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TECHNICAL NOTE

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Determination of Season at Death Using Dental Cementum Increment Analysis*†

ABSTRACT: Dental cementum anchors teeth into their sockets via the periodontal ligament. In mammals, dental cementum is laid down in alternating opaque and translucent bands representing winter (dormant) and summer (growth) seasons. Each pair of bands represents 1 year of life. Estimates of age at death based on counting pairs of bands are highly accurate. If the timing of the transition between winter and summer bands could be identified in humans, dental cementum increment analysis could be used to specify the season at death. This pilot project attempts to determine when these transitions occur and thus provide forensic anthropologists with a technique that would refine our estimates of postmortem interval. Extracted teeth were obtained from a local oral surgeon, embedded, sectioned, ground, polished, and examined under transmitted polarized light. The outermost increment was identified and measured along with other randomly chosen like bands. A transition from translucent to opaque bands was observed in teeth extracted in early October, while teeth extracted in early April exhibited nascent translucent bands. Further, significant correlations were observed between band thickness and number of days into either season, suggesting that band width increases as either season progresses. In this blind study, extracted teeth were effectively sorted into the spring/summer or fall/winter season 99% of the time.

KEYWORDS: forensic science, forensic anthropology, season at death, dental cementum, postmortem interval

Forensic anthropologists are often called upon to estimate time since death in decomposing and skeletonized human remains. Estimates are based on the overall condition of the remains, the presence of insect activity, and the decomposition microenvironment. Postmortem interval (PMI) estimates are usually expressed as broad ranges of months or years, especially when forensic anthropologists are not present at the time of recovery. Dental cementum increment analysis has the potential to help forensic anthropologists be much more specific in PMI determinations.

Dental cementum anchors teeth via the periodontal ligament to the alveolar bone. In cementum formation, hypermineralized layers of extracellular matrix alternate with less mineralized layers, creating alternating dark and light bands, analogous to tree rings. Cementum is first laid down immediately before the tooth erupts and additional layers are added throughout life. The main components of cementum layers are collagen bundles that become mineralized by hydroxyapatite crystals.

Research with comparative samples of known-age and known date-of-death individuals has demonstrated a consistent relationship between annual seasons and the formation of distinct increment types (1,2). The winter or arrested cementum increment appears under polarized light as an opaque band, while the summer or growth increment appears as a translucent band (Fig. 1).

Together these bands represent 1 year of an individual's life, providing an annual record of that person's life history. The total number of pairs of opaque and translucent increments provides a means of determining the individual's age at death within two and one half year ranges of error (1). To derive age at death, the number of pairs of bands is added to the age at which the tooth is known to erupt. In addition, cementum increments of increased thickness have been correlated with stressful events including pregnancy (3). Dirks et al. (3), for example, correlated increased increment thickness with menarche and first postweaning dry and rainy seasons in Ethiopian yellow baboons.

Zooarchaeologists have long used dental cementum increment analysis to estimate season at death in mammals (4,5), yet no published studies have tested this method in humans. The current study builds on Wedel and Peabody (6) and Wedel and Bowman (7) and examines one full calendar year's worth of samples in an attempt to identify the timing of increment formation in humans, and thus provide a method by which season at death could be determined in forensic cases.

Materials and Methods

Extracted teeth were obtained from patients of a local oral surgeon. Extraction was conducted for a variety of reasons ranging from orthodontics, periodontal disease, fitting for dentures, etc. Because the teeth were obtained from one dental office only, the donations came in sporadically at times, with only one donation for the month of December, 2004, and no donations for the month of January while the office was closed (Fig. 2). The cooperating oral surgeon was at his busiest in the months of October, March, and April.

From each individual, the dental office staff acquired informed consent according to procedures approved by the University of California Santa Cruz Human Subjects Committee. Staff members recorded the individual's date of birth and the date of the extraction, which is used here as a proxy for date of death. Recent studies involving human teeth (1,8–10) have indicated that no statistical difference exists in cementum accumulation of different teeth within a single individual. For this reason no specific tooth was required. The study sample was comprised of all tooth types, including incisors, canines, premolars, and molars.

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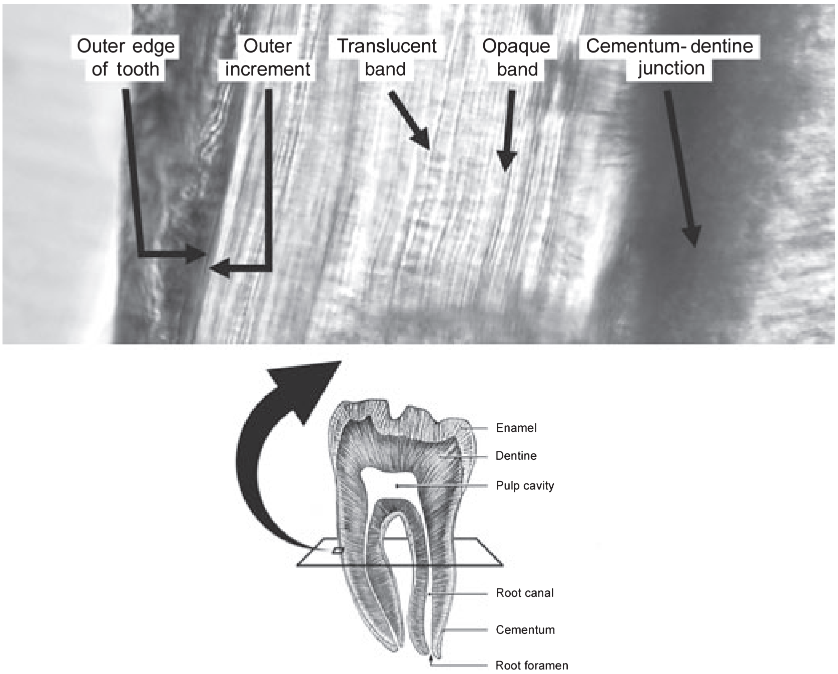

FIG. 1—*Transverse cross-section of human tooth root showing microstructures.*

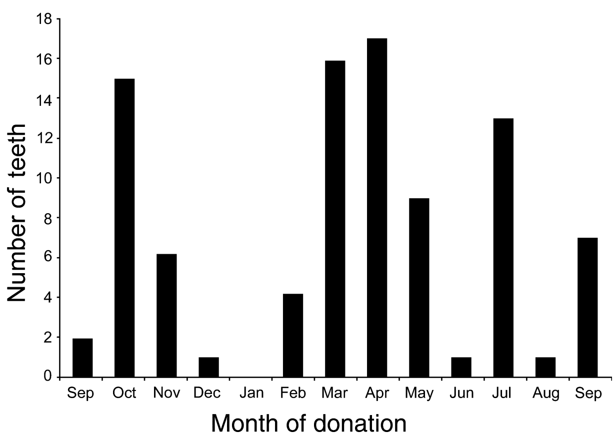

FIG. 2—*Number of teeth donated for each month of the study.*

TABLE 1—*Age distribution of samples.*

Age (years)	Number of Individuals
<20	11
20–39	33
40–59	26
60–79	11
>80	11

One hundred thirty-two teeth extracted from 100 individuals were donated for this study. Of these, 40 teeth were excluded, most (28) because they were the developing and un-erupted third molars of adolescents, in which the cementum increments have yet to begin forming. Two teeth were excluded because of incomplete accompanying demographic information; seven were excluded because gross caries obscured the increments; one exhibited *dens invaginitis* and thus abnormal histology, and the increments could not be distinguished in two teeth. Teeth were donated by individuals ranging from 15 to 90 years old (Table 1). Sex differences were not accounted for in this pilot study.

Each of the teeth included in the study was cleaned and embedded in Silmar S40™ (BP Chemicals, Inc., Cleveland, OH). Embedded teeth were sectioned to a thickness of 500 μm (verified by micrometer), mounted to petrographic glass slides, and manually ground and polished under water lubrication on a Buehler Metaserv 2000. The polished sections were viewed under 10× magnification and transmitted polarized light using an Olympus BX40™ light microscope (Olympus America, Inc., Center Valley, PA). Digital photographs were taken using a Nikon D70 SLR™ camera (Nikon, Melville, NY) mounted to the microscope. Once the outer nascent band was identified as either translucent or opaque, the widths of like bands were measured using the ruler function in Adobe Photoshop™ (Adobe Systems Inc., San Jose, CA) and the values were averaged in Microsoft Excel™. The thickness of the outer band was divided by this average increment thickness to determine the percentage growth. For example, if the outer emerging band was translucent, its width was divided by the average thickness of the other translucent bands present in the section (Fig. 3). Average band thickness differed minimally between individuals, but not significantly between age categories. All measurements were taken within an individual tooth, yielding percentage completion for the outermost increment. Data were analyzed in Microsoft Excel™ and SPSS™ version 14 (SPSS Inc., Chicago, IL). Statistical analyses included correlating percentage growth with date of extraction (death). Of particular interest were the marked transitions between growth and dormant bands.

Results

Figure 4 shows the percentages of increment completion for each day within the calendar year of the study for which teeth were available. The clear bars represent percentage completion for the translucent (growth) band, the dark bars represent percentage completion of the opaque (dormant) band. A marked transition between the

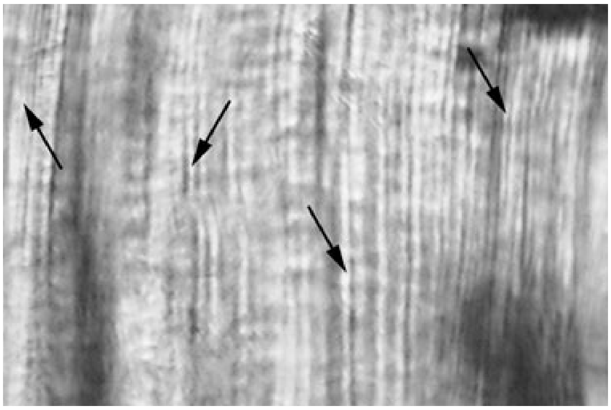

FIG. 3—*Transverse section of human tooth, sectioned to 500 μm, ground, and polished. External edge of tooth root at left. Arrows indicate translucent dental cementum increments.*

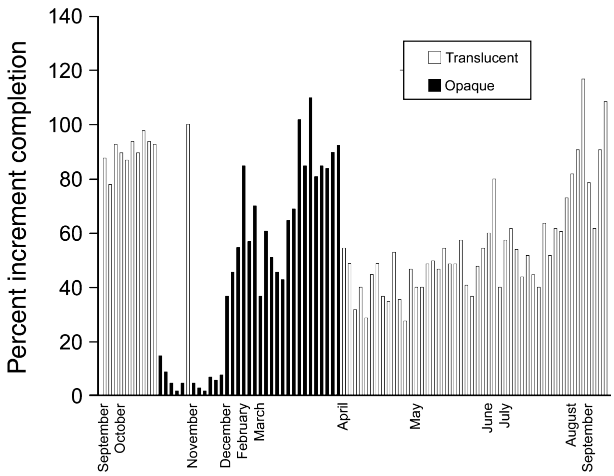

FIG. 4—*Percentages of increment completion from teeth extracted between September 2004 and September 2005.*

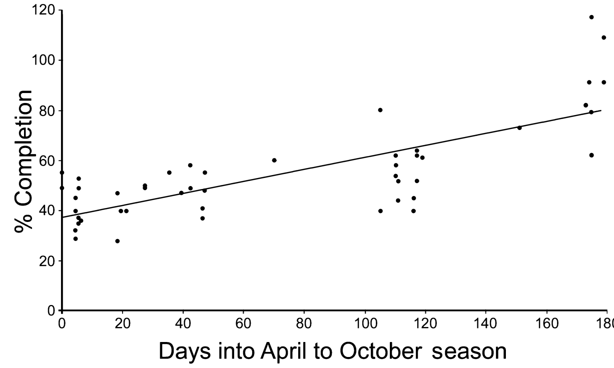

FIG. 5—*Percentage completion of translucent increment plotted against number of days into April to October season.*

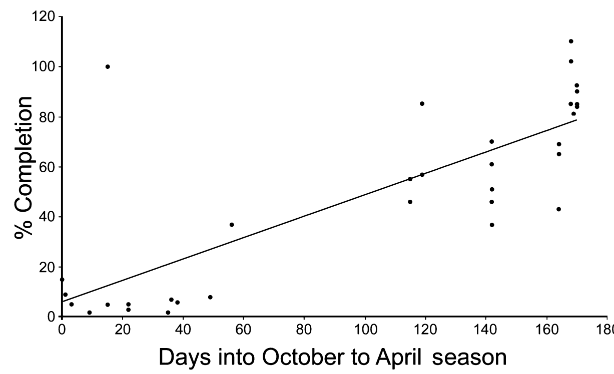

FIG. 6—*Percentage completion of opaque increment plotted against number of days into October to April season.*

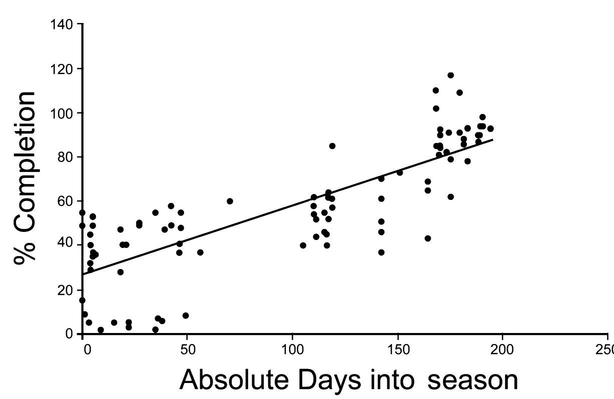

FIG. 7—*Percentage completion of either increment plotted against absolute number of days into either season.*

translucent and opaque bands occurred in late September/early October, while a less obvious transition occurred between late March and early April. The anomalous "complete" translucent increment from a tooth extracted on 10/27/04 is most likely a result of failure to identify the emerging outermost opaque increment.

Band width was strongly correlated with the number of days into the growth season. Average outer opaque band width was strongly correlated ($p < 0.0001$) with the number of days that had passed since 10/12/04, while outer translucent band width was strongly correlated with the number of days that had passed since 4/1/2005 ($p < 0.0001$) (Figs. 5 and 6). The scatter plots in Figs. 5 and 6 exhibit a significant linear relationship between increment thickness and days into the growth or dormant season. A highly significant outcome ($p < 0.0001$) was achieved when the percentage of completion for any band was correlated with absolute number of days into either season (Fig. 7). The trend line indicates a strong relationship between days into the season and increment thickness.

Discussion

Dental cementum increment analysis for estimations of season at death shows great potential for use in forensic anthropology. Teeth are very durable and are commonly recovered, even at death scenes where bone tissue quality is poor, advanced mummification or fragmentation is present, or cremation has occurred. The amount of time involved in embedding, sectioning, and preparing the samples for microscopic examination is moderate and requires several key pieces of lab equipment, namely a vacuum chamber and fume hood for embedding the samples, low speed saw and a grinder/polisher for preparing the sections, a transmitted polarized light microscope with an attached digital camera for photographing the increments, and a computer equipped with Adobe Photoshop™ for image analysis and SPSS™ for data analysis. In addition, some training by apprenticeship is invaluable in learning this technique.

For this method to be demonstrated reliable and precise, this pilot project needs to be expanded, with individuals of both sexes and all ages represented. Its application in forensic cases would be enhanced if ancestral population and reproductive history were also controlled for. Further, cementum increments of teeth extracted from living individuals versus cadavers of known dates of death need to be compared to insure that no differences exist. The effects of pathological conditions, such as hypercementosis, need to be assessed. Validation studies from geographically distinct samples also need to be completed because they may indicate differences in the transition times between the bands.

Conclusions

Teeth hold a wealth of information waiting to be extracted by the forensic scientist. From a single tooth, dental cementum increment analysis can help us determine the exact year and season in which an individual died. This study demonstrated that cementum increment analysis is 99% accurate in determining whether a tooth was extracted between October 12 and April 1 or April 1 and October 12. A tooth from a forensic case could then be sorted into fall/winter or spring/summer seasons. Further, since the cementum increments increase in thickness as each season progresses, estimates of season at death may be refined to four seasons a year. For example, if the winter or arrested band is 75% formed, the individual would have died in the second half of the October to April season, thus between January and April.

Forensic anthropologists could use this method along with the traditional macroscopic aging techniques and gross evidence of PMI. Take, for example, the hypothetical case of a skeleton estimated to be from an individual aged 35–50 who died more than 1 year ago. If dental cementum increment analysis were performed according to Wittwer-Backofen et al. (1) age at death method and the season at death method described here, a forensic anthropologist counting pairs of increments and identifying the outer increment could specify that the individual was 38 ± 2.5 years at death and died between April and October. Dental cementum increment analysis is a method that may allow forensic anthropologists to be much more precise in estimating PMI. Further, as no particular tooth is required for the technique to be useful, the method may be used in any case where teeth are recovered.

Acknowledgments

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