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NUMBER 4

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THE HUMAN BRAIN EVOLVING:

Paleoneurological Studies in Honor of Ralph L. Holloway

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FRONT COVER CAPTIONS

Center: Portrait of Ralph L. Holloway.
Upper left: A modern human brain.
Upper right: Ralph measuring landmarks on an endocast ca. 1976.
Lower right: Homo habilis cranium KNM-ER-1813 from Koobi Fora, Kenya (photo by Holloway).
Lower left: Ralph with an endocast of the Flores "hobbit" cranium.

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CHAPTER 16

PERIKYMATA COUNTS IN TWO MODERN HUMAN SAMPLE POPULATIONS

MICHAEL SHENG-TIEN YUAN

ABSTRACT

Many studies based on the perikymata, a dental enamel surface microstructure, have attempted to estimate the age-at-death and crown formation times on human and other fossil specimens. However, due to problematic assumptions, the small sample sizes, the wide range of perikymata count estimates, and the limited portions of dentition explored, considerable controversy has resulted. The collection of baseline information on perikymata counts for various hominid and hominoid dentitions represents an important step toward resolving the controversies.

The goal of this study is to establish a database of modern human perikymata counts of the maxillary third premolars for comparative purposes. The results demonstrated: 1) There is sexual dimorphism, though not statistically significant within the two populations: perikymata counts are higher in males. 2) There are no significant differences between right and left sides. 3) There are no significant differences for sex-combined samples between the two ethnic groups. 4) When data are pooled for the East Asian samples, the mean perikymata count for maxillary third premolars is 150 with a standard deviation of 25. 5) The perikymata counts are significantly correlated with their corresponding crown height. 6) Although the data on perikymata counts follow a normal distribution, the variation is high (coefficient of variation= 16 %). This study disputes the perikymata count application in anthropology and questions the interpretation of the ape affinity of australopithecines in hominid dental evolution.

INTRODUCTION

Teeth and their associated structures including the maxillary and mandibular bones and the masticatory musculature, provide one of the best vehicles for studying hominoid and hominid evolution (Dahlberg, 1971; Hillson, 1986; Aiello & Dean in Chapter 8, 1990; Hillson, 1996; Scott & Turner II, 1997).

Due to its high percentage of inorganic components (by weight: enamel, 95%; dentin, 70%, cementum 61%; vs. bone 45%; and by volume: enamel, 86%; dentin, 45%, cementum 33%; vs. bone 23%; see Schroeder, 1991, p. 73, Fig. 1.34) and the almost irreversible growth and development of the calcification process, the tooth itself, once formed, becomes a fossil-like material that is highly durable through time. During the past two decades, investigations of the morphological structures of the teeth, especially the microanatomy of enamel, have become prominent in studies of both hominoid taxonomy and hominoid ontogeny and phylogeny (see review of Macho & Wood, 1995; Mann et al., 1990a; Winkler & Swinder, 1991).

Many studies based on the microstructure of the dental enamel surface, especially the perikymata, and its internal enamel structures, the cross-striations and the striae of Retzius, have attempted to estimate crown formation time and age-at-death in hominoids (Boyde, 1963; Bromage & Dean, 1985; Dean et al., 1986; Bacon, 1987; Dean, 1987; Dean & Beynon, 1991; Stringer et al., 1990). In these studies, fossil teeth of Australopithecines, early (archaic) *Homo sapiens*, and Neanderthals were compared with those of *Homo sapiens* to reveal differences between taxa in crown maturation times and also to estimate age-at-death. The results of these investigations have stimulated several different views on how to infer patterns of human evolution based on dental mi-

croanatomy (Bromage & Dean, 1985; Dean et al., 1993; Mann et al., 1990a, 1990b, 1991).

Mammalian enamel ontogeny and comparative histology have been thoroughly reviewed by Boyde (1971, in Chapter 7, pp. 81-94; 1997) and Moss-Salentijn et al. (1997, in Chapter 1, pp. 5-30). Several widely-used textbooks on anatomy, oral development, and histology provide excellent reviews of human enamel structure. These texts include Avery (1994, see Piesco & Avery in Chapter IV, pp. 228-241), Ten Cate (1998, see Eisenmann in Chapter 10, pp. 197-217 and Chapter 11, pp. 218-235), Aiello & Dean (1990, in Chapter 7, pp. 106-132); Moss-Salentijn & Hendricks-Klyvert (1985, in Chapter 11, pp. 229-254, 1990), and Schroeder (1991, in Chapter 1, pp. 38-67). The physiologic and genetic interactions among enamel matrix proteins, minerals, and various components during the secretary and maturation phases, have also been extensively discussed in Chadwick & Cardew (1997).

In the following sections, several features of human enamel structure will be reviewed in detail. These include: 1) the histologic nature of the perikymata on enamel; 2) the lines of Retzius of enamel; 3) the cross-striations of the enamel prisms; and 4) the Hunter-Schreger bands. Additionally, the chronology of dental growth and development, human crown formation times, and the applications and the controversy surrounding the interpretation of hominid and hominoid dental remains based on studies of circadian and infradian characters will be discussed.

A number of attempts have been made to apply knowledge of the circadian and infradian incremental structures on dental enamel to explore the crown formation time (or crown maturation time), to estimate the age-at-death in developing individuals, and to reconstruct life history from the manifestation and counts of incremental structures. In addition, crown formation time and the rate of dental tissue formation, which were derived from the estimation of counts of incremental structures, may provide evidence for species differences in the hominoid and hominid evolution.

The cross-striations in enamel and perikymata counts on the crown surface are the most commonly used incremental structures to estimate crown formation time and age-at-death. Boyde (1963) first suggested that age could be estimated from prism cross-striation counts in non-living specimens. Bromage & Dean (1985) developed an ageing method based on perikymata counts alone, suggesting that lower permanent incisor crown formation started at 3 months of age, and then at approximately 6 months appositional enamel growth began, so that the first perikymata groove would appear at 9 months of age. They assumed a 7-day repeat interval to derive ages-at-death for uncompleted fossil dental crowns. Stringer et al. (1990) later carried out a study to test the age estimation from enamel layering using known age archeological specimens with the assumed cross-striation counts of suggested 7, 8, and 9-day repeat intervals. The best matching result was concluded by applying an 8-day repeat interval between Retzius lines for the age-at-death estimation. Dean & Beynon (1991) similarly applied the cross-striation counts and perikymata counts to estimate crown formation time and age-at-death in a child from the 18/19th century A.D. in London (Shown in Table 1-5).

Efforts have also been made to determine the perikymata counts of modern humans. Results of studies of modern humans should ideally and hypothetically be useful for inferring time of crown formation and age-at-death for fossil hominids, if accurate adjustments are applied. The results of studies of modern humans to date, however, have been very diverse. In addition, many of these studies do not provide sufficient information on the perikymata counts with which to compare to the posterior dentition. The range of human incisor perikymata counts based on a sample of 12 five-thousand-year-old immature human specimens from the Iranian site of Hasanlu is 75-157 with a mean of 116, a median of 118, and a standard deviation of 25 (Mann et al., 1990b, 1991). On the other hand, Bromage & Dean (1985) obtained perikymata counts ranging from 165-202 counts, with a mean of 188, in a sample of 10 unworn modern human lower incisors. Bacon (1987) also reported a range of 111-179 in a sample of 23 modern human incisors.

The collection of crown formation data during radiographic and histologic studies of dentition in modern humans, gorillas, and chimpanzees and the perikymata-derived crown formation time of the australopithecines, has made comparisons possible between extant and extinct hominoid species. For example, the combined upper and lower incisor crown formation time averages 4.21 years and ranges 3.71-4.71 years in *Homo sapiens* (Shellis, 1984). The investigation of *Pan troglodytes* provided crown formation time of the maxillary central incisor is reported to be 5.47 ± 0.24 years and that of the mandibular, 4.86 ± 0.37 years (Chandrasekera et al., 1993). The crown formation time of *Gorilla gorilla* based on the histological examinations revealed 4.0 and 3.6 years for maxillary and mandibular central incisors, respectively (Beynon et al., 1991). The mandibular central incisor formation times in *Australopithecus afarensis* and *Australopithecus africanus* were estimated 3 years and 3 years 1.1 months for specimens LH2 and Sts 24a respectively, utilizing the perikymata-derived crown formation times (Bromage & Dean, 1985).

A list of age-at-death estimate for various hominid fossil specimens using perikymata counts is summarized in **Table 1-5**. This approach has created a totally different interpretation of the fossil record, especially with regard to the affinity of australopithecines. Claiming the ape affinity of australopithecines based on the dental eruption pattern and estimation of age-at-death, perikymata counts played a key role in this ongoing controversy.

Nevertheless, we must note that these estimated results are based on the premises that: 1) all enamel microstructures are tightly correlated with their mutual periodicities; and 2) the dentitions are equally accounted for their developmental timings and sequences across the extant and extinct primate species.

Table 1-5. Perikymata count study on records.

Taxon	Specimen	Site	Teeth used	P.C.	Age-at-death Estimate (yr)	Ref.
A. afarensis	LH2	Laetoli, Tanzania	mand. R. 1st incisor	130	3.25	a
A. africanus	Sts24a	Sterkfontein, S.A.	max. R. 1st incisor	135	3.3	a
P. robustus	SK62	Swarkran, S.A.	mand. R. 1st incisor	57	3.35	a
			mand. L. 2nd incisor	64	3.48	a
P. robustus	SK63	Swarkran, S.A.	mand. R. 1st incisor	86	3.15	a
			mand. 1st incisor	75	3.98	g
			mand. L 2nd incisor	84	3.98	g
			mand. R canine	98	3.98	g
P. boisei	KNM-ER 1477	Koobi Fora, Kenya	mand. 1st incisor	92	2.5-3.0	c
P. boisei	KNM-ER 812	Koobi Fora, Kenya	mand. 1st incisor	86	2.5-3.0	c
P. boisei	KNM-ER 1820	Koobi Fora, Kenya	mand. 1st incisor	82	2.5-3.1	c
P. boisei	OH30	Olduvai, Tanzania	mand. 1st incisor	101	2.7-3.2	c
Early Homo	KNM-ER 820	Koobi Fora, Kenya	mand. L. 2nd incisor	105	5.3 (5.3-6)	a
Neanderthal	Gibraltar child	Devil's Tower, Gitraltar	max. 1st incisor	119	3.1	b
	Krapina 90		mand. R. 2nd incisor	205±10	4.4	e
	Krapina 91*			>100±4	-	e
	Krapina 93*			>107±2	-	e
	Krapina 94*		max. R. 1st incisor	>144±7	-	e
	Krapina 95*			>50	-	e
Homo sapien	2179	Spitalfield, London	max. 1st molar	85		
			max. 1st molar	120		
			mand. 1st incisor			
	197					
			mand. 2nd incisor	224	5.25	d
			mand. 2nd incisor	162		
			(?) canine	184		
			(?) canine	182		
		Hasanlu, Iran	(?) incisor	124±1	2.9	f
		(3000 BC)	(?) incisor	134±4	3.1	f
			(?) incisor	99±5	2.4	f
			max. R. 2nd incisor	128±4	2.9	e, f
			mand. R. 1st incisor	157±12	3.5	e, f
			(?) incisor	90±11	2.2	f
			max. R 1st incisor	75±7	1.9	e, f
			(?) incisor	134±2	3.1	f
			(?) incisor	103±1	2.5	f
			(?) incisor	93±1	2.3	f
		Island Field, USA	(?) incisor	148±7	3.3	f
		(AD 800)	(?) incisor	113+3	2.7	f

This table was based on the listed references. Modified from three sources: 1) Aiello & Dean, 1990, p. 131, Table 7.1; 2) Hillson, 1996, p. 179, Table 6.4.; and 3) Mann et al., 1991, p.180, Table 2.
(*) sign represents incomplete crown; (?) sign represents unknown dental location.
(P.C.): perikymata count; (max.): maxillary; (mand.): mandibular; (R): right; (L): left

Reference: *a. Bromage & Dean (1985)* *b. Dean et al. (1986)* *c. Dean (1987a)* *d. Dean & Beynon (1991)* *e. Mann et al. (1990b)* *f. Mann et al. (1991)* *g. Dean et al. (1993b)*

Although during the last decade researchers have repeatedly tried to assess crown formation times and the age-at-death in hominid fossil teeth, the use of perikymata counts in interpreting the fossil record remains problematic at various levels.

Some assumptions have been challenged, such as the true representation of the circadian rhythm of cross-striations, the correlation between the lines of Retzius and cross-striations especially in its notion of circaseptan rhythm, and the wide variation in the periodicity of perikymata counts within and between individuals (Mann et al., 1990b, 1991; FitzGerald, 1998; Risnes, 1998). In addition, during the cuspal, or appositional stage of enamel formation, lines of Retzius do not reach the tooth surface; only in enamel formed later at the imbricational stage, lines of Retzius are manifested at the surface of the crowns. Therefore, crown formation times may not have been precisely predicted, when the differential appositional enamel formation times were ignored or corrected by the estimation. Moreover, perikymata are variably expressed at the cervix of teeth, which increases the possibility of underestimation for age-at-death and crown formation time, even if the perikymata, the lines of Retzius, and the cross-striations were to be truly and exclusively correlated with their periodicity.

In the investigation of age-at-death, the results vary within the same individual. While Bromage & Dean (1985) estimated the age-at-death of a juvenile *Paranthropus robustus* SK 63 to be 3.15 years old, based on the perikymata counts of a mandibular right central incisor; Dean et al. (1993b) applied the histological cross-striation counts of a mandibular right canine and concluded that 4 years would be a more accurate estimate. The discrepancy between the estimates may result from either the high variability of the tissue studied or the inadequateness of the methodology itself.

A test was carried out by Stringer et al. (1990) to investigate the correlation between samples of known-age and the incremental ageing of the perikymata counts in the Spitalfields collection. Three estimates of age at death were calculated from each incisal perikymata count by using 7-day, 8-day, and 9-day periodicity for perikymata. The assumption of 7-day periodicity and early incisal calcification at about 3 months after birth (Dean et al., 1986) consistently underestimates age at death. The 8-day periodicity and an adjustment of a later initiation of calcification, about 6 months for lower central incisors and 9 months for upper central incisors, gives an agreement between real and estimated ages. The 9-day periodicity gives a poor agreement with the real ages. Though the study seems to provide a good match and evidence for their applicability, it also clearly demonstrates that no one choice of periodicity is likely to accurately reflect those of a whole population of individuals.

The controversy surrounding the use of perikymata counts as the ultimate tools of estimating crown formation times and age-at-death can only be resolved through further investigations.

The wide range of perikymata count estimates and very small sample sizes in previous studies have sparked much controversy surrounding the interpretations and inferences of results derived from perikymata counts. One of the major problems relates to the absence of important baseline information on the biological variation in human perikymata counts. We do not have sufficient comparative data across our own and relevant species. The taxonomic premises of such perikymata counts should be tested first in order to offer a true database for comparisons.

In this project, a scanning electron microscope (SEM) was used to determine the perikymata counts of the third premolars (symbol: P3 and clinically called the first bicuspids) in the maxillary region in two modern human samples collected from Taiwan and Japan.

The purpose of this study was to establish a database of modern human perikymata counts to facilitate comparisons by sex, ethnicity, and species, and to help resolve the perikymata counts controversy. This study was designed to achieve several goals which include the following:

1. to investigate the biological variation in modern human perikymata counts;
2. to determine the degree of sexual dimorphism in human perikymata counts;
3. to examine the ethnic differences of Chinese and Japanese or the regional differences between two isolated islands of Taiwan and Japan in their perikymata counts;
4. to analyze the strength of correlations between the perikymata counts and various dental dimensional parameters.

Methods

The dental samples were collected from two different geographic regions in Asia, which also had different ethnic compositions. One region was the Tainan City of Taiwan, Republic of China and the other was the Nagoya City of Japan. Both are the fourth largest cities in their own country.

Since the study applied ethnicity as one of the variables, the sample collection process excluded those specimens which were obtained from aborigines in Taiwan. Taiwan aborigines consist of nine tribes. They are widely dispersed, though most inhabit the remote mountains of central Taiwan, and are rarely seen in the highly populated coastal plain regions. They are believed to be more closely related to the Malay than to the Chinese both morphologically and genetically. Given that Taiwanese aborigines live quite distant from Tainan City, where the samples were collected, we assumed that the statistical error including aborigines in the study was quite low. The ethnic identification of the samples was

carried out by the dentists who collected them. If no screening had taken place during sample collection and the sampling had been random, sampling error would have been maximally 2 %.

The definition of Japanese here does not include Korean-Japanese descendants. The population of Japan was estimated 126.18 million as of July 1999. The ethnic groups in Japan includes 99.4% Japanese and 0.6 % other, consisting mostly of Korean descendants (CIA, 1999). Historically, many Koreans immigrated to Japan. As with the Chinese, Korean descendants usually maintained their own ethnic origin by holding on to their Korean family name.

The Ainu aboriginal Japanese who live in the Northern Japanese island of Hokkaido make up a very small percentage of the total population in Japan. Since they are morphologically distinctive and are distant from where our samples were collected, we can ignore the statistical bias of possibly inclusion of Ainu. Even if the Korean samples were accidentally included and the sampling had been random, there would only have been 0.6% error in statistic probability.

The samples were collected during a 5-year period from 1993 to 1998. Local dental clinics (predominantly orthodontic), of Tainan and Nagoya, assisted the dental sample collections for this project. The collected teeth were generally extracted during the course of orthodontic treatment. Since most of the orthodontic patients were juvenile or adolescent individuals, the age of the individual from whom dental specimens were extracted ranged mostly from 7 to 18 years old in our samples. Dental specimens from adults were excluded during the collection process, since attrition and abrasion would have occurred and been noticeable.

After the teeth were extracted, the samples were first divided into several subgroups according to ethnicity, ("Chinese" and "Japanese" as defined above); sex (male and female); side (right and left), and location (maxillary and mandibular). A total of 728 extracted modern human third premolars were collected: 439 from Taiwan and 289 from Japan.

Most of the specimens collected were well preserved in gross morphology with minimal attrition and abrasion. Specimens with obvious abnormality, such as microdontia, or major surface damage, such as fractured or chipped enamel, were excluded. Since most of the dental specimens were removed from individuals of young age during their ongoing apexogenesis (root apex formation), some of them exhibited incomplete root formation, which is in part characterized by an open apex.

All samples were then screened using a Zeiss dissecting microscope with a magnification of 50X to verify their surface morphology, especially the existence of the perikymata microstructure. Specimens were discarded when they either showed very little presence of perikymata or defective enamel on the buccal surface of the tooth. These criteria further reduced the sample size from 399 to 92 cases for the maxillary third premolars. The final perikymata SEM observations included a total of 92 cases of maxillary third premolars, in which 44 dental specimens came from Taiwan and 48 from Japan. The mandibular third premolar samples were stored away for later SEM investigation, and were not included in this research project.

In this study, three sets of dimensional parameters were directly measured from the dental specimens themselves, epoxy resin dental duplicates, and the dental plaster casts. All the measurements were performed by a Mitutoyo digimatic caliper. Each set of measurements included: 1) buccal crown height, 2) lingual crown height, 3) mesiodistal width, and 4) buccolingual width. For each measurement, three readings were carried out and were averaged to reach a final measurement for the record.

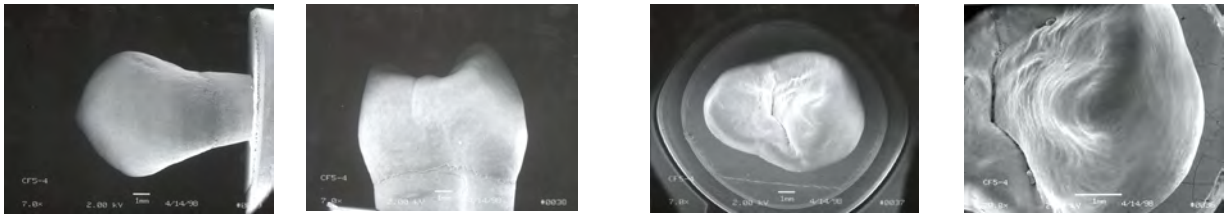
All the parameters were defined as the greatest dimensions during the assessment. Buccal and lingual crown heights were measured from the cervico-cemental junction (CEJ) to the buccal and lingual cusp tips. Buccolingual widths were measured between the most convex points on the buccal and lingual crown surfaces. Mesiodistal dimensions were measured between the mesial and distal contact points.

For SEM data collection Coltène PRESIDENT microSystem light body, a commercially available dental impression system, was utilized to produce replicas. Casts derived from these molds were made with Araldite 502. These casts were then coated and examined using an AMRAY 1850 field emission electron microscope.

The perikymata count observation was performed with the electron microscope operating at an acceleration potential of 2.00 KV with magnifications ranging from 7X to 2000X. In each case, a set of four reference micrographs was taken at the magnification of 7X to record its buccal, mesial, and occlusal views (Fig. 1) and at a magnification of 20X to record the occlusal view of the buccal cusp (Fig. 2). 40X to 50X magnification was used to provide adequate resolution for calculating perikymata counts (Fig. 3). Statistical analysis was carried out to calculate the sample size (N), means (M), standard deviations (SD), coefficient of variation (CV), range, minimum (Min.) and maximum (Max.) values, and other descriptive statistics such as skewness and kurtosis . Paired sample t-test, independent samples t-test, one-way Analysis of Variance (ANOVA), Scheffe's procedure for Post Hoc Comparisons, Pearson's correlation, and curve estimation for regression were also tested. A p value of ≤ 0.05 was considered to be significant.

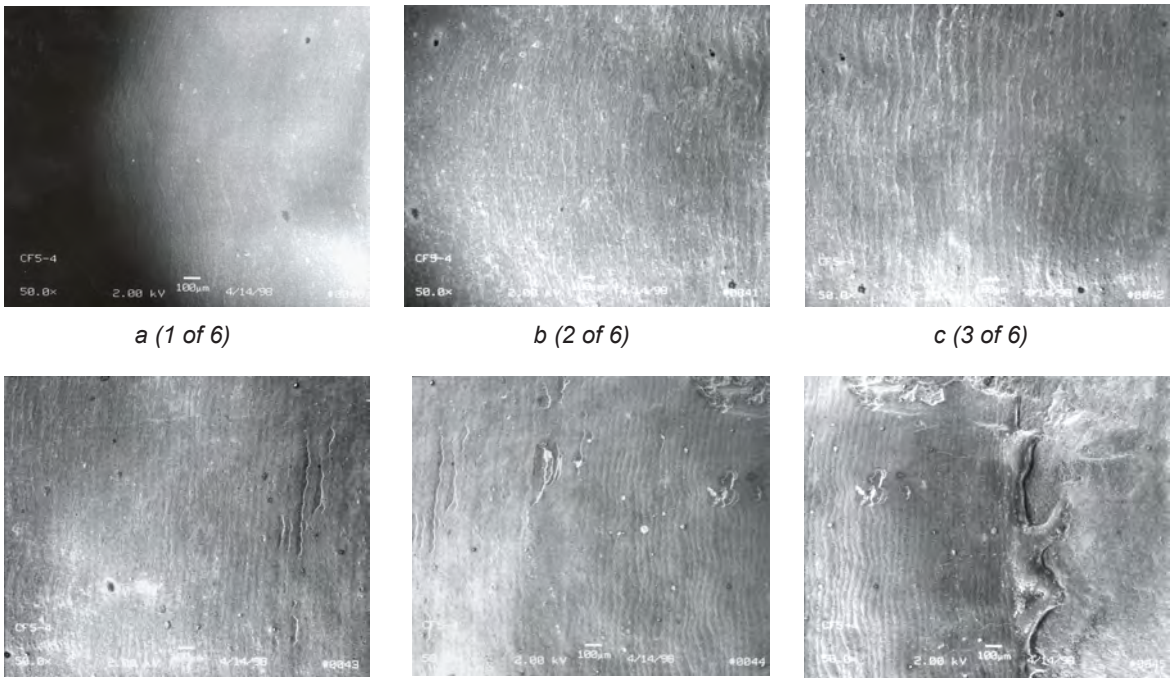
Results

When all cases were combined, the total sample size was 92. For the total sample, perikymata counts had a mean of 150, a standard deviation of 25, a coefficient of variation of 16%, minimum counts of 107, maximum counts of 209, a range of 102 counts, a median of 148, a mode of 140, kurtosis of - 0.275, and skewness of 0.437.



a: buccal view b: mesial view c: occlusal view d: buccal cusp

Fig. 1. A series of micrographs taken as references for the observation of perikymata. The specimen was obtained from a Chinese female. It is a right maxillary third premolar. (a), (b), and (c) are micrographs of buccal, mesial, and occlusal views respectively. (d) is the buccal cusp view. Note that on the (b) mesial view, the mesial groove was clearly seen in the middle of crown, dividing buccal and palatal cusps. (Photo© Michael S. Yuan)



a (1 of 6) b (2 of 6) c (3 of 6)

d (4 of 6) e (5 of 6) f (6 of 6)

Fig. 2. An example of a series of continuous micrographs on the buccal surface of a maxillary premolar. The micrographs are in a cusp-cervix sequence. (a): the buccal cuspal region; (b), (c), (d), & (e): regions between cusp and CEJ; and (f): the CEJ cervical region. (Photo© Michael S. Yuan)

Fig. 3. An example of a collage made from 6 continuous pictures illustrated at Figure 2-12. The collage shows perikymata ridges and grooves on the middle buccal surface of a human maxillary premolar. The cusp is on the left side of the collage, while the CEJ on the right. (Photo© Michael S. Yuan)

Table 1. Perikymata counts of the maxillary third premolar grouped by sex.

Group	N.	Mean	S.D.	C.V.	Range	Min.	Max.
Male	45	153.84	23.58	15.33%	93	111	204
Female	47	146.85	25.02	17.04%	102	107	209
Total	92	150.27	24.45	16.27%	102	107	209

T-test: t=1.378 (p= 0.171).

Table 2. Perikymata counts of the maxillary third premolar grouped by side.

Group	N.	Mean	S.D.	C.V.	Range	Min.	Max.
Right	46	153.87	24.91	16.19%	102	107	209
Left	46	146.67	23.70	16.59%	95	109	204
Total	92	150.27	24.45	16.27%	102	107	209

T- test: t=1.419 (p= 0.159).

Table 3. Perikymata counts of the maxillary third premolar grouped by ethnicity.

Group	N.	Mean	S.D.	C.V.	Range	Min.	Max.
Chinese	44	151.05	23.84	15.78%	100	109	209
Japanese	48	149.56	25.22	16.86%	97	107	204
Total	92	150.27	24.45	16.27%	102	107	209

T test: t=0.289 (p= 0.773).

The distribution is based on a scale of 5 counts per interval for perikymata count distribution. In the primary subgroups, there were no statistical differences in maxillary third premolar perikymata counts between the sexes and between the sides at the significance level of 0.05 (Tables 1 & 2). The variation was relatively high, since the coefficient of variation ranged between 15 to 18%.

Though no statistical differences were found, the mean differences showed that the males had more average perikymata than the females by 7 counts, and the right side had more counts than left side by 6. This suggests that males may require longer time to complete crown formation of the maxillary third premolar. Likewise, the right side may require longer time to complete crown formation as compared to the left.

Comparing ethnicities, there was also no significant difference in perikymata counts of the maxillary third premolar (Table 3). The means of the two subgroups, which were 151 and 150 counts, are practically identical. This result shows that there were no differences between two non-contiguous geographic regions of Taiwan and Japan, where samples were collected.

Further statistical analysis was performed to test the differences among the eight tertiary subgroups. In the tertiary subgroups, the perikymata counts of the maxillary third premolars demonstrated means ranging from 140 to l56 counts, standard deviations ranging from 20 to 30 counts, and coefficients of variation ranging from 20 to 30 %. Once again, there were no significant differences found among any of the tertiary subgroups (Table 4).

The statistical results suggests that given unknown modern East Asian maxillary third premolar specimens, one would not be able to identify their sex, side, and ethnicity by perikymata counts of the enamel microstructures. However, one can distinguish the right and left sides of the maxillary and mandibular third premolars based on their distinctive gross morphology (Kraus et al., 1969; Jordan, 1992). Results comparing buccal crown height, lingual crown height, mesiodistal width, and buccolingual width for all measurement except as follows: 1) there were significant differences between males and females, and right and left sides with regard to lingual crown height, 2) there was a significant difference between males and females with regard to buccolingual width, 3) there was a significant difference between Chinese-male and Japanese-female subgroups with regard to buccolingual width.

Measurements of the perikymata counts and crown dimensions of the maxillary third premolar were tested for correlations (Table 5). Perikymata counts of the maxillary third premolars were significantly correlated with buccal crown height. Such a high correlation makes sense since the perikymata counts were numbered on the buccal crown surface. The Pearson correlation coefficient (r) was calculated 0.475, which resulted in a coefficient of determination (r^2) of 0.23. Perikymata counts were also significantly correlated with the lingual crown height with a correlation coefficient of 0.304 and a r^2 of 0.09. However, perikymata counts were not correlated with the mesiodistal and buccolingual widths in our dental samples.

The dimensional parameters, i.e. the buccal crown height, lingual crown height, mesiodistal width, and buccolingual width, were mutually correlated with one

Table 4. Perikymata counts of the maxillary third premolar grouped by sex, side, and ethnicity (C: Chinese; J: Japanese; M: male; F: female; R: right; L: left).

Group	N.	Mean	S.D.	C.V.	Range	Min.	Max.
C-M-R	11	156.18	19.84	12.70%	62	127	189
C-M-L	12	151.50	26.76	17.66%	91	111	202
C-F-R	10	154.00	25.24	16.39%	91	118	209
C-F-L	11	142.73	23.99	16.81%	77	109	186
J-M-R	11	155.55	25.95	16.68%	68	119	187
J-M-L	11	152.36	23.96	15.73%	87	117	204
J-F-R	14	150.64	29.46	19.56%	97	107	204
J-F-L	12	140.25	20.44	14.57%	57	112	169
Total	92	150.27	24.45	16.27%	102	107	209

ANOVA F test: F= 0.634 (p= 0.727).

Table 5. Pearson correlation analysis of perikymata counts and crown dimensions (N = 92).

Group / r value	Periky. C.	Log Periky.C.	Bu.Cr.Ht.	Li.Cr.Ht.	Me.Di.Wd.	Bu.Li.Wd.
Periky. C.	1.000	0.995*	0.475*	0.304*	0.082	0.027
Log Periky. C.	0.995*	1.000	0.482*	0.318*	0.063	0.024
Buc. Cr. Ht	0.475*	0.482*	1.000	0.631*	0.396*	0.386*
Ling. Cr. Ht	0.304*	0.318*	0.631*	1.000	0.349*	0.391*
Mesiod. Wd.	0.082	0.063	0.395*	0.349*	1.000	0.716*
Bu. Li. Wd.	0.027	0.024	0.386*	0.391*	0.716*	1.000

** Correlation is significant at the 0.01 level (2-tailed).*

Abbreviations: *r: Pearson's correlation coefficient.*
Periky. C.: perikymata counts *Log Periky.C.: Log 10 base perikymata counts*
Bu. Cr. Ht.: buccal crown height. *Li. Cr. Ht.: lingual crown height.*
Me. Di. Wd.: mesiodistal width. *Bu. Li. Wd.: buccolingual width.*

another. The buccal crown height was significantly correlated with the lingual crown height with a correlation coefficient of 0.631 and a coefficient of determination of 0.369. The mesiodistal width was significantly correlated with the buccolingual width with a correlation coefficient of 0.716 and a coefficient of determination of 0.513.

Table 6 shows a correlation table of perikymata counts and its buccal crown dimension subcategorized by sex and ethnicity. All correlations are significant at the 0.01 level except the Chinese group which is significant at 0.05. The results demonstrate a higher correlation in males and in Japanese between perikymata counts and corresponding buccal crown height.

The means of perikymata counts, when rescaled in an increment of 1 mm, showed a consistent positive correlation with buccal crown height in both male and female samples (Table 7), and also in the sex-combined sample (Table 8). In Table 7, the mean counts clearly demonstrated an average of 15 increments corresponding to each mm height increase. A similar pattern is also shown in ethnicity, except that in the 10 mm buccal crown height group, Chinese showed a lower mean, while Japanese showed a much higher mean. This may be attributed to the low sample size.

Negative kurtosis value for most of the perikymata count observations corresponds to the flat, wide shape of the distribution. This large sample (n = 92) satisfies the power of statistical requirement. As noted, there is also an obvious right skewness along perikymata count and crown dimensions as all the results showed positive values.

As we apply correlation and curve estimation equation to explore the regression model between perikymata counts and crown dimensions, we should always bear in mind that such a model only applies to a limited range in reality. Data were transformed into log base 10 to provide a logarithmic approach in correlation with the parameters of crown dimensions. Although there does not exist much difference between the linear and log regression curve estimation between the perikymata counts and crown height, the log approach does fit better and serve as an accurate representation as the counts are one of the products of biological tissues.

Table 6. Pearson correlation analysis of perikymata counts and buccal crown height grouped by sex and ethnicity in a combined sample.

Category	sample size	correlation (R)	R square	p-value	Sig. level
Male	45	0.498	0.248	0.000	$p < 0.01$
Female	47	0.451	0.203	0.001	$p < 0.01$
Chinese	44	0.380	0.144	0.011	$p < 0.05$
Japanese	48	0.547	0.299	0.000	$p < 0.01$
Total	92	0.475	0.226	0.000	$p < 0.01$

Table 7. Mean of perikymata count based on the 1 mm interval of buccal crown height of the maxillary third premolar in East Asians.

Interval	N.	Mean	S.D.	C.V.	Range	Min.	Max.	Kurtosis	Skewness	Median
11 mm	1	180.0	-	-	0	180	180	-	-	-
10 mm	13	167.3	23.4	13.98%	69	140	209	-0.927	0.591	161.0
9 mm	50	152.9	23.9	15.63%	95	109	204	-0.177	0.331	149.5
8 mm	27	137.3	18.9	13.76%	73	107	180	-0.258	0.524	136.0
7 mm	1	119.0	-	-	0	119	119	-	-	-
Total	92	150.3	24.5	16.30%	102	107	209	-0.275	0.437	148.0

Table 8. Mean of male and female perikymata count based on the 1 mm interval of buccal crown height of the maxillary third premolar in East Asians.

Interval	Sex	N.	Mean	S.D.	C.V.	Range	Min.	Max.	Kurtosis	Skewness	Median
11 mm	M	1	180.0	-	-	0	180	180	-	-	-
	F	0	-	-	-	-	-	-	-	-	-
10 mm	M	8	167.0	22.0	13.17%	64	140	204	-0.631	0.470	164.0
	F	5	167.8	28.2	16.80%	67	142	209	-1.019	0.909	152.0
9 mm	M	24	155.7	23.2	14.90%	85	117	202	-0.409	0.554	151.0
	F	26	150.3	24.7	16.43%	95	109	204	0.041	0.233	149.0
8 mm	M	11	141.0	18.9	13.40%	69	111	180	0.790	0.565	140.0
	F	16	134.7	19.2	14.25%	63	107	170	-0.405	0.616	133.5
7 mm	M	1	119.0	-	-	0	119	119	-	-	-
	F	0	-	-	-	-	-	-	-	-	-
Subtotal	M	45	153.8	23.6	15.34%	93	111	204	-0.486	0.433	149.0
	F	47	146.9	25.0	17.02%	102	107	209	0.034	0.526	146.0

Discussion

The use of perikymata counts has been suggested for studies in estimating the age of death as well as the length of crown formation times. While this idea may be appealing, several problems stand in the way of an application of this methodology.

Age of death estimation is not feasible at this time for a number of reasons, some of which are inherent in the histological issues as described below. In addition, the degree of variability is high (maximal difference up to 1.75 years) in the radiographic and histological data on crown developmental times as shown in Table 9 modified from Reid et al. (1998).

The application of perikymata counts in estimating crown formation times requires several assumptions:

1. The full establishment of cross-striations as circadian rhythm;
2. The clear mathematical correlation of the counts of cross-striations to each interval between the line of Retzius, such as 5, 7, 8, 9, 10, 11, more, or irregular periodicities;
3. The correct correlation between lines of Retzius and perikymata.

How certain are we that the circadian rhythm of the cross-striations, the circaseptan rhythm or consistent periodicity of lines of Retzius, and correlations between

Table 9. Human crown initiation-(crown formation)-crown completion times (years).

		Moorrees et al.	Gustafson & Koch	Dean et al.	Reid et al.*
Tooth		(1963)	(1974)	(1993)	(1998)
Max.	I1	-	0.30-(4.20)-4.50	0.32-(3.15)-3.47	0.35-(4.08)-4.43
	I2	-	0.95-(4.15)-5.10	0.69-(3.72)-4.41	1.05-(3.61)-4.66
	C	(3.5)	0.40-(5.80)-6.20	0.38-(4.37)-4.75	0.75-(4.45)-5.20
	P3 b	(3.1-3.4)	1.75-(4.15)-5.90	1.67-(2.85)-4.52	1.85-(3.57)-5.42
	P4 b	(3.1-3.4)	2.15-(4.70)-6.85	2.41-(3.11)-5.52	2.65-(2.95)-5.60
	M1 mb	(2.1)	0.00-(3.10)-3.10	0.00-(2.41)-2.41	0.05-(2.83)-2.78
	M2 mb	(2.8)	2.85-(4.55)-7.40	2.92-(3.13)-6.05	2.80-(3.28)-6.08
	M3 mb	(2.8)	-	-	7.68-(3.27)-10.95
Mand.	I1	-	0.30-(3.90)-4.20	0.32-(3.10)-3.42	0.25-(3.52)-3.77
	I2	-	0.30-(4.20)-4.50	0.69-(3.72)-4.41	0.40-(4.20)-4.60
	C	(3.5)	0.35-(5.75)-6.10	0.38-(4.37)-4.75	0.55-(5.41)-5.96
	P3 b	(3.1-3.4)	1.75-(4.25)-6.00	1.67-(2.85)-4.52	1.85-(3.87)-5.72
	P4 b	(3.1-3.4)	2.25-(4.60)-6.85	2.68-(3.11)-5.79	2.65-(3.46)-6.11
	M1 mb	(2.1)	0.00-(3.00)-3.00	0.00-(2.67)-2.67	0.05-(3.39)-3.34
	M2 mb	(2.8)	2.85-(4.45)-7.30	-	2.90-(3.16)-6.06
	M3 mb	(2.8)	-	6.42-(3.16)-9.58	7.77-(3.09)-10.86

Adopted and modified from Reid et al., 1998, Journal of Human Evolution, p. 474,Table 5.
** Reid et al. (1998) was based on histological data; other results were assessed by radiograph.*
Abbreviations: (Max.): maxillary; (Mand.): mandibular; (b): buccal cusp; (mb): mesiobuccal cusp

lines of Retzius and perikymata are universally true across the extant and extinct primates?

1. Cross-striations

The overall evidence demonstrates that cross-striations characterize a 24-hourly or circadian rhythm (Mirura, 1939, Risnes, 1986, Bromage, 1991). The precise nature of the cross-striations is still unclear at present. The differences in the composition of hydroxy-apatite crystallites and the enamel matrix, as well as the degree of calcification have been proposed to explain the observable fact of cross-striations, but remain to be proven (Semmelink & Nygaard, 1982). While phases of the mineral secretion play the key roles in determining such periodicity, the consistency of the circadian rhythm may have some degree of variation (see Robison et al., 1997, for related issues in the mineral, water, protein, and enzyme distribution throughout the enamel maturation phases).

The difficulty in labeling enamel during its secretion and maturation phases has thus far made it difficult to establish beyond doubt that cross-striations represent a circadian rhythm in enamel formation. Nevertheless, evidence in other tissues such as dentin, bone, and cartilage make it quite reasonable to assume that they are indeed evidence of a circadian rhythm.

2. Relationship between cross-striations and lines of Retzius

Norman & Poole (1974) found in their transmission electron microscopic investigation that lines of Retzius appeared as gaps between rows of enamel crystals. They proposed that the lines of Retzius were a phenomenon of imperfect synchronizations of two or more circadian rhythms and suggested an eight-day periodicity. One example they cited was the study by Lewis & Lobban (1957) which explored the dissociation of diurnal rhythms in human subjects by restraining the subjects to live in abnormal time routines of 21, 24, & 27 hours per day. Surprisingly, they found that while the potassium secretion persisted in a 24-hour rhythm, the other variables adapted to the environmental alterations through time.

The numbers of cross-striations between the lines of Retzius have always been a confusing matter in debates. In the traditional examination of cross-striations, the methodology relies on ground sections of tooth enamel observed under light microscopes. One of the difficulties has been how to reduce the thickness to one or two enamel prisms, which would only be 10 to 15 µm, and still be able to observe the dark-light alternating bands under a light microscope.

Dean & Beynon (1991) recommended and performed their research with 100 µm to achieve a uniform section thickness. Bromage (1991) also applied dental

sections of 80 to 100 μm in the enamel labeling study on macaques. Nevertheless, the 100 μm thickness does impose a reading error by overlapping too many layers (about 15) of enamel prisms, therefore possibly leading to inaccurate estimations in cross-striation counts.

FitzGerald (1998) employed 158 anterior teeth of different sex and ethnicity to explore the periodicity between the lines of Retzius. The final dental sample size was 96. He reported a mean of 9.7 and a SD of 1.0 count of cross-striations between lines of Retzius. His effort in trying to dispute the generally believed circaseptan rhythm between lines of Retzius may not be reliable due to his methodology in choosing 100 μm thickness.

Although the reduction of section thickness was managed, Bullion (1987) commented that a poor reading resolution of cross-striations would result, if the enamel sections went under 40 μm in thickness. Therefore, the methodological constraints in tissue preparation and section observation would have introduced some degree of imprecision and inaccuracy in the aforementioned studies.

Moreover, in most of the illustrations in the literature, the counting of cross-striations is on the superficial or outer part of enamel prism and seldom on the deeper or inner part of the enamel prism. This is due to the difficulty in reading the cross-striations in the deeper enamel in which the Hunter-Schreger bands are more obvious. This optical phenomenon is created by the decussating layers of enamel prisms. The exaggerated curvilinear prism path may be the reason why the difficulty in reading the cross-striations occurs.

Moss-Salentijn et al. (1997, p.17) concluded, " In our opinion, all that can be stated at present is that the lines of Retzius in imbricational enamel exhibit an apparent periodicity. The difficulty of establishing the length of the period and the question whether this period is equal among hominids do not permit definitive statements at this time."

3) Correlation between lines of Retzius and perikymata

Perikymata are generally assumed to be the surface or external manifestations of the lines of Retzius of teeth. While this relationship has been well established for coronal and middle thirds of enamel (Kölliker, 1854; Pickerill, 1912; Risnes, 1984), the relationship is not as clearly defined in the cervical enamel. This is particularly troublesome since the distances between the cervical perikymata are progressively smaller, thus involving a relatively large proportion of the count (see Chapter 6, pp. 96-97 for the discussion on the perikymata count variations at the cervical regions).

In addition, the equivalence between the lines of Retzius in imbricational enamel and the perikymata on the enamel surface may not be a universal mammalian pattern. Skobe et al. (1985) have demonstrated that in carnivore enamel the lines of Retzius do not extend to perikymata grooves on the enamel surface.

Several methodological and theoretical assumptions based on the human perikymata counts of the permanent incisors and the radiographic observations on permanent dentition have long been employed as evidence in the application of perikymata counts in anthropological studies to estimate the crown formation time and age at death. We would like to point out again that such attempts have created numerous controversies and misleading results.

First, the currently widely applied assumptions were derived from small sample sizes that only used incisor teeth. The examples of modern perikymata count in human incisors, as was described in Chapter 1, includes two studies on modern humans by Bromage & Dean (1985, mean = 188, range = 165-202, n = 10, mandibular incisors) and Bacon (1987, mean = 145, range = 111-179, n = 23, specimen: incisors). There is one report by Mann et al. (1990b, 1991), using the archeological collection of 3000 B.C. from Hasanlu, Iran and A.D. 800 in Island Field, Delaware, U.S.A. This investigation disputed the aforementioned work (mean = 116, median = 118, SD = 25, range = 75-157, n = 12). In addition to the small sample sizes, none of these studies presented convincing results regarding sex, and ethnicity, nor were the other dental locations, such as canine, premolars, or molars, examined.

Second, Bromage & Dean (1985) verified their results as evidence for the perikymata count applicability in estimating the crown maturation times by the matched overlapping of the perikymata-derived crown maturation times with the radiographically documented crown maturation times. We emphasize, as did Mann et al. (1991), that while exercising these applications we must recognize the fact that since crown maturation times were derived, such an approach has to be considered as hypothetical and requires further proof in testing its accuracy.

Third, while perikymata counts may correspond to the incremental lines of the superficial imbricational enamel, the incremental lines in the appositional enamel can only be estimated, assuming that formation times can be derived from the counts of lines of Retzius or cross-striations. This indirect approach will expand the range of variation or reduce the correlations of the related dental microstructures, thus introducing more errors in estimation.

Fourth, as we examined the crown formation data, we noted that the growth and developmental timings for each tooth differed from one another. Therefore, as was done in this study, the subcomponents of the incisors, canines, premolars, and molars should be treated individually to obtain their respective perikymata counts, instead of pooling them together to form a much larger group for comparisons.

We should also not consider the upper and lower dentitions as essentially mirror images. In much of the literature, researchers tend to pool samples, which may lead to problems in increasing the biological variations and misrepresentation by a larger sample size. While

Table 10. Comparison of perikymata counts in this study vs. the lines of Retzius (LR) counts of Bullion (1987) of the maxillary third premolar.

Group	N.	Mean	S.D.	C.V.	Range	Min.	Max.
Bullion, 1987							
Sleeve LR	4	106.17	8.77	8.26%	20.67	94	114.67
Yuan, 2000							
Perikymata	92	150.27	24.45	16.27%	102	107	209

Significant p= 0.0007 (p < 0.01)

Mann et al. (1990b, 1991) cautioned researchers regarding the misleading conclusion of ape versus human affinity of the australopithecines by applying the perikymata counts to estimate the age of death, it was unfortunate that they grouped all the incisors together and failed to provide a true representation in the variations of each individual incisor. All incisors, whether maxillary or mandibular, central or lateral, have different crown maturation times.

This project, which explores human third premolars in order to establish a data set of perikymata counts, has been the only attempt so far in expanding our knowledge of human biological variations in perikymata counts beyond permanent incisors in the past decade. The sample size of 92 specimens in a single tooth location in permanent dentition, the maxillary third premolar, is also the largest observation ever made. This database will no doubt provide a reliable source for comparisons and further implications.

In this study, not only the sample size was expanded from the above mentioned 12 to 92 specimens, but also perikymata counts were established in a new dental location, maxillary third premolars. In addition, the variations in sex and ethnicity were fully explored. Moreover, the correlation among the perikymata counts and their corresponding crown dimensions were investigated. The results shown in this study not only help us understand the complexity of this phenomenon, but also dispute the present application of perikymata counts until further evidence of the correlation between the enamel microstructures is confirmed.

The perikymata counts of the maxillary third premolar in mixed sex and ethnicity of this East Asian population gives a mean count of 150, a standard deviation of 25, a coefficient of variation of 16%, a median of 148, a mode of 140, a range of 102 with minimum of 107 and maximum of 209.

The results demonstrated that there was neither sex differences between males and females, nor ethnic differences between Chinese and Japanese in perikymata counts. In addition, the results illustrated sexual dimorphism in that males had higher perikymata counts and larger crown dimensions than those of females.

The commonly cited reference in the study of cross-striations and lines of Retzius comes from Bullion (1987). Bullion counted the lines of Retzius of the appositional enamel (referred to as the dome-shaped lines of Retzius) and the imbricational enamel (referred to as the sleeve-shaped lines of Retzius, and is considered as the internal manifestation of perikymata) in a total sample of 48 unworn modern human teeth of 100 µm thickness ground sections (see Bullion, 1987, Chapter 7 for detailed descriptions and the summary of findings in Table 7.1, 7.2, 7.3, & 7.4). The sample mostly came from the Royal Lancaster Infirmary and local dental offices, UK, and partially contributed by Dean.

In Bullion's study, the maxillary third premolar dome-shaped lines of Retzius had average counts in a sample of 4 teeth of 39, 42, 36.33, and 43.33 respectively. The statistical results are n = 4, mean = 40.15, SD = 9.83, CV = 24.47%, range = 36.33-43.33. These reflect high variation.

The maxillary third premolar sleeve-shaped lines of Retzius had average counts in a sample of 4 teeth of 94, 106.67, 114.67, and 109.33 respectively. The statistical results are n = 4, mean = 106.17, SD = 8.77, CV = 8.26%, range = 94-114.67. These represent a very different pattern.

Bullion's results on dental examinations of the maxillary third premolar are reported 106 $\pm$ 9 (n = 4) for the counts of sleeve-shaped lines of Retzius. The result stands in contrast to what we have obtained in the result of perikymata counts in our sample. The 106 $\pm$ 9 counts of sleeve-shaped lines of Retzius demonstrate a major discrepancy to the corresponding counterparts of the 150 $\pm$ 25 (n = 92) perikymata count in this study. We find a significant difference between the two findings ($p < 0.01$) (see Table 10 for summary).

The discrepancy may be accounted for as a result of:

1. Observation errors. As Bullion (1987, p. 141) stated, "…under high magnification, they (lines of Retzius) had the appearance of 'fuzzy brown bands'… they often appeared in a haphazard pattern with none of the regularity observed under low magnification…" This phenomenon should have existed in all of the observations. The resolution needed to improve the detection or identification of these microstructures requires SEM investigation.

2. Small sample size. Bullion only investigated four maxillary third premolars as compared to the 92 specimens examined in this study. The small sam-

ple size may possibly lead to a biased result.

3. Pattern miscorrelation and misrepresentation. If there were no observation errors in Bullion's study, then the correlation between lines of Retzius and their external counterparts, perikymata, would have exhibited an unknown and more complicated pattern than what was previously concluded. As such, this opens a major theoretical gap for the implications of perikymata counts.

The notion of crown formation times and age-at-death estimation based on the perikymata counts should be re-examined.

As discussed above, significant issues remain in the interpretation of the three microstructures in enamel: cross-striations, line of Retzius, and perikymata. As we have seen in the methodology, (1) the estimations and countability of cross-striation counts between lines of Retzius, (2) the constraints in the thickness of section preparation and in the tissue observation, (3) the indefinite role of the curvilinear and decussating structure of the Hunter-Schreger band related enamel prisms, (4) the inconsistency in the deeper enamel layer for the lines of Retzius and cross-striations, these problems render the application of the perikymata counts as truly questionable.

It is obvious that too many unknown factors have contributed and played major and/or minor roles in determining the final outcome.

This study has demonstrated the value of a large data set. Such baseline information will serve not only the relevant anthropological field, but also several areas in biology, with a solid foundation to further examine the kinematics of ameloblasts and their principal product, enamel, during human development and the evolutionary history of hominid lineages.

Investigations of perikymata counts in great apes and monkeys are essential to hominid and hominoid dental anatomy comparisons, fossil record interpretations, and life history reconstructions. Without these data, we will not be able to accurately use the perikymata counts to estimate age in fossil primate species. The next step is to complete the East Asian mandibular third premolar perikymata count study in Chinese and Japanese, since the sample collection is available. The expansion of such a dataset should further include: 1) the comparisons of other teeth in the permanent dentition, and 2) the comparisons of other ethnic groups from different regions, e.g., Africa and Europe.

The controversies about the correlations between perikymata counts, lines of the Retzius, cross-striations, and Hunter-Schreger bands also require immediate attention. The perikymata counts will serve as a fundamental data set as more efforts are made to further explore these correlations.

Conclusion

Perikymata, the incremental lines on the dental crown surface, are of great interest to dental histologists and anthropologists. The use of perikymata counts in estimating the age at death and crown formation times has been a controversial issue in providing evidence for how we interpret the place of australopithecines in hominid evolution.

This study, which collected the largest sample size thus far, examined the perikymata counts at one of the posterior dental locations, the maxillary third premolar, rather than the previously studied incisors in the anterior dentition. A total sample size of 92 maxillary third premolars (P^3), including right and left sides from males (n = 45) and females (n = 47), were investigated for their perikymata counts and crown dimensions in two modern human populations, Taiwanese and Japanese.

The results dispute the utilization of perikymata counts in the estimation of age-at-death and crown formation times. A valuable database is reported here for further comparisons in resolving the controversy surrounding the use of human perikymata counts, based on a large sample of modern *Homo sapiens* teeth.

We conclude that:

1) A review of the relevant literatures on histology, periodicity, research methodology, and the correlations among the enamel microstructures, including cross-striations, lines of Retzius, Hunter Schreger bands, and perikymata, has provided sufficient scientific evidence to necessitate rethinking the assumptions and methodologies underlying many of the fossil interpretations. Clearly, we do not have enough accurate data on the times needed to form crowns. Additionally, there is variability in the number of cross-striations between lines of Retzius. These are sufficient reasons to warrant a moratorium on the use of perikymata for hominid evolution studies.

2) While there is sexual dimorphism in perikymata counts, it is not statistically significant within the two populations. The counts in males are higher than those of females in both Taiwanese and Japanese.

3) There are no differences in perikymata counts between the right and left sides.

4) There is no difference for either males or females between Taiwan and Japan in their perikymata counts.

5) Perikymata counts are significantly correlated to their corresponding buccal crown heights.

6) When data from the two populations were pooled as East Asians (n= 92), the mean perikymata count for maxillary third premolars is 150 with a standard deviation of 25. The perikymata counts in this study are very different from the commonly believed counterpart line counts of Retzius, investigated by Bullion (1987). This significant discrepancy verifies the cautions raised by Mann et al. (1990b, 1991).

7) Although the data on perikymata counts follow a normal distribution, the variation is high (coefficient of variation = 16 %). Such high variation provides

part of the evidence for disputing the perikymata count assumptions.

8) The presumption of applying perikymata counts in estimating age at death and crown formation times should not be encouraged, as this will only compound the unknown issues in dental biology and create misleading results in human evolution.

9) We demonstrated the strength of SEM in this study, just as in other studies in dental anatomy, such as Boyde (1990) and Risnes (1998, 1999). Scanning electron microscopy proves to be one of the most powerful tools in future research of enamel microstructures.

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