4. New perspectives on chimpanzee and human molar crown development

T.M. SMITH
Human Evolution Department, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig Germany
tsmith@eva.mpg.de

D.J. REID
Department of Oral Biology, School of Dental Sciences Newcastle University, Framlington Place Newcastle upon Tyne NE2 4BW, U.K.
D.J.Reid@newcastle.ac.uk

M.C. DEAN
Department of Anatomy and Developmental Biology University College London Gower Street, London WC1E 6BT, U.K.
ucgacrd@ucl.ac.uk

A.J. OLEJNICZAK
Human Evolution Department, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig Germany
olejniczak@eva.mpg.de

R.J. FERRELL
Assistant Professor Department of Sociology & Anthropology Howard University 2441 6th St. NW Washington, DC 20059
rferrell@howard.edu

L.B. MARTIN
Departments of Anthropology and of Anatomical Sciences Stony Brook University Stony Brook, NY 11794-4364 U.S.A.
lawrence.martin@stonybrook.edu

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Abstract

Previous histological studies of small samples of chimpanzee and human molars suggested similarities in crown formation time, which is surprising given substantial life history differences. As part of an on-going study of hominoid molar development, we report on the largest-known sample of chimpanzee and human molars, including re-evaluation of previously examined histological sections. Variation of incremental features within and between genera is examined, including Retzius line periodicity, daily secretion rate, and Retzius line number. Differences due to population-level variation and sexual dimorphism are also considered. Significant increasing trends in daily secretion rates were found from inner to outer cuspal enamel, ranging from approximately 3–5 microns/day in chimpanzees. Humans demonstrate slightly lower and higher mean values at the beginning and end of cuspal formation, respectively, but both genera show an overall average of approximately 4 microns/day. Retzius line periodicity ranges from 6–7 days within chimpanzees and 6–12 days within humans. Within upper molars, mesiopalatal cusps (protocones) show thicker cuspal enamel and longer crown formation time than mesiobuccal cusps (paracones). Within lower molars, mesiobuccal cusps (protoconids) show greater Retzius line numbers, longer imbricational formation time, and thicker cuspal enamel than mesiolingual cusps (metaconids), resulting in longer formation times. A negative correlation was found between Retzius line number and periodicity in the human sample, resulting in similar crown formation times within cusp types, despite the range of individual periodicities. Few sex differences were found, but a number of developmental differences were apparent among human populations. Cusp-specific formation time in chimpanzees ranges from 2–3 years on average. Within specific cusp types, humans show greater average formation times than chimpanzees, due to higher mean periodicity values and/or thicker cuspal enamel. However, formation time within specific cusp types varies considerably, and the two genera show overlapping ranges, which has implications for the interpretation of small samples.

Introduction

Recent histological studies of chimpanzee and human enamel crown formation have suggested a number of similarities in molar formation time (Reid et al., 1998a,b). This is unexpected given their life history differences, which include an almost two-fold difference in the age at M1 eruption (reviewed in Kelley and Smith, 2003; but see Zihlman et al., 2004). However, these studies were based on small samples, and included several individuals with moderate to heavy dental wear. Recent studies of hominoid anterior tooth formation have yielded evidence of developmental differences among taxa (e.g., Schwartz and Dean, 2001; Schwartz et al., 2001). Yet little is known about the variation in molar development within and among taxa; postcanine development had been described for only four individuals of *Pan*, two individuals of *Gorilla*, and a lone representative of *Pongo* (Beynon et al., 1991; Reid et al., 1998a; Schwartz et al., 2006). Recently, several researchers have analyzed large collections of diverse groups of modern humans and chimpanzees (Thomas, 2003; Smith, 2004; Reid and Dean, 2006; Reid and Ferrell, 2006; Smith et al., 2007). The current study aims to use these data to highlight and contrast some of the developmental variables in molar enamel within and between modern humans and our closest-living primate relatives. We also attempt to generate a broad comparative framework for the interpretation of developmental data on more limited fossil collections.

The specific objectives are (1) to determine the appropriate units of analysis for developmental variables (e.g., individual, molar, specific molar, specific cusp), and (2) to investigate variation in enamel development at hierarchical levels. Within cusp types and tooth types, we examine several developmental variables: cuspal daily secretion rate, Retzius line periodicity, Retzius line number, imbricational enamel formation time, cuspal enamel thickness, and cusp-specific enamel formation time. We also explore the potential influence of sex and population differences on these variables. Our final comparison is between chimpanzees and humans. This is of particular interest given previous studies that have suggested overall developmental similarities in enamel formation among hominoids, as well as known life history differences between these two genera.

Incremental Development

Tooth mineralization begins over the location of the future dentine horns, where cuspal enamel is secreted in an appositional manner by ameloblasts (enamel-forming cells) as they migrate away from the enamel-dentine junction (EDJ). Epithelial cells continue to differentiate into secretory ameloblasts along the future EDJ, permitting the growth of enamel through extension from the horn tip to the enamel cervix. Enamel development is characterized by the formation of short- and long-period incremental lines, representing rhythmic changes or disturbances in enamel secretion (Figure 1). Short-period lines, known as cross-striations, show a circadian repeat interval, and may be used to determine the daily secretion rate. Long-period lines are known as Retzius lines (or striae of Retzius), which run from the EDJ to the surface of the tooth and form perikymata. This region, referred to as imbricational enamel, includes both the lateral and cervical enamel. Within an individual dentition, a consistent number of cross-striations can be counted along enamel

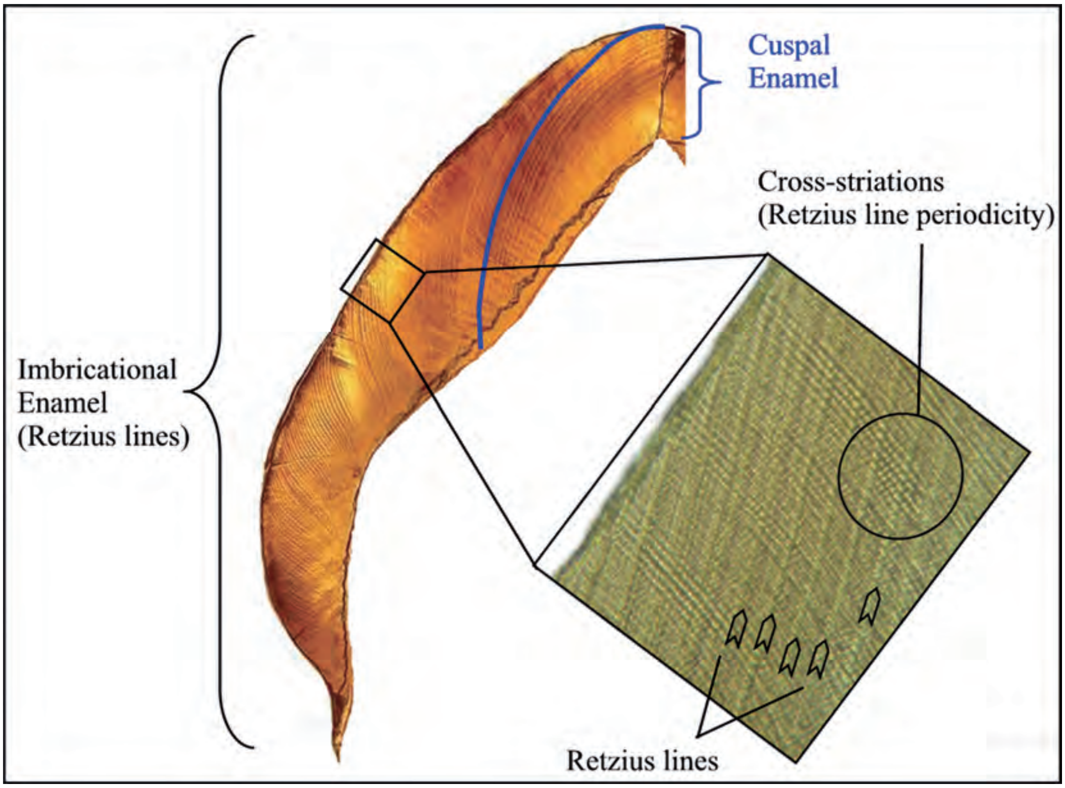

Figure 1. Incremental enamel development illustrated in a chimpanzee third molar. A portion of the enamel crown is shown divided into cuspal and imbricational areas. Cuspal enamel is defined (in blue) as the first formed enamel over the dentine horn that does not show Retzius lines running to the tooth surface. Imbricational enamel is characterized by the presence of Retzius lines running from the enamel-dentine junction to the surface of the tooth. At a higher magnification (lower right), daily cross-striations can be seen between Retzius lines; this consistent relationship is termed the Retzius line periodicity.

prisms between Retzius lines, (FitzGerald, 1998; Smith, 2004) and this number is known as the Retzius line periodicity.

From these developmental variables, the duration of crown formation may be estimated as the sum of cuspal and imbricational enamel formation times. Cuspal formation time is frequently estimated by dividing the linear cuspal enamel thickness by the average cuspal daily secretion rate (or cross-striation spacing). Imbricational formation time is often assessed by counting Retzius lines from the cusp tip to the cervix, and multiplying this number by the Retzius line periodicity. This yields a cusp-specific crown formation time. Because crown mineralization generally begins and ends at different times in different cusps, crown formation times derived from different cusps should not be directly compared (Reid et al., 1998a, b; Smith et al., 2007). Examination and registration of the first- and last-formed cusps are required to assess total crown formation time accurately.

Material and Methods

The chimpanzee sample consists of wild-shot, captive, and unknown provenience individuals from four collections, and includes *Pan troglodytes verus* and *Pan troglodytes* of unknown subspecies attribution (see Smith, 2004; Smith et al., 2007). Sex was unknown for the majority of individuals. A total of 269 histological sections of 134 molars from 75 individuals were prepared according to standard histological procedures (Reid et al., 1998a,b). The human sample consists of four collections of modern human molar teeth, including material from South Africa, Northern England, medieval Denmark, and North America (described further in Thomas, 2003; Reid and Dean, 2006; Reid and Ferrell, 2006). Sex was known for two of the four populations. Approximately 500 histological sections were generated from 420 molars of 365 individuals. Unfortunately, relatively few unworn or lightly worn cusps were available for the assessment of formation time. The approach taken in this study was intentionally conservative, as worn or missing enamel was not estimated for cusps showing more than very slight wear. Sections that were clearly oblique to an ideal plane or moderately to heavily worn were used only to determine the periodicity of Retzius lines (described below). Oblique sections were defined as those that did not show a relatively complete dentine horn tip, which reduces the accuracy of estimates (Smith et al., 2006).

Data are reported here for mesial cusps of maxillary and mandibular first through third molars, and includes the following variables: daily secretion rate, Retzius line periodicity, Retzius line number, and linear cuspal enamel thickness. In addition, cuspal and imbricational formation times were determined and summed to yield the cusp-specific crown formation time. Cuspal enamel formation was determined for both taxa with the use of taxon-specific regression equations requiring linear cuspal enamel thickness derived from a non-oblique plane of section (Dean et al., 2001). Imbricational (lateral and cervical) enamel formation time was determined as the number of Retzius lines multiplied by the individual's periodicity.

Data analysis included both parametric and rank-based (non-parametric) tests where appropriate. Rank-based statistical methods are appropriate given relatively small sample sizes or data that are not distributed normally, which often fail to meet the assumptions of parametric analyses. Rank-based tests are also appropriate because many of the data collected are discrete or categorical (non-continuous) (Conover, 1999). Comparisons of the developmental variables detailed below were made between mesial cusps, among molar types, between sexes, among populations, and between genera. Data on certain developmental variables are reported here for the larger human sample, where comparisons are more robust, but the trends are consistent with the chimpanzee sample unless indicated.

(1) For daily secretion rate (DSR), cross-striations were measured where possible in the cuspal enamel from the enamel near the dentine horn to the surface of the cusp (near the point where the first imbricational Retzius line meets the tooth surface), and were grouped into equivalent inner, middle, and outer zones (with a minimum of three sequential cross-striation measurements in a minimum of four areas per zone). Data were combined for all cusp and molar types, as Smith (2004) demonstrated in the chimpanzee sample that mean rates did not differ within zones among cusp types or molar types. Means, ranges, and standard deviations were determined for inner, middle, and outer cuspal enamel zones, and *t*-tests were performed to test for differences within zones between genera. Conover's (1999) adaptation of the Jonckheere- Terpstra test for trends was used to test for a gradient in rate from inner to outer cuspal enamel within each genus. Spearman's Rho is the statistic of choice for assessing the level of significance of the Jonckheere-Terpstra test, and is a more appropriate test for trends than the parametric ANOVA model (Jonckheere, 1954; Smith et al., 2005).

(2) Retzius line periodicity (the number of cross-striations between Retzius lines) was determined from each section when possible, including multiple sections and/or teeth per individual, to confirm the uniformity of this feature and the accuracy of the count. The mean, mode, and range were determined at the genus level. For the larger (and more variable) human sample, periodicity was plotted as a frequency histogram, and normality was tested using the one-sample Kolmogorov-Smirnov test for normality (Conover, 1999). Differences between the periodicities of the two known-sex human samples were assessed with the Mann-Whitney *U*-test (Sokal and Rohlf, 1995). Differences in periodicity among (combined-sex) human populations were assessed with the Kruskal-Wallis test, with population as the factor; if overall significance was achieved, then the multiple comparisons technique described by Conover (1999) was performed to determine which populations differed from one another. Differences between the periodicities of the two genera were assessed with the Mann-Whitney *U*-test.

(3) Differences between mesial cusps were tested in the four remaining developmental variables: Retzius line number, imbricational formation time, cuspal enamel thickness, and (cusp-specific) crown formation time. Means and ranges were determined for each variable in each cusp and tooth type, and two-sample *t*-tests were employed to assess differences between cusps, resulting in six comparisons for each of the four variables.

(4) Differences in the four aforementioned developmental variables were also examined among molar types. Kruskal-Wallis tests for molar differences were performed using molar type as the factor, testing each upper and lower mesial cusp type separately, resulting in four comparisons for each of the four variables. When significance was achieved, the multiple comparisons technique described above was performed in order to determine which molar types differed from one another significantly. The Jonckheere-Terpstra test for trends was also used to test for increasing or decreasing trends in mean variables along the molar row in both mesial cusp types.

(5) Differences in the four developmental variables were also examined between human populations. Kruskal-Wallis tests for population differences were performed using population as the factor, testing both mesial cusps in each upper and lower molar type separately, resulting in 12 comparisons for each of the four variables. When significance was achieved, Conover's (1999) multiple comparisons technique was performed to determine which populations differed from one another.
(6) The relationships between Retzius line periodicity and number was also examined within each mesial cusp and molar type in the human sample using Kendall's Coefficient of Concordance (Conover, 1999). The intention was to assess whether the negative correlation between these two variables reported by Reid et al. (2002) and Reid and Ferrell (2006) in canines from the Danish sample could be detected in a larger sample of diverse human molars.
(7) Finally, comparisons of the four developmental variables were made between chimpanzees and humans. However, because of the inequality of sample sizes (particularly the lack of maxillary *Pan* molars), any statistical results must be treated with due caution. Therefore, we only report the trends in mean differences, the relationships of the ranges of variables, and the patterning of developmental variables throughout the molar row.

Results

Daily Secretion Rate

Data on cuspal daily secretion rate (cross-striation spacing) are reported for inner, middle, and outer enamel zones in Table 1, along with the results of comparisons between genera.

Table 1. Mean molar cuspal daily secretion rate in Homo *and* Pan *(in microns/day), with t-test results for inter-generic differences*

	N	*Inner*	*Middle*	*Outer*	*Overall*
Homo	21	2.55	4.34	5.45	4.11
Pan	69	3.62	4.28	4.61	4.17
t	88	−10.886	0.524	7.112	−0.652
p	88	**<0.001**	0.602	**<0.001**	0.516

N is the total number of molar cusps sampled (above the line), or the degrees of freedom for the *t*-test (below the line). Inner, middle, and outer zones represent thirds in the enamel from the dentine horn to the cusp tip. Raw data are shown above the dotted line, and the results of a *t*-test for each specific zone are shown below the line. Significant differences are indicated in bold.

Humans showed a significantly lower mean value for inner enamel secretion rates, and a greater mean value for outer enamel rates, although the overall mean values were not significantly different. A statistically significant increasing trend in daily secretion rate from inner to outer enamel was found in both genera ($p < 0.001$ in both cases).

Retzius Line Periodicity

The Retzius line periodicity, or number of cross-striations between Retzius lines, is shown here for humans and chimpanzees (Table 2).

Table 2. Retzius line periodicity (in days) for Homo *and* Pan *individuals*

Taxon	*Group*	*N*	*Mean*	*Mode*	*Range*
Homo	South Afri.	121	8.6	8	6–12
Homo	Danish	61	8.4	9	7–11[1]
Homo	North. Eng.	83	8.1	8	6–11
Homo	North Am.	100	7.9	8	7–9
Homo	all	365	8.3	8	6–12
Pan	all	61[2]	6.4	6	6–7

For Group, South Afri. – South African, Danish – medieval Danish, North. Eng. – Northern England, North Am. – North American. N is the total number of individuals sampled. [1] The complete sample of anterior and posterior teeth from 84 Danish individuals reported in Reid and Ferrell (2006) ranged from 6–12 with a mean of 8.5. [2] It was not possible to determine conclusively the periodicity in 14 of the 75 individuals (see Smith, 2004).

No evidence was found to suggest that periodicity is variable within an individual's dentition when multiple teeth were compared. The human sample was plotted as a histogram (Figure 2); moment statistics indicate that this is a leptokurtic distribution skewed to the right. Further, the Kolmogorov-Smirnov statistic was significantly different ($p < 0.001$) from four hypothetical distributions (normal, uniform, exponential, and Poisson).

Of the two known-sex human samples (North American and South African), females were found to have a significantly higher periodicity than males in the South African sample only ($p < 0.010$). Nonetheless, sexes were combined for the following analyses, as sex was unknown for the remaining two human populations and the majority of the chimpanzee sample. Statistical differences were found among human groups ($p < 0.001$). Post hoc comparisons indicated that the South African sample showed a greater mean periodicity than all other groups, and the Danish sample was additionally greater than the Northern English and North American samples. When humans and chimpanzees were compared, humans showed a significantly greater mean periodicity ($p < 0.001$), despite the fact that the range of the chimpanzee sample fell entirely within the human range.

Variation Between Mesial Cusps

Results of comparisons of developmental variables between mesial cusps in the human sample are shown in Table 3. Within upper molars, mesiopalatal cusps (protocones)

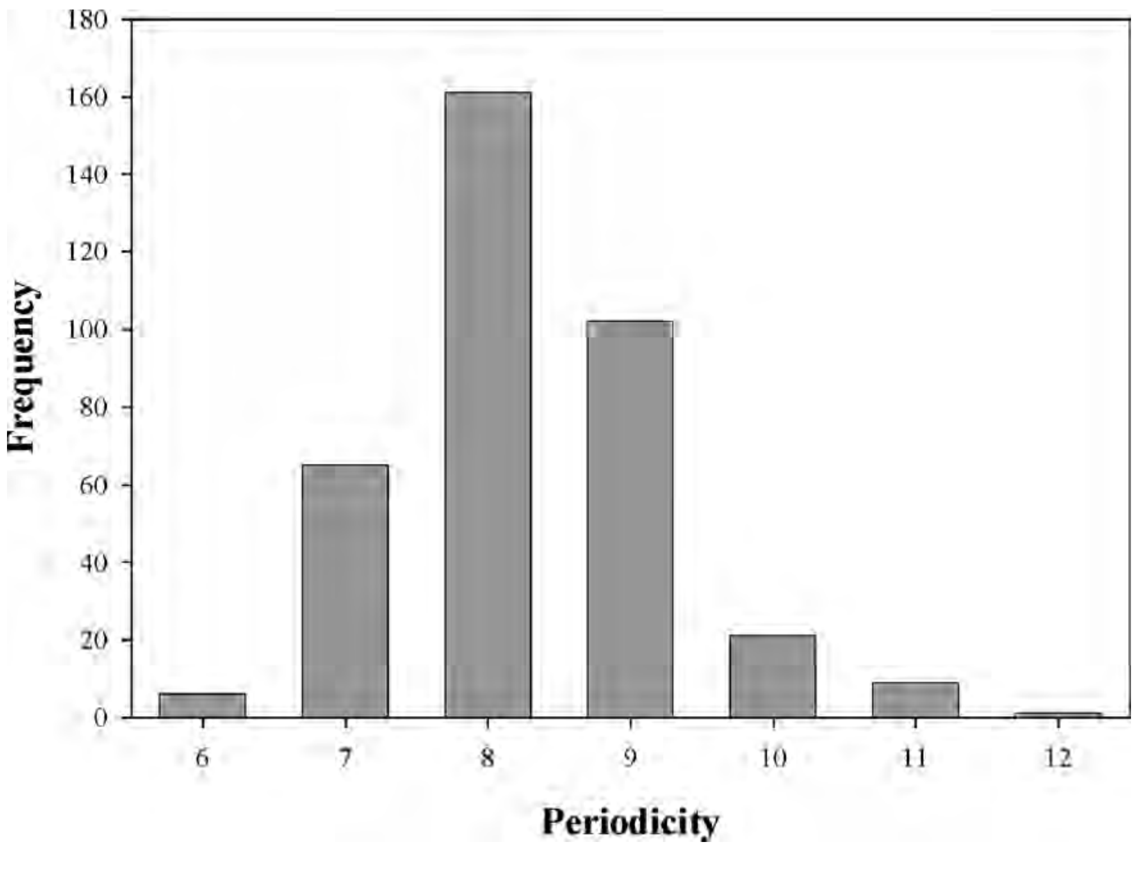

Figure 2. Histogram of human Retzius line periodicity data. Each value represents one of 365 individuals from diverse human populations. See text for details about the non-normality of this distribution.

Table 3. Mann-Whitney U-test comparisons of developmental variables between mesial cusps in Homo

Position	*Tooth*	*Statistic*	*Retz*	*Imb*	*Thick*	*CFT*
Upper	M1	*Z*	−0.092	−0.563	−4.272	−3.080
		p	0.927	0.573	**<0.001**	**0.002**
	M2	*Z*	−0.557	−0.832	−4.747	−3.337
		p	0.577	0.405	**<0.001**	**0.001**
	M3	*Z*	−1.166	−1.696	−4.782	−4.298
		p	0.244	0.090	**<0.001**	**<0.001**
lower	M1	*Z*	−5.855	−7.436	−2.293	−6.151
		p	**<0.001**	**<0.001**	**0.022**	**<0.001**
	M2	*Z*	−5.971	−6.822	−5.280	−6.580
		p	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	M3	*Z*	−3.920	−6.560	−4.389	−6.031
		p	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Z – the Mann-Whitney test statistic, *p* - *p*–value of the given comparison. The results of the comparison between mesial analogues are shown for the following variables: Retz – Retzius line number, Imb – imbricational formation time, Thick – cuspal enamel thickness, and CFT – crown formation time. Significant differences are indicated in bold.

had significantly thicker cuspal enamel and greater crown formation time than the respective mesiobuccal cusps (paracones). Mean values for Retzius line number and imbricational formation time were also greater in mesiopalatal cusps than in mesiobuccal cusps, although the differences were not significant. The opposite pattern was found in lower molars; mesiobuccal cusps (protoconids) had significantly greater numbers of Retzius lines, longer imbricational time, thicker cuspal enamel, and greater crown formation time than mesiolingual cusps (metaconids).

Variation Within the Molar Row

Results of comparisons of the four developmental variables among molars are shown in Tables 4 and 5, including the results of tests for trends in the molar row. Within upper molars, cuspal enamel thickness and crown formation time showed a significant increasing trend from first to third molars in both mesiopalatal and mesiobuccal cusps. Mean Retzius line number also increased posteriorly, although this was not significant. Within lower molars, both Retzius line number and imbricational formation time decreased from first to third molars in mesiobuccal cusps. Post-hoc tests revealed that these trends were generally the result of significant differences between first and second molars and between first and third molars. Significant increasing trends were also observed in cuspal enamel thickness and crown formation time in both mesial cusps in lower molars, largely due to significant increases between first and second and/or first and third molars.

In light of these trend results, a non-parametric correlation analysis was conducted between imbricational formation time and cuspal enamel thickness in the human sample, and a significant negative correlation was found in upper first molar mesiobuccal cusps ($p < 0.050$), and in all lower mesiobuccal cusps (M1: $p < 0.010$; M2: $p < 0.001$, M3: $p < 0.050$). Cusps with thicker cuspal enamel show shorter imbricational formation times. The result of this correlation appears to be that cusp-specific formation time is constrained to a degree within mesiobuccal cusps (although

Table 4. Kruskal-Wallis tests for differences among molar types in the Homo *sample*

Position	*Cusp*	*Statistic*	*Retz*	*Imb*	*Thick*	*CFT*
Upper	mb	*H*	1.793	3.370	49.702	23.579
		p	0.408	0.185	**<0.001**	**<0.001**
	mp	*H*	2.188	2.723	17.491	6.799
		p	0.335	0.256	**<0.001**	**0.033**
Lower	mb	*H*	12.154	37.191	49.267	6.128
		p	**0.002**	**<0.001**	**<0.001**	**0.047**
	ml	*H*	4.665	1.747	31.140	14.329
		p	0.097	0.418	**<0.001**	**0.001**

For upper molars, mb – mesiobuccal cusp, and mp – mesiopalatal cusp. For lower molars, mb – mesiobuccal cusp, and ml – mesiolingual cusp. H – the Kruskal-Wallis test statistic, *p* - *p*-value of the given comparison. Variables are defined in Table 3. Significant differences are indicated in bold.

Table 5. Kruskal-Wallis post-hoc comparisons for differences in developmental variables between molar types, with the directionality of significant trends indicated

Position	*Cusp*	*Var*	*M1 v. M2*	*M1 v. M3*	*M2 v. M3*	*JT*
Upper	mb	Thick	<	<	<	+
		CFT	<	<	–	+
Upper	mp	Thick	<	<	–	+
		CFT	<	<	–	+
Lower	mb	Retz	>	>	–	–
		Imb	>	>	–	–
		Thick	<	<	–	+
		CFT	–	<	<	+
	ml	Thick	<	<	–	+
		CFT	–	<	–	+

Cusp and Var- cusp type and developmental variables are defined in Tables 3 and 4. The direction of the sign below each bivariate comparison indicates which molar position is significantly greater. No significant difference is indicated by '–'. JT indicates the significant results of the Jonkheere-Terpstra test for trends ($p < 0.05$), with increasing trends illustrated by '+' and decreasing trends illustrated by '–'.

an overall increasing trend in cusp-specific crown formation time was significant from first to third molars in each case). It was not possible to test for similar trends within chimpanzee molars as sample sizes were too small.

Variation Within Homo

Sex differences in the four main developmental variables were examined within mesial cusps and molar types between the two known-sex human populations, and significant differences were found in only one of 64 comparisons. In this instance, the upper third molar mesiobuccal cusp in South African males showed a significantly greater imbricational formation time than females ($p < 0.050$), due to a greater number of Retzius lines. However, given the lack of consistent developmental differences between males and females, the sexes were combined for the subsequent analyses.

When human populations were compared, a complicated pattern of similarities and differences became apparent. Of the 48 comparisons of the four developmental variables, 18 comparisons showed significant differences among populations (Table 6). In general, no single cusp or tooth type showed more frequent inter-population differences, except for mesiobuccal cusps in upper third molars. The most frequent developmental variable to differ among populations was cuspal enamel thickness, in six of 12 comparisons, followed by imbricational formation time and cusp-specific crown formation time in five of 12 comparisons. Retzius line number appeared to be the least variable among populations, with differences in only two of 12 comparisons. Post-hoc tests showed that variation was found between several different population combinations (Table 7). Excluding any single population still resulted in a proportionate number of differences among the remaining populations. A few general trends were evident, as the North American sample consistently showed the thickest cuspal enamel in third molars (data were not available for first or second molars from this sample), and the Danish sample tended to show the thinnest cuspal enamel for most tooth positions. Despite these differences, the populations were combined, as the remaining issues concerned specific trends and generic differences.

Correlations Between Variables

Table 8 shows a highly significant negative correlation between Retzius line number and periodicity for each cusp and molar

Table 6. Kruskal-Wallis test for differences in developmental variables among populations of Homo

Pos	*Tooth*	*Cusp*	*Statistic*	*Retz*	*Imb*	*Thick*	*CFT*
Upper	M1	mb	*H*	2.349	4.793	1.705	1.792
			p	0.309	0.091	0.426	0.408
		mp	*H*	2.299	5.369	5.647	6.099
			p	0.317	0.068	0.059	**0.047**
	M2	mb	*H*	2.203	11.652	6.000	9.548
			p	0.332	**0.003**	0.050[1]	**0.008**
		mp	*H*	0.280	3.923	2.329	3.485
			p	0.870	0.141	0.312	0.175
	M3	mb	*H*	9.216	17.261	21.305	20.033
			p	**0.027**	**0.001**	**<0.001**	**<0.001**
		mp	*H*	0.992	0.978	22.005	8.407
			p	0.803	0.807	**<0.001**	**0.038**
Lower	M1	mb	*H*	1.232	8.893	7.703	1.170
			p	0.540	**0.012**	**0.021**	0.557
		ml	*H*	4.076	5.634	4.591	6.117
			p	0.130	0.060	0.101	**0.047**
	M2	mb	*H*	4.653	1.236	2.764	0.491
			p	0.098	0.539	0.251	0.782
		ml	*H*	9.147	8.980	9.776	2.668
			p	**0.010**	**0.011**	**0.008**	0.263
	M3	mb	*H*	5.535	10.787	13.228	3.147
			P	0.137	**0.013**	**0.004**	0.370
		ml	*H*	7.184	2.653	8.588	3.862
			p	0.066	0.448	**0.014**	0.145

Cusp, Statistic, and variables are defined in Table 3 and 4. Significant results are in bold. The degrees of freedom for each cusp and molar type comparison are 2 for first and second molars, and 3 for third molar comparisons (as the North American sample consisted of third molars only). [1]The post-hoc results for this comparison are also given in the following table.

Table 7. Kruskal-Wallis post-hoc comparisons for significant differences in developmental variables between populations

Pos	*Tooth*	*Cusp*	*Var*	*1 v. 2*	*1 v. 3*	*1 v. 4*	*2 v. 3*	*2 v. 4*	*3 v. 4*
Upper	M1	mp	CFT	n/a	<	–	n/a	n/a	–
	M2	mb	Imb	n/a	>	>	n/a	n/a	–
			Thick	n/a	–	>	n/a	n/a	–
			CFT	n/a	>	>	n/a	n/a	–
	M3	mb	Retz	>	>	–	>	–	–
			Imb	>	>	–	>	–	<
			Thick	<	–	–	>	>	>
			CFT	–	–	–	>	>	–
		mp	Thick	<	–	–	>	>	–
			CFT	<	–	–	–	–	–
Lower	M1	mb	Imb	n/a	–	–	n/a	n/a	<
			Thick	n/a	–	–	n/a	n/a	>
		ml	CFT	n/a	<	–	n/a	n/a	–
	M2	ml	Retz	n/a	<	<	n/a	n/a	–
			Imb	n/a	–	<	n/a	n/a	<
			Thick	n/a	–	>	n/a	n/a	–
	M3	mb	Imb	–	–	–	<	–	–
			Thick	–	–	–	–	>	–
		ml	Thick	–	–	–	–	>	–

Cusp and Var are indicated for Tables 3 and 4. Population codes are: 1- South African, 2- North American, 3- Northern England, 4- medieval Danish. The direction of the sign below the bivariate comparisons indicates which population is significantly greater. No significant difference is indicated by '–,' n/a indicates that comparisons were not possible due to the lack of first and second molar data for population 2 (North American).

Table 8. Correlations between Retzius line number and periodicity in Homo

Position	*Tooth*	*Cusp*	*N*	*Correlation*	*p-value*
Upper	M1	mb	26	−0.628	< **0.001**
		mp	19	−0.771	< **0.001**
	M2	mb	29	−0.611	< **0.001**
		mp	22	−0.776	< **0.001**
	M3	mb	81	−0.648	< **0.001**
		mp	68	−0.762	< **0.001**
Lower	M1	mb	34	−0.793	< **0.001**
		ml	50	−0.527	< **0.001**
	M2	mb	43	−0.679	< **0.001**
		ml	42	−0.560	< **0.001**
	M3	mb	50	−0.694	< **0.001**
		ml	41	−0.758	< **0.001**

Cusps are defined in Table 4. N equals the number of cusps (individuals). Correlation is Kendall's Tau correlation coefficient. Significant results are indicated in bold.

type in the combined human sample. The result of this correlation is that imbricational formation time is constrained in a given cusp and molar type. Individuals with a smaller number of Retzius lines have a greater periodicity, on average, than individuals with a greater number of Retzius lines.

Inter-Generic Comparisons

The final comparison is between human and chimpanzee developmental variables. Several trends are evident, and are summarized in Table 9. One of the notable differences between the two genera is in cuspal enamel thickness, which was generally two to three times greater in humans than in chimpanzees. Additionally, humans consistently showed longer mean crown formation times than chimpanzees, ranging from several months to more than a year of difference for certain cusp types. In general, there was little overlap in formation time ranges, although exceptions were found for certain cusp types and tooth types.

It also appeared that humans and chimpanzees showed differences in trends of developmental variables along the molar row (Figures 3 and 4). Humans commonly showed the greatest mean values in third molars (or less commonly in first molars), while chimpanzees appeared to show the greatest values in second molars (or less commonly in third molars). A particularly clear difference was apparent in imbricational formation time from lower first to third molars, which was greatest in human first molars, and least in chimpanzee first molars relative to their respective posterior molars.

Table 9. Summary of developmental variable comparisons between Pan *and* Homo

Variable	*Trend*	*Range Overlap*
Periodicity	*Homo* > *Pan*	*Pan* within *Homo*
Daily secretion rate		
Inner	*Homo* < *Pan*	little
Middle	*Homo* = *Pan*	*Homo* within *Pan*
Outer	*Homo* > *Pan*	partial
Overall	*Homo* = *Pan*	*Pan* within *Homo*
Retzius line number	*Homo* < *Pan*	partial
Imbricational time	*Homo* < *Pan*	partial
Cuspal thickness	*Homo* > *Pan*	none to little
Crown formation time	*Homo* > *Pan*	little

See text for description of variables.

Discussion

This study is a methodological analysis of enamel formation that has demonstrated that specific developmental variables should be assessed at differing hierarchical levels. Daily secretion rate does not vary within cusps or molar types, Retzius line periodicity varies at the individual (dentition) level, and the four additional developmental variables: Retzius line number, imbricational formation time, cuspal enamel thickness, and crown formation time all vary within cusps and among molar types (also see Smith et al., 2007 for additional data on distal cusps and total crown formation time in the chimpanzee sample). These factors each need to be considered when discussing variation in enamel developmental, or when making comparisons between molars. In light if this, some of the early work characterizing enamel development in naturally fractured hominid teeth should be viewed with caution (e.g., Beynon and Wood, 1987; Ramirez Rozzi, 1993).

The results of comparisons between mesial cusp pairs and among molar types may also have functional implications. As reported above, mesiopalatal cusps show significantly thicker cuspal enamel and longer crown formation times than mesiobuccal cusps in upper molars. Within lower molars, mesiobuccal cusps show significantly greater Retzius line numbers, longer imbricational formation times, thicker cuspal enamel, and longer formation times than mesiolingual cusps. This implies differential patterning in mesial cusp development between upper and lower molars, trends that are consistent with functional models of thicker enamel, and thus prolonged development, in principle or functional cusps (also see Reid et al., in 1998a; Suwa and Kono, 2005). When differences were examined among molar types, a similar pattern was revealed; increasing trends were found in cuspal enamel thickness and crown formation time from first to third molars

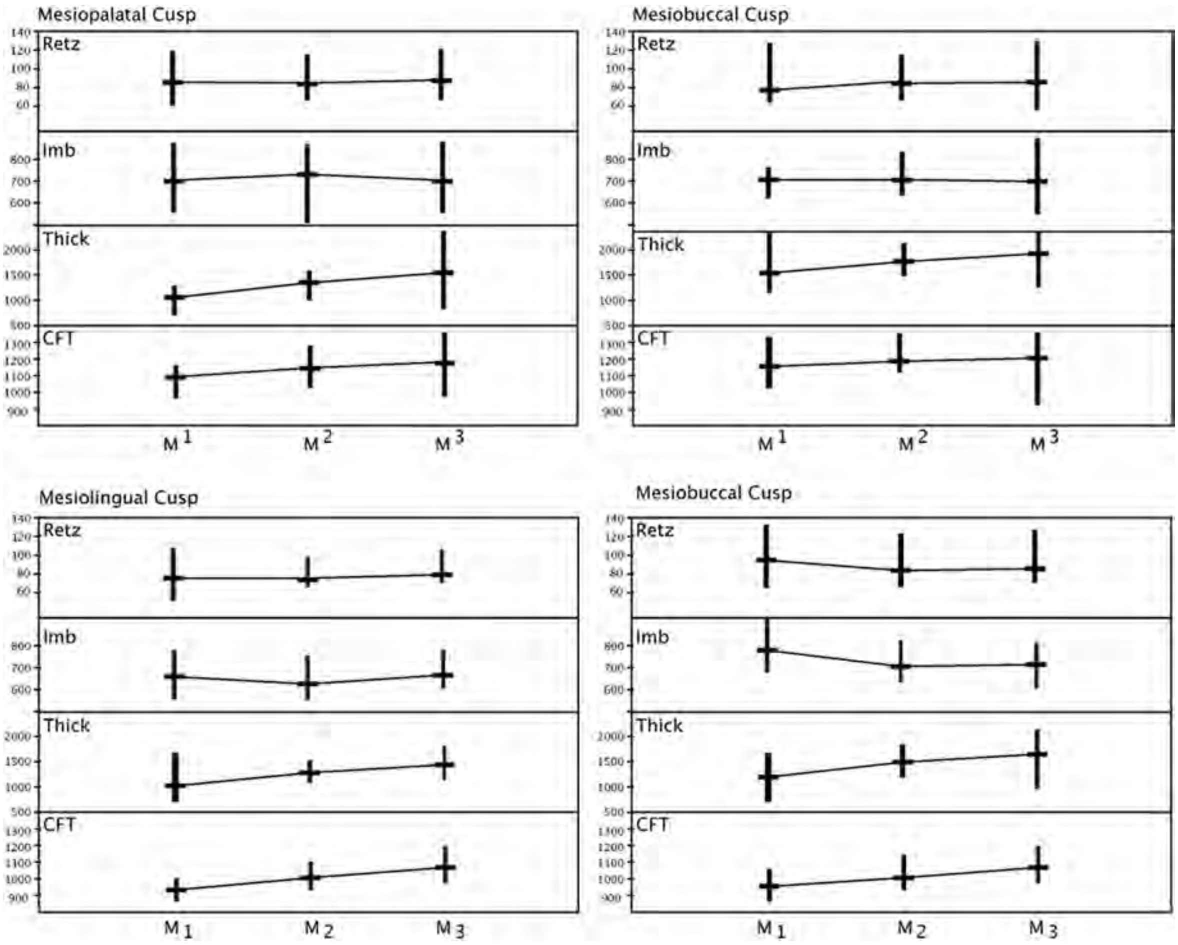

Figure 3. Anterior-to-posterior trends in developmental variables in human molars. Retz- Retzius line number, Imb- imbricational formation time (in days), Thick- cuspal enamel thickness (in microns), and CFT- crown formation time (in days). The range of values was plotted from left (M1) to right (M3) to show directional trends (upper molars above lower molars). Ends of vertical boxes represent 25% and 75% ranges of the data; the horizontal line is the mean.

in both mesial cusps for upper and lower molars. Smith et al. (2005) also showed an increasing trend for enamel cap area and/or average enamel thickness in mesial sections of the same sample of chimpanzees, which may relate to trends in overall tooth size throughout the molar row. Work in progress may shed more light on the relationship between tooth size and formation time variation.

Comparisons of developmental variables among human populations showed a complex pattern of developmental differences and similarities (also see Reid and Dean, 2006). Cuspal enamel thickness and cusp-specific crown formation time were most commonly different among populations. The North American third molar sample showed the most notable differences when compared to other populations, which may be due in part to their extremely thick enamel. If is unclear why this population showed a restricted range of periodicity values (including the lowest mean value) and very thick cuspal enamel. The most geographically similar European populations (Northern England and medieval Danish) showed relatively few differences, despite being separated by approximately 800 years. Future work is required to

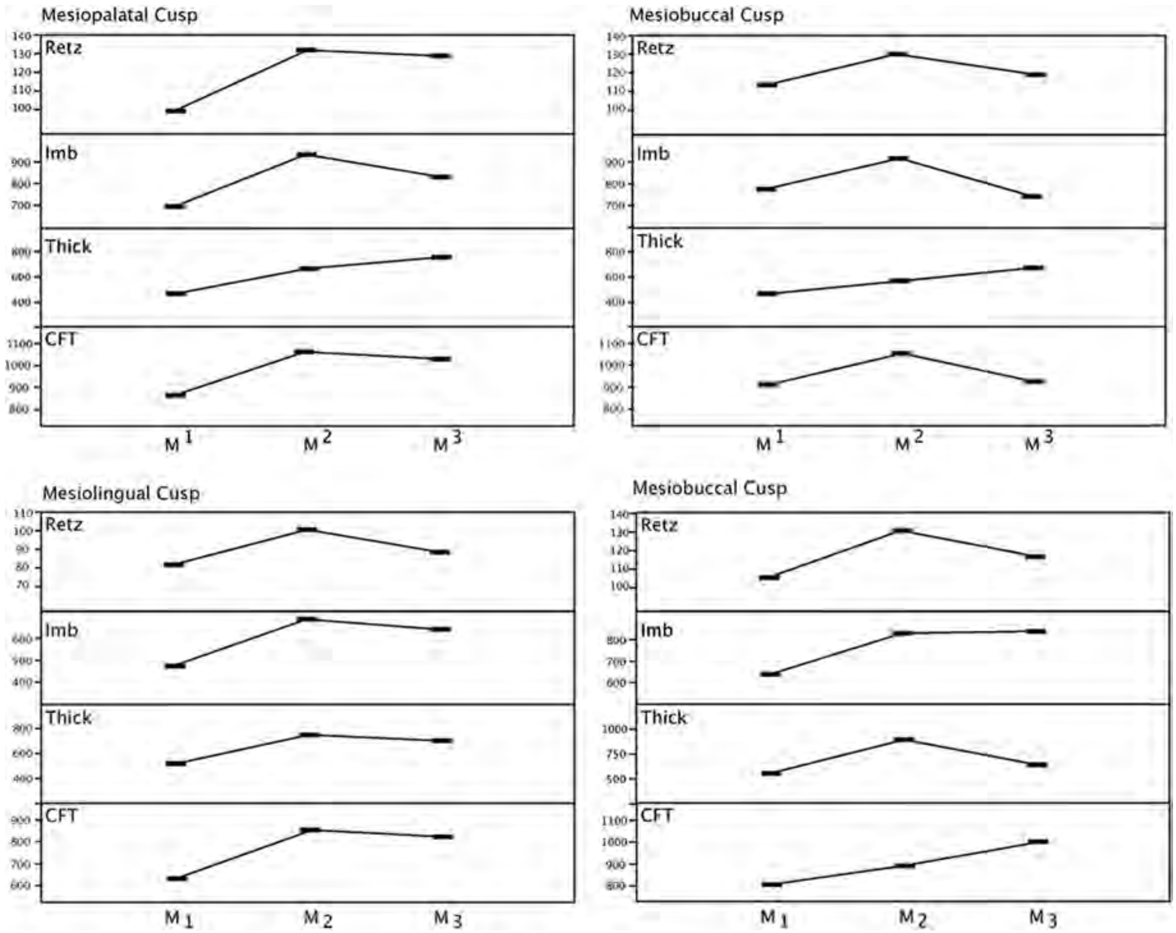

Figure 4. Anterior-to-posterior trends in developmental variables in chimpanzee molars. Retz- Retzius line number, Imb- imbricational formation time (in days), Thick- cuspal enamel thickness (in microns), and CFT- crown formation time (in days). The range of values was plotted from left (M1) to right (M3) to show directional trends (upper molars and lower molars). Due to the limited sample sizes, only mean values are given for each variable.

clarify the degree of variation among diverse human populations as well as among non-human primate sub-species/species groups. The influence of different developmental environments may also be a source of variation, as captive animals may show earlier ages of dental eruption than wild animals (Phillips-Conroy and Jolly, 1988; Zihlman et al., 2004). Additional studies controlling for these factors are required to facilitate a better understanding of how developmental variables may be influenced by phylogenetic, functional, or environmental factors.

An additional finding that warrants comment was the highly significant negative correlation between Retzius line number and periodicity in the human sample (also see Reid and Dean, 2006; Reid and Ferrell, 2006). This finding has serious implications for studies of perikymata, external manifestations of Retzius lines, and suggests that comparisons of perikymata numbers without knowledge of the individual periodicities may *not* provide information about actual differences in the rate or period of formation. For example, comparing counts of Retzius lines holding cusp and molar types constant could yield values that differ by as much as a factor of two, such as 70 lines and 140 lines, but the periodicities are likely to also differ by a factor of two, such as 12 and 6 days, respectively. In this example, what appears different on the surface of a tooth may actually represent the same developmental time. We advocate consideration of the taxon-specific periodicity range when making inferences about developmental rate or time from incremental lines on the surface of teeth.

Previous work on a large collection of hominoid canines by Schwartz and colleagues addressed issues of inter-generic differences and sex-differences (Schwartz and Dean, 2001; Schwartz et al., 2001), and the present study confirms a number of their conclusions. In terms of daily secretion rate, Schwartz et al. (2001) also found that humans have lower and higher values in the respective inner and outer cuspal enamel than these regions in chimpanzees. In regards to Retzius line periodicities, they also found that humans had a significantly greater mean periodicity than chimpanzees. However, Schwartz et al. (2001) reported periodicity ranges for humans and chimpanzees of 7–11 days and 6–9 days, respectively, which are different than the ranges found in this study. It is likely that differences between studies are due to the inclusion of outliers, which are present in very low frequencies. Cuspal enamel thickness in the former study was found to be greater in humans, also consistent with the present study. However, canine crown formation times were greater in chimpanzees, which appeared to be related to differences in canine crown height (Schwartz and Dean, 2001). Work by Reid and Dean (2000), Reid and Ferrell (2006), and Reid and Dean (2006) has shown some degree of overlap in canine formation time between humans and female chimpanzees, but not for male chimpanzees.

It is unclear why females in one of the two known-sex human populations in this study showed significantly greater Retzius line periodicity than males. Schwartz et al. (2001) did not find sex differences in their sample of great apes and humans, and in most instances males showed slightly higher mean values (although their female mean was higher in the human sample). It would be interesting to investigate periodicity in other samples of known sex (and known mass) humans and non-human primates. In conclusion, it appears that tooth size, sexual dimorphism, functional differences, and life history may impact developmental variation between chimpanzees and humans, particularly when anterior and posterior teeth are considered together.

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