Undulating Vertical Prism Decussation of *Pyrotherium* (Pyroteria, Mammalia) Molar

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**Abstract**

Most mammalian teeth are covered in enamel and its external tooth shape is determined by the phylogenetic background and the mode of mastication and occlusion. Characteristic microstructure also exists in the internal structure of enamel, and the characteristics of enamel prism and Hunter-Schreger bands (HSB) are well studied. *Pyrotherium* (Pyroteria) is an extinct Tertiary period mammal of South America, with unknown ecology and phylogeny. In previous study, we observed a dendritic streak with a thick, wavy trunk extending in the direction of the tooth axis. This is classified as a vertical HSB with some similarity to rhinoceros molar. We demonstrate a complex structure of the vertical HSB of *Pyrotherium* molar by using three-dimensional imaging of prisms based on serial SEM images. And also, we report the shape of the enamel prisms of *Pyrotherium* molar is keyhole-shaped with a head and tail, which is a predominantly found in humans and other animals, but in some parts it is similar to the ginkgo-shape found in proboscideans.

**Keywords:**
- enamel prism,
- Hunter-Schreger bands,
- *Pyrotherium*,
- 3D reconstruction

**Introduction**

The shape of enamel prisms and Hunter-Schreger bands (HSB) varies among the mammalian taxa. HSB in the enamel structure of the cheek teeth (i.e., molars and premolars) can be used to distinguish between the orders of mammals\(^1,2\), and recent studies have classified various HSB\(^3–5\). This study used the molars of *Pyrotherium* as experimental material. *Pyrotherium* was a large ungulate with an estimated body weight of 3.5 tons and a cranium length of more than 0.8 m that lived in the Tertiary Oligocene of South America\(^6–8\). Cheek teeth are bilophodont with two transverse lophs, and also interested in mastication mode\(^6,9\) (Fig. 1). Its phylogeny and ecology remains unclear due to the scarcity of remains discovered. Our previous study found that the HSB of *Pyrotherium* cheek teeth run vertically\(^4\), whereas horizontal HSB are predominant in mammals. In this study, we used reflected light microscopy, scanning electron microscopy (SEM), to closely investigate prism morphology (SEM) and three-dimensional imaging of the HSB based on serial SEM images.

**Materials and Methods**

**Materials**

Molar fragments of *Pyrotherium* were obtained from molar segments (MDH-no. 01998) received from the Department of Histology (Anatomy II), Nihon University School of Dentistry at Matsudo, Japan.

Some fragments were embedded in polyethylene resin and sectioned tangentially (parallel to the dentinoenamel junction (DEJ) or outer enamel surface (OES)), longitudinally (radial to the tooth axis), and horizontally (perpendicular to the tooth axis). After polishing and slight etching with 0.05 N HCl, the specimens were sputter-coated with Au-Pd.

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Light microscopy analysis

The specimens were analyzed under a reflected light microscope (SZ; Olympus Corp., Tokyo, Japan). The etching of the polished enamel surface provides a delicate morphology. When the light source is moved from one side to the other, the appearance of the surface changes based on the orientation and angle of the enamel prisms.

Scanning electron microscopy analysis

To investigate the microstructure of the molar enamel, we examined the polished sections with SEM (S2700 and S3400N; Hitachi Ltd., Tokyo, Japan), using conventional methods.

Three-dimensional reconstructions

To restore the three-dimensional course and profile of the enamel prisms, the sections were gradually ground down in 10-μm steps, tangentially to the DEJ, up to a depth of 50μm for SEM. We etched location marks on the enamel surface to aid image superimposition using an Er:YAG laser (Erwin Advel; Morita Corporation, Tokyo, Japan). The serial SEM images were adjusted using Photoshop Element functions (Adobe Systems Incorporated, San Jose, CA, USA). The outlines of ten adjacent prisms, corresponding to serial images, were traced and digitized via a graphics tablet (Intuos 3; Wacom, Saitama, Japan). Three-dimensional reconstructions were created with the “OZ” image rendering software (Rise Corporation, Sendai, Japan). In these reconstructions, each prism was rendered in a different color and could be viewed from any perspective.

Results

We found that the thickness of *Pyrotherium* molar enamel varied between the mesial and distal margins of the lophs (Fig. 1). The thin margin of the enamel formed comb-like fine ridges (Pl. 1–1), which were continuous with the lateral surface of the molars. We observed loose wavy extension on the lateral surface along the tooth axis (Pl. 1–2). The thick margin of enamel had advanced attrition with a flat occlusal surface. The striae, which appeared slightly uneven, were seen from the DEJ to about 2/3 of the layers (Pl. 1–3). Three of the specimen surfaces (tangential, horizontal, longitudinal section) were simultaneously polished and observed, and we found particularly irregular dendritic streaks along the tooth axis in the tangential section (Fig. 2). Irregular striae were observed in both horizontal and longitudinal sections. In the horizontal section, several thick streaks extended from the DEJ, and the streaks became indistinct on around 1/3 of the enamel surface. However, multiple thin striae extended laterally in the deep layer near the DEJ in some parts (Pl. 1–4). In the longitudinal section, we observed both broad striae and numerous fine striae extending laterally (Pl. 1–5). When we observed the tangential cross-section using reflected light microscope, streaks of irregular widths were aligned loosely along the tooth axis, but the boundary was not clear in some parts. There was also a difference in the density of the streaks and the thick streaks were connected by thin and thread-like side-branches (Pl. 1–6).

When we observed the tangential section at low magnification with a scanning electron microscope, the streaks showed the same intensity as observed with reflected light microscope (Pl. 2–1). Enlarging the region of the clear boundary, we found the cross-sectional shape of the enamel prisms were different across the boundary (Pl. 2–2). There was enamel prism meandering, even in the wide streak regions (Pl. 2–3). We found different...
Plate 1
1. Thin margin of the enamel. Note the comb-like ridges. D; dentin. Scale bar = 1 mm.
2. Lateral surface of the molar. Note the wavy extension along the tooth axis. Scale bar = 1 µm.
3. Thick margin of enamel. Note the internal enamel structure. D; dentin. Scale bar = 1 mm.
4. Horizontal section observed by reflected light microscope. D; dentin. Scale bar = 500 µm.
5. Longitudinal section observed by reflected light microscope. Extending numerous fine striae. D; dentin. Scale bar = 500 µm.
6. Tangential section observed by reflected light microscope. Streaks of irregular width were lined up loosely along the tooth axis. Threadlike side branches connected the thick streaks. Scale bar = 500 µm.
rows of enamel prisms with different cross-sectional shapes, forming indistinct boundaries (Pl. 2–4). Groups of prisms of different directions confirmed the clear border in the horizontal section (Pl. 2–5). In the longitudinal section of the indistinct boundary area, there were some portions where the cross-sectional form of the enamel prisms gradually changed and some portions appeared to be fused (Pl. 2–6). In the tangential section, there were densely packed regions of enamel prisms with various orientations and deformed shapes (Pl. 3–1) and, in particular, rows of enamel prisms with a flattened morphology, which formed lines reminiscent of cracks (Pl. 3–2). Both in tangential sections (Pl. 3–3) and horizontal sections (Pl. 3–4), the cross section of the enamel prisms had a keyhole shape, with a head and tail in the stable region, but in some regions the cross section was ginkgo leaf shaped.

We constructed three-dimensional SEM images of continuously polished specimens at the site of convoluted enamel prisms (Fig. 3). The letters A through K indicate individual enamel prisms, which are assigned different colors. The start and end points of the same enamel prisms are indicated by A-A’ to K-K’. Changes in the shape and relative position of the enamel prisms were remarkable, and there was a substantial twist in the enamel prisms themselves.

**Discussion**

*Pyroterium* is a large ungulate from the Tertiary Oligocene of South America (Fig. 1), and its phylogeny and ecology remains a mystery. Their cheek teeth are bilophodont with two transverse lophs, in which enamel elliptically surrounds dentin by attrition. There is a substantial difference in the degree of attrition between the two margins, demonstrated by the difference in thickness. The thin margin of the enamel forms comb-like fine ridges, whereas the thick enamel margin forms the occlusal surface by attrition. These structural differences indicate the two margins have different functions in mastication (9). The occlusal surface at the thick margin, which has a slight ruggedness, shows the internal structure of the enamel and corresponds to the structure we found in the polished surface of the horizontal section. In 1/3 of the surface enamel, the enamel prisms radiate, increasing their thickness and widening the occlusal surface. The cause of such changes in the behavior of ameloblasts during enamel formation is unclear.

In the tangential section of the molar enamel, we observed a dendritic streak with a thick, wavy trunk extending in the direction of the tooth axis. This is classified as a vertical HSB, but it is very different from typical rhinoceros molar enamel (10–12). Our previous study named this an “Undulating vertical HSB” (4), and it can also be expressed as a pyrotherian modification of HSB (9). The reflected light microscopic observation of this complex dendritic structure, also described as a feather-like structure (9), revealed different shades of light because of the different inclination of the prisms constituting the line. This is demonstrated by the different cross-sectional shapes of the prisms observed using electron microscopy. The cross-sectional shape of the enamel prisms is keyhole-shaped with a head and tail, which is a predominantly found in humans and other animals (1, 13), but in some parts it is similar to the ginkgo-shape found in proboscideans. Although the considerably irregular morphology is observed in the boundary area, the reason why the cross-sectional shape of enamel prism is similar to that of human and elephant with different phylogenetic relationship is unknown.

Where the boundary of the bands is clear is either
Plate 2
1. Tangential section (SEM). Note same intensity as observed with reflected light microscope.
2. Tangential section (SEM). Enlarging the region of the clear boundary. Prisms with different orientations meet at the boundary.
3. Tangential section (SEM). Meandering of the enamel prisms in the wide streak region.
4. Tangential section (SEM). Different rows of enamel prism with different cross-sectional shapes forming indistinct boundaries.
5. Horizontal section (SEM). Different orientations of enamel prism forming distinct boundaries.
6. Longitudinal section (SEM) of indistinct boundary area. Enamel prisms gradually changed shape and appeared to be fused.
where enamel prisms with very different inclinations come into contact or where the inclination rapidly changes. The place where the boundary is indistinct is where the inclinations of the enamel prisms changes more gradually. The fact that the cross-sectional shape of the enamel prisms is remarkably deformed and appears to be flattened indicates that a rapid change in orientation and direction occurred.

Figure 3 presents a three-dimensional reconstruction of the boundary region constructed using SEM images obtained from serial tangential sections, reveals that the cutting shape collapses and irregularly deforms according to the orientation of the enamel prisms. In addition, the positional relationships between the prisms distinctly changes and deforms along the path by bending, twisting, and crossing each other. The characteristics of the enamel prisms at the border of the vertical HSB in *Renoceros* molar have been previously examined (10) and determined that the enamel prisms were twisted and bent, and the positional relationships between the prisms changed, and the morphology was distorted as if pressed from the circumference. Moreover, the study results indicated that the rapid change in the direction of the enamel prisms and the change in shape led to the striae, containing few prisms, to form the clear boundary of the vertical HSB.

However, the reconstructed image of the *Pyrotherium* molar boundary region is so complex that it is difficult to follow the orientation of individual enamel prisms. It is exceptionally complicated compared to all 3D images of vertical HSBs published so far (14).

Enamel prisms run continuously through the DEJ to the enamel surface, forming external tooth morphology.
peculiar to each species. HSB indirectly displays the course of enamel prisms and the three-dimensional motion of ameloblasts; moreover, it can indicate the formation process of the external form (15–21). Enamel structure is the basis of tooth morphology and reflects the function of the tooth, while it is also involved in resistance to tooth fracture (22, 23); it is primarily an indication of the adaption of teeth to diet and mastication.

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Conflicts of interest
The authors have no potential conflicts of interest.

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