Dental Histology

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Introduction

Because they are highly mineralized, teeth are one of the best preserved and most commonly-recovered elements in archaeological and fossil assemblages. They have inspired more than a century of comparative studies of hominin tooth size and shape (e.g. Dubois 1892; Keith 1913; Weidenreich 1937; Hooijer 1948; Le Gros Clark 1950; Wolpoff 1971; Brace et al. 1991; Wood et al. 1991; Bailey 2002; Irish and Guatelli-Steinberg 2003; Hanihara and Ishida 2005; Hanihara 2008). Additional valuable information is recorded on outer surfaces and inner aspects of the dental hard tissues (enamel, dentine, and cementum) that make up tooth crowns and roots, providing a permanent record of growth. Initial study of dental histology, or microscopic tooth structure, predates the fields of archaeology and evolutionary biology by several centuries. The innovative microscopist Anthony Leeuwenhoeck first described the structure of enamel in the 1600s, noting that it was made of longitudinal 'pipes' (enamel prisms) that appeared as 'globules' when viewed end-on (Leeuwenhoeck 1677-1678). During the 1800s and early 1900s, microscopic investigations revealed the tubular nature of dentine and the presence of successive temporal lines in enamel and dentine (reviewed in Dean 1995; Smith 2006). By the 1940s, American and Japanese teams had experimentally demonstrated the presence of circadian structural features, as well as the neonatal (birth) line, which allows one to relate developmental time to chronological (calendar) age in juvenile dentitions. These *incremental features* form the basis of a growing area of anthropological study that is illuminating aspects of human evolutionary developmental biology, as well as the health and demography of past human populations.

Tooth development in humans begins before birth and continues throughout adolescence. Like many biological systems, the formation of dental hard tissue is characterized by a circadian rhythm, which manifests in the enamel and dentine and remains unchanged in these tissues for millions of years after death. Enamel is secreted by cells known as ameloblasts, which differentiate at the enamel-dentine junction and migrate outward towards what becomes the surface of the crown. The tracks left by these individual cells are known as *enamel prisms*. These prisms show

cross-striations that result from the circadian rhythm of enamel secretion (Figure 1) (Bromage 1991; Smith 2006). The successive positions of the advancing front of forming enamel are preserved as long-period incremental structures termed *Retzius lines*, which contact the enamel surface and form circumferential rings known as *perikymata* (illustrated below). Cross-striations and Retzius lines are also frequently referred to as short- and long-period structures due to their respective 24-hour and greater than 24-hour rhythms. An important relationship exists between these types of internal lines, since the long-period line *periodicity* (repeat interval or number of days between long-period lines) can be determined only by counting cross-striations between Retzius lines. This value is believed to be the same for all teeth in an individual's dentition, although it may vary among individuals (FitzGerald, 1995, 1998). Modern human and fossil hominin periodicities range from 6 - 12 days (FitzGerald 1998; Smith 2008; Antoine et al. 2009); tentative evidence suggests that fossil hominin ranges were once as wide as 5 - 13 days (Smith et al. 2015). Dentine is produced by cells known as odontoblasts that generate *dentine tubules*, and shows daily incremental lines known as *von Ebner's lines* (equivalent to cross-striations), long-period structures known as *Andresen lines* (equivalent to Retzius lines), and *periradicular bands* (equivalent to perikymata) (reviewed in Dean 1995; Smith 2008; Smith and Reid 2009). Tooth cementum shows annual incremental features known as *cementum annulations* (e.g., Kay et al. 1984; Lieberman 1994; Wittwer-Backofen et al. 2004), but due to the difficulty of accurately identifying a full series of these lines in adult teeth (Renz and Radlanski 2006; Wittwer-Backofen et al. 2008), they are rarely used in archaeological studies.

Insert Figure 1 here

Counts and measurements of incremental features have been used to determine the timing of tooth formation, stress experienced during development, and the age at death in juvenile fossil humans and apes (reviewed in Dean 2006; Smith 2008; Hillson 2014). Archaeological applications, which are reviewed below, have mainly focused on aspects of human developmental variation, determinations of age at death, and defects of enamel formation. These data may provide insight into population-level developmental variation, mortality rates/demographic structure, and living conditions of prehistoric cultures, respectively. Dental microstructure analyses also hold promise for the integration of temporal and chemical information locked inside teeth (e.g., Sponheimer et al. 2006; Humphrey et al. 2007, 2008a, b; Richards et al. 2008; Austin et al. 2013). In the subsequent sections, common methods of histological preparation and analysis are presented (Table 1), followed by a review of archaeological applications of dental histology and a case study illustrating how these methods may be used to detail the dental development, weaning process, and age at death of a juvenile Belgian Neanderthal.

Methods of Study

Insert Table 1 and Figure 2 here

Impressions and Casts

Histological study typically requires careful preparation of high-resolution impressions of external tooth surfaces, along with physical sectioning and generation of thin (histological) sections. In both cases high-resolution microscopy (with optical magnification factors of 200-500X) is needed to image and quantify incremental features on tooth surfaces or from histological sections. Recently, a new non-destructive approach (*virtual histology*) has been developed using synchrotron phase-contrast X-ray imaging (Figure 2; reviewed in Smith and Tafforeau 2008; Tafforeau and Smith 2008; Le Cabec et al. 2015), which is currently possible at the European Synchrotron Radiation Facility in Grenoble, France, the Swiss Light Source in Villigen, Switzerland, and Elettra in Trieste, Italy. In the following section, aspects of conventional sample preparation are reviewed, followed by descriptions and illustrations of the various imaging techniques commonly used to assess microscopic tooth structure.

Initial analytical steps consist of macroscopic or stereoscopic photography, followed by micro-computed tomography (micro-CT) of particularly valuable samples. It is advisable to create a careful record prior to physical sectioning; micro-CT provides a means of digitally archiving a sample in three dimensions, which can be virtually sectioned in advance to guide physical sectioning, or quantified volumetrically and physically reproduced with a 3D printer (e.g., Tafforeau 2004; Macchiarelli et al. 2006; Olejniczak et al. 2007; Smith et al. 2007a; Smith and Tafforeau 2008; Smith et al. 2009). Original tooth crown surfaces are often difficult to image directly due to the transparency of enamel; light tends to reflect poorly, complicating the resolution of fine surface details (Hillson 1992a). Marks et al. (1996) detail a method whereby tooth surfaces can be temporarily enhanced by coating with ammonium chloride (performed within a fume hood). Alternatively, tooth surfaces can be cleaned with ethanol or acetone, and molded with high-resolution dental impression materials, such as Struers' (Struers Inc., Westlake, Ohio) Repliset or Coltène Whaledent's (Coltène/Whaledent Inc., Cuyahoga Falls, Ohio) light body systems (polyvinyl silicone). This facilitates production of epoxy replicas (casts) for preserving a copy and for efficiently visualizing hypoplasias, fine external growth lines (perikymata), and microwear.

Impression materials can be administered via a handheld dispenser (or gun) with disposable tips that mix the base and catalyst, or via a two-stage process (termed the *Beynon technique* by Hillson [1992a] after oral biologist David Beynon). In the latter case, a first impression is made with a 'coarse' impression material (such as Coltène Whaledent's soft body putty), which serves after hardening as a template for a finer impression made by lightly coating the tooth with Coltène's light body, and rapidly placing it inside the soft body impression to polymerize. Guatelli-Steinberg and Mitchell (2003) note that an advantage of the Repliset system over the use of Coltène is the stability of impressions (or molds) under an electron beam, rendering them directly useful for scanning electron microscopy. These authors also demonstrate that the Repliset impression material has a slight advantage in resolution over the Coltène Whaledent light body impressions.

After dental impressions have hardened, replicas can be produced with the use of slow curing low viscosity resin. Various resins (e.g. Epo-Tek 301 resin: Rose 1983; Spurr resin: Beynon 1987; Araldite resin: Hillson 1992a) that have been employed have similar properties, including stability under an electron beam. Replicas can be colored by the addition of commercially-available resin or acrylic paint coloring agents, or their surfaces can be coated with ammonium chloride, graphite, gold, silver, or palladium to enhance the appearance of hypoplasias and incremental features (reviewed in Beynon 1987; Hillson 1992a). Often an electron microscopy sputter-coater is used to lay down a very thin coating of carbon or metal, which reduces the transparency of epoxy casts, and facilitates either stereomicroscopy or scanning electron microscopy (detailed below).

Insert Figure 3 here

Physical Sectioning

When permissible, additional developmental information can be obtained by thin sectioning via cutting, grinding, and polishing, yielding a thin slice (approx. 0.1 mm) that allows transmission of light and visualization of internal features. Archaeological dental remains, especially the dentine and cementum, are particularly vulnerable to diagenetic alteration (e.g. Bell et al. 1991). These tissues may appear very brittle or soft and chalk-like (Hillson 1996), and are liable to fragment when force is applied. In order to protect samples during the sectioning process, teeth are typically coated with successive layers of cyanoacrylate (super-glue) or embedded in a slow-curing resin (e.g. Epo-Tek 301) or methylmethacrylate polymer. Methylmethacrylate (MMA) has been lauded for its ability to penetrate and fill very small spaces within samples (Boyde 1989), including dentine tubules that are approximately 0.001 mm in diameter. It can be removed by soaking samples in a dichloromethane, which will soften and eventually dissolve the MMA. However, epoxy resins may be preferable for embedding since they are less toxic than MMA and do not require the use of a fume hood.

Once coated or embedded, dental samples are slowly cut with a diamond-tipped blade mounted on a peripheral or annular saw. The sample is then advanced approximately 0.5 to 1.0 mm, and a second cut is made (Figure 3). This method results in a “thick section” of approximately 0.2 to 0.7 mm, which is bonded to a microscope slide, slowly ground down to a final thickness of approximately 0.1 mm, lapped or polished with a fine-grain suspension solution (e.g. 1.0 micron Alumina), cleaned with an ultrasonicator, dehydrated in an alcohol series, cleared in xylene, and covered with a cover slip and mounting medium (typically xylene-based, such as DPX [Fluka Chemicals]). After the mount dries, the thin section is ready for microscopic imaging. Additional descriptions of histological preparation procedures may be found in Hillson (1996), Marks et al. (1996), Reid et al. (1998a,b), Caropreso et al. (2000), and Füsün et al. (2005); details of ultrathin section preparation may be found in Gray and Opdyke (1962), Murphy and McNeil (1964), and Neal and Murphy (1969).¹

An added advantage of sectioning teeth is the potential to sample internal surfaces for light or heavy isotopes, trace elements, enamel proteins, or preserved

DNA in concert with analyses of dental development (e.g., Lakonis Neanderthal molar: Richards et al. 2008; Nielsen-Marsh et al. 2009; Smith et al. 2009; Scladina Neanderthal molar: Smith et al. 2007a; Nielsen-Marsh et al. 2009; Austin et al. 2013). Sectioned teeth are of great value for these complimentary approaches, since the internal aspects of tooth crowns, particularly the enamel portion, are subject to less diagenetic modification than are external surfaces (Schoeninger et al. 2003). Following sectioning, the tooth can be reconstructed using color-matched dental restorative materials (Figure 4; also see Smith et al. 2009: 114, Figure 4) or dental sticky wax (e.g. Schwartz and Dean 2001: 273, Figure 1).

Insert Figure 4 here

Microscopic Imaging

Incremental features in histological sections have traditionally been assessed with polarized (transmitted) light microscopy. This technique is ideal for revealing the birefringent (optically varied) properties of enamel and dentine, and it often enhances microstructure clarity (Figure 1; also see Schmidt and Keil 1971; Boyde 1989). Modern image analysis systems include transmitted light microscopes (outfitted with polarizers and analyzers) coupled via high-resolution digital cameras to workstations with image analysis software. Most commercially-available software packages include tools for performing linear measurements and manual counts of incremental features. Unfortunately it has not yet been possible to automate incremental feature analysis because of subtle inhomogeneities of dental microstructure (which is also true for virtual histology).

Insert Figures 5 and 6 here

Additional forms of incremental feature analysis include stereomicroscopy and scanning electron microscopy (SEM), which are particularly effective for visualizing features such as hypoplasias and perikymata on tooth surfaces (Figure 5). Scanning electron microscopy is also useful for imaging microstructure in sectioned and polished teeth (Figure 6) or on developing enamel surfaces (e.g. Boyde and Jones 1983; Boyde et al. 1988; Boyde 1989). An advantage of SEM over traditional stereoscopic imaging is the improved depth of focus at high resolution in secondary electron mode. Furthermore, back-scattered SEM yields density-dependent images of a single plane, as opposed to the optical averaging of light microscopic imaging of 0.1 mm sections (roughly the thickness of 20 prisms stacked longitudinally). Another technique that has been applied to human and ape dental material is confocal microscopy (Figure 7; also see Boyde and Martin 1987; Dean 2004), which is particularly valuable for naturally-fractured teeth that are nearly flat, facilitating sub-surface microstructure imaging. Confocal microscopy has been applied to the study of hominin enamel secretion rates, periodicities, crown formation times (e.g. Lacruz et al. 2006, 2009), and enamel-prism packing patterns (e.g. Boyde and Martin 1987).

Insert Figure 7 here

Archaeological Applications

Crown Formation Time and Estimation of Age at Death

Bullion (1987) completed the first doctoral dissertation on the study of incremental enamel structures in an archaeological population, followed by FitzGerald (1995), Antoine (2000), and Thomas (2003), who detailed aspects of incremental growth and the prevalence of developmental stress. Several other studies have focused on the age of individuals at death, which may provide insight into the mortality rates, demography, and living conditions of ancient cultures. Archaeological applications of tooth histology include studies of the British Spitalfields collection (Stringer et al. 1990; Dean and Beynon 1991; Dean et al. 1992; Antoine et al. 1999; Hillson et al. 1999; Antoine 2000; Antoine et al. 2009), as well as other medieval British collections (Boyde 1963; Bullion 1987; FitzGerald 1995; Huda and Bowman 1995), medieval French individuals (Reid et al. 1998a), medieval Danish individuals (Thomas 2003; Reid and Ferrell 2006; Smith et al. 2007b), imperial Roman children (FitzGerald et al. 1999; FitzGerald and Saunders 2005; FitzGerald et al. 2006), ancient Greek infants (FitzGerald and Hillson 2009), and prehistoric Native Americans (e.g. Rose et al. 1978; Goodman et al. 1980; FitzGerald 1995; Simpson 1999).

These studies provide information on human developmental variation, which is critical for consideration of individual dentitions or isolated teeth from living or fossil hominins. Currently it appears that crown formation times do not differ greatly between modern and recent archaeological European populations (e.g. Reid et al. 1998a; Antoine 2000; Smith et al. 2007b). Smith et al. (2007b) compared molar crown formation times among three modern populations and medieval Danish individuals, and found few differences between the modern and medieval specimens. No differences were found between crown formation times in the Danish and northern European samples. Less is known about histological development in non-European archaeological populations. Given developmental variation among modern northern European and southern African anterior and premolar teeth (Reid and Dean 2006; Reid et al. 2008), it is likely that additional archaeological samples will demonstrate similar regional variation. In a study of the oldest-known *Homo sapiens* juvenile from northern Africa, Smith et al. (2007c) reported long crown formation times and dental eruption ages that were quite similar to those of modern European children. Current evidence suggests that the slow-growing modern developmental condition is unique to *Homo sapiens*; recent data on a second fossil *Homo sapiens* individual and several juvenile Neanderthals supports this conclusion (reviewed below and in Smith et al., 2010).

Permanent first molars in apes and humans begin forming a few weeks before birth, permanently recording the birth process as an accentuated line known as the neonatal line (Rushton 1933; Schour 1936). Identification of the neonatal line in first molars, or calculation of post-natal delay in other teeth, allows age at death to be estimated in juveniles whose dental development is incomplete. This approach has been applied most often to fossil hominins (reviewed in Smith 2008: 218, Table

3, also see Smith et al. 2015), but also to humans from recent archaeological contexts in limited cases (e.g. Boyde 1963; Huda and Bowman 1995; Antoine 2000). FitzGerald and Hillson (2009) examine the postnatal survivorship of Greek infants in a cemetery population based on the presence and position of the neonatal line in deciduous teeth (which begin forming several months before birth). Similarly, Schwartz et al. (2010) analyzed the presence or absence of the neonatal line in 50 deciduous teeth from Carthage in order to assess whether individuals were likely to have been sacrificed. It appeared that roughly half the sample died before one to two weeks after birth, leading the authors to conclude that other factors likely contributed to the death of perinatal individuals in this particular cemetery.

Studies of age at death in the known-age Spitalfields material have also been used to demonstrate the high degree of accuracy ($\pm 2\%$) of histological methods (Stringer et al. 1990; Antoine 2000; Antoine et al. 2009). Although there are potential applications of tooth histology for forensic identification, very few published cases exist (likely because pinpointing age at death is time consuming and technically demanding). Katzenberg et al. (2005) present a case study where the remains of an unknown infant were assessed through dental histology, historical records, and DNA analysis. Histology yielded an age at death of 4.8 to 5.1 months, supporting putative historical identification of a 5-month-old female (see also a similar study by Skinner and Anderson 1991). Precise reconstruction of juvenile age at death represents one of the most valuable applications of dental histology, particularly when compared to other commonly employed anthropological aging methods.

Developmental Stress

The most frequent application of tooth histology in archaeology is the detection of developmental stress. External manifestations of stress are known as *hypoplasias* on tooth crowns (Berten, 1895; reviewed in Hillson and Bond 1997; Simpson 1999; Hillson 2014) and *accentuated rings* on tooth roots (Smith and Reid 2009). Internal manifestations of developmental stress in enamel and dentine are termed *accentuated lines* (or, less commonly, Wilson bands in enamel [see FitzGerald and Saunders 2005] and lines of Owen in dentine [see Dean 1995]). Numerous studies have reported on the frequency and timing of hypoplasias in archaeological populations and fossil hominins, a subject beyond the scope of this review (see references in Goodman and Rose 1990; Hillson 1992b, 1996; Katzenberg et al. 1996; Skinner 1996; Hillson and Bond 1997; King et al. 2002; Guatelli-Steinberg 2004; Hillson 2014). Relatively less attention has been paid to the frequency or causation of internal accentuated lines (but see references in Thomas 2003; FitzGerald and Saunders 2005; Witzel et al. 2008; Smith and Boesch 2015).

Unfortunately both hypoplasias and accentuations arise from non-specific stresses experienced during development, limiting the utility of these features for reconstructions of past environments and population health. Numerous studies have posited relationships between these features and vitamin D deficiency (rickets), hypothyroidism (hypocalcemia), acute dehydration, starvation, exanthematous fevers or other systematic disturbances, severe trauma, weaning,

parturition, psychological disorders, seasonal resource availability, disease cycles, or rainfall patterns (e.g. Boyde 1970; Nikiforuk and Fraser 1979; Skinner 1986; Bowman 1991; Simpson 1999; Bracha 2004; Skinner and Hopwood 2004; Schwartz et al. 2006; reviewed in Goodman and Rose 1991; Katzenberg et al. 1996; Guatelli-Steinberg 2001; Dirks et al. 2002; FitzGerald and Saunders 2005; Guatelli-Steinberg and Benderlioglu 2006; Hillson 2014; Smith and Boesch 2015). This area of study would benefit from additional systematic or experimental studies of defect expression from a range of pre- and postnatal conditions in order to provide a framework for interpreting the significance of these features in archaeological and fossil populations.

A substantial debate has ensued in the archaeological literature about the most appropriate approach to determining the timing or age of hypoplasia formation (reviewed in Goodman and Rose 1991; Hillson and Bond 1997; Goodman and Song 1999; Reid and Dean 2000; Martin et al. 2008; Ritzman et al. 2008). The standard approach for much of the past century has been to divide the tooth crown into equally-spaced and equally-timed divisions (assuming constant linear growth rates). However, it is clear that primate teeth do not grow at a constant rate, as evidenced by varying extension rates along the enamel-dentine junction and changing perikymata spacing along the tooth surface. Furthermore, the first-formed enamel in the cuspal regions, which forms over several months to more than a year, is not represented by perikymata on the tooth surface, complicating attempts to assign ages to positions on the tooth surface without knowledge of internal development.

In contrast to decades of previous archaeological studies, Reid and Dean (2000) have shown that no relationship exists between tooth crown height and the total time of formation. They initially proposed a set of developmental standards from 115 northern European anterior teeth for approximate ages of crown surface deciles from the cusp tip to the cervix (Reid and Dean 2000: 138, Figure 1). This was followed by standards for southern African anterior teeth and molars from both populations (Reid and Dean 2006). It is important to note that since anterior tooth development varies among human populations (Reid and Dean 2006), histological information will be most effectively applied to the populations from which the standards are derived. Recent studies have explored differences in the timing of defects using traditional models and histological information (Martin et al. 2008; Ritzman et al. 2008), showing deviations from several months to more than a year among methods.

A number of studies have used patterns of enamel hypoplasias or accentuated lines to match teeth forming at the same time (e.g. Gustafson 1955; Boyde 1963; King et al. 2002; Schwartz et al. 2006; Smith et al. 2007a). This aspect of hard tissue registry allows for more precise estimates of the timing of crown initiation and completion than dissection or radiographic techniques and is also critical for estimates of age at death in older juvenile material (detailed in the case study below). Kelley (2008) illustrates an interesting application of enamel

hypoplasia matching in a potential birth cohort of the (presumably) rare ape species *Griphopithecus* from the Miocene locality of Paşalar (Turkey). Based on similar perikymata counts and the presence of an identical pattern of linear enamel hypoplasias on nine incisors, he suggested that the individuals attributed to this taxon were all at the same maturational stage, and that they experienced the same two stressful episodes simultaneously. From this, he inferred that these animals were part of the same cohort, that they experienced birth seasonality, and that they all died at the same time (based on identical amounts of wear). This elegantly demonstrates how incremental features can be used to reconstruct not only the “intimate history of the individual,” as Gysi (1931) notes, but also the history of a group of individuals and the environmental conditions they experienced.

Case Study: Scladina Juvenile Neanderthal

This case study documents the determination of crown formation time in the Scladina juvenile Neanderthal (Toussaint and Prison 2006), in addition to identification of developmental stress, leading to an estimate of the age at death (originally published in Smith et al. 2007a) as well as the age at weaning (Austin et al. 2013). Following the preparative methods detailed above (photography, micro-CT scanning, molding and casting), the upper first molar of an associated dentition was cut and a histological section prepared (Figures 1, 3, 4). This tooth was sectioned to determine the long-period line periodicity and to register chronological and developmental time via the neonatal line. Birth occurred approximately 13 days after cusp initiation, and crown formation time was determined by tracking days of growth along enamel prisms (according to the procedure described in Boyde [1963, 1990]) and adding this to the number of Retzius lines multiplied by their periodicity (Figure 8). The formation time of this first molar cusp is approximately 872 days, and subtraction of the prenatal enamel yields an age at crown completion of 2.35 years. A record of post-natal developmental stress was also determined; a particularly marked accentuated line formed at approximately 435 days of age, followed by a second accentuated line at 875 days of age in the root of both mesial cusps.

Insert Figure 8 here

All associated teeth were micro-CT scanned, molded and cast, allowing counts of long-period incremental features on tooth crowns and roots, and quantification of the cuspal enamel thickness. Crown formation times were calculated from these variables and the long-period line periodicity of 8 days from the sectioned molar (Smith et al. 2007a: Supplementary Information, Table S1; periodicity confirmed virtually by Smith et al. 2014). These data were compared with data on incremental development in modern humans from northern England and southern Africa, as well as a large sample of Neanderthals (Reid and Dean 2006; Smith et al. 2007b; Guatelli-Steinberg and Reid 2008; Reid et al. 2008), revealing particularly short post-canine crown formation times in the Scladina individual. This pattern is due to thin cuspal enamel and rapid rates of crown extension in

Neanderthals (Smith et al. 2007a, 2010), yielding teeth similar in size to those of living humans that form more rapidly. Finally, a sequence of developmental stress was mapped across the dentition, which allowed registry of teeth forming at the same time, and estimation of the age at death.

Developmental stress in the enamel and dentine of the first molar at approximately 435 and 875 days of age was matched to hypoplasias on anterior teeth (Smith et al. 2007a: 20222, Figure 1). A third developmental stress event was identified and matched at 1779 days of age in the developing lower third premolar and upper canine, and subsequent developmental time was added to establish that the individual was approximately 8 years old at death (~2939 days). This registry of stress across the dentition allowed the establishment of crown initiation and completion ages, yielding an overall developmental chronology for this young Neanderthal (Smith et al. 2007a: 20223, Figure 2).

When the Scladina juvenile is compared to modern humans, its tooth calcification stages and eruption status appear to be advanced by several years over modern juveniles at the same chronological age (Smith 1991; Liversidge 2003). For example, the Scladina second molar had advanced beyond clinical (gingival) occlusion by 8 years of age; second molar eruption occurs on average at 10-13 years of age in global human populations. It is clear that from these data and other studies that age at death in Neanderthals should not be assessed by comparison with modern human standards, particularly those derived from European populations. Furthermore, this individual has shed light on the on-going debate about Neanderthal life history, illustrating a developmental pattern that appears to be intermediate between that of fossil *Homo sapiens* and *Homo erectus* (Smith et al. 2007a). More recent studies employing virtual histology have confirmed this pattern of rapid dental development in other Neanderthals (Smith et al. 2010), implying that the prolonged childhood and slow developmental schedule of modern humans is, in fact, unique to our species.

Finally, the Scladina juvenile also represents the first fossil hominin for which a precise age at weaning has been reported (Austin et al. 2013). By integrating information on the timing of first molar incremental growth with patterns of the incorporation of barium, a trace element enriched in breast milk, Austin et al. (2013) demonstrated barium/calcium patterns in enamel that appear to coincide with periods of placental nutrition, exclusive breastfeeding for 7 months, and supplementation followed by an abrupt cessation of nursing at 1.2 years of age. While this fossil was particularly well preserved, having previously yielded enamel proteins (Nielsen-Marsh et al. 2009) and mitochondrial DNA, biogenic elemental patterns and diagenetic indicators suggest that tooth histology and elemental mapping can be employed to document the timing of early life diet transitions in fossil enamel. Importantly, this approach may be extended to test theories about changes in the timing of human weaning (reviewed in Smith, 2013; Reynard and Tuross, 2015; also see Beaumont et al. 2015).

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Footnotes

¹ An illustrated description of the sectioning process using Logitech equipment is available in PDF format (by permission of Logitech Ltd, Glasgow, Scotland, UK): http://tanyasmith.fas.harvard.edu/files/tanyasmith/files/thin_section_prep_0.pdf

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Table 1. Methods for studying tooth growth and development.

Technique	Application	Exemplars
<i>Preparation</i>		
High-Resolution Impressions	Replicate surface microstructure (perikymata, hypoplasias from molds or casts)	Beynon, 1987; Hillson, 1992a
Thin Sectioning	Prepare samples (~0.1 mm thick) for light microscopy	Reid et al., 1998; Antoine et al., 2009
<i>Imaging</i>		
Transmitted Light Microscopy	Image dental thin sections from a controlled section plane	Gustavson, 1959; Schmidt and Keil, 1971
Stereomicroscopy	Image surface microstructure with focal depth	Smith et al., 2007a
Scanning Electron Microscopy - Secondary Electrons (SE)	Same as stereomicroscopy, higher resolution possible	Boyde et al., 1988; Hillson and Bond, 1997; King et al., 2002
- Backscattered Electrons (BSE)	Image cut & polished internal surfaces based on density differences & interfaces	Boyde and Jones, 1983; Witzel et al., 2009
Confocal Microscopy	Image subsurface microstructure to approx. 0.1 mm depth	Boyde and Martin, 1987; Dean, 2004
Fluorescent Light Microscopy	Illuminate experimental labels (or fluorescent antibiotics)	Bromage, 1991; Dean et al., 1993
Profileometer	Register variation in surface topography at microscopic scale	Hillson and Jones, 1989; King et al., 2002

Radiography

- Flat Plane Radiography

Image internal tissues based on density differences

Moorrees et al., 1963; Beynon et al., 1998

- Micro-computed Tomography Absorption Based

Image density differences in 3D w/ near-micron resolution, quantitative mineralization w/synchrotron

Hayakawa et al., 2000; Smith and Tafforeau, 2008

Synchrotron Phase Contrast

Image internal interfaces based on phase contrast effect

Tafforeau et al., 2006; Tafforeau and Smith, 2008

Image external surfaces with Phong's algorithm and lighting effects

Le Cabec et al. 2015

Figures

Figure 1. Polarized light images of enamel and dentine microstructure in the Scladina Neanderthal upper first molar.

A) Long-period Retzius lines in enamel (arrowed); B) overview of the embedded block of the Scladina right upper first molar (prior to reconstruction) showing the position of higher magnification images in A) and D); C) Retzius lines (arrowed) and

cross-striations in enamel (light and dark bands indicated by brackets); D) Andresen lines (arrowed) and von Ebner's lines in dentine (light and dark bands indicated by brackets). Modified from Smith et al. (2014).

Figure 2. The first fossil hominin assessed with virtual phase-contrast X-ray imaging: the Jebel Ihroud 3 mandible.

A) The North African juvenile fossil mandible showing the location of the incisor tooth enamel (white box) sampled with the Grenoble synchrotron. B) Close up of enamel fragment, with the area of interest (on right) shown in the white box. C) Synchrotron image showing Retzius lines (white arrows) with 10 daily cross-striations between them (white brackets). The scale bar is 0.2 mm. Modified from Smith et al. (2007c).

Figure 3. Preparation of the initial thick section of the Scladina Neanderthal upper first molar.

A. The embedded tooth mounted to the cutting arm of the Logitech APD1 annular saw prior to the initial cut. B. Sectioned tooth showing the 'thick section' still attached to the distal half of the embedded block. Modified from Smith et al. (2014).

Figure 4. Image of the Scladina Neanderthal first molar before (left) and after (right) sectioning.

Note a small portion of the root was removed prior to sectioning for ancient DNA analysis; this was not lost due to the physical sectioning process. Modified from Smith et al. (2014).

Figure 5. Stereomicroscopic imaging of dental material.

Left – the Le Moustier palate on the base of the Olympus SZX 9 stereomicroscope. Right – image of a sputter-coated epoxy cast of a premolar viewed with stereomicroscopy. Note the horizontal long-period perikymata encircling the tooth crown. The scale bar is 2 mm long.

Figure 6. Scanning electron micrograph of cut, etched, and polished fossil ape enamel.

Note the relatively straight course of enamel prisms from the bottom right of the image toward the tooth surface (beyond the top left of field), and the fine light and dark bands that represent daily cross-striations spaced 4.1 microns (0.0041 mm) apart on average. The scale bar is 0.1 mm long (modified from Smith et al. 2004).

Figure 7. Spinning-disk white light confocal images of Neanderthal enamel and dentine, illustrating various incremental features and growth disturbances in a naturally-fractured sample (shown in the center of image).

Figure 8. Case study: Scladina Neanderthal dental development.

The neonatal line is indicated by the first blue line on the lower left (0), with subsequently calculated time indicated for a series of stress events as 153 days, 227 days, 348 days, and 435 days post-natal age. The stress event at 435 days (1.2 years) was the most marked until later stressors at 875 and 1779 days of age (not shown). The scale bar is 1 mm long. Modified from Smith et al. (2007a; 2014).