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

3

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27 **Abstract**

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

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

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

53 **Introduction**

54 Primate tooth enamel grows incrementally (Boyde, 1979, 1989). Each increment is marked
55 by a growth line, as in shells and trees. One type of marking are Retzius lines (Retzius,
56 1837), which emerge on the outer lateral enamel surface as perikymata (e.g., Goodman and
57 Rose, 1990). Retzius periodicity (RP) is the number of days of enamel growth between
58 adjacent lines. The Havers-Halberg Oscillation (HHO) hypothesis proposes that RP of
59 permanent teeth is a manifestation of an underlying biorhythm that regulates growth, is
60 associated with adult body mass, and is related to life history traits when compared between
61 mammalian species (Bromage et al., 2009, 2012). The underlying cause of the biorhythm is
62 unknown, though experimental research on domesticated pigs implicates resting metabolic
63 rate as an important influence (Bromage et al., 2016).

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

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

86 In the second stage of this study we compare the timing of Retzius lines between
87 deciduous and permanent teeth of great apes. We report the first deciduous RP values ($n=4$)
88 for modern orangutan (*Pongo pygmaeus*), gorilla (*Gorilla gorilla*), and chimpanzee (*Pan*
89 *troglodytes*). These values are compared to new data for permanent teeth ($n=18$) from these
90 species, as well as to previously published values. Even though the deciduous and permanent
91 teeth are not from the same individuals, we can still determine if deciduous RP's are
92 encompassed within the range of RP's for permanent teeth from each species. The present
93 study will also contribute to a new baseline comparative data set for great ape deciduous
94 teeth. Retzius periodicity of permanent teeth is often compared between fossil and modern
95 hominoids to gain insights into the evolution of dental development (e.g., Beynon et al.,
96 1998; Schwartz et al., 2003; Mahoney et al., 2007), but rarely do such analyses include RP of
97 deciduous teeth.

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102 **The timing of Retzius lines in humans and great apes**

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

113 Modern orangutan permanent teeth have a range of RP's between eight to 11 days, with a
114 mode of 9 or 10 days (Beynon et al., 1991a; Dean, 2000; Schwartz et al., 2001; Kelley and
115 Schwartz, 2010; Smith, 2016). Amongst modern gorillas, RP lies between seven to ten days,
116 with a mode of eight (females) and nine days (males) (Beynon et al., 1991a; Schwartz et al.,
117 2001; Kelley and Schwartz, 2010). The RP of modern chimpanzee permanent teeth might be
118 as low as five days (Smith et al., 2010), but the majority of values range between six to nine
119 days (Reid et al., 1998; Schwartz et al., 2001), with a mode of six or seven days (Schwartz et
120 al., 2001; Smith et al., 2007). No study has reported the RP of great ape deciduous teeth.

121

122 **Enamel hypoplastic defects**

123 Disruptions to ameloblast activity during the secretory phase of enamel development can lead
124 to hypoplastic defects that are retained in a tooth crown (Zsigmondy, 1893; Kreshover, 1940;
125 Guatelli-Steinberg, 2001 for a review). Hypoplastic defects, which are classified by their
126 morphology as furrow, pit, or plane-type, can be visible from the external surface depending

127 upon the angle that Retzius lines emerge in outermost enamel (Hillson and Bond, 1997;
128 Guatelli-Steinberg et al., 2012). These defects correspond with a range of non-specific
129 stressors in humans, including nutritional deficiencies (vitamin D and calcium), infectious
130 diseases, fevers, and congenital syphilis (Sarnat and Schour 1941; Sweeney et al., 1971;
131 Purvis et al., 1973; Norén et al., 1978; Nikiforuk and Fraser 1981; Goodman et al., 1987;
132 May et al., 1993; Hillson et al., 1998; Berdal et al., 2005; Bossù et al., 2007). Unlike a
133 localised hypoplasia (Goodman and Rose, 1990), these systemic events can disrupt enamel
134 growth in all forming crowns at the same time.

135 Hypoplastic enamel can be less mineralized, softer, and contain smaller hydroxyapatite
136 crystallites, relative to normal enamel (Suckling et al., 1989; Batina et al., 2004). An altered
137 microstructure implies that ameloblasts did not recover from the stress event that occurred
138 during enamel secretion, and this affected subsequent maturation (Suckling et al., 1989;
139 Batina et al., 2004). Hypoplastic enamel can also be as hard as normal enamel, indicating
140 that maturation resumed after the defective secretory phase (Suckling and Purdell-Lewis,
141 1982; Suckling et al., 1989). Thus, disruptions to ameloblast activity can either be temporary
142 or more sustained, which might relate in part to the stage of cell activity (Suga, 1989).

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152 **Materials and methods**

153 Five human juvenile skeletons with erupted dm^2 ($n=5$) and erupting maxillary $M1$'s ($n=4$)
154 were selected (Table 1). The skeletons dated to the medieval period (11th to 15th Century AD)
155 in England (Hicks and Hicks, 2001) and are curated in the Skeletal Biology Research Centre,
156 University of Kent, UK. The accession numbers are NGB 1988, Sk27; NGA 1989, Sk102,
157 178, 665, 671. One dm^2 retained evidence of a hypoplastic defect, which was systemic, as
158 we observed a corresponding defect in cervical enamel of dm^1 from the same maxilla.

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

169 Thin sections of 18 ape permanent teeth were selected from the Elliot Smith Collection.
170 These were a mix of maxillary and mandibular permanent first, second, and third molars of *P.*
171 *troglodytes* ($n=8$: accession numbers CA-11, CA-13D, CA-14, CA-14A two slides, CA-14E,
172 CA-19B, D-Case), permanent premolars and molars of *G. gorilla* ($n=6$: accession numbers
173 HT41-89 two slides, HT42-89, HT44-89, UCL-CA-18, UCL-CA-4), and permanent
174 premolars and molars of *P. pygmaeus* ($n=4$: accession numbers HT-162/88 two slides, HT-
175 166/88, HT-1/91). No permits were needed to examine the deciduous or permanent slides.

176

177 Sample preparation

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

188

189 Microscopy

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

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

203 dentine horn, excluding lateral and cervical enamel. The word ‘cusp’ (e.g., protocone, or
204 metacone cusp) refers to the first formed enamel over the dentine horn to the last formed
205 enamel at the cervix.

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

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

218

219 **Average enamel thickness**

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

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228 **Results**

229 **RP in human deciduous and permanent enamel**

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

234

235 **RP in a crown with hypoplasia**

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

242

243 **RP in great ape deciduous and permanent enamel**

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

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

254 **Discussion**

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

266

267 **RP in human deciduous and permanent enamel**

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

278

279 Several factors contribute to primate tooth enamel thickness. One is RP, which we have
280 shown. The number of active ameloblasts, their secretory life span and the time taken to
281 form regions of a crown, as well as the rate these cells secrete enamel matrix, also relate to
282 enamel thickness (Macho, 1995; Grine and Martin, 1998; Dean, 2000; Dean et al., 2001;
283 Mahoney, 2011). It is not surprising therefore that RP correlates with the time required to
284 form dm² paracone cusp enamel (Mahoney et al., 2016). Shorter total crown formation times,
285 thinner enamel, and lowered RP's of deciduous compared to permanent teeth (Mahoney,
286 2011, 2012, 2016; Reid and Dean, 2006) are consistent with this idea. RP also correlates
287 with permanent canine lateral enamel formation time (Reid and Ferrell, 2006), though this
288 might relate to the duration of enamel extension. Whether there is also an association
289 between the length of the enamel-dentin junction and enamel thickness of permanent canines,
290 when for example smaller are compared to larger teeth, has yet to be determined.

291 Two additional analyses were undertaken to further explore RP and the amount, and rate,
292 of enamel deposition within enamel 'layers'. The distance between two adjacent Retzius lines
293 in 14 human dm²'s, from different individuals, was compared to RP from the same teeth (Fig.
294 3). RPs were observed and measured in homologous locations, in outer lateral post-natal
295 enamel, within each of the crowns. The distance between lines was significantly and
296 positively correlated with RP (Pearson's $r=0.940$, $p<0.000$). Thus, higher RP's are associated
297 with thicker enamel 'layers' in this sample of teeth because there are a greater number of
298 days - more cross striations - between each 'beat' of the biorhythm. However, thicker enamel
299 'layers' between Retzius lines of higher periodicity were not accompanied by a clear change
300 in the rate that ameloblasts secrete enamel. Mean DSRs in mid to outer lateral enamel of
301 molars with RP's of four to seven days ranged between 3.44-4.20 μm (one outlier of 5.10 μm),
302 overlapping with mean DSRs of 3.50-4.50 μm from molars with RP's of nine to 11 days.
303 These data suggest, if the rate that enamel matrix is deposited between adjacent Retzius lines

304 varies only slightly, higher RP's, combined with ameloblasts that have longer secretory life
305 spans, should lead to thicker enamel on molar crowns. Our results imply that, when secretion
306 rates are constrained, RP variation appears to be a major contributor to enamel thickness,
307 when equivalent enamel regions from one tooth type are compared between individuals.

308 Retzius periodicity was calculated high in outer lateral enamel and compared to RP low in
309 outer cervical enamel of the same section, for three permanent second molars. Retzius
310 periodicity did not change between these locations in each molar (RP of 7, 8, and 10 in each
311 molar respectively). This makes sense, because here - unlike the comparison of RP between
312 14 dm²'s above - secretion rates are not constrained, as they vary greatly from one enamel
313 region to the next in human permanent molars (e.g., Lacruz and Bromage 2006, their Table
314 2). In the three molars examined here, DSR's ranged between 4.65µm and 5.09µm high in
315 outer lateral enamel, and between 2.58µm to 3.10µm low in outer cervical enamel. The
316 spacing of Retzius lines, as well as their surface manifestation as perikymata, also become
317 compressed in cervical compared to lateral enamel (e.g., Beynon, 1991b; Dean and Reid,
318 2001; Reid and Ferrell, 2006; Guatelli-Steinberg et al., 2007). Thus, the narrow enamel
319 layers that form towards the end of a crown's growth period, do so slowly, leading to the
320 same RP as the thicker enamel layers of lateral regions, which form relatively faster and
321 earlier on in crown growth. In each of these enamel regions, the number of cross striations
322 between adjacent Retizus lines remains constant, even though the amount of enamel
323 deposited, and the spacing between the lines, changes. Thus, the relationship between RP
324 and enamel layers is much weaker when DSRs are more variable. Our results imply that the
325 timing of Retzius lines does not vary within a 'healthy' molar crown.

326 Factors that contribute to enamel thickness are not constant from one tooth type to the
327 next, when compared along the row (e.g., Mahoney, 2015). Given that RP can be associated
328 with enamel thickness, then there is reason to suspect that these associations will also not

329 transfer unchanged from one tooth type to the next, in any one individual. That is,
330 relationships between RP, and enamel growth and thickness, are likely to be *relative*, within a
331 tooth type. For example, large portions of enamel forming at the same time in different
332 deciduous teeth, such as maxillary lateral incisors and first molars, might have equivalent
333 RPs that are associated with very different developmental pathways. Ameloblasts secrete
334 enamel at an accelerated rate in deciduous incisors but have a shortened secretory life span,
335 leading to a thinner enamel **crow**n, compared to molars (Mahoney, 2010, 2011, 2012, 2013).
336 Theoretically, accelerated ameloblast secretion rates of incisors could produce thickened
337 enamel layers, relative to enamel layering in molars with the same RP's that have slower
338 secretion rates. Thicker enamel layers of deciduous incisors would then be associated with a
339 thinner incisor enamel **crow**n, when compared to deciduous molars (also see developing the
340 HHO below).

341

342 **RP and hypoplasia**

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

354 'beat' of the underlying biorhythm, after recovering from a stress event that led to a
355 hypoplastic defect.

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

364

365 **RP in great ape deciduous and permanent enamel**

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

374 Lower RPs from ape deciduous teeth are consistent with the proposal that RP may be
375 linked to enamel thickness, and at least one underlying enamel growth mechanism, formation
376 time. The orangutan dm₂ AET of 0.53 mm (0.4 to 0.5mm: Zanolli et al., 2015) extends
377 below the lowermost AET of 0.77mm from permanent molars of this species (Skinner et al.,
378 2015). Further analysis of the dm₂ reveals a mesio-buccal cusp formation time of 396 days
379 (see Mahoney, 2011 for method), which lies outside the lowermost formation time of 1006

380 days reported from an analysis of six permanent M1 mesio-buccal cusp's of *P. pygmaeus*
381 (Smith, 2016). The gorilla dm₂ AET of 0.54mm extends below the lowermost AET of
382 0.79mm for permanent molars (Skinner et al., 2015). Further analysis of the dm² reveals a
383 mesio-buccal cusp formation time of 366 days (see Mahoney, 2011 for method), which is less
384 than the formation time of 843 to 891 days reported for two permanent M1 mesio-buccal
385 cusps of *G. gorilla*. No study has reported AET for permanent maxillary canines from *Pan*.

386

387 **Developing the HHO**

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

397 Enamel thickness increases along the human tooth row, from first to third permanent
398 molars (Grine, 2005). We have shown RP is one major contributor to enamel thickness when
399 DSR variation is constrained. Whether RP is associated with enamel thickness when
400 compared between analogous regions along the molar row from the same individuals has yet
401 to be determined. Future studies might incorporate an assessment of RP, enamel thickness,
402 DSR's and the length of time over which ameloblasts secrete enamel. One such approach
403 would be to count the total number of cross-striations along an enamel prism, calculate DSRs
404 along the prism length, and then assess how those numbers correspond with RP. Based upon

405 our findings, it would seem likely that all three variables, RP, DSR, and the length of time
406 over which ameloblasts secrete enamel, need to be considered and incorporated into
407 predictions about how these factors affect enamel thickness.

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

416

417 **CONCLUSION**

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

427

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700 **Table 1. Retzius periodicity in humans**

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

701 Sk = Skeletal number. *Hypoplastic.

702 Tooth types: Udm2, upper second deciduous molar,

703 UM1, upper first permanent molar.

704

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706

707 **Table 2. Retzius periodicity in great apes**

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

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

712

713

714 **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

721 DSR = mean daily enamel secretion rates in outer enamel.

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

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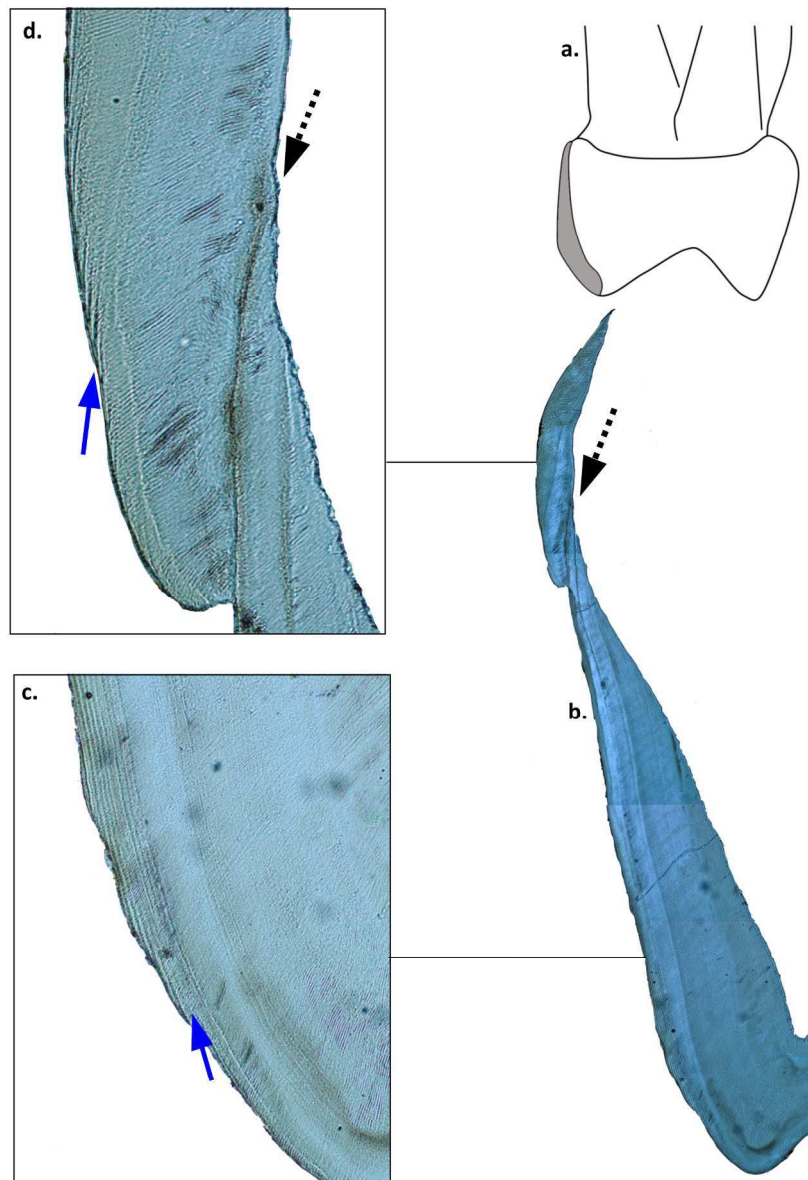


Fig. 1 Hypoplastic defect and Retzius periodicity (zoom in to see Retzius lines). (a) Human deciduous maxillary second molar mesio-lingual enamel highlighted in grey. (b) The same region imaged using a polarizing lens. Dashed arrow points to a hypoplastic defect associated with an accentuated marking. 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)

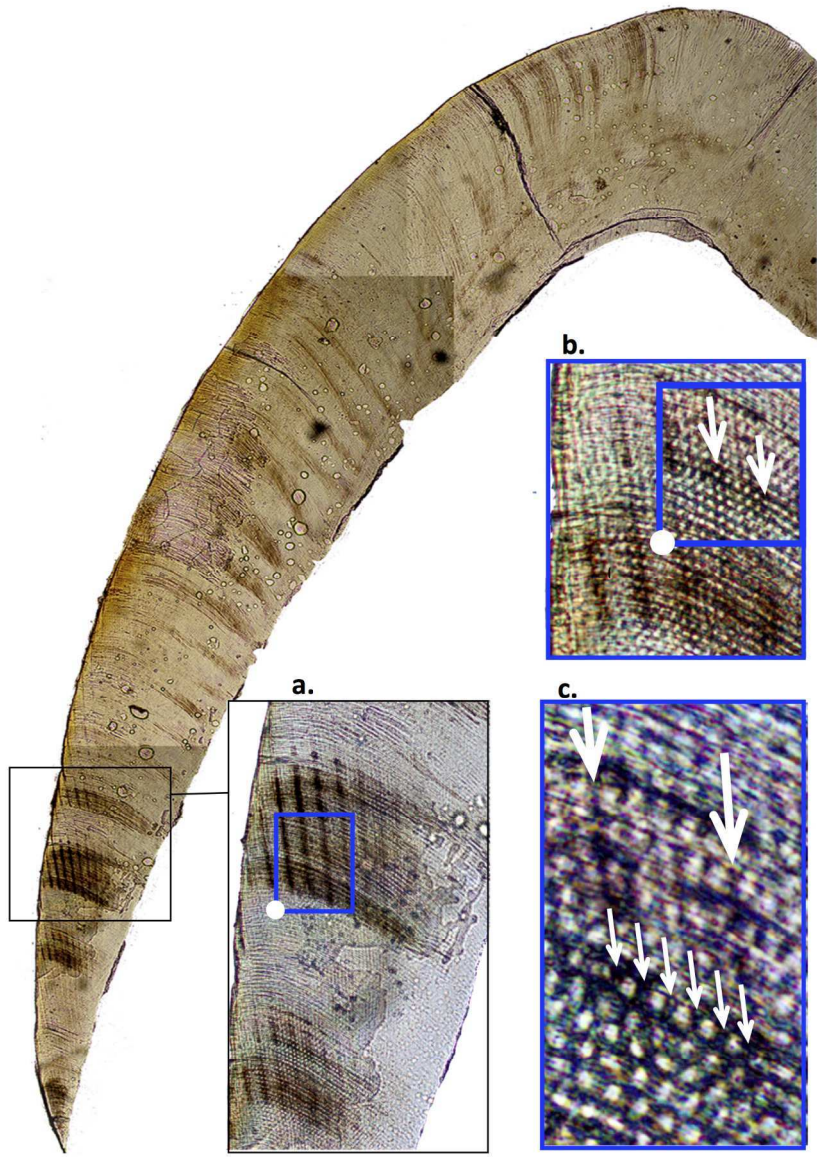


Fig. 2 Retzius periodicity in a juvenile orangutan lower second deciduous molar. (a) Retzius lines in cervical enamel. Magnification = 4x. (b) Daily cross striations. White arrows point to the first and last cross striation between two adjacent Retzius lines (zoom in to see). Magnification = 20x. (c) 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)

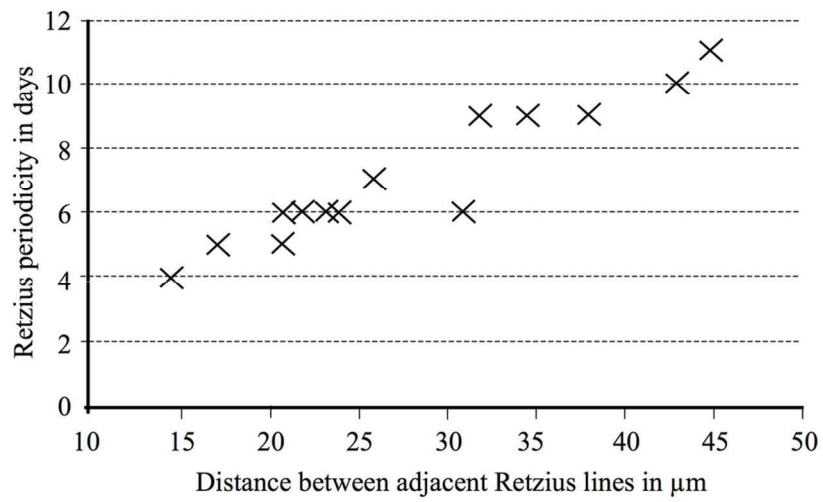


Fig. 3 Scatter plot of dm2 Retzius periodicity against Retzius line spacing. There is a significant ($p < 0.000$) and positive correlation between the two variables.

Fig. 3

102x67mm (300 x 300 DPI)

View Only