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USING CEMENTUM HISTOLOGY TO ESTIMATE AGE IN THE PALEOGENE MAMMAL CORYPHODON

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INTRODUCTION

Acellular extrinsic cementum is a continuously growing tissue that anchors the tooth root to the alveolar bone and has the potential to record life history traits such as age of an individual (Klevezal, 1996). Cementochronology offers an alternative to bone histology in determining the age of fossil individuals (Newham et al., 2019). In contrast to bone, cementum growth layers do not remodel nor get resorbed. In this study, we aim to use cementochronology to age the fossil mammal Coryphodon. The Paleocene to Eocene Coryphodon is one of the first placental mammals to reach large body size (over 500 kg) after the K-Pg extinction event and is hypothesized to have undergone dwarfing in the Eocene as a response to increased temperature and a drier, more seasonal climate (Uhen & Gingerich, 1995). Whether Coryphodon extended or accelerated its development to achieve larger body sizes and/ or if the rapid climatic shifts impacted growth rate and longevity are unknown. The Bighorn Basin in Wyoming is an ideal area to study changes of life history traits in fossil communities. The Fort Union and Willwood Formation strata are extensive in the Bighorn Basin. These strata preserve a dense fossil record and high-resolution carbon and oxygen record at the Paleocene-Eocene boundary, which is known for sudden faunal turnover and climate change (Gingerich, 2003).

The Paleocene-Eocene Thermal Maximum (PETM) is an abrupt global warming event ~56 Mya caused by the release of thousands of petagrams of carbon most likely released from thermogenic methane from organic materials and accumulated carbon stock in

Antarctic permafrost (McInerney & Wing, 2011). The most notable consequences of the PETM on mammals are speciation, diversification, and body-size evolution (Clyde & Gingerich, 1998; Gingerich, 2003). At Wasatchian-0, several species dwarf and later rebound concurrently with the duration of the PETM (Secord et al., 2012). Bergmann's Rule states that body-size decreases with warming temperatures and increases with cooling temperatures by the optimization of body size through thermoregulation and availability of food sources. This could explain dwarfing in Coryphodon, but more sampling and testing are needed. No previous research exists to explain how growth rates and lifespan may have changed to cause dwarfing. Cementochronology will be used to estimate ages for collected Coryphodon specimens and evaluate usefulness in comparison to bone histology. To do so, we sampled Coryphodon specimens from several Wasatchian and Clarkforkian biozones in the Bighorn Basin of Wyoming.

BACKGROUND

Cementum is a dental tissue that surrounds the tooth root and attaches it through the periodontal ligament to the alveolar bone. Gonçalves et al. (2004) describes three principal types: acellular afibrillar cementum, cellular intrinsic fiber cementum, and acellular extrinsic fiber cementum. Acellular afibrillar cementum forms in minor amounts along the cementoenamel junction with little known significance. Cellular intrinsic cementum mineralizes collagen fibers parallel to the root surface containing cementocytes on the apical portion. This type grows faster and aids in repairing resorption lacunae and fractures. Acellular extrinsic fiber cementum is found on the cervical, middle, and some of the apical portion of the root. This type serves the purpose of anchoring the root through the peridontal ligament to the alveolar bone. The part of the peridontal ligament that connects to the cement is called Sharpey's fibers—short, connective collagen bundles oriented perpendicular to the root surface. Both cellular and acellular cementum grow continuously in concentric layers around the root. Light and dark bands are caused by a variation in relative mineralization and collagen orientation from seasonal variations in nutrition, biomechanics, and cyclic hormone factors. (Lieberman, 1994).

The seasonal banding pattern of cementum is useful for estimating age. Cementochronology has been used to estimate age in humans in forensic and archeological investigations (Foster, 2017). One of the first comprehensive reviews of cementochronology in extant mammals can be found in Klevezal (1996). Since then, cementochronology has also been applied to estimate ages of fossil mammals and for determining mortality and seasonality information in the fossil record (Stutz, 2002). In one study, the lifespans of Jurassic stem-mammals were determined using incremental tooth histology to calculate basal metabolic rates (Newham et al., 2019).

METHODOLOGY

Coryphodon specimens were collected from the Clarkforkian to Wasatchian biozones of the Fort Union and Willwood Formations in the Bighorn Basin of Wyoming. Of these, six teeth were sampled for histology, including four canines, two premolars, and one incisor. In addition, four teeth were sampled from the Yale Peabody Museum (YPM). These consist of two canines (YPM 14723, 16131), and two premolars (YPM 14723, 17800).

Specimens were photographed for photogrammetric reconstruction before sampling. Each specimen was placed in a lightbox and photos of each sample were taken at low, moderate, and high angles by rotating a stage, from which photogrammetric models can be created. In addition to photogrammetry, the external morphology of the specimens was preserved through molding and casting before cutting the teeth to make ground thin sections. The roots were sampled near the apical one third because the most acellular extrinsic cementum mineralizes there (Newham et al., 2019). Several samples were also cut longitudinally, but cross sections were found to better depict the layers of cementum. Next, samples were smoothed and polished using sandpaper and mounted to slides with epoxy. The samples were again cut and ground to a thickness of 100 μ m or less until optimal viewing conditions were realized Thin sections were photographed using an Axiocam 503 camera under magnification on a Zeiss Axioimager Z2, and montaged with Zen2 software.

The montages were imported into Adobe Illustrator CS6 for analysis. The cementum could be identified starting at the cemento-dentin junction as visible growth layer groups with countable annulation lines. The preservation quality assessed for each photo was determined based on the presence of Sharpey's fibers and lacunae from cementocytes formed during cementogenesis. Excellently preserved specimens include both preservation features with countable cementum lines. Moderately well preserved specimens lack the detail in preservation but have visible lines. Poorly preserved specimens are unusable with no preservation of lines, lacunae, or Sharpey's fibers. In Illustrator, each image was locked in the first layer and an additional layer was used for tracing the lines of growth. For CORY 19-29, an additional layer was added for uncertain lines as the cementum lines for this sample are irregular and more difficult to follow. Lines were traced using the pencil tool and various colors were used to correlate the same lines across long layers. The lines were counted to find an estimated age for each Coryphodon individual.

RESULTS

Below we describe each specimen, from best to worst preserved.

Excellently preserved Specimens

CORY 19-29 (Fig. 1A) is a canine. The central region consists of the dentin of the canine. The cementum is preserved between the dentin and alveolar bone. Both the dentin and cementum preserve clear growth layers. The concentric rings of the dentine are cross-



Figure 1. Tooth histology of excellent specimens A) canine (CORY 19-29), B) canine (YPM 14723), C) premolar (YPM 14723), and D) canine (YPM 16131). Individual growth layers indicated by arrows. Enlarged area to the right of each image. Examples of dentin, cementum, cementoenamel junction, cementocyte lacunae, Sharpey's fibres, and alveolar bone are indicated.

cut perpendicularly by lines extending from the center. The cementum is identified in this sample by its wavy pattern of lines caused by uneven growth and spotted texture from the presence of cementocyte lacunae. Evidence of Sharpey's Fibres is left behind by the long dark lines cross cutting growth layers. This specimen has a line count of 33 demonstrating that Coryphodon had a lifespan extending past three decades.

YPM 14723 (Fig. 1B) is another canine. Similar to CORY19-29, the cementum is identified surrounding the inner dentin and shows preservation of cementocytes and Sharpey's fibres. The outer half of the growth layers are well preserved and have an estimated count of 22 lines. Unfortunately, the innermost layers are not well preserved enough to be countable. At a minimum, this individual exceeded two decades in age, but may have lived much longer as suggested by the overall thickness of the cementum layer.

YPM 14723 (Fig. 1C) is a premolar of the same specimen as the canine above. The cementum is clearly distinguishable between the dentine and bone. Both the cementum and dentine preserve incremental growth layers. The cementum is identified surrounding the dentin and shows preservation of cementocytes and Sharpey's fibres. Similar to the canine the outer part of cementum is much better preserved than the inner part (with each part being about equal in thickness). The outer part preserves up to 28 layers.

YPM 16131 (Fig. 1D) is a canine and was cut in a longitudinal section. The dentin is similar to the previous two samples, but the cementum noticeably changes character around the sides of the tooth. Some areas had flat, even lines while others were too dark, too close together, or not well preserved. The dentin is preserved well and lacunae and Sharpey's fibres are present. A minimum of six lines are present.

Moderately well preserved Specimens

Intermediate specimens are categorized by having some visible lines but are not as well preserved. The two canines (CORY 19-17, CORY 19-32) assigned to this category are shown in Figure 2. Both specimens show some lines in the cementum and dentine, but the lines cannot be consistently traced. CORY 19-32 preserves at least four cementum lines but the junction between the dentin and cementum is not visible. CORY 19-17 is even more poorly preserved. Although some lines are visible in the dentin the cementum-dentin junction is not recognizable and the cementum can only be identified by its generally wavy nature.

Poorly preserved Specimens

Poor specimens are completely diagenetically altered and are unusable (Fig. 3). No biological structures are visible in the tooth including the cementum in CORY 19-35 (canine), CORY 19-53 (incisor and premolar), and YPM 17800 (premolar).



Figure 2. Tooth histology of intermediate specimens A) canine (CORY 19-17) and B) canine (CORY 19-32). Individual growth layers indicated by arrows.

CONCLUSION

This study demonstrates that cementochronology is a useful tool in vertebrate paleontology to determine ages for individuals. Cementum histology is an alternative to bone histology when bones are incomplete, decomposed, or not present. Cementum also offers several advantages as it is not usually subject to resorption, is not vascularized, and has a very regular, incremental pattern (Stutz, 2002). Three of the specimens sampled herein were deemed sufficiently preserved to give reliable age estimates based on preservation of Sharpey's fibres and lacunae. Specimens studied had just a few to almost 30 growth layer groups. This is the first study to demonstrate that Coryphodon reached several decades in age. Based on these data, we can start to understand how changes in lifespan might have influenced changes in body size of Coryphodon during the Paleogene. Overall, cementochronology has great potential to be a useful method for studying body size evolution in the fossil record.



Figure 3. Tooth histology of poor specimens A) canine (CORY 19-35), B) incisor (CORY 19-53), C) premolar (CORY 19-53), D) premolar (YPM 17800).

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