Revised Proposal to Study Transvaal Museum Juvenile Hominin Dental Material

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Introduction

The aim of this study is to use a combination of traditional and state-of-the-art techniques to accurately evaluate dental development and age at death for several South African juvenile hominins at key developmental stages. Multiple lines of evidence suggest that Plio-Pleistocene hominin ontogeny differs from modern humans (e.g., Bromage and Dean, 1985; Smith and Tompkins, 1995; Dean et al., 2001), although few data on tooth formation times and ages of molar eruption are available for precise comparisons. Fossil hominin estimates almost ubiquitously incorporate data from great apes and humans, leading to circular comparisons with living taxa and potentially inaccurate results. For example, histological reassessments of age at death in three fossil juveniles originally studied in the 1980s have reported later estimates of molar eruption and/or ages at death in each case, ranging from differences of a few months to more than a year (Stringer & Dean, 1997; Lacruz et al., 2005; Smith et al., in review), demonstrating the value of re-examining other juveniles with improved methods. This project is also particularly timely as recent studies report that first molar eruption ages and rates of root formation derived from captive great apes may be accelerated relative to wild populations (Zihlman et al., 2004; Kelley and Schwartz, 2010; Smith et al., in press). However, Plio-Pleistocene hominins have been estimated to erupt their first molars at the same ages as captive apes (Dean, 1987; Beynon & Dean, 1988; Dean et al., 1993). If substantiated, these results would imply that life histories in Plio-Pleistocene hominins were more rapid than those of wild great apes (Kelley and Schwartz, 2009).

The proposed study takes advantage of a powerful synchrotron imaging technique that permits *virtual histology* (Tafforeau, 2004; Tafforeau et al., 2006; Tafforeau & Smith, 2008), revealing internal microscopic growth lines accurately and non-destructively. This technique permits analysis of incremental features in tooth enamel and dentine, as well as the neonatal (birth) line (Figure 1), which may be seen in highly mineralized fossils that are millions of years old (Tafforeau et al., 2006; Tafforeau & Smith, 2008). In combination with virtual data on cuspal enamel thickness and the developmental status of unerupted teeth, this approach may be used to characterize dental development and age at death in material that is unavailable for traditional physical sectioning, and may also be used to provide novel information on previously-studied fossils. This technique is currently only possible at the European Synchrotron Radiation Facility in Grenoble, France, and has permitted the study of dozens of hominin fossils thus far (Appendix 1). Virtual histology cannot be performed with conventional medical or laboratory tomographic scanners as several special properties of synchrotron X-rays are required (Tafforeau et al., 2006; Tafforeau & Smith, 2008). Unfortunately there are no synchrotrons in South Africa.

The first application to the study of fossil hominins was performed at the European Synchrotron Radiation Facility for the Jebel Irhoud *Homo sapiens* juvenile fossil, leading to a precise age at death estimate (Smith et al., 2007), which was followed by the imaging of nine more juvenile Middle Paleolithic hominins (Smith et al., in review), and several isolated Plio-Pleistocene teeth (Tafforeau and Smith, 2008). We have also applied this method to study the Sterkfontein individual Stw 151 and the Drimolen hominin teeth, and the initial results for *Paranthropus* and early *Homo* dental development are very promising. In order to accurately characterize tooth formation in a broader sample, and to provide precise ages at death for key developmental stages, we request permission to study the following individuals from the Transvaal Museum Collection: SK 61 (mandible), SK 62 (mandible), Sts 2 (maxilla and canine crown), Sts 24 (right maxilla and associated upper and lower incisors), KB 5223 (4 permanent elements – I1, I2, C, M1), and TM 1536 (mandible). Two of these individuals were originally studied by Bromage and Dean (1985) (Sts 24 & SK 62), and we also intend to assess the accuracy of the original assigned ages based on expanded information about internal dental development. Additional hominin material has also been requested from the University of Witwatersrand (MLD 2; contact: Bernhard Zipfel) and from the Tanzanian National Museum and House of Culture (LH 2, 3, 6, 21a, OH 30, OH 13; contact: Charles Musiba). Decisions are expected about these requests within the next few days or weeks.

Research Protocol

1. *Photographic recording of developing teeth and generation of impressions*

A Coolpix 4500 digital camera with a drop-in lens for stereomicroscopy will be employed to capture the developmental stage of each tooth. Following this, dental impressions and peels will be made of each permanent tooth crown with Coltene President soft putty and Struers' Repliset impression materials to assess long-period line numbers (e.g., perikymata). Standard protocols of impression generation will be employed to ensure that fragile surfaces are handled carefully and any small remnants of impression materials are removed from the original fossils.

2. *Synchrotron imaging of select developing tooth crowns (in Grenoble, France)*

Samples chosen for high resolution synchrotron imaging will be hand-carried from Pretoria to Grenoble, France, where they will be deposited in a fire-proof safe until scanning. A special carry case (e.g., Pelican 1500 hard-shell case) will be provided for this trip. We will cover the costs to enable a staff member of the Vertebrates Department at the Transvaal Museum to accompany the fossils at all times, including shuttle-services, return airfare, subsistence, accommodation and passport/visas if required. In collaboration with Paul Tafforeau (Beamline Scientist, European Synchrotron Radiation Facility), the teeth will be micro-CT scanned with 30, 5, and 0.7 micron resolutions employing both absorption and phase-contrast synchrotron imaging techniques over the course of 6 days of beamtime (divided into 2 or 3 trips). This technique is non-destructive and provides results that are far superior to laboratory micro-CTs, particularly for highly re-mineralized samples.

The combination of developmental information preserved on the surfaces of the teeth and the internal features imaged non-destructively with the synchrotron will permit a highly precise estimate of the age at death and formation time of the teeth. This will allow age estimates by Bromage and Dean (1985) to be more precisely determined and will provide a more accurate account of dental development in these juveniles. The new data on *Australopithecus* and *Paranthropus* teeth will facilitate important insight into variation in Plio-Pleistocene hominin development and life history (detailed further below). Copies of the data will be made available to the museum upon request.

3. Timeline

We will prepare an official proposal for beamtime should this request be approved, which would ensure free scan time and logistical support. The initiation of this project is contingent upon receiving beamtime from the European Synchrotron Radiation Facility, which is granted over six-month-periods that may be applied for every six months. The Co-PIs have a successful history of obtaining official beamtime, so it can be anticipated that access will be obtained for a first visit in fall 2010 or the first half of 2011. Should official proposals fail to be accepted, we will employ Dr. Tafforeau's in-house beamtime for access in 2011 or early

2012. An application for financial support has been submitted to the National Science Foundation, which may be revised and resubmitted this fall if necessary. The analysis of all samples is expected to take 1-2 years after scanning, depending on the number of final samples and planned employment of a postdoctoral fellow. We anticipate that we will prepare several publications for submission to high-impact journals (e.g., Science, Proceedings of the National Academy of Science USA), as well as specialist journals (e.g., Journal of Human Evolution, Journal of Anatomy). We will credit the Transvaal Museum in all publications arising from this project.

4. *Significance and expected results*

Modern humans have an unusual life history with an early age at weaning, long childhood, late first reproduction age, short interbirth intervals, and long lifespan (Bogin, 1990). Despite more than 80 years of speculation about fossil hominin ontogeny, the evolution and origin of modern development remain unknown. Fossilized dental remains are important indices of maturity as tooth formation varies less than skeletal development, and their completion signifies the onset of adulthood. This project will employ non-destructive synchrotron X-ray imaging to comprehensively assess dental development in several Plio-Pleistocene taxa, including more than 20 juveniles of *Australopithecus afarensis*, *Paranthropus boisei*, *Australopithecus africanus*, *Paranthropus robustus*, and early *Homo* from southern and eastern Africa using synchrotron virtual histology. Tooth formation and molar eruption will be compared with recently expanded samples of great ape and human dentitions, which provide important insight into variation within and among populations and species. Precise histologically-derived estimates of age at death will be regressed against predicted ages using chimpanzee and human developmental models, enabling the first independent assessment of ontogenetic similarities among these taxa.

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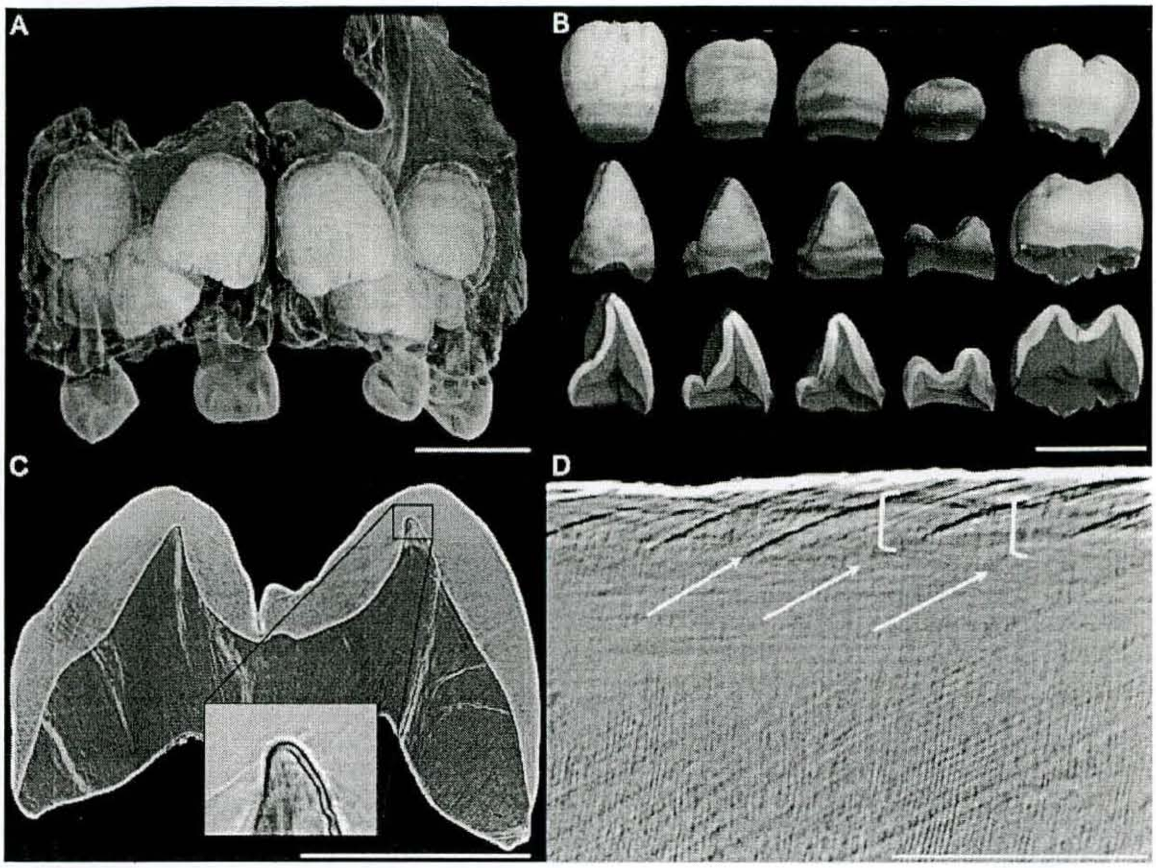
Figure 1. Fossil hominin dental development revealed by synchrotron imaging.



A) Synchrotron microCT scan (31.3 μm voxel size) showing central incisors in light blue, lateral incisors are in yellow, canines are in pink, and third premolars are in green. B) Isolated elements and cross-sectional slices showing the degree of permanent tooth calcification. The scale bar in A & B is 10 mm. C) Synchrotron phase contrast image (4.95 μm voxel size) of the upper first molar used to count long-period lines. Scale bar is equal to 5 mm. The inset shows the neonatal line just above the conical dentine horn tip of the paracone; this cusp was estimated to begin forming 17 days prior to birth. D) Synchrotron phase contrast image (0.678 μm voxel size) of the 8 day long-period line periodicity; 8 daily lines (in brackets) can be seen between long-period Retzius lines (arrows). Scale bar is equal to 0.2 mm.

Appendix 1. Hominin material imaged at the European Synchrotron Radiation Facility.

Fossil Taxon	Individual	Institution	Reference
Homo sapiens			
	Irhoud 3	University Mohammed V-Agdal	Smith et al. 2007
	Qafzeh 10	Tel-Aviv University	Smith et al., in review
	Qafzeh 15	“”	“”
Homo neanderthalensis			
	Engis 2	Direction de l'Archéologie, MRW	“”
	Gibraltar 2	Natural History Museum (London)	“”
	La Quina H18	National Archaeology Museum Paris	“”
	Krapina Max B	Croatian Natural History Museum	“”
	Krapina Max C	Croatian Natural History Museum	“”
	Scladina	Direction de l'Archéologie, MRW	“”
	Le Moustier 1	Museum fur-Vor- und Fruhgeschichte	“”
South African *Homo*			
	DNH 35	University of Witwatersrand	unpublished
	DNH 39	“”	“”
	DNH 62	“”	“”
	DNH 67	“”	“”
	DNH 70	“”	“”
	DNH 71	“”	“”
	DNH 83	“”	“”
	Stw 151*	“”	“”
Paranthropus robustus			
	DNH 84	“”	“”
	DNH 44	“”	“”
	DNH 47	“”	“”
	DNH 60	“”	“”
	EM 1009	Natural History Museum (London)	Tafforeau and Smith, 2008
	EM 2368	“”	“”
Australopithecus africanus			
	EM 1011	“”	“”
Australopithecus sp.			
	Malapa H1	University of Witwatersrand	unpublished
Sahelanthropus tchadensis			
	TM 266-01-60-1	Poitiers University	Brunet et al., 2005