study is relatively small and poor preservation of six samples makes it even smaller, we can preliminarily conclude that estimated ages on cementum bands are relatively correlated with those based on the crown height model by the paired test. Therefore, this experiment proves that the information of season of death is readable in many of the teeth. That means the technique is a potential powerful tool for us to explore the prehistoric seasonality of equid utilization at Pleistocene sites. And the sections from wild Asian horses should contribute to the analysis of perissodactyls in the future by providing more comparative specimens. A large number of isolated and fragmentary teeth, which are hard to study by traditional methods (e.g., tooth eruption and wear stages) and have often been ignored by archaeologists, might be used via this approach to recover useful information in the future.

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