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Distribution patterns of elements in dental enamel of *G. blacki*: a preliminary dietary investigation using SRXRF

Yating Qu · Changzhu Jin · Yingqi Zhang · Yaowu Hu · Xue Shang · Changsui Wang

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Abstract We measured the elemental mappings in dental enamel of *Gigantopithecus blacki* ($n = 3$) using synchrotron radiation X-ray fluorescence (SRXRF) to understand the dietary variation during the time of tooth eruption. In order to account for the effects of diagenesis on the variation of elements in these fossil teeth, we compared the Fe and Mn elemental distribution and levels in dental enamel of *G. blacki* with that of a single modern pig tooth and found no differences. The observation of the variations of Sr, Ca and RE (rare earth elements) distribution in the incremental lines reveals that the plant foods utilized by *G. blacki* from the early Pleistocene or the middle Pleistocene had varied during the formation of dental enamel, possibly caused by the change of living environment or food resources. The variations of elemental distribution in different incremental lines are very promising to understand the nutritional and physical stress of *G. blacki* during the tooth eruption and environmental adaptations.

Y. Qu · Y. Hu (✉) · X. Shang · C. Wang
Lab of Human Evolution, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China
e-mail: ywhu@gucas.ac.cn

Y. Qu · Y. Hu · X. Shang · C. Wang
Department of Scientific History and Archaeometry, School of Humanities, University of Chinese Academy of Sciences, Beijing 100049, China

C. Jin · Y. Zhang
Key Laboratory of Evolutionary Systematics of Vertebrates, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China

1 Introduction

Gigantopithecus is an extinct giant primate and since only teeth and jaw bones have been recovered, the phylogenetic classification and evolution of *Gigantopithecus* remains controversial. One particular species of *Gigantopithecus* is *G. blacki*, and more research is needed to better understand its own evolutionary history as well as its evolutionary relationship with contemporary humans [1]. The main cave localities where the fossils of *G. blacki* were discovered include Longgupo, Jianshi, Bijie, Liucheng, Bama, Wuming, Daxin, Mohui, and Chongzuo in southern China, and Tham Khuyen in northern Vietnam [2]. It is believed that *G. blacki* lived until the early to middle Pleistocene, but recent discoveries in the Mulan Mountains (unpublished data) near Chongzuo City, Guangxi Provence, China suggests that *G. blacki* survived until the late Pleistocene, and was contemporary to the archaic modern humans also found there [3]. A greater understanding of the *G. blacki* diet and how this may have varied due to changing environmental conditions is critical to deciphering how and why *G. blacki* went extinct, and if it was in competition for dietary resources with early modern humans.

Giant teeth are the most frequent remains found of *G. blacki*. It is generally acknowledged that incremental lines in enamel, including the daily cross striations and the striae of Retzius (SR), are the markers of the individual's growth [4]. The SR in the inner enamel represent longer time periods, which vary in species. The periodicity of SR is determined from counts and measurements of daily cross striations between adjacent striae [5]. Dean and Schrenk found that the cross striation spacing of *G. blacki* was between 3.8–6 μm, corresponding to a periodicity of 11 days [6], which is longer than other hominoids, such as *Australopithecus afarensis*, *Australopithecus africanus*, *Paranthropus*

Table 1 Samples information sorted by location, age and tooth type

Lab number	Sample number	Sites	Age (Ka)	Scanned areas (mm^2)	Type of teeth
G1	GCSD0410-245	Sanhe Cave	1200–1600	1.45	premolar
G2	GCSD0410-246	Sanhe Cave	1200–1600	1.28	premolar
C1	CLMH0904-091	Hejiang Cave	310	0.65	molar
P1		Shaanxi province	Modern	1.03	molar

robustus and *Homo neanderthalensis*, but within the ranges of the modern humans and *Pongo pygmaeus* [7–9]. Since the periodicity of enamel formation is correlated to growth rate, understanding the process of enamel biomineralization of *G. blacki* can reflect nutritional and physical stress during tooth formation.

Investigation of the elemental distribution in bones and teeth from archaeological sites can provide important information (health, diet, diagenesis, growth patterns, etc.) about humans and animals, and various analytical techniques have been used such as: nuclear microprobe [10], Particle Induced X-ray Emission (PIXE) and Particle Induced Gamma-ray Emission (PIGE) [11], Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [12], and Synchrotron Radiation X-ray Fluorescence (SRXRF) [13]. Of these techniques, the SRXRF has the most potential in archaeological research, since it is non-destructive and provides high quality elemental distribution maps that can be related to growth stress, diagenesis or pollution in an individual specimen [13–15].

The focus of the study presented here is the use of SRXRF to determine the elemental distribution in dental enamel of three *G. blacki* individuals and a single modern pig (for comparison) to explore the elemental variations along the incremental lines of dental enamel, aiming to understand the nutritional and physical stress of *G. blacki* during tooth eruption and environmental adaptations.

2 Materials and methods

2.1 Materials

A lot of fossil teeth of *G. blacki* were excavated from Sanhe Cave and Hejiang Cave, located in the north-east part of Chongzuo City in Guangxi, south China. Based on biostratigraphic records and geomagnetic analysis, 56 fossils from Sanhe Cave were dated to the early Pleistocene, about 1200 to 1600 Ka [16, 17], while fossils from Hejiang Cave were dated to the middle Pleistocene, about 310 Ka (unpublished data).

Two tooth samples from Sanhe Cave and one from Hejiang Cave were selected for SRXRF mapping. In addition, to investigate the diagenetic effect of the burial environment on the elemental composition of *G. blacki* dental enamel, one modern pig tooth from Shaanxi province China was collected for comparison. In total, four samples, including three *G. blacki* teeth and one pig tooth, were analyzed by SRXRF (Table 1).

2.2 Sample preparation

In order to limit the amount of physical destruction of these precious fossil samples, SRXRF was only applied to previous broken or cracked sections of dental enamel. The areas that were scanned include: G1—flank of dental crown, G2—occlusal surface, C1—bottom of dental crown, and P1—cusp. Teeth were first cleaned in an ultrasonic bath (distilled water) for 10 minutes to remove contaminants. Next the broken areas of dental enamel were polished by emery paper to make the surface plane smooth. Finally, the samples were again washed in distilled water, and dried at room temperature.

2.3 Elemental mapping by synchrotron radiation X-ray fluorescence analysis

The SRXRF mapping was performed in the workstation of beamline 15U1 at the Shanghai Synchrotron Radiation Facility (SSRF), China. The monochromator was Si (111) and the monochromatic photons were 18 keV. The fluorescence spectrum was recorded with a single element silicon detector (Vortex-EX, made by SII-Nano Technology, USA). The distance from sample to detector was approximately 4 cm. The exact beam size was 5×20 μm (5 μm in the x-axis, 20 μm in z-axis) and the step was 0.02 mm. The sampling time for each point was 7.2 seconds, and the mapping was obtained with a matrix size of 512×512 pixels.

Elemental distribution was obtained from the plane scan analysis along the dental enamel from the surface enamel to the enamel dentine junction (EDJ). Selected areas of the dental enamel of each sample are expressed through bidimensional (x, y) scanning. The surface enamel to the EDJ stands for the x-axis from zero to the positive in samples G1, C1 and P1, but stands for the y-axis from zero to the positive in sample G2. The axes represent the number of the points analyzed. The chemical elements detected by SRXRF are determined by the energy, such as Sr, Ca, Zn, Fe, Mn, Br, As, Pb, Cl, and RE (Rare Earth Elements). The different color of every element indicates the variability of the elemental composition. The elemental mapping is displayed in the Figs. 1–4.

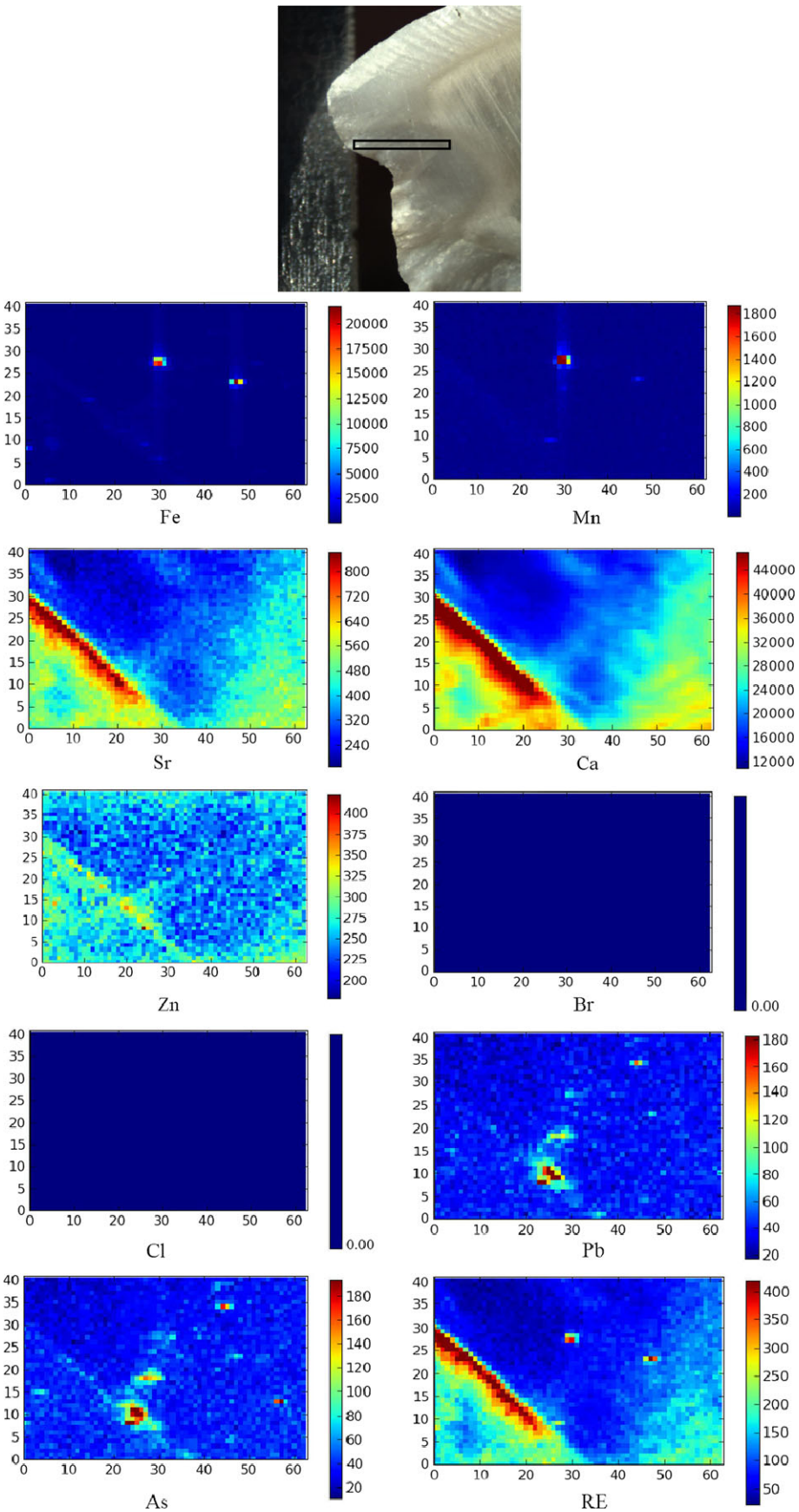

Fig. 1 The elemental mapping in dental enamel of P1

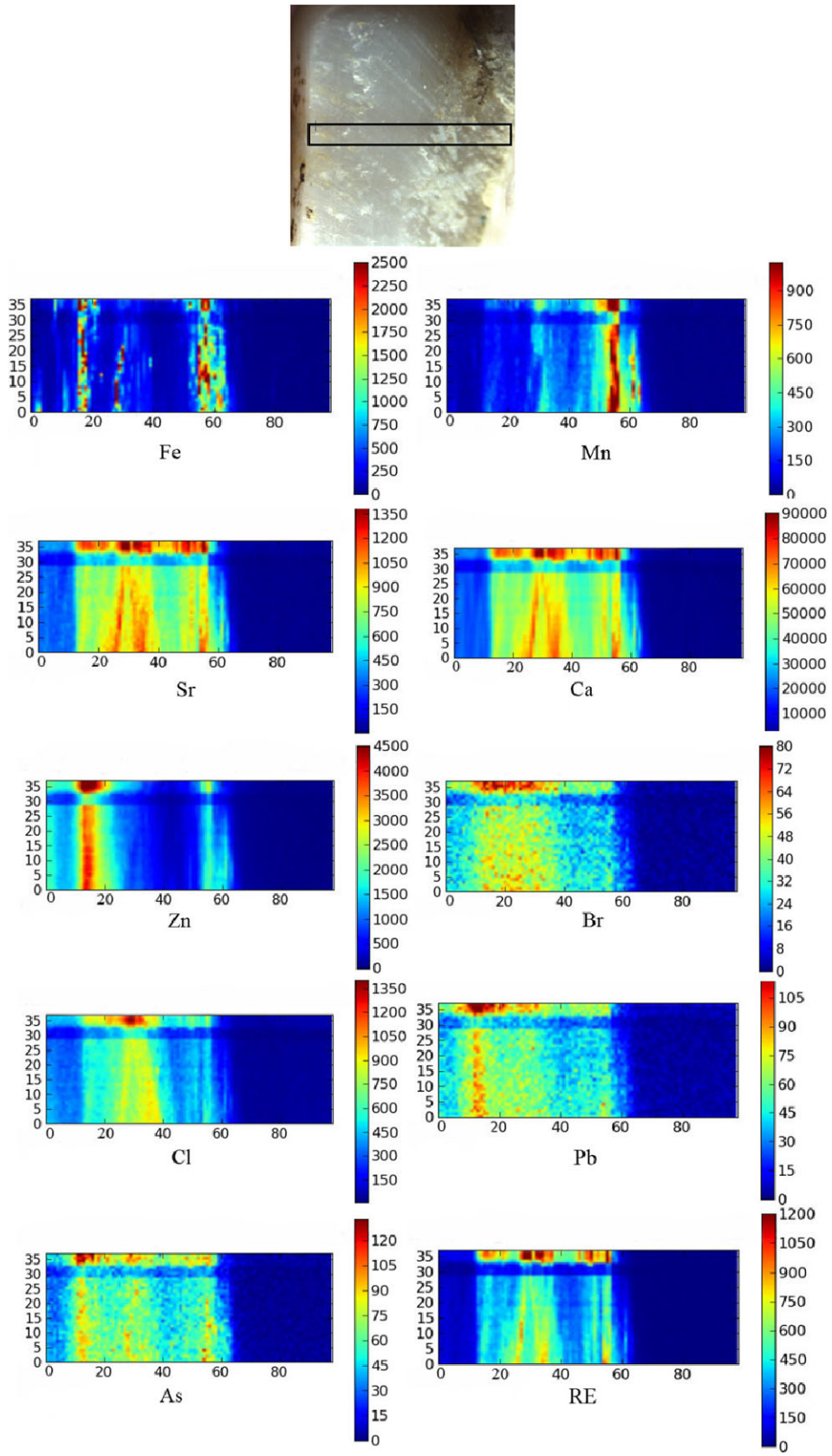

Fig. 2 The elemental mapping in dental enamel of G1

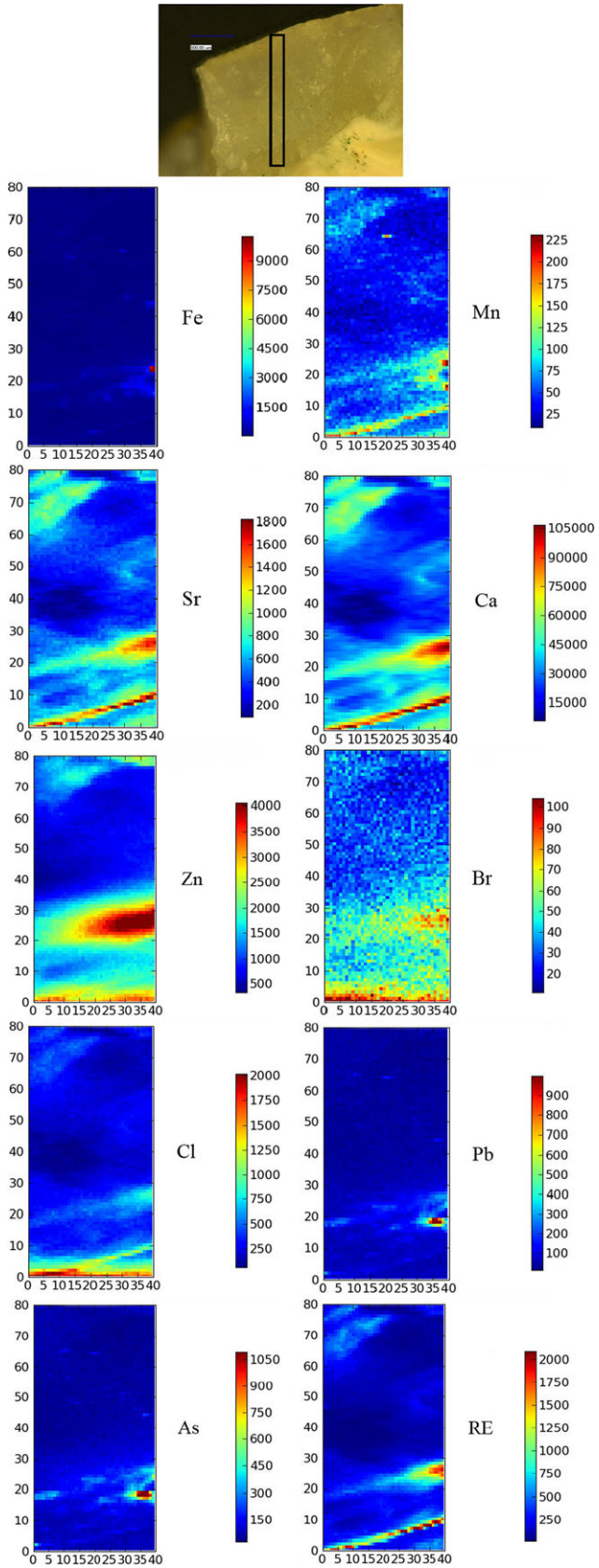

Fig. 3 The elemental mapping in dental enamel of G2 (Scale bar, 500 μm)

3 Results and discussions

3.1 The elemental distribution in the dental enamel of a modern pig tooth

Diagenetic effects on bones or teeth buried in sediments will cause an enrichment or deletion of elements within them [18]. In particular, the elements Fe, Al, Mn and Cu can be enriched due to contamination from sediments [15, 19, 20]. In order to better understand the effects of diagenesis on the variation of elements in fossil teeth detected by SRXRF, a tooth from modern pig is used for comparison in Fig. 1.

Generally, the contents of Fe, Al, Mn and Cu in bones or teeth can be enriched due to the contamination from the sediments nearby [15, 19, 20]. Figure 1 shows no correlation between Fe and Mn, which suggests that these elements are biogenetic not diagenetic. This finding provides important information that can be used to understand the types of contamination that could be found in the fossil dental enamel of *G. blacki*.

The composition of dental enamel is 97 % hydroxylapatite [$Ca_{10}(PO_4)_6(OH)_2$], and Ca is one of the most important elements [21, 22]. Strontium (Sr) has the ability to substitute for Ca since they have similar chemical properties, and according to the principle, "You are what you eat" [23], the concentration of Sr or barium (Ba) in dental enamel will correspond to the foods consumed by an organism. In general, a diet high in plants will correlate to higher concentrations of Sr or Ba in the dental enamel hydroxylapatite [24]. However, the concentration of Sr or Ba can also be influenced by plants with different Ca contents so care must be used in interpretation of the results [25–27]. In addition to dietary studies, the concentration of rare earth elements in dental enamel can also be used to determine migration patterns and the environment in which an organism lived [28].

Since SRXRF is unable to detect Ba, Sr has become the main element to examine dietary variation during tooth formation. In Fig. 1, the variations of Ca, Sr and RE show quite similar trends. Since the mapping area of the dental enamel extends from the surface to the EDJ, this variation in the above elements is likely the result of natural processes during the biomineralization and not the result of diagenetic effects. The increasing Sr content can be interpreted that more Sr-enriched foods were consumed during tooth formation. This finding suggests that more plants or more Ca enriched plants were included in the pig's diet. The enhanced RE indicates that the living environment of the pig had changed or the sources of its food had varied. In contrast to the Ca, Sr and RE results, the other elements analyzed in Fig. 1 do not show distinct patterns that can be related to diet and will not be discussed further.

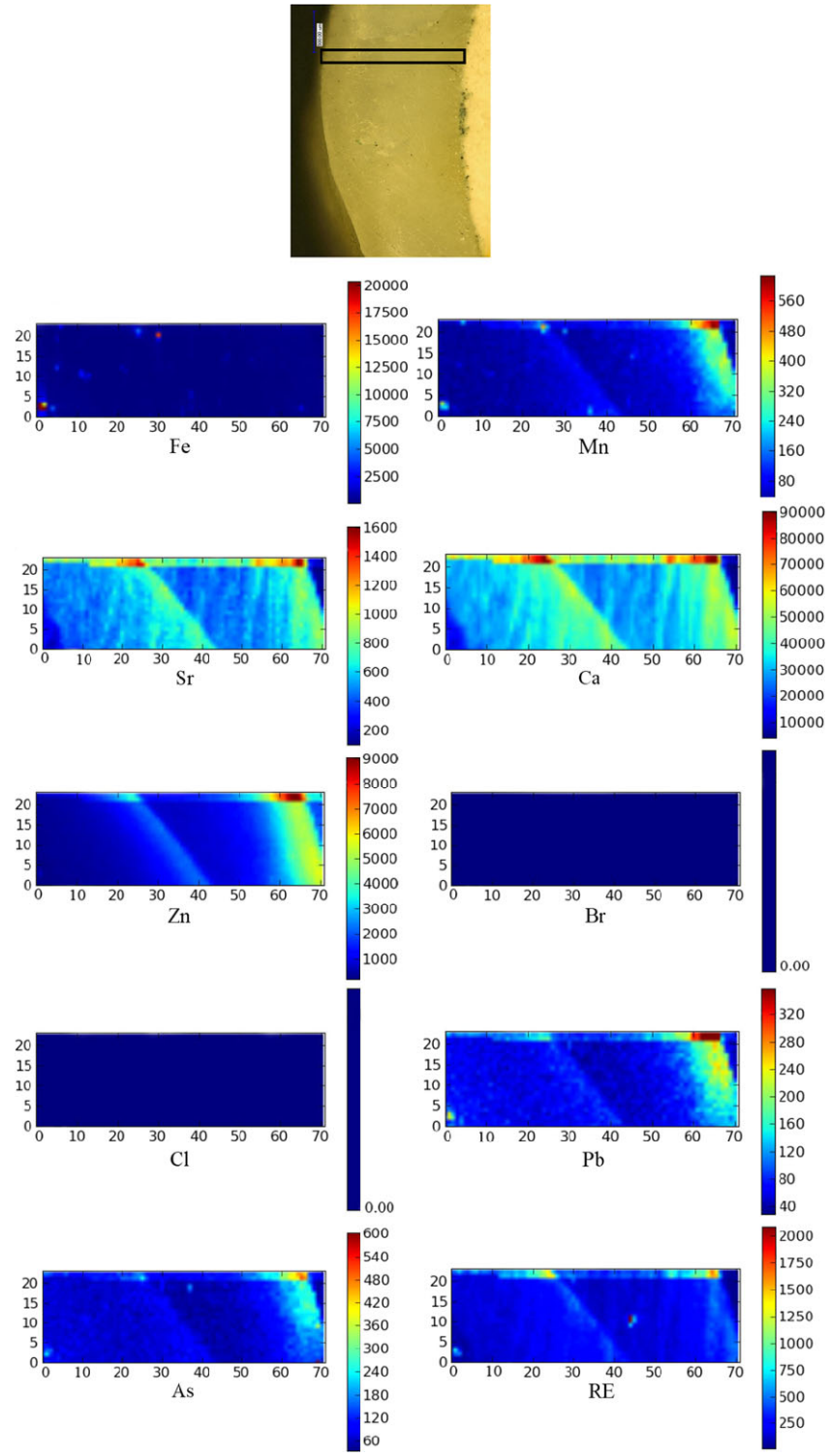

Fig. 4 The elemental mapping in dental enamel of C1 (Scale bar, 500 μm)

3.2 The elemental distributions in the dental enamel of *G. blacki*

In order to verify if the dental enamel of the three *G. blacki* specimens was diagenetically altered, the correlation between the elements Fe and Mn must be determined [20]. In Figs. 2–4, no Fe or Mn correlation is observed and this indicates that these teeth have not experienced alteration by diagenesis even though they are aged fossils.

It has been stated that the variations of the elements in dental enamel from the surface to the EDJ must be caused by the change of living environment or food re-

sources during the tooth information. Base on the periodicity of SR, we can discuss the periodically dietary changes of *G. blacki*, even the daily changes, which is very essential for understanding the nutritional and physical stress of *G. blacki*.

The enamel formation times and the number of SR for *G. blacki* were calculated by the relative enamel thickness, the spacing of daily cross striations (the spacing is from 3.8 μm to 6 μm with the mean of 4.9 μm) [6], and the periodicity of SR (the periodicity was 11 days). For example, the relatively scanned enamel thickness of G1 is about 1.96 mm (step 0.02 mm × 98 points = 1.96 mm), so the enamel formation times is ≈400 days (1.96 mm × 1000/4.9 μm = 400), and the number of SR is 36 (400 days/11 days = 36). According to the above formula, the scanned enamel formation times of G2 and C1 are ≈326 days and 289 days, respectively, and the number of their SR is 29 and 26, respectively.

In Fig. 2, in the sections of the enamel near the surface and near the EDJ, the contents and distributions of Sr, Ca and RE are symmetrical, while the contents of Sr, Ca and RE are increased in the inner part of the dental enamel. In Figs. 3 and 4, there are also clear patterns of Sr, Ca and RE contents in the inner part of enamel. The increased Sr and Ca content suggests that the individual consumed more plants or more Ca enriched plants, and the increased RE indicates that the living environment of the individual changed or the sources of its food had varied during these time spans of dental formation. In contrast to the Ca, Sr and RE results, the other elements analyzed in Figs. 2–4 do not show unique patterns that can be related to diet and will not be discussed further.

Previous studies about the diet of *G. blacki*, have been based on the analysis of dental morphology [6, 29, 30], bone chemistry [31], phytoliths on the surface of enamel [32], and dental pathology [33], and suggest that *G. blacki* subsisted on tough or fibrous foods, including bamboo, grasses, fruits and seeds. In general, the leaves of plants are rich in calcium, especially the calcicole (Lamiaceae, Rosaceae, Rubiaceae, Oleaceae, etc.) [34–36]. Hence, the observed variations of Sr and Ca in the incremental lines imply that *G. blacki* consumed more Ca enriched plants or more leaves during this period. This finding suggests that the plant foods utilized by *G. blacki* from the early Pleistocene or the middle Pleistocene varied over the course of tooth eruption, and this is possibly linked to environmental changes or a variation in food resource use. Our findings indicate that the variations in elemental distribution in dental enamel of *G. blacki* likely indicate correlations with dietary changes during the tooth eruption, reflecting the nutritional and physical stress during growth.

4 Conclusion

Research focused on the diet of *G. blacki* is a key for possibly understanding the causes of its extinction. The technique of SRXRF permits a new angle for us to understand their dietary changes during the tooth growth. Based on the comparison with the elemental distribution patterns of a modern pig, the variations of Sr, Ca and RE in the incremental lines of *G. blacki* dental enamel reveal that the plant foods utilized by *G. blacki* varied during the tooth eruption, possibly caused by a change of living environment or the variations of the food resources. However, it is important to stress that these results are preliminary and limited by small numbers of samples and a single modern reference. In the near future, we expect to collect and analyze more samples and references, including teeth from *G. blacki*, contemporary primates, modern primates and archaic humans, to further shed light on the possible dietary variation and competition among them. Additionally, we will use fluorescence microtomography with synchrotron radiation (SR-XFCMT) to explore the elemental distributions in the entire dental enamel to better understand dietary changes during tooth eruption in more detail.

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