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Intraspecific variation in M_1 enamel development in modern humans: implications for human evolution

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Abstract

The timing and sequence of enamel development, as well as enamel thickness, was documented for individual cusps (protoconid, hypoconid, metaconid, entoconid) in 15 unworn permanent lower first molars (M_1s) from a sample of modern human juveniles. These data were compared with previously published data for modern and fossil species reported in the literature.

Crown formation in all teeth was initiated in the protoconid and completed in the hypoconid. These cusps had significantly longer formation times (2.91 and 2.96 yrs, respectively) than the metaconid and entoconid (2.52 and 2.38 yrs, respectively), as well as thicker enamel, and each represented between 92–95% of the total crown formation time. Rates of enamel secretion in all cusps increased significantly from 2.97 μm in the inner enamel to 4.47 μm in the outer enamel. Two cusps of one individual were studied in more detail and did not follow this typical trajectory. Rather, there was a sharp decrease in the middle of enamel formation and then a slow recovery of secretion rates from the mid- to outer enamel. This anomalous trajectory of enamel formation is discussed in the context of other nondental tissue responses to illness. Neither secretion rates nor periodicity differed significantly when compared between the cusps of each molar.

Differences in cusp formation times, initiation, and completion suggest a relationship between the rates of enamel formation and enamel thickness. This fits with expectations about the mechanics of the chewing cycle and general lower molar morphology. A comparison with similar data for some nonhuman primates and fossil hominoids suggests this relationship may hold true across several primate taxa. Other aspects of enamel growth differed between this human sample and certain fossil species. The lower molars formed slowly over a longer period of time, which may reflect the extended growth period of modern humans. The methodological approach adopted in this study is discussed in the context of that used in other studies.

Keywords: Dental development; Daily enamel secretion rates; Periodicity; Histology

Introduction

Understanding dental development in extant species is fundamental to understanding dental development in an evolutionary context. Yet, there is much to learn about intraspecific variation in modern human molar development from studies of enamel histology. These internal microscopic structures within teeth retain a record of growth in the form of incremental markings. Counts and measures of these markings can provide detailed chronological information about the way a tooth formed, such as the sequence and timing of enamel development (Boyde, 1963, 1990; Bromage, 1991; Dean et al., 1993, Dean, 1998; Reid et al., 1998a,b). Because of this, histological methods are sometimes used to examine dental development in fossil species. Data of this kind needs to be interpreted by comparisons with extant species, through which insights can be gained into the evolution of different growth patterns (e.g., Bromage and Dean, 1985; Dean, 1987; Ramirez Rozzi, 1993; Macho et al., 1996; Beynon et al., 1998; Dean et al., 2001; Kelley et al., 2001; Dean and Schrenk, 2003; Schwartz et al., 2003, 2007; Smith et al., 2003, 2004; Mahoney et al., 2007). Yet, while there are some good data about intraspecific variation in the sequence and timing of molar cusp formation in modern humans, mostly obtained using these methods, there is still much more to be learned (Reid et al.,

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1998a; Reid and Dean, 2006). Variations in cusp initiation and formation times are still poorly documented aspects of enamel growth and presently reduce the scope for comparative interpretations.

The aim of the present study was to document developmental variation in 15 unworn permanent, erupted, lower first molar crowns (M_1s) from modern human juveniles. Pre- and postnatal cusp formation times, daily rates of enamel secretion, Retzius line periodicity, the sequence of cusp initiation and completion, as well as enamel thickness at the cusp tip, were compared within and between the cusps of each molar tooth type. Total crown formation time (CFT) was also calculated. Comparisons were made within and between the cusps, and with data from extant and fossil species drawn from the published literature.

Background

Amelogenesis and growth lines

Amelogenesis, enamel formation, occurs through a continuous presecretory, secretory, and maturation process. During the presecretory part of the process, cells of the inner dental epithelium differentiate into enamel forming cells, ameloblasts, initially at the tip of the cusp, and then subsequently towards the cervix along the enamel-dentine junction (Nanci, 2003). The secretory part of the process commences as fully differentiated ameloblasts secrete enamel matrix proteins containing 90–95% amelogenins, a heterogeneous group of low-molecular-weight proteins, and 5–10% nonamelogenins, a less well known group including ameloblastin and tuftelin (Boyde, 1989; Eisenmann, 1998; Nanci, 2003). Enamel proteins play a crucial role in organizing and controlling the orientation of calcium phosphate crystals (hydroxyapatite) within the crystallites that make up the enamel prisms (Berkovitz et al., 2002). Enamel grows in thickness as secretory ameloblasts deposit incremental layers of enamel as they move away from the enamel-dentine junction (EDJ) toward the future outer enamel surface. At the same time, ameloblasts continue to differentiate, or extend, along the EDJ towards the enamel cervix. The path of the ameloblasts determines the path of prisms in the fully formed tissue. A prism, therefore, is a bundle of hydroxyapatite crystallites oriented with respect to the long axis of the prism path (Boyde, 1989). Abrupt differences in the orientation of crystallites within adjacent prisms define the boundaries between prisms (Eisenmann, 1998). The secretory process ends as the full thickness of enamel is attained, at which stage the newly formed enamel is only partially mineralized (65% water, 20% organic material, 15% hydroxyapatite by weight; Berkovitz et al., 2002).

Maturational ameloblasts undergo a morphological change at the end of the secretory phase but continue to secrete calcium and phosphate ions that are continuously incorporated into the maturing enamel and account for the increasing dimensions of the crystallites and increasing hardness of the enamel. Maturational ameloblasts also remove degraded proteins (primarily amelogenins) and water to make space for these ions (Berkovitz et al., 2002; Nanci, 2003). Fully mature enamel is almost entirely a calcified tissue (95–96% by weight corresponding to 88–90% by volume).

The forming enamel front is subject to periodic changes and regular variations in ameloblast activity. These variations produce short- and long-period incremental (growth) lines in the enamel (Fig. 1). Short-period growth lines, or cross-striations, represent a daily circadian rhythm in secretory ameloblast activity (see Dean, 1995 and FitzGerald, 1998 for reviews), and although their etiology is not entirely clear, some suspect (Darling, 1958; Driessens et al. 1984; Boyde, 1989) that they may reflect regular variations in mineral composition [or shifts in hydrogen ion concentration (i.e., pH)]. Under transmitted light, these variations along the prism alter the refractive index of enamel, making them visible as cross striations. Long-period growth lines, or Retzius lines, mark the layers of enamel produced by the secretory ameloblasts, which occur every 6–12 days in modern humans (Schwartz et al., 2001; Reid and Ferrell, 2006). These layers are visible under transmitted light within the enamel cusp, also referred to as appositional enamel, but emerge as perikymata on the outer surface of imbricational enamel (Dean, 1987; Risnes, 1990; Shellis, 1998). Short- and/or long-period growth lines have both been used to calculate rates of enamel secretion, Retzius line periodicity, and cusp formation times. These are discussed in detail in the methodology.

Episodes of systemic stress occur during enamel formation and produce accentuated growth lines. Internally, some of these lines are known as Wilson bands, and on the crown surface they appear as a type of hypoplasia (Gustafson, 1959; Hillson and Bond, 1997; also see Fig. 1, where a Wilson band is followed by a surface hypoplasia). Given that prenatal enamel does not normally contain markedly accentuated lines, the first line is thought to mark a brief period of disruption to enamel secretion during birth. This line is known as the neonatal line (Rushton, 1933; Schour, 1936; Christens and Kraus, 1965; Kraus and Jordan, 1965; Beynon et al., 1991a; Berkovitz et al., 2002; Schwartz et al., 2005). In studies of dental development the location of the neonatal line is an important indicator for calculating prenatal and postnatal enamel formation times. Accentuated growth lines have also been used to determine the growth sequence between the teeth or cusps of the same individual (e.g., Tagiguchi, 1966; Reid et al., 1998a,b; Antoine, 2001).

The sequence and timing of molar cusp development in modern humans

Few studies of permanent M_1 enamel histology have determined the growth sequence (initiation and completion) between the mesial (protoconid, metaconid) and distal (hypoconid, entoconid) cusps, or differences in formation times between these cusps. A study of five M_1s showed that the protoconid was the first cusp to initiate formation, and this was followed by either the hypoconid or metaconid (Antoine, 2001). A study of one M_1 also recorded prenatal cusp initiation in the protoconid, and documented postnatal cusp completion in the hypoconid (Reid et al., 1998a). As part of their study, Reid and Dean

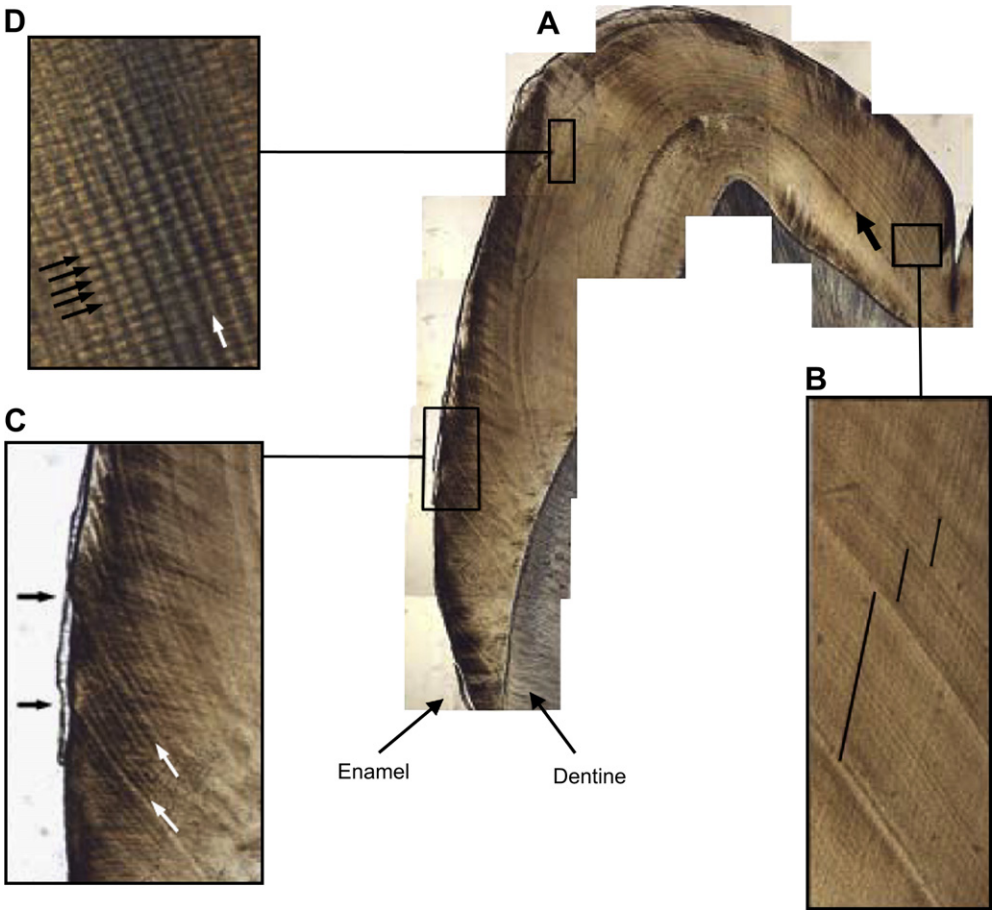

Fig. 1. Incremental lines. A. Thin section of the entoconid (10×). Thick black arrow points in the direction of an accentuated incremental line (Wilson band) in the appositional enamel. B. Close-up of inset, showing less marked incremental lines. From the bottom left corner to the top right corner, the markings occur in approximately eight, and two × three weekly intervals (calculated by dividing the enamel thickness between adjacent lines by a local daily secretion rate giving the number of days between adjacent lines; 20×). The black lines follow the pathway of the prisms between the accentuations. C. White arrows point in the direction of two accentuated lines, which emerge on the outer enamel surface as a type of hypoplasia (black arrows). Retzius lines run in the same direction as the white arrows. D. Cross-striations (40×). White arrow points in the direction of the enamel prisms. Black arrows point to cross-striations.

(2006) reported differences in formation times between mesial cusps from M_1s in two contemporary human populations. However, formation times from direct counts of enamel incremental lines (see Materials and methods) for the distal cusps are poorly represented in the literature (e.g., Reid et al., 1998a), and a statistically valid sample size is still lacking.

Numerous studies have calculated the time taken for a human enamel cusp to form using histological methods (e.g., Komai, 1942; Boyde, 1963; Kajiyama, 1965; Dean and Beynon, 1991; Dean et al., 1992, 1993; Reid et al., 1998a; Antoine, 2001; Reid and Dean, 2006). Sometimes, regression equations, rather than histological methods, are used to estimate appositional enamel formation time from enamel thickness (e.g., Dean et al., 2001). Nonlinear regression, such as polynomial equations (Dean et al., 2001), rather than linear regression is sometimes considered more appropriate, particularly for some biological processes (Anemone and Watts, 1992). Comparisons of formation times derived from these different equations have not been evaluated in the literature.

Total molar crown formation time (CFT) differs from cusp formation time because no one cusp records the beginning and end of molar growth. The difference in developmental time between the cusps has been estimated from surface counts of perikymata in fossil hominoids (e.g., Ramirez Rozzi, 1993), and calculated from histological methods in one human molar (Reid et al., 1998a). Therefore, including total CFT in this study will facilitate developmental comparisons between fossil and extant species (e.g., Lacruz et al., 2006).

Rates of appositional enamel secretion in studies of dental development have often been calculated for gross regions within a cusp, such as inner, middle, or outer enamel, where each region corresponds to approximately one third of the total appositional enamel thickness (e.g., Schwartz et al., 2005). Far less is known about the trajectory of enamel secretion over weekly or monthly increments within these regions (e.g., Beynon et al., 1998; Dean, 1998), or indeed if growth rates for any given region of a particular cusp differs from the same region within another cusp of the same molar.

Retzius line periodicity (see Methodology) has been shown to remain constant in anterior teeth (FitzGerald, 1998), and between the anterior and posterior teeth of four individuals (Reid et al., 1998a). Like rates of enamel secretion, consistency

between cusps is now usually assumed but not often independently documented.

Modern human first molar enamel thickness at the cusp tip

Linear enamel thickness measured from thin sections can differ between modern human M_1 mesial cusps. In general, and depending on the plane sectioned, enamel is thicker on the protoconid than the metaconid (Shillingburg and Grace, 1973; Khera et al., 1990; Schwartz, 2000a; Reid and Dean, 2006; Smith et al., 2006), though this can vary at the cusp tip (Kono et al., 2002; Grine, 2005; Suwa and Kono, 2005). Differences in enamel thickness between molar cusps have been related to functional differences during chewing. For instance, the buccal cusps (protoconid, hypoconid) of mandibular molars, the so-called functional cusps, are mainly involved in crushing and grinding food during chewing, whereas the so-called nonfunctional lingual cusps (metaconid, entoconid) largely shear food (Kay and Hiiemäe, 1974; Kay, 1977). Thicker enamel in the functional cusps of both mandibular and maxillary molars may provide increased resistance to wear, to greater mechanical loads, and resistance to fracture (Shillingburg and Grace, 1973; Gantt, 1977; Molnar and Gantt, 1977; Grine and Martin, 1988; Khera et al., 1990; Macho and Thackeray, 1992; Macho and Berner, 1993, 1994; Spears and Macho, 1995; Schwartz, 2000a,b; Gantt et al., 2001; Grine, 2005).

Several studies have reported linear measurements taken from thin sections of the distal cusps of human M_1s (Shillingburg and Grace, 1973; Grine and Martin, 1988; Shellis et al., 1998; Smith et al., 2006). Of these, the study undertaken on the largest sample size ($n = 16–20$) was by Shillingburg and Grace (1973), who showed that enamel thickness did not increase from the mesial to the distal cusp tips. In contrast, a recent three dimensional micro-CT based study reported thicker enamel over the hypoconid cusp tip compared to the protoconid (Kono et al., 2002).

Materials and methods

The dental sample

Fifteen erupted but unworn permanent M_1s were selected from human juvenile skeletons recovered from nine archaeological sites dating to the British Bronze Age (2,300–700 BC; Table 1; Fig. 2). Unworn molars from juveniles were chosen so that appositional formation times did not need to include an estimate for worn enamel.

Table 1
The skeletal sample ($n = 15$)

Archaeological site	Skeleton number
Scotland	
West Fenton, East Lothian[a]	11
Ardachy, Bunessan, Mull[b]	14
Home Mains Farm, Inverness[c]	31
Harveston Cottage, Catterline[d]	36
Nunraw Mains, East Lothian[a]	48
England	
Porton, Wiltshire[e]	57
Aldro, Yorkshire[f]	59
Garton Slack, Yorkshire[f]	60
Painsthorpe Wold, Yorkshire[f]	75
Aldro, Yorkshire[f]	80
Aldro, Yorkshire[f]	82
Garton Slack, Yorkshire[f]	87
Garton Slack, Yorkshire[f]	100
Garton Slack, Yorkshire[f]	105
Aldro, Yorkshire[f]	116

[a] Childe et al. (1944).
[b] Mitchell (1897).
[c] Brown (2003).
[d] Small et al. (1988).
[e] Unpublished.
[f] Mortimer (1905).

Sample preparation

Each molar was replicated prior to removal for sectioning, and an epoxy cast was prepared (e.g., Mahoney, 2007). Contaminants were removed from the enamel surface of each molar using ethanol and cotton wool. An impression of the enamel surface was taken using a rubber-based, addition-curing silicone (Colténe President Jet, lightbody). The outer surface of the impression was surrounded with Colténe President Putty for stability. A cast of the inside of the impression was produced using an epoxy resin (Araldite MY 753, hardener HY 956, Ciba-Geigy).

Standard histological procedures were followed (e.g., Schwartz et al., 2005). The molars were embedded in polyester resin to reduce the risk of splintering while sectioning. Using a diamond-wafering blade saw (Buehler® Isomet low speed), longitudinal sections between 180–200 μm were taken through the mesial cusp tips and dentine horns of each molar. A second section was made through the distal cusp tips. Each section was mounted on a microscope slide, lapped to 100–120 μm using a graded series of grinding pads (Buehler®), polished with a 0.3 μm aluminium oxide powder, placed in an ultrasonic bath to remove surface debris, dehydrated through a series of alcohol baths, cleared (using Histoclear®) and mounted with a cover slip using a xylene-based mounting medium (DPX®). Each section was examined using polarized light microscopy (Zeiss Axioskop 40), and an image was taken (Q-Imaging Micropublisher 5.0 RTU, 1999–2008). Aspects of enamel microstructure and thickness were recorded using image analysis software (Image-Pro Express, 1993–2005).

Daily rates of enamel secretion

Two methods were used to calculate daily secretion rates (DSR) in the appositional enamel. The first method was applied to the entire sample. Daily secretion rates were calculated by dividing the appositional enamel into three regions of equal thickness (inner, middle, and outer; see Fig. 3). Daily enamel secretion rates were measured along the long axis of

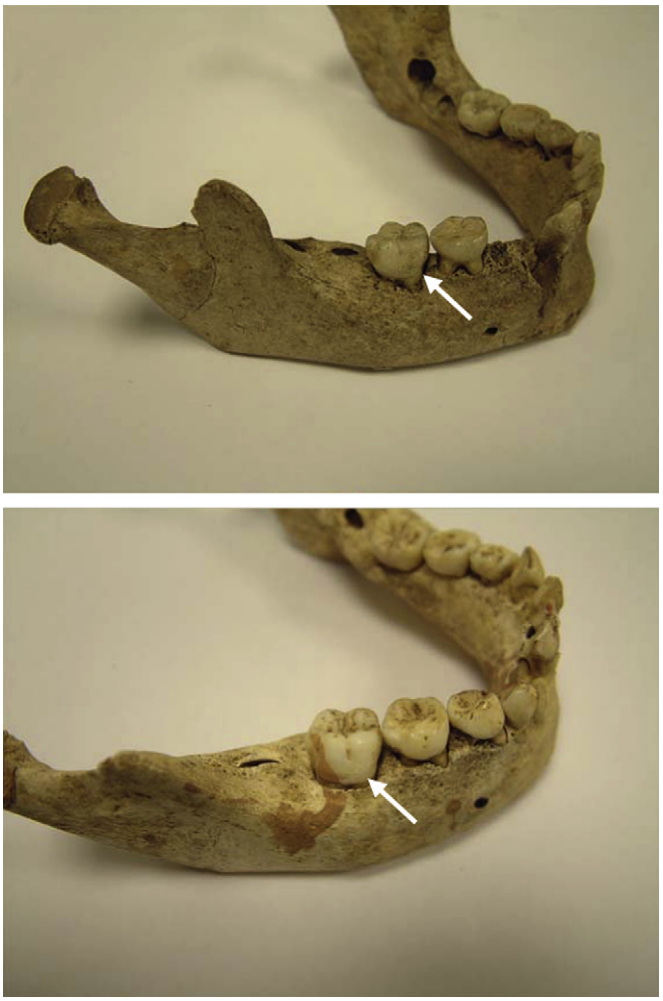

Fig. 2. View of erupted but unworn permanent M_1 (white arrows) of skeleton numbers 11 and 14.

an enamel prism at a magnification of 40× and 60×, around the center of each region. A distance corresponding to five days of enamel secretion was measured, and then divided by five to yield a mean daily rate. The procedure was repeated a minimum of six times in each region, which allowed a grand mean value and standard deviation (sd) to be calculated.

The second method used to calculate DSRs was applied to two cusps of one molar belonging to skeleton number 82. The hypoconid and entoconid appositional enamel was subdivided into regions, each measuring 60 microns (approximately three weeks of growth). Within each 60 micron region, five days of enamel secretion was measured along the long axis of a prism, and then divided by five to yield a daily rate within each region. The procedure was repeated a minimum of six times in and around the center of each region to produce a grand mean value and sd. These data were used to examine variation in DSRs at greater resolution for this one individual. The mesial cusps belonging to this skeleton were not suitable for this methodology because they did not show enamel prisms and cross-striations running over long distances.

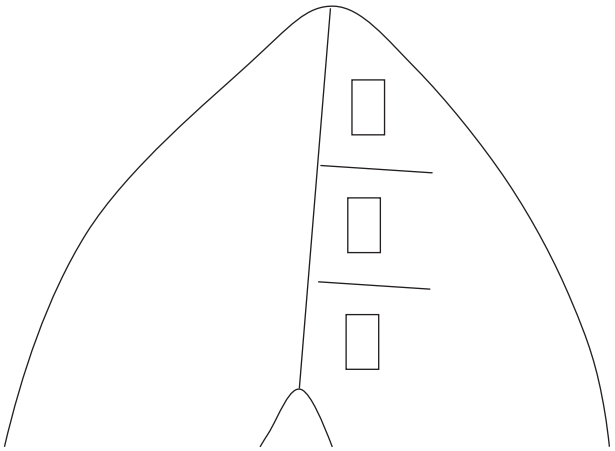

Fig. 3. Measuring appositional cross-striation spacing. The sketch is based upon Beynon et al., (1991b) and illustrates the appositional enamel, subdivided into three equal regions, producing, from bottom of figure, inner, mid, and outer zones. Measurements are taken in the center, and either side of center, in each region (indicated by the squares). A mean value and standard deviation are then calculated for each region (see description in Materials and methods).

Appositional formation times and enamel thickness

Appositional formation times were calculated using the following formula: ([{enamel thickness × correction factor}/mean daily rate of secretion]; Schwartz et al., 2003). A correction factor of 1.05 was used because decussation was not marked in this sample (e.g., Schwartz et al., 2003). Enamel thickness was measured from the tip of the dentine horn to the position of the first Retzius line at the tooth surface (measurement referred to as the cusp tip). The grand means for the DSR from each region (see above) were averaged to produce an overall mean DSR for the whole thickness of appositional enamel. The appositional enamel thickness was then divided by the overall mean DSR to yield the time taken to form the appositional enamel.

One linear regression equation was calculated from the data for enamel thickness and appositional formation times (four cusps combined). The upper and lower bounds of the 95% confidence interval were also calculated. Appositional enamel formation times calculated from enamel thickness/DSR (above) were compared to formation times estimated from the linear equation calculated in this study, and a polynomial equation taken from the literature (Dean et al., 2001).

Retzius line periodicity, imbricational, and cusp formation times

Retzius line periodicity is the number of days of enamel formation observed between two adjacent striae of Retzius. Periodicity was determined in this study by counting the number of cross-striations between adjacent Retzius lines in the imbricational enamel. Imbricational enamel formation time was calculated by multiplying the number of Retzius lines by the periodicity. Where adjacent Retzius lines were indistinct, enamel prism lengths were divided by the average

secretion rates in the region to estimate imbricational enamel formation times (for a description see Mahoney et al., 2007). The total enamel formation time in each cusp was calculated by summing the time taken to form the appositional and imbricational enamel. Appositional and imbricational formation times, as well as enamel thickness measurements for each cusp, are included in the tables so that others may reuse the data and construct their own analyses.

Prenatal enamel formation times

Prenatal enamel formation time was calculated by locating the position of the neonatal line. The enamel thickness between this line and the dentine horn was measured and divided by a local DSR, taken around the center of the prenatal enamel. The neonatal line is the first markedly accentuated line in the cusp enamel, though it is not always present.

Sequence of cusp growth

The sequence of cusp growth (initiation and completion) was determined by locating the position of the neonatal line in the protoconid, and the position of subsequent accentuated growth lines. The time elapsed between the neonatal line and each accentuated growth line was calculated by dividing the enamel thickness by local DSRs, thus determining a chronology of growth disturbances. The chronology of disturbances was then sought in and matched between the remaining cusps.

Total crown formation times

Once the growth sequence was established (above) the total CFT was calculated as the protoconid formation time summed with the period of additional and final growth that is recorded in the hypoconid (but which is missing from the protoconid).

Statistical procedures

Paired-samples t-tests were used to localize significant differences in DSRs between the inner, mid, and outer enamel within and between each cusp. This test was also used to identify differences in appositional enamel thickness, and appositional and imbricational formation times between cusps. This test assumes that the differences calculated for each pair have a normal distribution (Norusis, 1993), and this was checked with a one-sample Kolmogorov-Smirnov test. Pearson's correlation coefficient was used to measure the strength of the association between enamel thickness and appositional formation time.

Results

Daily enamel secretion rates

Average DSRs increased significantly from the inner to the outer enamel in each cusp (Tables 2a–2c). No significant differences occurred between the inner, mid, and outer enamel, when compared between cusps. Recalculating average DSRs subdivided by successive 60-μm regions for the hypoconid appositional enamel from skeleton number 82 shows a gradual increase from 2.51 μm to 3.80 μm over a period of 216 days (Table 3a; Fig. 4). Following this, the DSR dropped to 2.91 μm over 39 days. The DSR then gradually returned to 3.81 μm over 105 days. Recalculating average DSRs subdivided by 60 μm regions for the entoconid from skeleton number 82 shows an increase in DSR from 2.55 μm to 3.73 μm over 168 days (Table 3b). The DSR then dropped to 2.84 μm over 39 days. The DSR then gradually returned to 3.79 μm over 108 days.

Table 2a
Mean molar cusp daily enamel secretion rates (in μm per day)

	Inner	Mid	Outer
Mean	2.97	4.15	4.55
Min	2.38	3.36	3.64
Max	3.59	4.96	5.45
±1SD	0.51	0.56	0.61

Enamel thickness and appositional formation times

Buccal cusp tip enamel thickness was significantly greater than that of the lingual cusps (Tables 4a and 4b). The longer appositional formation times for the buccal cusps differed significantly from the shorter formation times for both the lingual cusps (Tables 5a and 5b). Correlations indicate a significant and strong positive association between appositional enamel thickness and formation time when the data for the four cusps

Table 2b
Mean molar cusp daily enamel secretion rates by cusp (in μm per day)

Cusp	Min	Max	Mean	±1SD
Prd[a]				
Inner (13)	2.43	3.64	3.08	0.55
Mid (15)	3.44	4.97	4.14	0.52
Outer (15)	3.91	5.90	4.60	0.69
Med[b]				
Inner (13)	2.33	3.78	3.01	0.48
Mid (13)	3.23	5.16	4.26	0.54
Outer (13)	3.56	5.58	4.61	0.64
Hyd[c]				
Inner (15)	2.24	3.67	2.86	0.51
Mid (15)	3.37	4.89	4.13	0.62
Outer (14)	3.47	5.12	4.41	0.50
End[d]				
Inner (12)	2.55	3.29	2.93	0.47
Mid (14)	3.40	4.83	4.09	0.54
Outer (14)	3.63	5.23	4.59	0.67

Pre-natal DSR = 2.04 μm ± 0.33 (11).
[a] Protoconid
[b] Metaconid
[c] Hypoconid
[d] Entoconid

Table 2c
Comparing daily enamel secretion rates within cusps

Cusp[a]	Inner vs. Middle			Inner vs. Outer			Middle vs. Outer		
	t	df	p	t	df	p	t	df	p
Prd	−7.518	12	0.000*	−7.307	12	0.000*	−4.249	14	0.001*
Med	−5.523	12	0.000*	−6.482	12	0.000*	−3.830	12	0.003*
Hyd	−6.691	14	0.000*	−7.279	13	0.000*	−1.421	13	0.179
End	−5.474	11	0.002*	−3.850	11	0.012*	−3.573	13	0.007*

* = Significant difference; p values of 0.000 are rounded and indicate at least $p < 0.0001$.
[a] Abbreviations the same as in Table 2b.

is combined (Pearson's $r = 0.820$; $p = 0.0001$). When subdivided by cusps the association is still strong for the protoconid (Pearson's $r = 0.793$; $p = 0.001$), and entoconid (Pearson's $r = 0.829$; $p \leq 0.0001$), and weaker but still significant for the metaconid (Pearson's $r = 0.746$; $p = 0.003$) and hypoconid (Pearson's $r = 0.699$; $p = 0.004$).

One linear regression equation was calculated from the data for the four cusps, where y is the number of days of enamel formation, and x is enamel thickness in μm:

$$y = 58.292 + (0.226x).$$

The upper and lower bounds of the 95% confidence interval are:

$$\text{lower bound} : y = 2.160 + (0.183x)$$

$$\text{upper bound} : y = 114.424 + (0.269x)$$

Retzius line periodicity, imbricational, and cusp formation times.

Periodicity was recorded in more than one cusp for ten individuals. No variation occurred between the cusps of these individuals (Table 6). Peridocity varied between individuals, ranging between 6 to 9 days, with a mode of 7. The longer imbricational formation times for the buccal cusps differed significantly from the shorter formation times for the lingual cusps (Tables 5a and 5b). A significant difference also occurred between the imbricational formation times of the metaconid and entoconid. Summing the appositional and imbricational formation times gave a mean cusp formation time of 1,062 days (2.91 yrs) for the protoconid, 1,082 days (2.96 yrs) for the hypoconid, 919 days (2.52 yrs) for the metaconid, and 868 days (2.38 yrs) for the entoconid (Table 5a).

Prenatal growth, sequence of cusp growth, total CFT

Prenatal enamel growth was initiated in the protoconid between 10 to 48 days before birth in eleven individuals (Table 5a). The sequence of cusp initiation and completion was recorded for five of these individuals (Fig. 5A–E). For these five individuals, no evidence was found for prenatal enamel formation in any other cusp. Following the protoconid, growth initiated after birth in either the hypoconid (min = 7 days; max = 63 days) or the metaconid (min = 27 days; max = 43 days). The entoconid was consistently the last cusp to initiate (min = 58 days; max = 93 days). The hypoconid was consistently the last cusp to complete formation in each individual crown (min = 950 days; max = 1,182 days). The sequence of cusp completion varied between the entoconid (min = 874 days; max = 1,086 days), the metaconid (min = 813 days;

Table 3a
Enamel secretion rates in the hypoconid cuspal enamel of skeleton no. 82[a]

Inner enamel			Mid enamel			Outer enamel		
ED[b] (μm)	DSR[c]	DPR[d]	ED	DSR	DPR	ED	DSR	DPR
60	2.51 ± 0.03	24	480	3.60 ± 0.13	17	900	3.01 ± 0.26	20
120	2.73 ± 0.13	22	540	3.71 ± 0.14	16	960	3.31 ± 0.20	18
180	3.10 ± 0.11	19	600	3.79 ± 0.08	16	1020	3.49 ± 0.11	17
240	3.33 ± 0.16	18	660	3.75 ± 0.24	16	1080	3.61 ± 0.37	17
300	3.37 ± 0.19	18	720	3.80 ± 0.23	16	1140	3.63 ± 0.33	17
360	3.49 ± 0.22	17	780	3.41 ± 0.20	18	1200	3.81 ± 0.21	16
420	3.53 ± 0.13	17	840	2.91 ± 0.21	21	1260	3.80 ± 0.43	16
	3.15 ± 0.14[e]	135[f]		3.56 ± 0.18	120		3.52 ± 0.28	121

[a] The data in Table 3 was derived from prism lengths and DSRs, for successive 60 μm regions, to give an estimate of 376 days for appositional formation time. Recalculating the data in Table 3 using the formula [(enamel thickness × correction factor)/mean daily rate of secretion]; Schwartz et al., 2003; also see description in the methodology] gives a similar estimate of 388 days.
[b] Enamel depth.
[c] Mean daily rate of enamel secretion for each 60 μm region of enamel ± 1SD.
[d] Days per region = number of days taken to form each 60 μm region of enamel (enamel depth/DSR).
[e] Mean daily secretion rate for the inner enamel ± 1SD.
[f] Number of days taken to form the inner enamel.

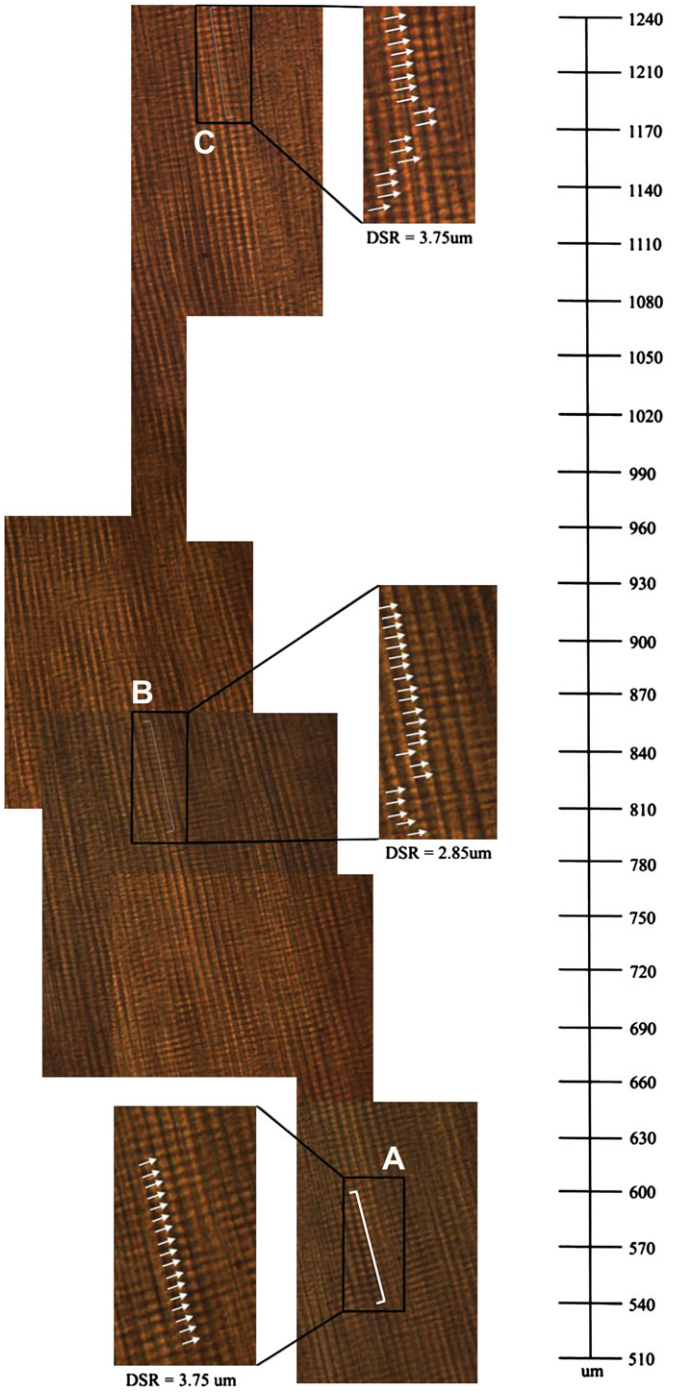

Fig. 4. Daily cross-striations from skeleton number 82. The figure is a continuous 730 μm sequence of hypoconid enamel (from 510 μm to1, 240 μm, not including the outermost 20 μm of enamel) from before, during, and after the region of reduced DSRs. It is created from a montage of adjacent and overlapping images taken at a magnification of 40×, with inserts (A, B, C) at 60×. A. At a distance of 540–600 μm from the dentine horn it took 16 days to form a 60 μm region of enamel (DSR = 3.75 μm). B. At a distance of 800–860 μm from the dentine horn, in the region where there is a reduction in DSRs, it took 21 days to form a 60 μm region of enamel (DSR = 2.85 μm). C. At a distance of 1,180–1,240 μm from the dentine horn it took 16 days to form a 60 μm region of enamel (DSR = 3.75 μm).

max = 1,096 days), and the protoconid (min = 885 days; max = 1,076 days). No single cusp recorded the total CFT.

Total CFT was recorded for the same five individuals. When the period of additional enamel growth recorded in the hypoconid (mean = 88 days; min = 52 days; max = 161 days), was summed with the protoconid formation time (mean = 1,034 days; min = 920 days; max = 1,106 days), it gave a mean total CFT of 1,121 days. For these five individuals, the mean cusp formation times represented 95% (hypoconid), 92% (protoconid), 83% (metaconid), and 77% (entoconid) of the mean total CFT.

Discussion

Enamel thickness

The thicker enamel over the buccal cusp tips compared to the lingual cusps is consistent with previous findings and with established ideas about the relationship between enamel thickness and mechanical function during the chewing cycle. This has also been noted in *Pan*, where thicker buccal enamel also takes longer to form (Reid et al., 1998b). The correlation between formation time and enamel thickness in this study supports this idea because a longer period of development is required for the thicker enamel of the buccal cusps. Initiation and completion of molar crown growth in these cusps would also facilitate a longer period of development.

There was no trend in molar enamel thickness in the distal cusps with respect to the mesial cusps, which is consistent with the few studies that have compared these locations (see Shillingburg and Grace 1973 and Smith et al., 2006). However, this finding may reflect the location of the linear measurement through the cusp tip. Maximum enamel thickness measurements have, for example, been shown to differ between the lateral surface of mesial and distal cusps in M_1s (Kono et al., 2002). Given that enamel thickness also differs between the lateral surfaces of mesial cusps in mandibular (Grine, 2005; Suwa and Kono, 2005) and maxillary molars (Macho and Berner, 1993, 1994; Schwartz, 2000b), it seems likely that this latter location, rather than the cusp tip, may show a more consistent trend.

Growth sequence: enamel initiation and completion

The sequence of cusp initiation in the protoconid between one and seven weeks before birth and final enamel completion of the crown in the hypoconid reflects the findings of a previous histological study of one human molar (Reid et al., 1998a). The status of calcification at birth is, however, quite variable and can include from one to four lower first molar cusps (Christensen and Kraus, 1965). The variability in cusp initiation between the hypoconid and metaconid seen in this study has also been reported in a study of five human molars (Antoine, 2001). Histological studies report a similar period of prenatal enamel growth in M_1 from *Pan* ($n = 8$; 10–3 weeks), *Gorilla* ($n = 1$; 2 weeks), *Hylobates* ($n = 1$; at least 16 days), and *Pongo* ($n = 1$; 12–16 days; Beynon et al.,

Table 3b
Enamel secretion rates in the entoconid cuspal enamel of skeleton no. 82[a,b]

Inner Enamel			Mid Enamel			Outer Enamel		
ED (μm)	DSR	DPR	ED	DSR	DPR	ED	DSR	DPR
60	2.55 ± 0.04	24	480	3.69 ± 0.10	16	900	3.41 ± 0.09	18
120	2.62 ± 0.09	23	540	3.73 ± 0.10	16	960	3.63 ± 0.18	17
180	3.13 ± 0.29	19	600	3.27 ± 0.11	18	1020	3.79 ± 0.20	16
240	3.31 ± 0.23	18	660	2.84 ± 0.11	21	1080	3.87 ± 0.17	15
300	3.40 ± 0.12	18	720	3.05 ± 0.07	20	1140	3.95 ± 0.17	15
360	3.51 ± 0.10	17	780	3.22 ± 0.28	19			
420	3.52 ± 0.23	17	840	3.37 ± 0.19	18			
	3.15 ± 0.16	136		3.31 ± 0.14	128		3.73 ± 0.16	81

[a] The CFT for the entoconid in Table 5a, includes an additional three days as an estimate of the formation time for the final 11 μm of enamel thickness (i.e., total enamel thickness = 1,151 μm).

[b] Accentuated markings were not present in the hypoconid and entoconid so the sequence of cusp growth could not be calibrated and registered between the cusps (see Materials and methods). However, the timing of the reduced DSRs in the mid enamel of both the hypoconid and entocond makes sense in view of what is known about the sequence of cusp growth and difference in the timing of cusp initiation for this sample, assuming that the reduced DSRs in the mid enamel of both cusps is a response to the same event. For example, if the greatest reduction in DSRs in the mid enamel of both cusps is used as a marker, then this occurred between a minimum and maximum enamel depth of 601 μm (186 days) and 660 μm (207 days) in the entoconid. The greatest reduction in the mid enamel of the hypoconid occurred at a minimum and maximum enamel depth of 781 μm (234 days) and 840 μm (255 days), respectively (Table 3a.). If the maximum value for the entoconid is subtracted from the minimum value for the hypoconid this gives 27 days, which is the minimum number of days that the hypoconid could have recorded the reduction in DSRs before the entoconid. If the minimum value for the entoconid is subtracted from the maximum for the hypoconid this gives 69 days, which is the maximum number of days that the hypoconid could have recorded the reduction in DSRs before the entoconid. These values (27–69 days) lie close to the difference in the timing of the hypoconid and entoconid cusp initiation recorded for five individuals in this sample (30–64 days; Table 5a; Fig. 5A–E).

1991a; Dirks, 1998; Schwartz et al., 2006; Smith et al., 2007a). While few have reported prenatal enamel formation times for fossil hominoids, those who have suggest an equivalent period of growth in M_1s (Mahoney et al., 2007). The length of the prenatal growth period in hominoids contrasts with the advanced state of prenatal calcification seen in some prosimians, which along with other aspects of dental development, has been linked to early weaning and a largely folivorous diet (Schwartz et al., 2005, 2007).

Table 4a
Linear appositional enamel thickness (in μm)

Cusp[a]				
No.	Prd	Med	Hyd	End
11	1240	1144	1297	963
14	1434	1212	1260	947
31	1255	1268	1200	1079
36	1534	1294	1300	1066
48	1465	1271	1506	1428
57	1355	1196	1579	1300
59	1696	1271	1386	1493
60	1006	1035	1193	1025
75	1548	1093	1609	1311
80	1231	1093	1297	1216
82	1179		1260	1151
87	1691	1487	1562	1309
100	1451	1377	1350	1303
105	1593	1255	1352	1155
116	1271		1270	1301
Mean	1397	1230	1361	1203
Min	1006	1035	1193	947
Max	1696	1487	1609	1493
±1SD	199	123	138	165

Average pre-natal protoconid enamel thickness = 56 μm.
[a] Abbreviations the same as in Table 2b.

Cusp and total crown formation times

The difference in formation time between each of the mesial cusps compares well with previous findings for modern humans (Reid et al., 1998a; Reid and Dean, 2006), and *Gorilla* (Schwartz et al., 2006), while the difference in formation time between the distal cusps has only previously been reported for *Pan* (Reid et al., 1998b; Smith et al., 2007a). Like *Pan*, no single cusp represented the total period of crown formation, though the buccal cusps represented a greater percentage of the total CFT (95–92%) compared to the lingual cusps (83–77%). The additional and final enamel growth period recorded in the hypoconid compared to the protoconid was equal to 88 days (mean value). The variation in cusp initiation times and cusp enamel formation times recorded in this study indicates that comparisons between modern human and both living

Table 4b
Comparing appositional enamel thickness

Cusp[a]	t	df	p
Prd vs. med	4.662	12	0.001*
Prd vs. hyd	0.856	14	0.406
Prd vs. end	4.106	14	0.001*
Med vs. hyd	−3.229	12	0.007*
Med vs. end	0.631	12	0.540
Hyd vs. end	4.681	14	0.000*

* = Significant difference.
[a] Abbreviations the same as in Table 2b.

Table 5a
Formation times for lower first molar cusps in days

No.	Cusp[a]							
	Prd		Med		Hyd		End	
	Apo[b]	Imb[c]	Apo	Imb	Apo	Imb	Apo	Imb
11	378 (−18)	640	334	524	403	681	277	497
14	405 (−27)	686	329 (42)	637	357 (25)	734	252 (88)	580
31	391	685	398	588	358	803	334	630
36	383 (−21)	566	347 (43)	558	351 (63)	675	281 (93)	548
48	369 (−30)	715	313	610	400	717	348	477
57	380 (−27)	723	321 (32)	631	474 (7)	701	365 (71)	650
59	449 (−48)	744	321	693	363	728	412	588
60	259 (−35)	661	296 (43)	474	341 (27)	582	277 (86)	511
75	343 (−10)	745	245	595	378	713	296	447
80	310	701	288	526	356	651	319	462
82[d]	336 (−15)	777	–	–	376	773	348	692
87	464	667	398	602	436	710	370	553
100	350 (−35)	756	336 (27)	733	334 (8)	783	334 (58)	516
105	405	675	343	503	356	706	319	455
116	365 (−32)	602	–	–	358	638	385	491
Mean	372	690	328	590	376	706	328	540
Cusp formation[e]	1,062 (2.91 yrs)		919 (2.52 yrs)		1,082 (2.96 yrs)		867 (2.38 yrs)	
Min	920 (2.52 yrs)		770 (2.11 yrs)		923 (2.53 yrs)		743 (2.04 yrs)	
Max	1,193 (3.27 yrs)		1,069 (2.93 yrs)		1,175 (3.22 yrs)		1,026 (2.81 yrs)	
±1 SD	74 (0.20 yrs)		88 (0.24 yrs)		69 (0.19 yrs)		95 (0.26 yrs)	
Prenatal formation	−27 (0.07 yrs)							
Total crown formation[f]	1,121 (3.07 yrs)							

[a] Abbreviations the same as in Table 2b.
[b] Appositional enamel formation time. Prenatal enamel growth is given for the protoconid (in brackets) for eleven individuals. A prenatal DSR could not be recorded in four individuals. The subsequent postnatal initial calcification of the metaconid, hypoconid, and entoconid, is also given (in brackets) for five individuals. For example, no. 14 showed 27 days of prenatal enamel growth in the protoconid. Following this, growth began in the hypoconid 25 days after birth.
[c] Imbricational enamel formation time.
[d] A correction factor was not used when calculating appositional formation times for no. 82 because of the absence of decussation (also see Fig. 4). The hypoconid and entoconid appositional formation time was taken from Table 3a–b.
[e] Appositional and imbricational times summed giving a mean formation time.
[f] Mean total molar crown formation is calculated for five individuals (nos. 14, 36, 57, 60, and 100; see Materials and methods and Fig. 5A–E), using data from Table 5a, giving a total crown formation time for these individuals, respectively, of 1,143 days (protoconid = 1,091 days summed with the additional and final growth in the hypoconid compared to the protoconid = 52 days), 1,110 days (protoconid = 949 days + additional hypoconid growth = 161 days), 1,209 days (protoconid = 1,103 days + additional hypoconid growth 106 days), 985 days (protoconid = 920 days + additional hypoconid growth 65 days), and 1,160 days (protoconid = 1,106 days + additional hypoconid growth = 54 days).

and fossil nonhuman primate species need to be made in a cusp-specific and molar tooth-type-specific way.

Differences between mesial cusp formation times in lower molars have been reported for several Miocene hominoids. Longer protoconid formation times compared to the metaconid have been reported for an M_1 from *Sivapithecus parvada* (Mahoney et al., 2007), second molars from *Afropithecus turkanensis* and *Dryopithecus laietanus* (Kelley et al., 2001;

Table 5b
Comparing lower first molar cusp formation times

Cusp[a]	Apo			Imb		
	t	df	p	t	df	p
Prd vs. med	3.941	12	0.002*	6.059	12	0.000*
Prd vs. hyd	−0.270	14	0.791	−1.204	14	0.249
Prd vs. end	3.277	14	0.006*	7.153	14	0.000*
Med vs. hyd	−3.298	12	0.006*	−8.018	12	0.000*
Med vs. end	0.444	12	0.665	2.767	12	0.017*
Hyd vs. end	3.802	14	0.002*	9.390	14	0.000*

* = Significant difference.
[a] Abbreviations the same as in Table 2b.

Table 6
Retzius line periodicity (in days)[a]

No.	Prd	Med	Hyd	End
11		8		8
14	9			9
31		8	8	
36	7			
48	6			6
57	7	7		
59		8		
60	6			
75	8	8		
80	7		7	
82			9	9
87	7			
100		9		
105		7	7	
116	9		9	9
Mode = 7	Min = 6	Max = 9		

[a] Periodicity was recorded in the imbricational enamel avoiding the cervical region. Abbreviations the same as in Table 2b.

Smith et al., 2003), and third molars from *Gigantopithecus blacki* and *Graecopithecus freybergi* (Dean and Schrenk, 2003; Smith et al., 2004). This variation, together with thicker enamel (*Graecopithecus*, *Sivapithecus*, *Afropithecus*) and earlier cusp initiation (*Graecopithecus*, *Gigantopithecus*) in the protoconid compared to the metaconid, suggests that some of the fossil species may display a relationship between rates of enamel formation and enamel thickness that may ultimately reflect the functional mechanics of the chewing cycle.

The mean protoconid formation time recorded for other contemporary northern European populations (3.25 yrs; Reid and Dean, 2006) is more than the mean protoconid formation time calculated for the M_1s here (2.91 yrs; Table 5a). The difference in mean formation time appears mainly to be due to the slightly thinner appositional enamel in the present study (mean = 1,397 μm; Table 4a), compared to the contemporary European sample (mean = 1,573 μm) of Reid and Dean (2006) that formed in a slightly shorter period of time (1.02 yrs and 1.27 yrs, respectively).

The range of protoconid formation times recorded for the M_1s overlaps only at the upper end of the range of M_1 protoconid formation times recorded for *Pan* (2.01–2.61 yrs), and

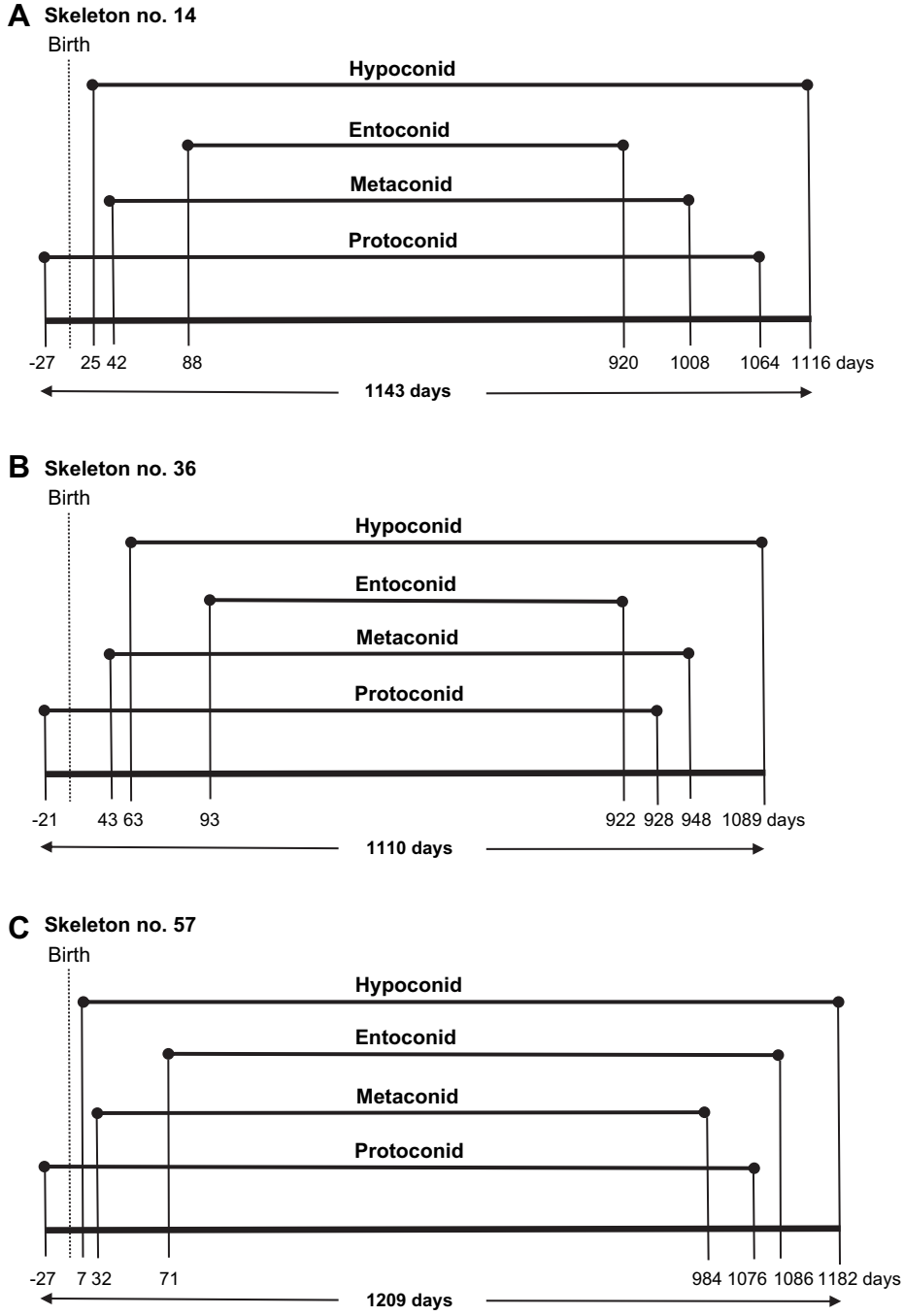

Fig. 5. A–E. Lower first molar total crown formation time and sequence of cuspal growth (data taken from Table 5a.). The figures show the initiation and completion of cusp growth, and total formation time (all in days) for five individuals: skeleton numbers 14 (A), 36 (B), 57 (C), 60 (D), and 100 (E).

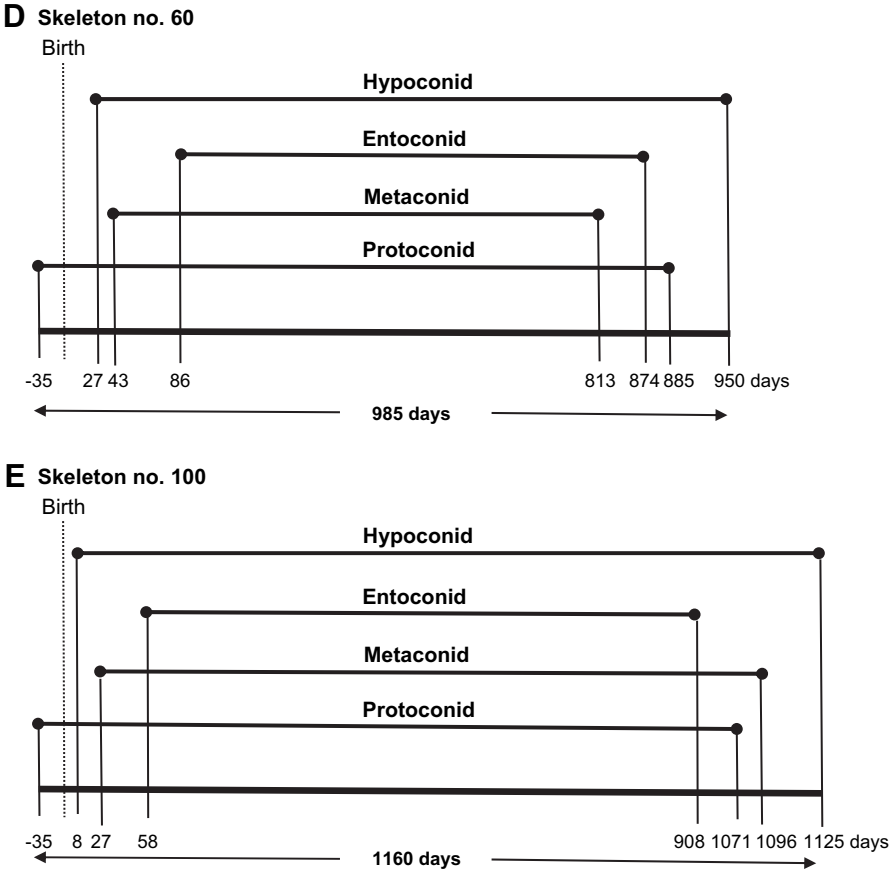

Fig. 5. (*continued*).

are above the known values recorded for M_1 in *Gorilla* (2.31 yrs), as well as the fossil hominoids *Proconsul heseloni* (1.20 yrs), *Lufengpithecus hudienensis* (2.11–2.18 yrs), and *Sivapithecus parvada* (2.40 yrs; Beynon et al., 1998; Schwartz et al., 2003, 2006; Mahoney et al., 2007; Smith et al., 2007a). The longer formation times in the human sample compared to some nonhuman primates might reflect a delay in other aspects of dental development, such as molar eruption times (e.g., Smith et al., 1994). When compared to fossil hominoids, the longer formation time, together with a slower rate of enamel secretion during the early stages of cusp growth (Mahoney et al., 2007), suggests differences in enamel growth that might reflect the extended growth period seen in modern humans (see Dean et al., 2001). It has been demonstrated previously that first molar eruption and life history schedules are correlated (e.g., Smith, 1989; Smith and Tompkins, 1995), and it now seems increasingly likely that other aspects of the timing and sequence of molar development, even at a histological level, might also correlate tightly with life history schedules, although this remains to be fully evaluated (see Macho, 2001; Kelley and Smith, 2003; Dean, 2006; Smith et al., 2007b).

Appositional enamel protoconid formation times calculated from enamel thickness/DSR for the M_1s in Table 5a were compared to formation times derived from the linear regression equation (this study) and a polynomial equation

Table 7
Comparing estimates of CFT for the protoconid

ET/DSR[a]	Linear equation[b]				Polynomial equation[c]			
CFT	Lower CI[d]	CFT	Upper CI[e]	R[f]	Lower CI	CFT	Upper CI	R
378	222	339	448	−40	340	383	427	+5
405	265	382	500	−23	376	428	480	+23
391	232	342	452	−50	343	387	432	−4
383	283	405	527	+22	393	448	505	+55
369	270	389	509	+20	381	434	488	+65
380	250	366	479	−16	362	410	459	+30
449	313	442	571	−7	417	481	545	+32
259	186	286	385	+26	290	325	360	+66
343	285	408	531	+65	395	451	509	+108
310	227	336	446	+26	338	381	424	+71
336	218	325	432	−12	327	369	410	+41
464	312	440	569	+24	416	480	544	+16
350	268	386	505	+36	379	431	484	+81
405	294	418	543	+13	402	461	520	+56
365	235	346	456	−20	346	391	436	+26

[a] (Enamel thickness × correction factor)/mean daily rate of enamel secretion = CFT. Taken from Table 5a.
[b] This study.
[c] Dean et al. (2001).
[d] Lower bound of 95% confidence interval for linear equation.
[e] Upper bound of 95% confidence interval for linear equation.
[f] Residual.

taken from Dean et al. (2001). The linear equation either over or under-estimated the formation time by an average of 26 days (Table 7). The polynomial equation differed by an average of 45 days. The polynomial equation almost always overestimated the formation times for this sample, and it was always higher than the estimate derived from the linear equation. This difference between the equations is emphasized by the plot of the formation times in Fig. 6. The estimates derived from the linear equation lie on the lower bound of the confidence interval for the polynomial equation. There is also a difference in the confidence intervals derived from each equation. The intervals for the linear equations encompass much lower and higher formation times compared to the intervals for the polynomial formula. Therefore, both equations give similar estimates of formation for this sample, although the confidence intervals for the linear equation produced a much broader range of values than the polynomial formula. Overall, given the use of the predictive aspect of each equation in the literature (i.e., CFT from enamel thickness), the comparison in this study suggests that either may be used to predict a CFT, but the estimates derived from the upper and lower confidence intervals for the linear equation are probably too broad to be practically useful.

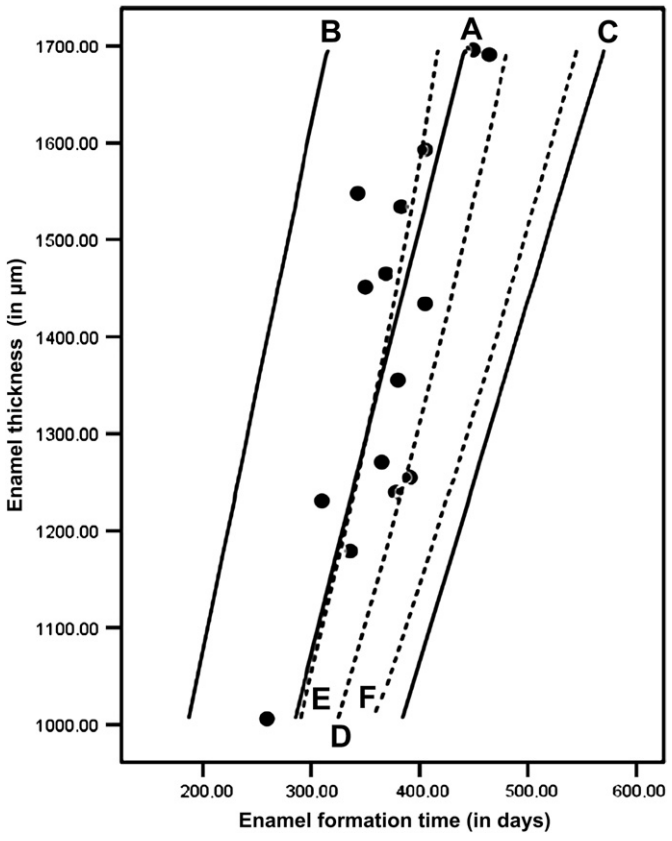

Fig. 6. Linear and polynomial regression estimates of CFT. The 95% confidence interval bands are the boundaries of all possible straight lines, not the recorded values. You can be 95% confident that the two confidence bands enclose the true best-fit linear regression line, leaving a 5% chance that the true line is outside those boundaries. Recorded values can still lie outside of these lines. A: linear regression equation estimate of CFT from enamel thickness. B: lower bound of 95% confidence interval. C: upper bound of 95% confidence interval. D: polynomial regression equation estimate of CFT from enamel thickness. E: lower bound of 95% confidence interval F: upper bound of 95% confidence interval. Black circle: protoconid enamel thickness and CFT taken from Tables 4 and 5a.

Daily secretion rates

The significant increase in cross-striation spacing from the inner to the outer enamel follows the clear pattern reported for extant and extinct hominoids (e.g., Mahoney et al., 2007). Overall, growth rates were slightly faster in the lower first molar inner enamel, and slightly slower in the outer enamel compared to other modern human samples, although each value still lies within the range of these previous studies (Table 8). These slight differences could be an artifact of the recording method used here, where measurements from around the center of the inner, mid, and outer enamel regions contributed to the grand mean values for each (Fig. 3). These would differ from other studies, for example, if the mean value was calculated directly adjacent to the EDJ or at the inner most region of the mid enamel.

The duration of the sharp reduction and slow recovery of DSRs seen in the M_1 belonging to skeleton 82 is almost identical in the hypoconid and entoconid. However, this trajectory occurs at different times during the growth of each cusp. For instance, the maximum reduction in secretion rates occurs in the hypoconid between 27 to 69 days before it occurs in the entoconid (see Table 3b footnote for calculations). This difference between the cusps makes sense in view of what is known about the sequence of cusp growth for five individuals in this sample. For these five, the hypoconid initiated between 30 to 64 days before the entoconid (Table 5a; Fig. 5A–E). Therefore, the difference in the timing, as well as the similarity in the duration of the anomalous enamel trajectory in the two cusps from skeleton 82 suggests that this may have been a response to the same event.

The sharp reduction and slow recovery of DSRs seen in the two M_1 cusps belonging to skeleton 82 does not follow the trend reported for modern humans, nor the trajectory recorded

Table 8
Daily enamel secretion rates in extant hominoids (in µm per day ± 1 SD)

Species	s*	*n*	Inner	Middle	Outer
Homo sapiens	a	1	2.66 ± 0.15	3.44 ± 0.25	5.50 ± 0.85
	b	11–15	2.7 ± 0.4	4.3 ± 0.5	5.1 ± 0.7
	c	10	2.80 ± 0.43	4.50 ± 0.55	5.20 ± 0.58
	d	15	2.97 ± 0.51	4.15 ± 0.56	4.55 ± 0.61
Pan troglodytes	e	73	3.62 ± 0.42	4.24 ± 0.50	4,62 ± 0.49
	f	3	3.92 ± 0.28	4.46 ± 0.44	4.72 ± 0.34
Gorilla gorilla	g	1	3.37 ± 0.49	5.37 ± 0.08	5.47 ± 0.08

*Sources are: a = Dean (1998). Data for M_2 divided into inner, mid, and outer regions, with an average and standard deviation calculated for each region. Overall mean from Dean (1998); b = Beynon et al. (1991b); c = Lacruz and Bromage (2006); d = this study (Table 2a); e = Smith et al. (2007a). Data for M_1–M_3 combined; f = Reid et al. (1998a). Mean DSR and sd. calculated for the occlusal enamel from the four M_1 cusps; g = Schwartz et al. (2006).

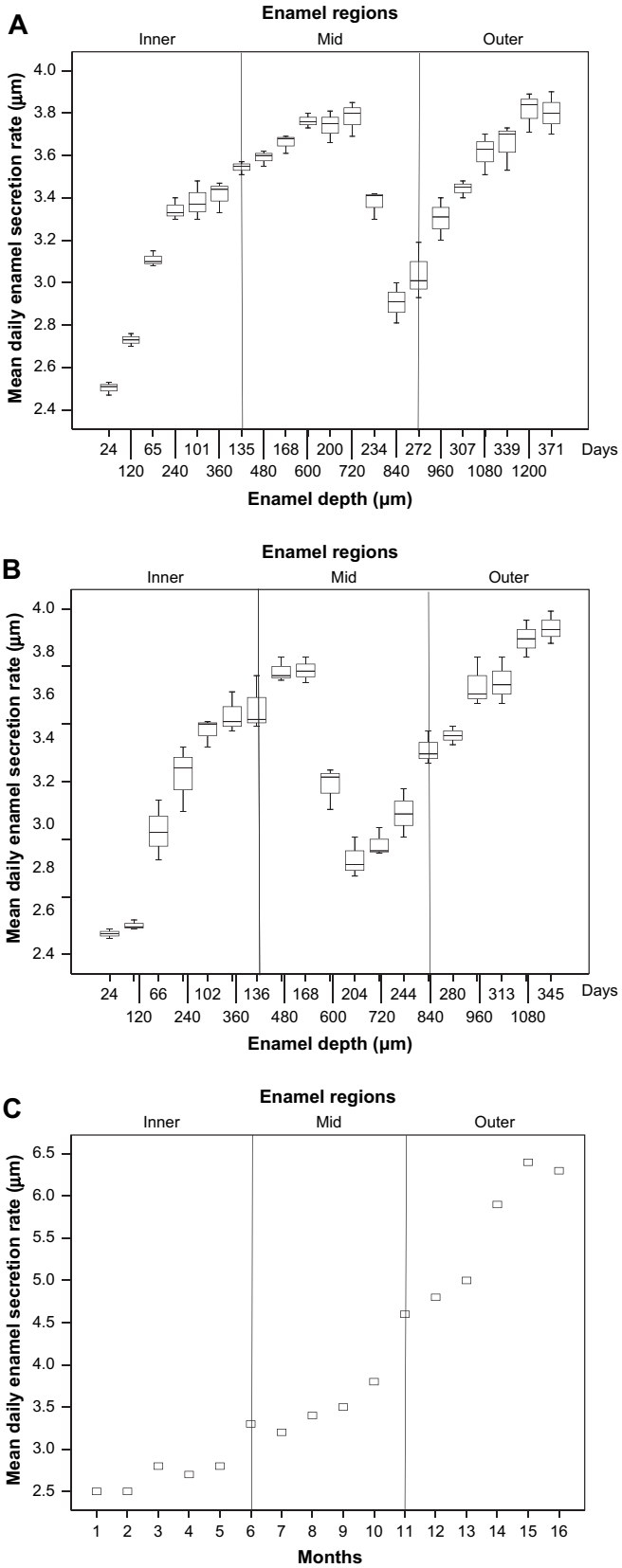

Fig. 7. Mean enamel secretion rates (Data taken from Table 3a.) in the hypoconid cuspal enamel of skeleton number 82. The pattern of enamel secretion in the M_1 (this study) differs from the results for an M_2 (Dean, 1998; see Fig. 7c). There was a sharp decrease in the rate of enamel secretion approximately half way through the period of cuspal growth in the M_1, which was not seen in the M_2. Instead, the M_2 showed a progressive increase in the rate of enamel formation throughout the course of cuspal growth. The anomalous trajectory seen in the M_1 is discussed in the text. B. Mean enamel secretion rates (Data taken from Table 3b.) in the entoconid cuspal enamel of skeleton number 82. C. Mean enamel secretion rates (Data taken from Dean (1998: his Table 1)) in a human second molar.

in a second molar (Dean, 1998; also see Fig. 7A–C). Nor is it likely that the reduction in DSRs in the M_1 reflects intradian increments of 8 or 12 hours, rather than a circadian 24 hour increment, because they are not half those measured previously. Instead, the trajectory of enamel growth reported here resembles the trajectory described for modern human and Neandertal deciduous molars, where a reduction in DSRs across the neonatal line was followed by a slow return to maximum rates of secretion (Macchiarelli et al., 2006). A reduction in the amount of secreted enamel matrix (inferred from a reduction in spacing between Retzius lines) has also been shown to correspond to a surface hypoplasia in wild boar and domestic pigs (Witzel, et al., 2006), while a recovery in enamel secretion after a systemic insult (increased fluoride) has been documented for roe and red deer (Kierdorf and Kierdorf, 1997). Like these studies, the M_1 in this study may also have retained a record of a systemic event that produced the reduction in enamel secretion in response to, for example, the type of juvenile illness that corresponds to some types of hypoplasia (e.g., Eliot et al., 1934; Pindborg, 1982). The subsequent return to normal rates of matrix secretion over a period of 15 weeks could then be described as a type of 'catch-up growth', which is commonly seen in nondental skeletal and somatic growth (Prader et al., 1963). 'Catch-up growth' occurs often after periods of juvenile illness (e.g., Osborne and Mendel, 1916; Williams et al., 1974; Lee and Myers, 1979; Williams, 1981).

Unlike the deciduous teeth described by Macchiarelli et al. (2006), the sudden reduction and then gradual recovery in matrix secretion in the M_1 cusps described in this study was not associated with an accentuated marking (which, in their case, was the neonatal line) visible in polarized transmitted light microscopy. Therefore, it may be that this method of recording the rate of enamel formation through the entire cuspal enamel is one that is able to distinguish between events that are either associated with accentuated markings, and other events that may effect secretion rates but leave no accentuated marking. Clearly some enamel defects are more marked than others, even within the same tooth (compare accentuations in Fig. 1, box a–c).

Summary and conclusion

When compared to other extant and fossil hominoids, the modern human sample studied here showed both similarities and differences in cusp growth. The relationship between enamel formation rates and enamel thickness in the lower first permanent molars resembled that reported in previous studies of fossil hominoids and followed expectations that fit with what is known about the mechanics of chewing. All of the hominoids for which there is comparable data shared similar prenatal enamel formation times to those calculated for modern human M_1s in this study. In contrast to all fossil species (except Neandertals) where similar data has been published, this modern human sample, like others, had longer cusp formation times and a slower rate of enamel secretion, leading to a longer total crown formation time. These differences may reflect the extended growth period seen in modern humans. With this in mind, the considerable variation observed in enamel formation times between molar cusps indicates that inter- and intraspecific comparisons should be restricted to identical cusp types. Otherwise, variation that exists between individual cusp formation times is liable to be misinterpreted in an evolutionary context. The consistency in periodicity and DSRs when compared between the cusps of each molar indicates that either may be calculated for comparative purpose from any of the four first molar cusps.

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