Enamel biorhythms of humans and great apes: the Havers-Halberg Oscillation hypothesis reconsidered

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Abstract

The Havers-Halberg Oscillation (HHO) hypothesis links evidence for the timing of a biorhythm retained in permanent tooth enamel (Retzius periodicity) to adult body mass and life history traits across mammals. Potentially, these links provide a way to access life history of fossil species from teeth. Recently we assessed intra-specific predictions of the HHO on human children. We reported Retzius periodicity (RP) corresponded with enamel thickness, and cusp formation time, when calculated from isolated deciduous teeth. We proposed the biorhythm might not remain constant within an individual. Here, we test our findings. RP is compared between deciduous second and permanent first molars within the maxillae of four human children. Following this, we report the first RP's for deciduous teeth from modern great apes (*n*=4), and compare these to new data for permanent teeth (*n*=18) from these species, as well as to previously published values. We also explore RP in teeth that retain hypoplastic defects.

Results show RP changed within the maxilla of each child, from thinner to thicker enameled molars, and from one side of a hypoplastic defect to the other. When considered alongside correlations between RP and cusp formation time, these observations provide further evidence that RP is associated with enamel growth processes, and does not always remain constant within an individual. RP of five days for great ape deciduous teeth lay below the lowermost range of those from permanent teeth of modern orangutan and gorilla, and within the lowermost range of RP's from chimpanzee permanent teeth. Our data suggest associations between RP and enamel growth processes of humans might extend to great apes. These findings provide a new framework from which to develop the HHO hypothesis, which can incorporate enamel growth along with other physiological systems. Applications of the HHO to fossil teeth should avoid transferring RP between deciduous and permanent enamel, or including hypoplastic teeth.

KEY WORDS: Retzius lines, enamel growth, life history, biorhythms.

Introduction

Primate tooth enamel grows incrementally (Boyde, 1979, 1989). Each increment is marked by a growth line, as in shells and trees. One type of marking are Retzius lines (Retzius, 1837), which emerge on the outer lateral enamel surface as perikymata (e.g., Goodman and Rose, 1990). Retzius periodicity (RP) is the number of days of enamel growth between adjacent lines. The Havers-Halberg Oscillation (HHO) hypothesis proposes that RP of permanent teeth is a manifestation of an underlying biorhythm that regulates growth, is associated with adult body mass, and is related to life history traits when compared between mammalian species (Bromage et al., 2009, 2012). The underlying cause of the biorhythm is unknown, though experimental research on domesticated pigs implicates resting metabolic rate as an important influence (Bromage et al., 2016).

This study builds upon our recent work in which we tested intra-specific predictions of the HHO on human children (Mahoney et al., 2016). We reported that the modal and range of RP's from human deciduous teeth were lower compared to those calculated for human permanent teeth. Based upon this comparison, we suggested that RP might not remain constant within humans, though we did not calculate the periodicity of Retzius lines for deciduous and permanent teeth from the same individuals. We also reported that RP correlated with the reconstructed activity of enamel forming cells (secretory ameloblasts). The total amount of enamel deposited, and the time required by ameloblasts to form a human deciduous second maxillary molar cusp (dm^2), were both correlated with RP. Correlation between RP and enamel formation time has been noted previously, within a sample of human permanent canines (Reid and Ferrell, 2006), and during inter-specific comparisons of permanent first molars (M1) from extant and fossil hominoids (Mahoney et al., 2007). These correlations led us to suspect that RP might be related to some enamel growth processes.

The present study further investigates the possible links between RP and enamel growth. First, we compare RP between human deciduous and permanent molars within the maxillae of four human children. If the hypothesis that RP changes between these tooth types, from thinner to thicker enamel is correct (Mahoney et al., 2016), then the timing of this growth rhythm should not remain constant within each maxilla. A deciduous molar from a fifth maxilla retained evidence of disturbed enamel growth in the form of a hypoplastic defect (see below). Relationships between non-specific pathology and RP have not been examined previously. Yet, if, as we suspect, RP is linked to enamel growth, then perhaps disturbed enamel growth will be associated with RP in a deciduous crown.

In the second stage of this study we compare the timing of Retzius lines between deciduous and permanent teeth of great apes. We report the first deciduous RP values (*n*=4) for modern orangutan (*Pongo pygmaeus*), gorilla (*Gorilla gorilla*), and chimpanzee (*Pan troglodytes*). These values are compared to new data for permanent teeth (*n*=18) from these species, as well as to previously published values. Even though the deciduous and permanent teeth are not from the same individuals, we can still determine if deciduous RP's are encompassed within the range of RP's for permanent teeth from each species. The present study will also contribute to a new baseline comparative data set for great ape deciduous teeth. Retzius periodicity of permanent teeth is often compared between fossil and modern hominoids to gain insights into the evolution of dental development (e.g., Beynon et al., 1998; Schwartz et al., 2003; Mahoney et al., 2007), but rarely do such analyses include RP of deciduous teeth.

The timing of Retzius lines in humans and great apes

Retzius periodicity of modern human permanent teeth lies between a lowermost value of six days and an uppermost value of 12 days, with modes between seven to nine days depending upon the sample (Schwartz et al., 2001; Reid and Dean, 2006; Reid and Ferrell, 2006; Mahoney, 2008). The periodicity of 34 human deciduous teeth ranged between four to 11 days with a mode of six days (Mahoney et al., 2016). The lowered modal and range of RP values in this sample of isolated deciduous teeth, compared to permanent teeth, suggests the timing of Retzius lines might not remain constant within humans. However, one study reported that RP of a deciduous molar was the same as that observed in a permanent molar from the same mandible (Mahoney, 2012). Thus, it is still unclear if RP changes between these tooth types in modern humans.

Modern orangutan permanent teeth have a range of RP's between eight to 11 days, with a mode of 9 or 10 days (Beynon et al., 1991a; Dean, 2000; Schwartz et al., 2001; Kelley and Schwartz, 2010; Smith, 2016). Amongst modern gorillas, RP lies between seven to ten days, with a mode of eight (females) and nine days (males) (Beynon et al., 1991a; Schwartz et al., 2001; Kelley and Schwartz, 2010). The RP of modern chimpanzee permanent teeth might be as low as five days (Smith et al., 2010), but the majority of values range between six to nine days (Reid et al., 1998; Schwartz et al., 2001), with a mode of six or seven days (Schwartz et al., 2001; Smith et al., 2007). No study has reported the RP of great ape deciduous teeth.

Enamel hypoplastic defects

Disruptions to ameloblast activity during the secretory phase of enamel development can lead to hypoplastic defects that are retained in a tooth crown (Zsigmondy, 1893; Kreshover, 1940; Guatelli-Steinberg, 2001 for a review). Hypoplastic defects, which are classified by their morphology as furrow, pit, or plane-type, can be visible from the external surface depending upon the angle that Retzius lines emerge in outermost enamel (Hillson and Bond, 1997; Guatelli-Steinberg et al., 2012). These defects correspond with a range of non-specific stressors in humans, including nutritional deficiencies (vitamin D and calcium), infectious diseases, fevers, and congenital syphilis (Sarnat and Schour 1941; Sweeney et al., 1971; Purvis et al., 1973; Norén et al., 1978; Nikiforuk and Fraser 1981; Goodman et al., 1987; May et al., 1993; Hillson et al., 1998; Berdal et al., 2005; Bossù et al., 2007). Unlike a localised hypoplasia (Goodman and Rose, 1990), these systemic events can disrupt enamel growth in all forming crowns at the same time.

Hypoplastic enamel can be less mineralized, softer, and contain smaller hydroxyapatite crystallites, relative to normal enamel (Suckling et al., 1989; Batina et al., 2004). An altered microstructure implies that ameloblasts did not recover from the stress event that occurred during enamel secretion, and this affected subsequent maturation (Suckling et al., 1989; Batina et al., 2004). Hypoplastic enamel can also be as hard as normal enamel, indicating that maturation resumed after the defective secretory phase (Suckling and Purdell-Lewis, 1982; Suckling et al., 1989). Thus, disruptions to ameloblast activity can either be temporary or more sustained, which might relate in part to the stage of cell activity (Suga, 1989).

Materials and methods

Five human juvenile skeletons with erupted dm^2 (n=5) and erupting maxillary M1's (n=4) were selected (Table 1). The skeletons dated to the medieval period (11th to 15th Century AD) in England (Hicks and Hicks, 2001) and are curated in the Skeletal Biology Research Centre, University of Kent, UK. The accession numbers are NGB 1988, Sk27; NGA 1989, Sk102, 178, 665, 671. One dm^2 retained evidence of a hypoplastic defect, which was systemic, as we observed a corresponding defect in cervical enamel of dm^1 from the same maxilla.

Thin sections of four deciduous teeth from great apes were chosen for this study. One deciduous second mandibular molar (dm_2) from *P. pygmaeus* and *G. gorilla*, and one deciduous mandibular canine (dc_1) from *P. troglodytes* were selected from the Elliot Smith Collection, housed in the Anatomy Lab, University College London, UK. These sections were selected because it was possible to accurately reconstruct RP. The apes were wild shot specimens from the 1920's. Thin sections from these specimens were first prepared for a paper on tooth wear by Aiello and colleagues (1991). The accession numbers are (*Orangutan*) J56-E, (*Gorilla*) CA1F-1472-E, and (*Pan*) CA20A-2-36. Another dc_1 from *P. troglodytes* (906-11-73) was selected from a collection of primate sections held at The Ohio State University.

Thin sections of 18 ape permanent teeth were selected from the Elliot Smith Collection. These were a mix of maxillary and mandibular permanent first, second, and third molars of *P. troglodytes* (n=8: accession numbers CA-11, CA-13D, CA-14, CA-14A two slides, CA-14E, CA-19B, D-Case), permanent premolars and molars of *G. gorilla* (n=6: accession numbers HT41-89 two slides, HT42-89, HT44-89, UCL-CA-18, UCL-CA-4), and permanent premolars and molars of *P. pygmaeus* (n=4: accession numbers HT-162/88 two slides, HT-166/88, HT-1/91. No permits were needed to examine the deciduous or permanent slides.

Sample preparation

The human molars were prepared using standard methods (e.g., Mahoney 2008). Each tooth was embedded in polyester resin to reduce the risk of splintering while sectioning. Using a diamond-wafering blade (Buehler® IsoMet 4000 precision saw), sections were taken through the outermost enamel cusp tip, the tip of the dentin horn, and the most cervical enamel extension. Each section was mounted on a microscope slide, and lapped (Buehler® Eco-Met 300) using a graded series of grinding pads (ranging in grit size from P400 to P1200) to reveal incremental lines. Each section was polished with an aluminum oxide powder (Buehler® Micro-Polish II: 0.3μm) placed in an ultrasonic bath to remove surface debris, dehydrated through alcohol baths, cleared (Histoclear®), and mounted with a coverslip using a xylene-based mounting medium (DPX®).

Microscopy

All sections were examined at magnification (20-60x) using a high-resolution microscope (Olympus® BX51). Images were captured with a microscope digital camera (Olympus® DP25) and analyzed in CELL® Live Biology imaging software. RP's for human juveniles were recorded over a five-year period. Each slide was recorded four times. If values were not the same from one recording to the next, then the slide was not included in this study.

We calculated RP in post-natal lateral enamel, avoiding cervical enamel immediately adjacent to the tooth cervix, because the 'packing' effect of Retzius lines in this region makes it difficult to calculate their periodicity. In humans, dm^2 lateral enamel forms from about three months after birth, to around the end of the first post-natal year (see Mahoney, 2015 for data; and discussion in Mahoney et al., 2016). A neonatal line, the marker between pre-, and post-natal enamel, was located in cuspal enamel of the great ape dm_2's (which can be seen in the corresponding Figure of the orangutan dm_2 reported in the Results section). Cuspal enamel forms before lateral enamel. The word 'cuspal' refers to enamel that forms over the dentine horn, excluding lateral and cervical enamel. The word 'cusp' (e.g., protocone, or metacone cusp) refers to the first formed enamel over the dentine horn to the last formed enamel at the cervix.

A neonatal line, with a corresponding accentuated marking in dentin, was located towards the end of cuspal enamel growth in the chimpanzee dc_1 from the UCL collection. RP was calculated for this dc_1 from Retzius lines that were present in lateral enamel, just after the neonatal line. A neonatal line was not present in the chimpanzee dc_1 from The Ohio State University collection. We recorded Retzius lines in the most apical lateral enamel of this tooth.

The number of daily enamel growth increments (cross-striations) was counted along a rod between two adjacent Retzius lines of one human molar, the orangutan deciduous molar, and two ape permanent molars. Cross striations correspond with a circadian rhythm (Lacruz et al., 2012; Zheng et al., 2013). For all other sections, RP was calculated by measuring the distance between Retzius lines of lateral enamel. The measurement was divided by average local daily enamel secretion rates (DSRs) (Mahoney, 2008 for a methodology).

Average enamel thickness

Average enamel thickness (AET) was calculated by dividing the area of the enamel crown by the length of the dentin-enamel junction (DEJ), which provides the average straight-line distance in mm between the DEJ and outer enamel surface (Martin, 1983, 1985).

Results

RP in human deciduous and permanent enamel

Human RP data are in Table 1. In the maxillae of three children, RP increased from dm^2 with a lower mean AET of 0.69mm to M^1 with a higher mean AET of 1.01mm. In one maxilla, RP decreased from 10 days in a dm^2 with an AET of 0.89mm to eight days when compared to M^1 with an AET of 0.81mm.

RP in a crown with hypoplasia

Figure 1 illustrates the enamel defect and Retzius lines. The average distance of 14.5μm between two adjacent lines in mesio-buccal cusp lateral enamel, before the defect formed, divided by a local average DSR of 3.81μm, gave an RP of four days. When the analysis was repeated on an equivalent region of the mesio-lingual cusp it gave an RP of four days. The average distance of 21μm between two Retzius lines in cervical enamel, after the defect formed, divided by a local average DSR of 4.10μm, gave an RP of five days.

RP in great ape deciduous and permanent enamel

Retzius periodicity data for great apes are in Table 2. Figure 2 illustrates a direct count of cross striations between adjacent Retzius lines in the mesio-lingual cusp of the orangutan dm_2, which was five days. Periodicity for the mesio-lingual cusp of the gorilla dm_2 was five days. When the analysis was repeated on the mesio-buccal cusp of the gorilla molar it gave a count of five days. RP of the chimpanzee dc_1 from the UCL collection was five days. The periodicity of the dc_1 from the Ohio collection was either five or six days.

Retzius periodicity of permanent teeth ranged between 10 to 12 days for *P. pygmaeus*, seven to eight days for *G. gorilla*, and five to eight days for *P. troglodytes*. The one uppermost value of 12 days for *P. pygmaeus* extends the know range of RP's from permanent teeth for this species by one day.

Discussion

The present study builds upon our previous work by showing that in humans, within the same individual, RP can change from deciduous to permanent teeth. Our data also suggests that this may be the case in great apes, although RP differences between deciduous and permanent teeth of the same individuals would be necessary to confirm this hypothesis. Our study further suggests that RP can change on either side of a hypoplastic defect, where both a higher RP and an increase in daily secretion rates can occur after the defect has formed. Combined, these observations indicate that if RP is a systemic rhythm governed by supra-chiasmic nuclei (in the hypothalamus), then it appears that it does not always remain constant over an individual's lifespan, as previously assumed (Bromage et al., 2009). Instead, the timing of Retzius lines within an individual will either remain constant (Mahoney, 2012), or vary by up to three days, from deciduous to permanent teeth (Table 1, and Table 3).

RP in human deciduous and permanent enamel

An increase in RP from deciduous to permanent molars from the same individual is consistent with our previous finding, that the timing of Retzius lines is associated with enamel thickness (Mahoney et al., 2016). However, in one maxilla where RP decreased from a deciduous to a permanent molar, the dm^2 was slightly larger with thicker enamel compared to M^1. Normally, dm^2 has an AET that is less than M^1. Sometimes though, permanent first molars can be slightly smaller than their deciduous precursors (Moorrees and Reid, 1964), and their range of AET values can overlap (dm^2 range= 0.42-1.04; M^1 range= 0.82-1.21; Skinner et al., 2015; Mahoney et al., 2016). These data suggest that RP can change with age for human children when enamel is thicker, or thinner, in later forming permanent molars, relative to deciduous molars.

Several factors contribute to primate tooth enamel thickness. One is RP, which we have shown. The number of active ameloblasts, their secretory life span and the time taken to form regions of a crown, as well as the rate these cells secrete enamel matrix, also relate to enamel thickness (Macho, 1995; Grine and Martin, 1998; Dean, 2000; Dean et al., 2001; Mahoney, 2011). It is not surprising therefore that RP correlates with the time required to form dm^2 paracone cusp enamel (Mahoney et al., 2016). Shorter total crown formation times, thinner enamel, and lowered RP's of deciduous compared to permanent teeth (Mahoney, 2011, 2012, 2016; Reid and Dean, 2006) are consistent with this idea. RP also correlates with permanent canine lateral enamel formation time (Reid and Ferrell, 2006), though this might relate to the duration of enamel extension. Whether there is also an association between the length of the enamel-dentin junction and enamel thickness of permanent canines, when for example smaller are compared to larger teeth, has yet to be determined.

Two additional analyses were undertaken to further explore RP and the amount, and rate, of enamel deposition within enamel 'layers'. The distance between two adjacent Retzius lines in 14 human dm^2's, from different individuals, was compared to RP from the same teeth (Fig. 3). RPs were observed and measured in homologous locations, in outer lateral post-natal enamel, within each of the crowns. The distance between lines was significantly and positively correlated with RP (Pearson's r=0.940, $p<0.000$). Thus, higher RP's are associated with thicker enamel 'layers' in this sample of teeth because there are a greater number of days - more cross striations - between each 'beat' of the biorhythm. However, thicker enamel 'layers' between Retzius lines of higher periodicity were not accompanied by a clear change in the rate that ameloblasts secrete enamel. Mean DSRs in mid to outer lateral enamel of molars with RP's of four to seven days ranged between 3.44-4.20μm (one outlier of 5.10μm), overlapping with mean DSRs of 3.50-4.50μm from molars with RP's of nine to 11 days. These data suggest, if the rate that enamel matrix is deposited between adjacent Retzius lines varies only slightly, higher RP's, combined with ameloblasts that have longer secretory life spans, should lead to thicker enamel on molar crowns. Our results imply that, when secretion rates are constrained, RP variation appears to be a major contributor to enamel thickness, when equivalent enamel regions from one tooth type are compared between individuals.

Retzius periodicity was calculated high in outer lateral enamel and compared to RP low in outer cervical enamel of the same section, for three permanent second molars. Retzius periodicity did not change between these locations in each molar (RP of 7, 8, and 10 in each molar respectively). This makes sense, because here - unlike the comparison of RP between 14 dm^2's above - secretion rates are not constrained, as they vary greatly from one enamel region to the next in human permanent molars (e.g., Lacruz and Bromage 2006, their Table 2). In the three molars examined here, DSR's ranged between 4.65μm and 5.09μm high in outer lateral enamel, and between 2.58μm to 3.10μm low in outer cervical enamel. The spacing of Retzius lines, as well as their surface manifestation as perikymata, also become compressed in cervical compared to lateral enamel (e.g., Beynon, 1991b; Dean and Reid, 2001; Reid and Ferrell, 2006; Guatelli-Steinberg et al., 2007). Thus, the narrow enamel layers that form towards the end of a crown's growth period, do so slowly, leading to the same RP as the thicker enamel layers of lateral regions, which form relatively faster and earlier on in crown growth. In each of these enamel regions, the number of cross striations between adjacent Retizus lines remains constant, even though the amount of enamel deposited, and the spacing between the lines, changes. Thus, the relationship between RP and enamel layers is much weaker when DSRs are more variable. Our results imply that the timing of Retzius lines does not vary within a 'healthy' molar crown.

Factors that contribute to enamel thickness are not constant from one tooth type to the next, when compared along the row (e.g., Mahoney, 2015). Given that RP can be associated with enamel thickness, then there is reason to suspect that these associations will also not transfer unchanged from one tooth type to the next, in any one individual. That is, relationships between RP, and enamel growth and thickness, are likely to be *relative*, within a tooth type. For example, large portions of enamel forming at the same time in different deciduous teeth, such as maxillary lateral incisors and first molars, might have equivalent RPs that are associated with very different developmental pathways. Ameloblasts secrete enamel at an accelerated rate in deciduous incisors but have a shortened secretory life span, leading to a thinner enamel crown, compared to molars (Mahoney, 2010, 2011, 2012, 2013). Theoretically, accelerated ameloblast secretion rates of incisors could produce thickened enamel layers, relative to enamel layering in molars with the same RP's that have slower secretion rates. Thicker enamel layers of deciduous incisors would then be associated with a thinner incisor enamel crown, when compared to deciduous molars (also see developing the HHO below).

RP and hypoplasia

A change in the timing of Retzius lines, from one side of a hypoplastic defect to the other in a deciduous crown suggests that RP can be modulated by local systemic stress events. A period of 'catch up growth' in enamel secretion, after a period of reduced secretion, has been documented previously (e.g., Macchiarelli et al., 2006; Mahoney, 2008), but an increase in RP after a hypoplastic lesion is a new observation. We observed greater spacing between Retzius lines in cervical enamel after a hypoplastic defect, which also has been reported for enamel of domestic pig and wild boar (Witzel, et al., 2006; 2008 see their Fig 8a). Slightly accelerated average DSRs in cervical compared to lateral enamel were also unexpected, because like permanent teeth, rates usually decrease towards the end of the growth period in deciduous crowns (Mahoney, 2011). Taken together, greater distance between Retzius lines, and accelerated secretion rates, suggest that ameloblasts deposited more enamel between each 'beat' of the underlying biorhythm, after recovering from a stress event that led to a hypoplastic defect.

One futher analysis was undertaken to explore RP in three isolated permanent teeth that retained evidence of hypoplastic defects (Table 3). In two of these teeth, RP changed, increasing from one side of the defect to the other. Like the hypoplastic deciduous tooth, secretion rates also accelerated after the defect formed in two permanent teeth, and this was combined with a slower beat of the biorhythm leading to a higher RP and an increased spacing between Retzius lines. These preliminary data from a few teeth imply that ameloblast secretion rates and the underlying biorhythm can both respond to systemic non-specific pathology.

RP in great ape deciduous and permanent enamel

Retzius periodicity of deciduous teeth from *P. pygmaeus* and *G. gorilla* extends below the lowermost RP's we observed in permanent molars from these species (Table 2), as well as those reported previously (Schwartz et al., 2001; Kelley and Schwartz 2010). RP's of two deciduous canines from *P. troglodytes* lie within the lower range of RP's from permanent teeth (see our Table 2; Schwartz et al., 2001; Smith et al., 2010). Clearly, the extent of similarities or differences in RP of deciduous and permanent enamel from great apes has yet to be determined. Nevertheless, the deciduous RP's are all low, compared to RP's from permanent teeth of great apes.

Lower RPs from ape deciduous teeth are consistent with the proposal that RP may be linked to enamel thickness, and at least one underlying enamel growth mechanism, formation time. The orangutan dm_2 AET of 0.53 mm (0.4 to 0.5mm: Zanolli et al., 2015) extends below the lowermost AET of 0.77mm from permanent molars of this species (Skinner et al., 2015). Further analysis of the dm_2 reveals a mesio-buccal cusp formation time of 396 days (see Mahoney, 2011 for method), which lies outside the lowermost formation time of 1006 days reported from an analysis of six permanent M1 mesio-buccal cusp's of *P. pygmaeus* (Smith, 2016). The gorilla dm_2 AET of 0.54mm extends below the lowermost AET of 0.79mm for permanent molars (Skinner et al., 2015). Further analysis of the dm^2 reveals a mesio-buccal cusp formation time of 366 days (see Mahoney, 2011 for method), which is less than the formation time of 843 to 891 days reported for two permanent M1 mesio-buccal cusps of *G. gorilla*. No study has reported AET for permanent maxillary canines from *Pan*.

Developing the HHO

More work is needed to understand the interaction between the different factors that contribute to enamel thickness, and the timing of Retzius line. Perhaps crown extension in height combined with enamel thickness and crown formation time will show some associations with RP, given the stretching of the ameloblast sheet that has been demonstrated and modeled previously (Shellis, 1984). Disentangling these relationships will benefit the development of the HHO. For example, lower RP's were associated with longer lateral enamel formation times within a sample of permanent canines, while higher RPs were related to longer cusp formation times within a sample of deciduous molars (Reid and Ferrell, 2006; Mahoney et al., 2016).

Enamel thickness increases along the human tooth row, from first to third permanent molars (Grine, 2005). We have shown RP is one major contributor to enamel thickness when DSR variation is constrained. Whether RP is associated with enamel thickness when compared between analogous regions along the molar row from the same individuals has yet to be determined. Future studies might incorporate an assessment of RP, enamel thickness, DSR's and the length of time over which ameloblasts secrete enamel. One such approach would be to count the total number of cross-striations along an enamel prism, calculate DSRs along the prism length, and then assess how those numbers correspond with RP. Based upon our findings, it would seem likely that all three variables, RP, DSR, and the length of time over which ameloblasts secrete enamel, need to be considered and incorporated into predictions about how these factors affect enamel thickness.

Future studies might explore associations we have reported across primates. For example, AET of human permanent molars ranges between 0.67 to 2.30mm (Olejniczak et al., 2008) which coincides with a range of RP's between six and 12 days. AET of *Pan* molars ranges between 0.58 and 0.94mm (Skinner et al., 2015), which coincides with an RP of five to nine days. If RP is linked to permanent enamel thickness, and, or, underlying enamel formation processes, then these different ranges might be expected. Given that enamel thickness relates to lifespan and high-wear diets across primates (Pampush, 2013), such analyses may potentially reveal new ways to explore the timing of life history traits.

CONCLUSION

Our data have shown that RP can change within human children. Preliminary insights suggest great ape dentition might follow a similar pattern. When these data are considered alongside altered RP's within a crown, from one side of a hypoplastic disruption to the other, as well as correspondence between RP and the amount of enamel deposited within an 'enamel layer', it suggests that the timing of Retzius lines is linked to enamel growth. If RP is as a measure of an underlying systemic biorhythm that affects multiple physiological systems (Bromage et al., 2012), then we conclude that the influence of the biorhythm extends to enamel growth, can be modulated by local stress events, and may even express differently in enamel of different thickness and, or, in teeth with contrasting secretion rates and formation times.

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Table 1. Retzius periodicity in humans

	RP in days	
Sk	**Udm2**	**UM1**
27*	4 to 5	
102	6	7
178	9	10
665	7	10
671	10	8

Sk = Skeletal number. *Hypoplastic.
Tooth types: Udm2, upper second deciduous molar,
UM1, upper first permanent molar.

Table 2. Retzius periodicity in great apes

	RP in days							
Species	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
<u>Deciduous</u>								
P. troglodytes	1	1[a]						
G.gorilla	1							
P. pygmaeus	1							
<u>Permanent</u>								
P. troglodytes	1	4	2	1				
G. gorilla			5	1				
P. pygmaeus						3		1[b]

a= The RP of this lower deciduous canine was either 5 or 6 days. b= The RP calculated in the lateral enamel of the mesio-buccal cusp of this premolar was 12 days. When the analysis was repeated in the mesio-lingual cusp lateral enamel of the premolar it gave an RP of 12 days.

Table 3. Retzius periodicity and daily secretion rates in hypoplastic teeth

Enamel Region	LI2		LC1		LC1	
	RP days	**DSR** μm	**RP** days	**DSR** μm	**RP** days	**DSR** μm
Before defect	8	4.43	6	3.57	11	4.25
During defect	8	3.51	6	3.36	11	3.57
After defect	10	3.93	8	3.99	11	4.02

DSR = mean daily enamel secretion rates in outer enamel.

Tooth types: LI2, lower lateral permanent incisor, LC1, lower permanent canine.

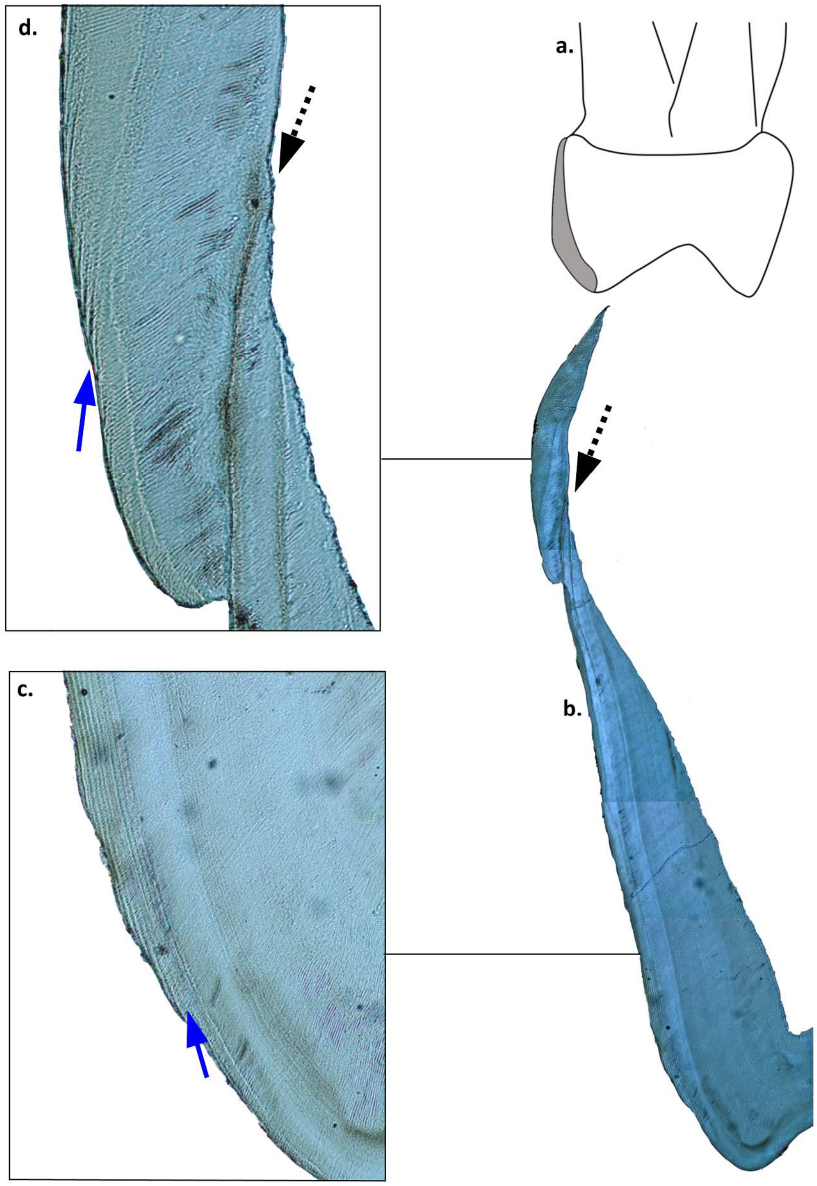

Fig. 1 Hypoplastic defect and Retzius periodicity (zoom in to see Retzius lines). (a) Human deciduous maxillary second molar mesio-lingual enamel highlighted in grey. (b) The same region imaged using a polarizing lens. Dashed arrow points to a hypoplastic defect associated with an accentuated marking. Magnification = 4x. (c) Blue arrow points to Retzius lines that formed before the hypoplastic defect. Magnification = 20x. (d) Blue arrow points to Retzius lines that formed after the hypoplastic defect. The stress event did not prevent secretory ameloblasts from recovering, as these cells had a functional Tomes process (separate rods are visible) that deposit enamel at a slightly accelerated rate.

Fig.1

166x240mm (300 x 300 DPI)

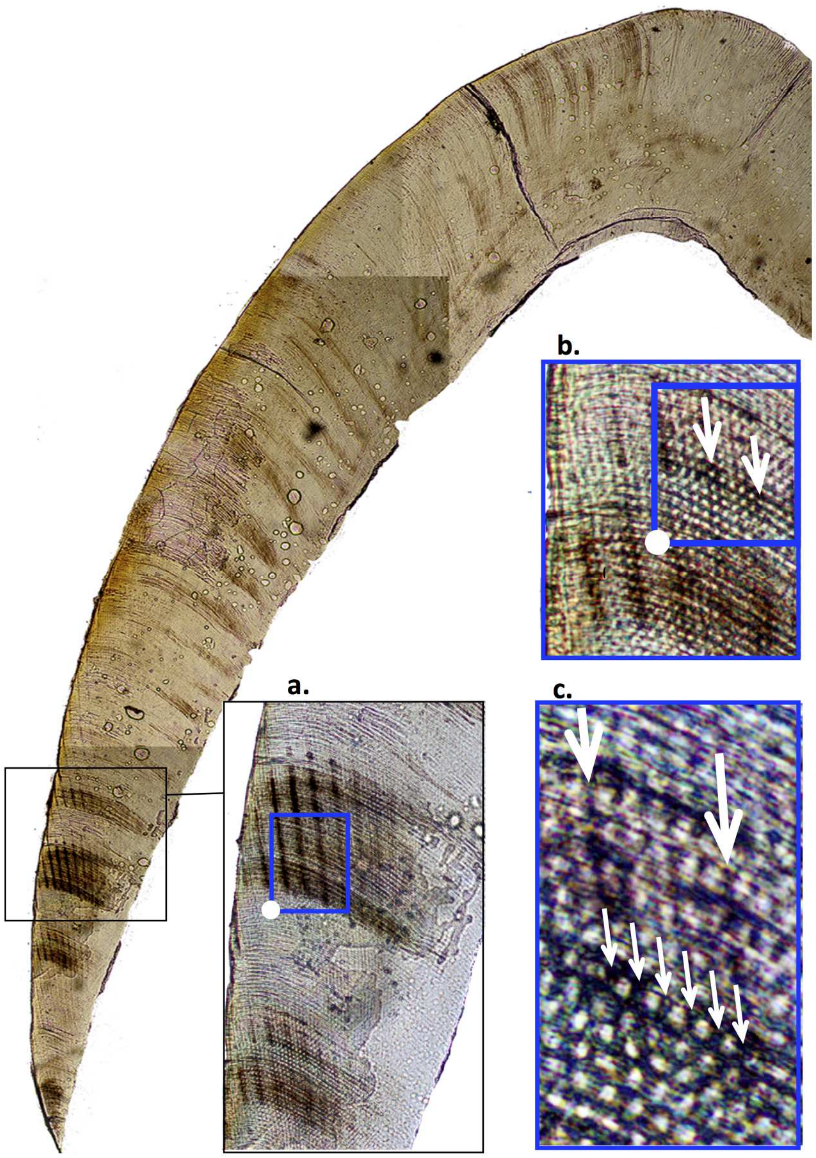

Fig. 2 Retzius periodicity in a juvenile orangutan lower second deciduous molar. (a) Retzius lines in cervical enamel. Magnification = 4x. (b) Daily cross striations. White arrows point to the first and last cross striation between two adjacent Retzius lines (zoom in to see). Magnification = 20x. (c) Large white arrows point to the same two adjacent Retzius lines. Smaller white arrows point to cross striations, corresponding to five days of enamel secretion. Magnification = 60x.
Fig.2
195x277mm (300 x 300 DPI)

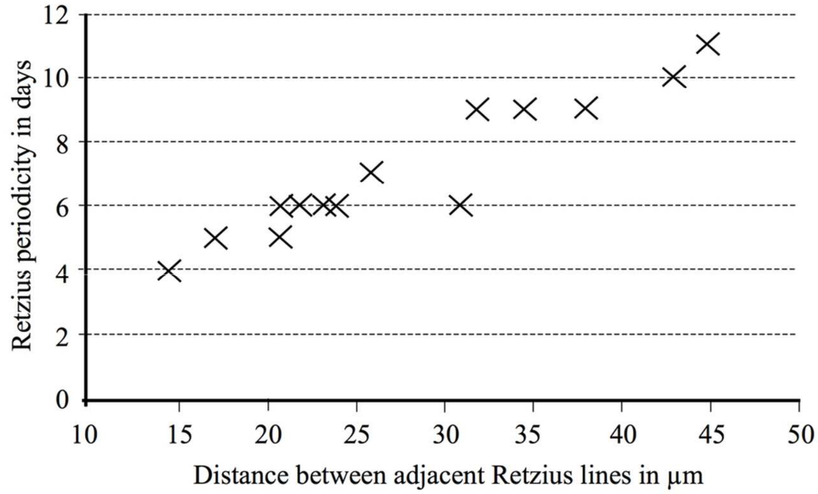

Fig. 3 Scatter plot of dm2 Retzius periodicity against Retzius line spacing. There is a significant ($p < 0.000$) and positive correlation between the two variables.
Fig. 3
102x67mm (300 x 300 DPI)