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## Growth of Neanderthal infants from Krapina (120-130 ka), Croatia

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## Abstract

Modern humans have a slow and extended period of childhood growth but to what extent this ontogenetic pathway was present in Neanderthals is debated. Dental development, linked to the duration of somatic growth across modern primates, is the main source for information about growth and development in a variety of fossil primates, including humans. Studies of Neanderthal permanent teeth report a pace of development either similar to recent humans or relatively accelerated. Neanderthal milk teeth, which form and emerge before permanent teeth, provide an opportunity to determine which pattern was present at birth. Here we present a comparative study of the prenatal and early postnatal growth of five milk teeth from three Neanderthals (120,000-130,000 years ago) using virtual histology. Results reveal regions of their milk teeth formed quickly before birth, and over a relatively short period of time after birth. Tooth emergence commenced towards the earliest end of the eruption schedules displayed by extant human children. Advanced dental development is consistent with expectations for Neanderthal infant feeding.

# 1. Introduction

Modern humans have an extended period of childhood growth that is unique amongst modern primates [1-3]. Slow somatic growth during the childhood years likely evolved in response to low adult mortality and the time and energy required to develop a large brain that attains extensive cognitive abilities [4-6]. Neanderthals shared a common ancestor with modern humans [7] and had an adult cranial volume that lay towards the larger end of the range reported for recent humans [8]. How close the Neanderthal trajectory of childhood growth was to living humans is debated.

Almost nothing is known about the prenatal ontogeny of Neanderthals. Infant skeletons are represented in the fossil record [9-13] but there are few palaeobiological studies of Neanderthals with estimated ages of less than one year [14-17] leading to limited knowledge about the growth of Neanderthals in the months before and year after birth. Analyses of teeth provide an alternative way of accessing information about growth schedules in fossil hominins [18-23]. Dental development is linked to the duration of somatic growth across modern primates [24-27] though caution has to be exercised as tooth eruption ages can vary greatly within a species [28]. Milk (deciduous) teeth start to form before birth. Emergence of the first deciduous tooth has a strong correlation with brain weight [27]. Thus, building deciduous dental chronologies around the age at which Neanderthals are born can provide unique information about their developmental pathways and pace of life history.

Deciduous dental development (enamel growth rates, formation times, tooth emergence age) can be reconstructed from histology. There are no histology data for Neanderthal deciduous central incisors. Small fragile incisors in the process of eruption are rarely recovered within intact jaws making it nearly impossible to reconstruct the age at which deciduous teeth started to emerge for Neanderthal infants. Deciduous enamel extension rates are lacking for all Neanderthal tooth types. Enamel daily secretion rates (DSR) of three Neanderthal deciduous molars [22,29], and prenatal DSRs near the enamel-dentin junction (EDJ) of one mandibular lateral incisor [29] were similar to those of modern humans. Much more is known about Neanderthal permanent dental development, which is debated. Development of permanent teeth can be similar to modern humans [18-20,22] or lie towards the accelerated end of the human range [21,23,30-31] suggesting Neanderthal childhoods may have been relatively advanced. It is unknown which of these developmental patterns was present in Neanderthal at birth.

Here, we reconstruct the development of Neanderthal deciduous teeth from four months before birth to six months after birth. We apply non-destructive synchrotron radiation computed microtomography (SR  $\mu$ CT) to five isolated deciduous teeth of three Neanderthals from Krapina (120-130 ka) in Croatia [30, 32-34]. We reconstruct deciduous enamel and dentin secretion and extension rates, and enamel formation times. We employ an alternative approach to the problem of

Neanderthal deciduous incisor emergence age based upon root length. Support for our approach is provided by laboratory-based X-ray  $\mu$ CT analyses of emerging permanent premolars and a canine for Krapina Neanderthals (see electronic supplementary material, Analyses of K52 Mandible B and K47 Maxilla C). We compare our data for Neanderthal deciduous teeth to those of recent modern humans and explore our findings against the pace of Neanderthal infant life history especially infant feeding.

## 2. Materials and methods

Krapina is a sandstone rock shelter dated to 120-130,000 years ago [32-34]. Neanderthals are preserved at the site and only Mousterian tools form the archaeological assemblage. More than 200 isolated Neanderthal permanent and deciduous teeth were recovered [35]. Gorjanović-Kramberger [32] identified the level in which many Neanderthal specimens were found. None of the teeth used in the present study have a recorded stratigraphic level. Wolpoff [30] inventoried and identified each tooth. He attributed many deciduous and permanent teeth to the same individuals, designated Krapina Dental Persons (KDP), based primarily on occlusal wear and interlocking interproximal facets.

### (a) Dental samples

One Neanderthal maxillary right deciduous central incisor, two mandibular right deciduous lateral incisors, and two maxillary, one right and one left, deciduous first molars were selected (Table 1; see electronic supplementary material, Supplementary figures). We incorporated an enamel DSR from one Neanderthal deciduous second molar from La Chaise-de-Vouthon, France [22] into our calculations for the K181 and 183 deciduous molars. Comparative enamel extension rates were calculated for British ( $n=9$  upper deciduous first molars), Australian [36] ( $n=30$  deciduous incisors), and Canadian ( $n=7$  upper deciduous first molars) teeth. Existing thin sections of Medieval and Roman deciduous teeth ( $n=13$  upper incisors,  $n=17$  upper molars) were reused [37-39]. Comparative enamel DSRs, formation times, and deciduous incisor emergence ages were taken from published literature.

**Table 1.** Neanderthal deciduous samples and the reconstructed aspects of dental development

Individual	Tooth id	Tooth	Reconstructed dental development	
			<i>Prenatal</i>	<i>Postnatal</i>
KDP1	K21	Right di <sup>1</sup>	enamel extension enamel secretion dentin secretion	formation time emergence age
	K11	Right di <sub>2</sub>	enamel secretion	root extension
KDP21	K181	Right dm <sup>1</sup>		formation time
KDP22	K16	Right di <sub>2</sub>	enamel secretion	
	K183	Left dm <sup>1</sup>	enamel extension formation time	formation time

di<sup>1</sup>=upper deciduous central incisor. Di<sub>2</sub>=lower lateral incisor. Dm<sup>1</sup>=upper first molar.

## (b) Synchrotron radiation-based 3D X-ray imaging

Single Neanderthal teeth were analysed via SR $\mu$ CT imaging operated at the SYRMEP beamline [40] of the Elettra Sincrotrone laboratory in Basovizza, Trieste, Italy. Full methodological details for the SR $\mu$ CT measurements can be found in the electronic supplementary material, Supplementary methods (see Synchrotron radiation-based 3D X-ray imaging). Samples were imaged in propagation-based phase contrast mode. Scans were produced with a 3.0  $\mu$ m pixel size to visualize the neonatal line, and a higher spatial resolution of 0.9  $\mu$ m pixel size to visualize daily cross striations (see electronic supplementary material, Supplementary figure S8 for cross striations).

## (c) 3D image processing and analysis

Virtual images were generated by importing volumes into ImageJ (version 1.52a) using the ‘reslice’ tool to generate a stack of images in the buccolingual plane, passing through the tip of the dentin horn. Section planes were reoriented if needed using Avizo Lite 9.0.0 software (Thermo Fisher Scientific, USA) (see electronic supplementary material, Supplementary figure S9 for a virtual slice of K11 di<sub>2</sub>). The image stack was processed through the ‘ZProject’ average intensity function of ImageJ varying the range of the stack to obtain different virtual thicknesses. High-pass filters were applied to enhance visualization of growth lines. Measurements of the K52 mandible B and K47 Maxilla C were taken on 2D virtual slices generated from 3D volumes of laboratory X-ray  $\mu$ CT scans using Avizo Lite 9.0.0. The 3D visualization of the reconstructed and processed data were obtained with VGStudio MAX 2.0 (Volume Graphics, Germany).

## (d) Enamel and root extension rates

Extension rates measure the speed at which a tooth crown gains height. Neanderthal extension rates were recorded on virtual slices in ImageJ. Comparative human rates were recorded from thin sections. Full methodological details for calculating extension rates, and creating thin sections, can be found in electronic supplementary material, Supplementary methods. We used two standard methodologies to calculate enamel extension rates from a portion of the EDJ length divided by the time required by ameloblasts to form a corresponding portion of an enamel prism located between the EDJ and neonatal line [38,41]. The first method provided an average extension rate over a set period of time leading up to birth. We applied this method to the K21 deciduous incisor and K183 deciduous molar. The second method captured rates from an earlier period before birth. We applied this second method to the K183 molar but not the K21 incisor because the neonatal line was present in the deciduous incisor lateral enamel, not cuspal enamel. One initial root extension rate was calculated for the K11 deciduous lateral incisor that was incorporated into our estimates of deciduous incisor emergence age.

### (e) Enamel and dentin secretion rates

Enamel DSRs measure the amount of new matrix deposited by ameloblasts in 24-hours between two adjacent cross striations. Neanderthal enamel DSRs were recorded on 2D virtual slices in ImageJ using standard methods [42] for deciduous incisor cuspal enamel that was subdivided into three regions of equal thickness (inner, mid, and outer). Cross striations were not clearly preserved in the outer cuspal enamel region of the K21 crown so we moved to the outer lateral region to calculate DSRs for this region of this tooth. Prism lengths were measured across four to six adjacent cross striations and subdivided to gain a mean DSR. This was repeated several times so that a grand mean DSR could be calculated for a region. Enamel cross striations were not visible in Neanderthal deciduous molars. One coronal dentin DSR was calculated for the K21 incisor and incorporated into the calculation of root extension for this tooth (see electronic supplementary material, Supplementary methods).

### (f) Enamel formation times

Postnatal formation times for the Neanderthal dm<sup>1</sup> protocone cusps of K181 and K183, and the K21 deciduous upper central incisor, were reconstructed from extension rates and postnatal EDJ length in ImageJ. Prenatal formation time was reconstructed for the K183 dm<sup>1</sup> protocone by dividing the length of cuspal enamel prisms between the dentin horn and neonatal line, by the mean enamel DSR from the La Chaise Neanderthal deciduous molar [22]. The prenatal formation time was added to the postnatal formation time to give the total protocone cusp formation time for the K183 deciduous molar. We could not calculate prenatal formation time for the K21 deciduous incisor due to slight wear of the cusp.

### (g) Estimated incisor emergence ages

Human deciduous incisors partially emerge (defined as cusp mid-way towards full eruption [43]) during the early stages of root formation with a root length that is between one half and the full height of the crown [43]. We estimated a range of partial emergence ages for the K21 deciduous incisor by combining the time taken to form these two fractions of root with postnatal crown enamel formation time. These data are compared to partial, and gingival emergence age (cusp tip starts to appear through the gums), for modern human children. Gingival emergence precedes partial emergence and it is included here for a comparative context.

### 3. Results

#### (a) Enamel extension rates

The K21 upper deciduous incisor crown (figure 1a) extended in height at an average rate of 31.06  $\mu\text{m}/\text{day}$  for 44 days leading up to birth (figure 1b). This rate lies above the uppermost range of rates we calculated for the same enamel region in upper deciduous central incisors of modern humans (figure 1c; and see electronic supplementary material, Supplementary tables, Table S2).

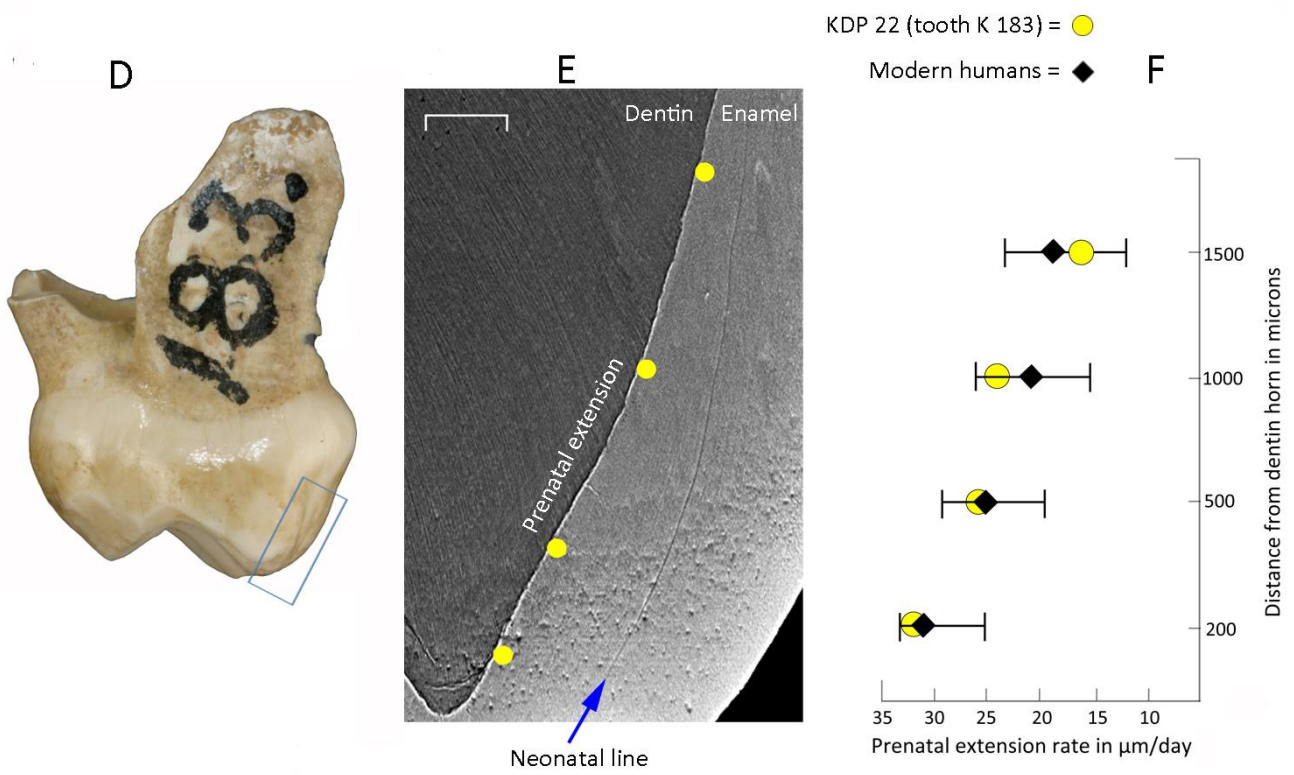
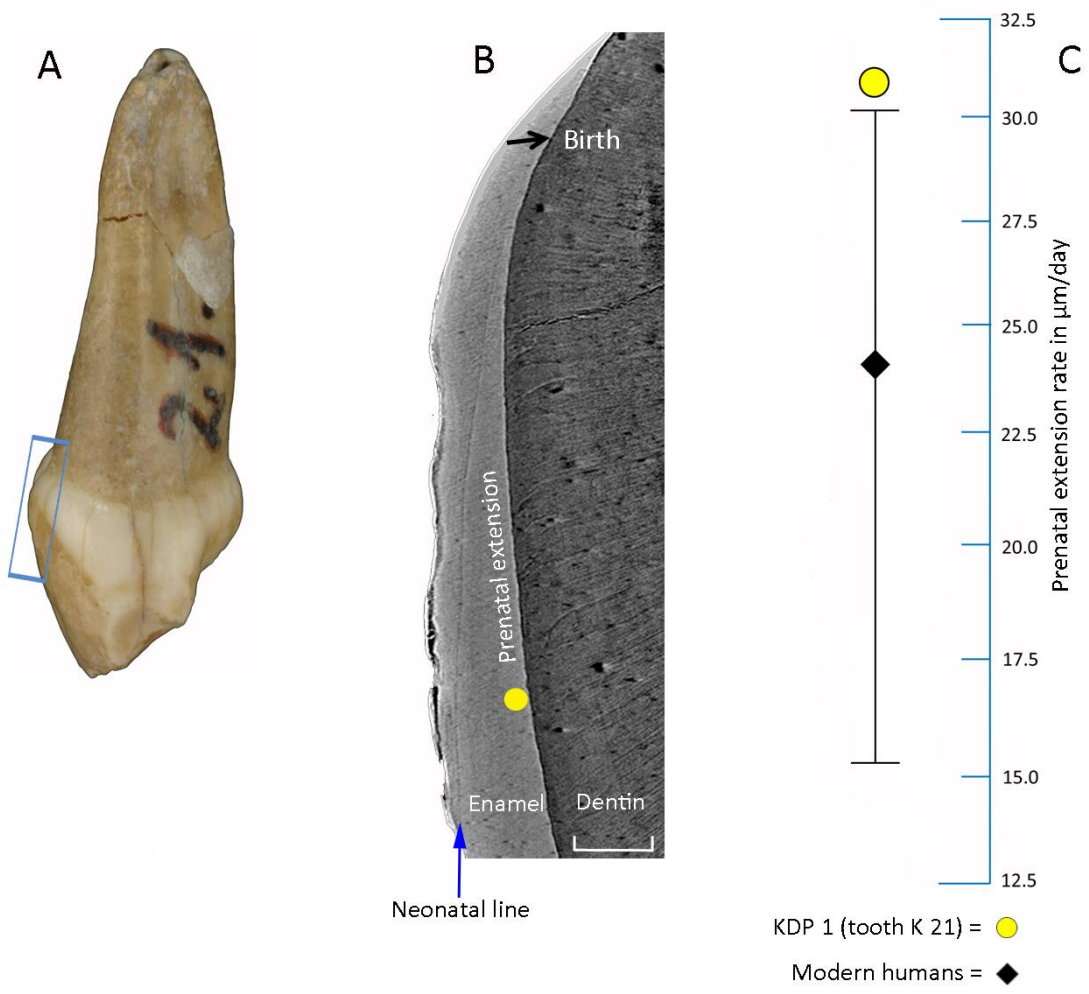
The K183 upper deciduous first molar crown (figure 1d) extended in height at an average rate of 14.33  $\mu\text{m}/\text{day}$  over a 60-day period leading up to birth. The average rate, and range of values that we calculated for the K183 deciduous molar, lies within the range of extension rates from an equivalent region of modern human upper deciduous first molars (electronic supplementary material, Supplementary Table S2). Additional exploration of extension rates in the K183 molar (electronic supplementary material, Supplementary Table S3) revealed a mean value of 32.03  $\mu\text{m}/\text{day}$  at 200  $\mu\text{m}$  away from the dentin horn decreasing to a mean of 16.68  $\mu\text{m}/\text{day}$  at 1500  $\mu\text{m}$  away from the horn (figure 1e-f). These additional rates for the K183 deciduous molar confirmed our original finding by revealing a growth trajectory that was indistinguishable from modern human deciduous molars. The decrease in extension rates as newly activated enamel cells moved away from the dentin horn (figure 1f) is similar to modern human deciduous molars [38].

#### (b) Root extension rate

The initial root extension rate of 30.29  $\mu\text{m}/\text{day}$  for the K11 deciduous lateral incisor lies above the uppermost initial root extension rate of modern human deciduous lateral incisors that ranges between 18.80 to 24.96  $\mu\text{m}/\text{day}$  [44].

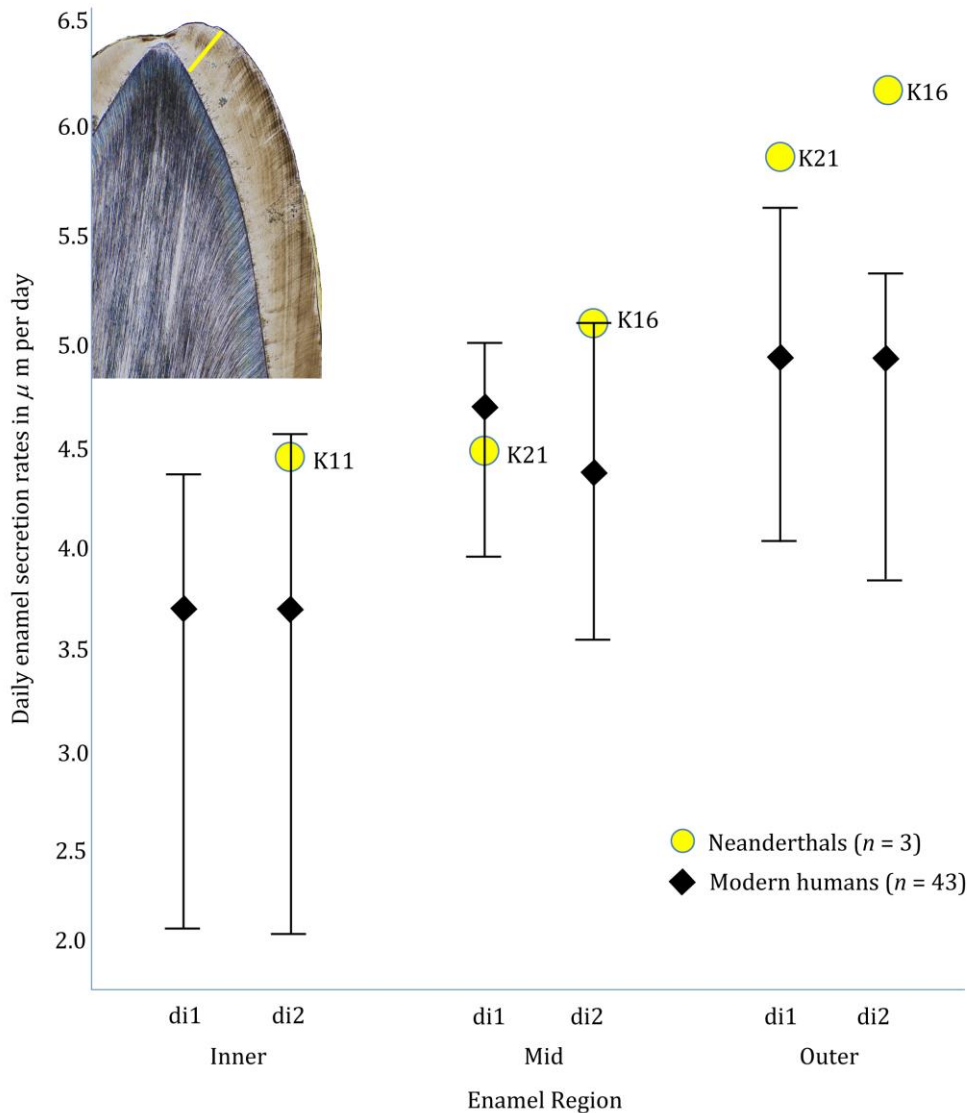
**Figure 1. Prenatal enamel extension rates.** (A) K21 maxillary deciduous central incisor, mesial view. Rectangle highlights region of interest imaged through SR $\mu$ CT. (B) Buccal-lingual virtual histological section. Scale bar is 200 microns. Isotropic voxel size = 3.0  $\mu\text{m}$ ,  $\delta/\beta=20$ , reformatted slice thickness=15  $\mu\text{m}$ . Yellow dot indicates EDJ 44 days before birth. Blue arrow points to neonatal line. Back arrow points to the location that the neonatal line intersects with the EDJ at birth. (C) Yellow dot represents average rate new ameloblasts were recruited along the EDJ over a period of 44 days leading up to birth. Mean values for modern comparative samples (Australian  $n=29$ ; Medieval British  $n=13$ ) represented by black diamond, with line showing min and max values. See electronic supplementary material, Supplementary Table S2. (D) K183 maxillary deciduous first molar, mesial view. Rectangle highlights region of interest imaged through SR $\mu$ CT. (E) Buccal-lingual virtual histological section. Scale bar is 200 microns, isotropic voxel size = 3.0  $\mu\text{m}$ ,  $\delta/\beta=20$ , reformatted slice thickness=15  $\mu\text{m}$ . Rates calculated for starting points commencing 200, 500, 1000 and 1500  $\mu\text{m}$  away from the dentin horn, represented by yellow dots. (F) Yellow dots represent extension rate for K183 compared to upper deciduous first molar extension rates for modern comparative sample (Canadian  $n=7$ ). Black diamond represents human mean with line illustrating max and min values. See electronic supplementary material, Supplementary Table S3.





### (c) Enamel secretion rates

Figure 2 illustrates that mean prenatal enamel DSRs lay between 4.50 to 6.20  $\mu\text{m}/\text{day}$  for the three Neanderthal deciduous incisors. Rates from deeper within the Neanderthal deciduous incisor enamel were slower and overlap with those of extant humans (figure 2). Rates increased from the inner to outer enamel regions of the Neanderthal deciduous incisors, which is also similar to extant humans [37,38]. However, the lowermost range of DSRs from the outer enamel region of the K21 and K16 deciduous incisors extends above the uppermost range of DSRs for this enamel region from modern humans (see electronic supplementary material, Supplementary Table S4).



**Figure 2. Prenatal enamel secretion rates.** Neanderthal rates obtained from SR $\mu$ CT data reconstructed with an isotropic voxel size of 0.9  $\mu\text{m}$ . Rates are for cuspal enamel (illustrated in the inset figure by the yellow line on the image of a thin section through a deciduous tooth crown) except DSRs from the outer region of K21 di<sup>1</sup> which were recorded in lateral enamel. Modern human values are previously published mean DSRs (black triangles) and min, max values produced from thin sections [37,39]. See electronic supplementary material Supplementary Table S4.

#### (d) Dentin secretion rate

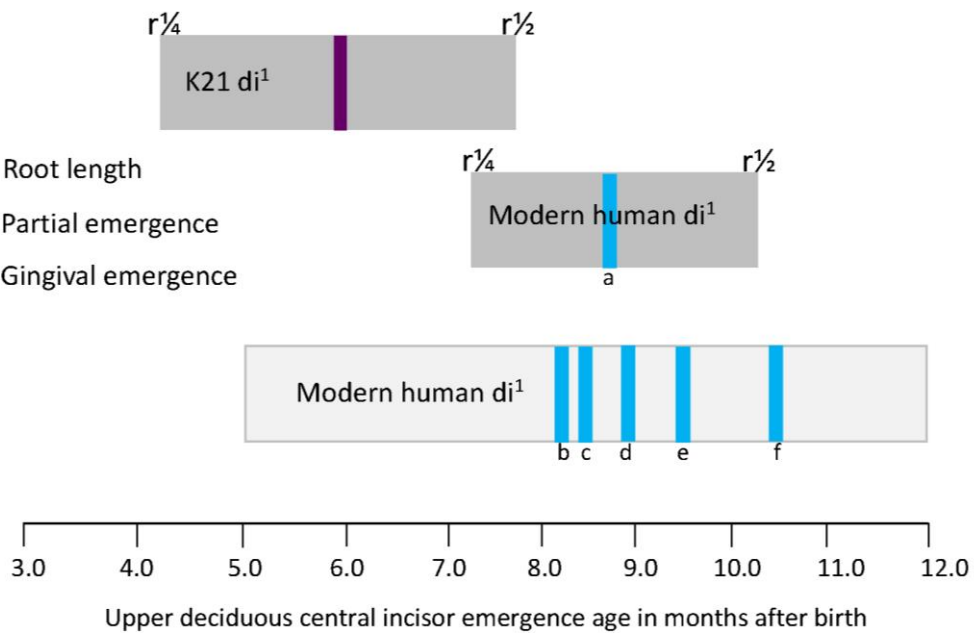
Odontoblasts in the K21 incisor crown secreted dentin matrix at an average daily rate of 3.90  $\mu\text{m}/\text{day}$ .

#### (e) Enamel formation times

The postnatal formation time of 19 days for the K21 deciduous incisor crown is low compared to modern human incisors (see electronic supplementary material, Supplementary Table S5). The postnatal formation time of the K183 and K181 deciduous molars was 123 and 181 days respectively which lies below the postnatal formation times reported for modern human maxillary deciduous first molars that range between 221 to 333 days (see electronic supplementary material, Supplementary Table S6). The total protocone cusp formation time of 310 days for the K183 deciduous first molar lies below the modern human range of protocone formation times between 336 to 510 days [38]. Enamel formed for 129 days before birth in the K183 protocone which is similar to the period of prenatal enamel formation seen in human deciduous first molars [38].

#### (f) Estimated age at deciduous incisor emergence

Partial emergence ages for the K21 upper deciduous incisor lie between 4.27 to 7.89 months after birth (figure 3; see electronic supplementary material, Supplementary Table S7). Our highest estimated partial emergence age for K21 overlaps with the lowest end of the modern human partial emergence range that lies between 7.23 to 10.03 months after birth [45]. Our estimated mean partial emergence age for K21 lies towards the lower end of the range that is typical for the gingival emergence of modern human incisors. Support for our approach is provided by additional analyses of two emerging permanent premolars and one permanent canine from Krapina Neanderthals which revealed a root-to-crown ratio that is similar to modern human permanent canines and premolars that are at an equivalent stage of eruption (see electronic supplementary material, analyses of K52 Mandible B and K47 Maxilla C).



**Figure 3. Estimated deciduous incisor emergence age.** Partial emergence ages are given for the K21 upper deciduous central incisor when the root length is between half ( $r\frac{1}{4}$ ) and the full length of the crown ( $r\frac{1}{2}$ ). The mean of the two values is indicated by a vertical purple line. Equivalent root lengths, and partial emergence ages, are shown for a British population with the mean value indicated by a light blue line. Gingival emergence, which precedes partial emergence, is illustrated for modern human populations with mean values indicated by light blue lines. a=Britain. b=Japan. c=Australia. d=Iceland. e=Papua New Guinea. f=Nigeria. Modern human emergence ages are taken from the published literature (see electronic supplementary material, Supplementary Table S7).

## 4. Discussion

We reconstructed the development of five deciduous teeth for three Neanderthals recovered from the Krapina rock shelter (120-130 kyr) in Croatia. The rate and duration of tooth formation was calculated, and for the first time, we estimated the age at which a Neanderthal maxillary deciduous central incisor emerged. Our data indicate that the deciduous dental development of these Neanderthals was advanced relative to extant humans.

### 4.1 Tooth formation rates and times

Regions of the Neanderthal deciduous incisors formed relatively quickly before birth. The K21 deciduous incisor extended in height rapidly (figure 1c), and prenatal enamel cells approached the outer crown surface at a faster rate compared to enamel DSRs of modern human deciduous incisors [37-39,46]. These accelerated rates ensured that the K21 deciduous incisor was formed mainly before birth, requiring only a short formation time after birth. A short postnatal enamel formation period has been reported for a deciduous incisor of the Fumane 2 upper Paleolithic modern human infant (39-42

ka) [29], which differs to longer enamel growth periods reported for extant humans [37]. Relatively rapid formation rates have also been reported for Neanderthal permanent anterior teeth [21,47].

There are several potential reasons why deciduous incisors of Neanderthals and extant humans might form at different rates. Neanderthal deciduous incisor crowns have a greater average crown dentin volume ( $\text{mm}^3$ ) leading to a greater EDJ surface area ( $\text{mm}^2$ ) compared to deciduous incisors of extant humans [48]. This means that newly activated enamel cells would have to spread over a larger surface area in Neanderthal deciduous incisors. To do so would require rapid extension rates if parts of the tooth formed over relatively short periods of time as in the K21 deciduous incisor crown. The different ontogenetic trajectories of Neanderthal and modern human jaws [49] is another potential reason. The rate roots grow in length is likely limited by the space available within a developing jaw [50]. Jaws of two Neanderthal children from Dederiyeh, aged 1 to 2 and 1.5 to 2.5 years respectively, had larger anterior tooth crypts compared to extant human children of a similar age [51]. Relatively large tooth crypts would provide more space for roots to develop, which might facilitate relatively fast initial root extension rates such as those we observed in the K11 deciduous lateral incisor.

The Neanderthal deciduous molars formed at a slow and ‘human-like’ rate. Slow prenatal enamel extension of Neanderthal deciduous molars (see figure 1f) compared to fast prenatal enamel extension of Neanderthal deciduous incisors (figure 1c) is not unusual. It is equivalent to the variation in enamel extension that occurs along the deciduous tooth row of modern humans [38]. Cross striations were not visible in the Krapina deciduous molars so we could not calculate enamel DSRs, but enamel secretion rates within the range of modern humans have been reported for Neanderthal deciduous molars from Nadale 1 (70 ka) [29], Fumane 1 [29], and La Chaise [22]. All of these data indicate Neanderthal deciduous molars form at a rate that is similar to that of extant humans.

The Neanderthal deciduous molars were formed relatively soon after birth. This can be clearly seen in the K183 deciduous molar. Growth commenced *in utero* at about the same time as a modern human deciduous first molar, but the Neanderthal deciduous molar was formed by the end of the sixth postnatal month which is early compared to extant humans [38,52]. The K181 deciduous first molar displayed the same foreshortened period of postnatal growth (see electronic supplementary material, Supplementary Table S6). Thus, these Neanderthal deciduous molars formed slowly but over shorter periods of postnatal time, which has also been reported for the Fumane 1 deciduous second molar [29]. This makes sense, as less time would be required to produce the thinner enamel of Neanderthal deciduous molars [53,54] given that the rate at which enamel cells secrete enamel in this tooth type is similar to that of modern humans. These comparisons of deciduous formation rates and times between Neanderthals and modern humans illustrate the importance of considering histology variables together, as interpretation of one without the other could lead to different conclusions.

## 4.2 Tooth emergence ages

Wolpoff [30] was the first to suggest that dental eruption was advanced for Neanderthals of Krapina, which he based upon the greater wear of mandibular third compared to permanent first and second molars. His suggestion was supported by the emergence age of the Krapina Maxilla B permanent first molar that lay within the faster half of the modern human range [21]. Our data for the K21 deciduous incisor is consistent with these findings. Regions of the K21 deciduous incisor formed quickly over a short period of time so that the tooth was ready to emerge at the earliest end of the modern human range (figure 3). We were unable to reconstruct emergence ages for the K181 and K183 deciduous molars because root growth lines were not visible, but their short postnatal enamel formation times are consistent with a relatively advanced eruption schedule. Not all Neanderthals display an advanced schedule of dental eruption. A permanent first molar of the La Chaise Neanderthal emerged towards the later end of the human range [22]. The permanent lateral incisor of the El Sidron child (49 ka) from Spain had clearly attained alveolar emergence at an age that is typically seen in modern human children of the same age [19]. It is possible therefore that the wide range of eruption ages reported for modern humans [45] was probably present in Neanderthals as well.

## 4.3 Interpreting deciduous dental development at Krapina

The transition into supplementary feeding for human infants is facilitated through a series of physiological changes involving the oral cavity and lips, tongue and jaw movements, the ability to swallow non-liquid foods and enhanced motor skills [55-59]. The emergence of deciduous incisors is tied into these developments as they act as sensory receptors enabling human infants to cut into or break off foods, and subsequently chew harder foods as molars emerge [55,57,58]. If Krapina infants followed a similar course of development, then the early emergence of the K21 deciduous incisor implies that they could have started to process more demanding supplementary foods at a slightly younger age. This idea fits in with direct evidence of diet obtained from trace element analyses of the Fumane 1 Neanderthal that detected foods other than maternal milk from the age of four months [29], which is early compared to the recommended age of 6 months for a human infant [60]. Higher brain growth rates by their second year [14] might have generated large energetic costs for Neanderthals [5]. These could have been offset for Krapina infants by their ability to process more demanding supplementary foods at a relatively early age, thereby providing the increased energy rapid brain growth demanded.

Other factors could have contributed to the advanced eruption schedule of the Krapina Neanderthals. Higher mortality rates among young Neanderthal adults [61] may have led to advanced maturation of juveniles [62] that facilitated early eruption schedules. While nursing is essential to

infant survival, it can potentially lead to parent-offspring conflict over maternal energy resources and reproductive success [63]. Parent-offspring competition might favor selection for an advanced eruption schedule and weaning process.

## 5. Conclusion

The pace of Neanderthal growth has been heavily debated. Evidence from permanent teeth suggests a course of development that was either slow and similar to modern humans or was relatively advanced. Our study contributes to this debate by showing that three Neanderthals from Krapina had a pattern of deciduous dental development that was advanced compared to a typical modern human infant. Regions of the Krapina Neanderthal deciduous teeth formed quickly, or over shorter periods of time after birth, and a deciduous incisor emerged at the advanced end of the modern human schedule. We infer from this evidence that the year after birth was a period of relatively advanced somatic growth for the Neanderthal infants of Krapina.

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