Investigating the micro-scale structures of macro-scale dinosaur bones using synchrotron radiation-based X-ray micro-tomography

Introduction

Understanding the biology of dinosaurs is facilitated by assessing the histological microstructures (HMSs) of fossilized bones. Synchrotron radiation-based X-ray micro-tomography (SXMT) was employed at SPring-8 **BL28B2** to visualize the HMSs of dinosaur bones through tomographic images [1].

Analyzing dinosaur bones

HMSs provide numerous insights into the growth rate, age, and physiology of vertebrate bones [2]. For example, a growing bone contains numerous vascular canals (less than 1 mm in diameter) that provide nutrients to the bone. A young bone also exhibits secondary osteons as sites for the deposition of new bone materials. Additionally, a long bone accumulates the lines called the lines of arrested growth (LAGs). Each LAG is much less than 0.1 mm in width and is deposited each year in most dinosaurs with irregular growth rates (Fig. 1). What is important about these HMSs is that they may be preserved in fossil bones. Therefore, they can be used to investigate the growth rate, age, and physiology of fossilized and extinct animals, including dinosaurs.

However, although the usefulness of HMSs in dinosaur research is unquestionable, there are limitations. In the traditional analytical technique to observe HMSs, samples must be cut and ground into thin sections (typically 0.03 mm thick) to be analyzed under a petrological microscope. Thus, this technique damages the samples and is difficult to apply to the specimens under conservation. This is where SXMT becomes very useful.

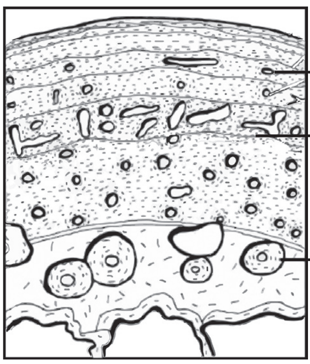

Fig. 1. Schematic of HMSs observed in a dinosaur bone (modified from Ref. 2).

Studying dinosaur growth at SPring-8

Using SXMT, HMSs in fossil bones can be visualized without damaging them. In the SXMT technique, an X-ray beam with a large flux and high coherence can penetrate dense and large fossilized bones and highlight subtle compositional differences in various HMSs. Previously, the capacity and advantages of SXMT at SPring-8 have not been thoroughly addressed. Therefore, this technique was applied to dinosaur limb bones to demonstrate its effectiveness.

In the study by Imai *et al.* two femora (thigh bones) of *Fukuiraptor* were used as samples. *Fukuiraptor* is a carnivorous dinosaur found in Fukui, Japan (Fig. 2) that lived approximately 120 million years ago, and the largest individual measured approximately 4.5 m in length [3]. Fossil bones assignable to *Fukuiraptor* are designated as natural treasures, making it difficult to conduct HMS studies using the traditional (destructive) technique. These dinosaur fossils are good candidates for SXMT. The BL28B2 was selected for the experiments because it has a large available field of view (~3 cm) and X-ray energy (~200 keV), which are ideal for large and dense dinosaur bones. The femora were approximately 20 cm long and 3 cm wide at their shafts. Special permission was granted to cut and grind these samples to compare the results of the traditional technique with those of SXMT.

For each experiment, approximately 20 tomographic images of the femoral shaft were acquired in each experiment. Figure 3 shows a comparison of the images obtained using traditional and SXMT techniques. Thin-section images obtained using the traditional technique exhibited vascular canals, numerous secondary osteons, and LAGs. Tomographic images captured using SXMT showed identical HMSs. On tomographic images, extensive distribution of vascular canals and many secondary osteons indicated that the femur was actively growing. Additionally, there were in total of four LAGs in the femur, suggesting that it belonged to a *Fukuiraptor* individual that was four years old at the time of death. Therefore, this study argues that at the age of four, *Fukuiraptor* was immature and its bones were still growing. These observations and arguments demonstrate the effectiveness of SXMT in HMS analyses of dinosaur bones.

Applications of SXMT to HMSs in fossil bones have only recently begun at SPring-8. The experiments were successful in that HMSs in *Fukuiraptor* femora were visualized without damaging the samples [1].

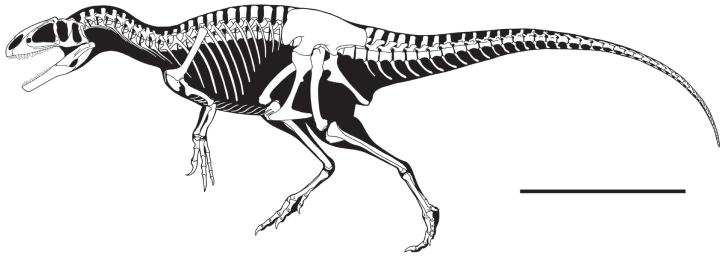

Fig. 2. Skeletal reconstruction of *Fukuiraptor kitadaniensis*. Scale bar = 1 m. Artwork by G. Masukawa.

The potential of this technique is not limited to evaluating dinosaur growth. For example, the pathways of the blood vessels within fossil bones that supply nutrients to particular tissues can be traced. Additionally, SXMT enables large-scale HMS analysis of multiple samples, in which numerous histological sections of numerous bones can be obtained. However, it is hoped that more researchers and students will apply this technique to their specimens to expand its potential. Numerous dinosaur species and types of HMS studies can be used to increase our understanding of these peculiar animals.

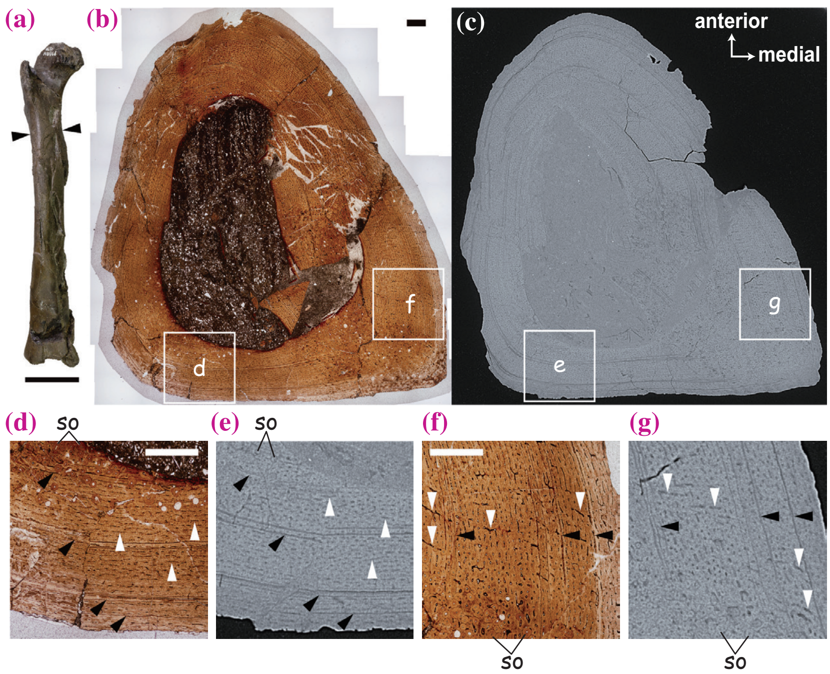

Fig. 3. Exterior **(a)**, transverse thin sections **(b, d, f)** and tomographic images **(c, e, g)** of the shaft of a *Fukuivenator* femur. Arrowheads in (a) indicate the level at which the thin section and tomographic images was obtained. Rectangles in (b) and (c) indicate areas magnified in d–g. Black arrowheads in (d–g) indicate LAGs. White arrowheads in (d), (e), (f), and (g) indicate vascular canals. Thin-section images and corresponding tomographic images are in the same scales. Abbreviations: so, secondary osteon. Scale bars = 20 mm for (a) and 1 mm for (b), (d), and (f).

Takuya Imai

Institute of Dinosaur Research, Fukui Prefectural University

Email: ti.star.gtw@gmail.com

References

[1] *T. Imai, S. Hattori, M. Hoshino and K. Uesugi: J. Synchrotron. Rad.* ***30*** *(2023) 627.*
[2] A. M. Bailleul *et al.*: PeerJ. **7** (2019) e7764.
[3] Y. Azuma and P. J. Currie: Can. J. Earth Sci. **37** (2000) 1735.