Towle, I and Irish, JD

Recording and interpreting enamel hypoplasia in samples from archaeological and palaeoanthropological contexts

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Ian Towle a,b,*, Joel D. Irish b,c

a Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, 9016, New Zealand

b Research Centre in Evolutionary Anthropology and Palaeoecology, School of Biological and Environmental Sciences, Liverpool John Moores University, L3 3AF, United Kingdom

c Evolutionary Studies Institute and Centre for Excellence in PaleoSciences, University of the Witwatersrand, Private Bag 3, WITS, 2050, South Africa

Number of text pages: 13

Number of reference pages: 6

Number of figures: 5

Number of Tables: 2

Declarations of interest: none

Abstract

Enamel hypoplasia is often split into several macroscopic categories, including pit, localised, linear and plane-form defects. All types have been considered a sign of 'non-specific stress' during dental development in archaeological, as well as palaeoanthropological and other samples. There is growing evidence suggesting many defects may not be caused by illness or malnutrition during childhood, instead relating to trauma to the developing tooth, genetic conditions or specific environmental factors, i.e., may not be associated with 'stress' to the individual. In this study all types of macroscopic enamel hypoplasia were recorded, including pitting, linear, plane and localised type defects, in three extant primate species and three fossil hominin species. The aim is to compare the characteristics and prevalence of different types of enamel hypoplasia among species and discuss potential differences in aetiology. The results show that samples have diverse prevalences of different kinds of defects, and pitting, linear and localised defects likely have different aetiologies. Additionally, dental characteristics (e.g., tooth morphology, developmental timing/speed and enamel structure) heavily influence the likelihood of specific types of enamel hypoplasia forming. In sum, studies that include only one type of enamel hypoplasia, or focus on one tooth type, to generate a 'stress' rating for a sample may miss relevant information when comparing groups. Instead, it may be beneficial to record different types of defects separately, for all teeth, and then consider how genetic, environmental and tooth property factors may influence population differences.

Key words: Dental defects; stress; dental development; Amelogenesis imperfecta; localised enamel hypoplasia; pitting enamel hypoplasia; linear enamel hypoplasia; fossil hominins; primates

1. Introduction

Enamel hypoplasia is defined as the reduction of enamel thickness caused by cessation or diminution of ameloblast function during the secretory stage of enamel formation (Guatelli-Steinberg, 2015; Ten Cate, 1994; Xing et al., 2015; Goodman et al., 1987; Guatelli-Steinberg et al., 2004; Hillson, 2014; Lukacs et al., 2001; Eversole, 1984). Defects are often characterised into four broad categories, pit-form (PEH), plane-form, linear-form (LEH), and localised hypoplasia (Guatelli-Steinberg, 2015; Pindborg, 1970; Seow, 1990; Hillson & Bond, 1997; Skinner et al., 2016). However, splitting defects into these categories can sometimes be difficult (e.g., Odgen et al., 2007; Towle et al., 2018; Ioannou et al., 2016).

Pitting enamel hypoplasia (PEH) can be broadly defined as numerous circular to oval defects that cover an extended area of a crown. Pits can be anything from small circular pin like defects up to vast irregular depressions (Hillson & Bond, 1997; Skinner, 1996). Additionally, some pits form rows around the circumference of a crown, or are associated with plane-form defects, whilst others are much more randomly scattered (Goodman & Rose, 1990; Hillson & Bond, 1997; Lauc et al., 2015; Towle and Irish, 2019). Each pit is created due to cessation/diminution of ameloblast activity, but it is not clear why only some ameloblasts are affected along the plane of a brown stria of Retzius during formation. Small pits are created when only a few ameloblasts stop forming enamel matrix, with large pits involving hundreds (Guatelli-Steinberg, 2015). The enamel between pits often appears normal, and exposed Tomes' process pits can frequently be observed within pits (Hillson, 2014; Hillson & Bond, 1997). There is debate in the literature to whether PEH is caused by different factors than LEH, or if it is just a consequence of the crown position and tooth involved (Hillson, 2014; Hillson & Bond, 1997; Goodman & Rose, 1990; Lovell & Whyte, 1999).

Linear enamel hypoplasia (LEH) are bands of reduced enamel on a tooth's crown, and are the most common type of enamel hypoplasia reported in the literature (e.g., Dobney & Ervynck, 2000; Goodman & Armelagos, 1985; Guatelli-Steinberg, 2004; Guatelli-Steinberg & Lukacs, 1999; Skinner et al., 2015). Anterior teeth tend to have a higher prevalence of LEH, likely due to enamel property and morphology differences, although defects may also be harder to detect macroscopically in posterior teeth (Goodman & Rose, 1990; Hillson & Bond, 1997; Guatelli-Steinberg, 2003; Bocaege et al., 2010; Hassett, 2012). LEH has been directly

associated with malnutrition and disease in clinical and animal studies, with a variety of other disturbances during development also considered in archaeological and other studies, with deeper/wider LEH defects usually linked to more severe events (Goodman & Rose 1990; Guatelli-Steinberg & Benderlioglu, 2006; McGrath et al., 2018; Hillson, 2014). The age the individual was when a LEH defect formed can be accurately found, through several different techniques that calculate the developmental timing of grooves (e.g., Goodman & Armelagos, 1985; Reid & Dean, 2000; Cares Henriquez and Oxenham, 2019).

Localised hypoplasia is characterised by isolated irregular depressions that do not extend around a crown, with usually only one or two continuous defects on the tooth (Skinner et al., 2016; Suckling, 1980; Skinner and Skinner, 2017; Suckling et al., 1983; Suckling et al., 1986; Skinner et al. 2014; Skinner & Newell, 2003). The aetiology of many types of localised defects is related to direct trauma to the tooth during development, usually associated with crypt fenestration (Suckling, 1980; Skinner and Skinner, 2017; Suckling et al., 1983; Suckling et al., 1986). In particular, insufficient growth space in the maxilla and mandible has been associated with localised defects termed crypt fenestration enamel defects (CFEDs) (Skinner et al. 2014). Localised hypoplasia is common in certain groups but scarce in others, with deciduous canines in certain primate species/populations commonly affected (Skinner et al., 2016; Skinner & Newell, 2003; Halcrow and Tayles, 2008; Jančová et al., 2019). Although crypt fenestration may be a common cause of localised defects, other processes can cause localised defects, with specific genetic mutations linked to what would typically be recorded as localised enamel hypoplasia (e.g., Hart et al., 2003).

Plane-form enamel hypoplasia occurs when enamel matrix formation ceases, either completely, or in part. This creates an area of a crown with little or no enamel deposition (Hillson & Bond, 1997; Krenz-Niedbała & Kozłowski, 2013; Ogden et al., 2007; Towle et al., 2017). Hillson (2014) described these defects as extreme linear defects, with one perikymata significantly widened. Similarly, plane-form hypoplasia is often reported in the literature as part of other types of defects, in particular PEH or localised defects (Guatelli-Steinberg, 2003; Littleton & Townsend, 2005; Skinner et al., 2016; Towle et al., 2018). These defects are often found alongside other severe enamel defects, including those associated with conditions such as congenital syphilis (e.g., Ioannou et al., 2016).

Significant research into enamel hypoplasia took place during the early to mid-20th century. Research that utilised rat and mouse models were particularly common (e.g., Kreshover, 1960; Schour & Massler, 1945), as well as later studies on sheep and pig (e.g., Suckling and Cutress, 1977; Suckling et al., 1983; Witzel et al., 2006). These studies highlighted that nutritional deficiencies can lead to hypoplastic defects. Enamel hypoplasia has also been studied in a variety of human populations, with defect frequencies varying substantially in both deciduous and permanent dentitions (Goodman & Rose, 1990; Hillson, 2014; Moggi-Cecchi et al., 1994; Pisanty & Garfunkel, 1977; Purvis et al., 1973; Seow, 1990; Skinner & Newell, 2003). Methods used to record enamel hypoplasia varies between studies. Researchers often only record LEH frequencies (e.g., Guatelli-Steinberg, 2003, 2004; Miszkiewicz, 2015). Whereas other studies record all hypoplastic defects (e.g., Goodman et al., 1980, 1984; Goodman & Armelagos, 1985; Ogilvie et al., 1989). Similarly, some researchers only record enamel hypoplasia on certain teeth, with anterior permanent teeth usually favoured (e.g., Infante & Gillespie, 1974; Lovell & Whyte, 1999). When PEH is included in a study it is often not clear if this includes defects found as part of LEH grooves (e.g., Goodman et al., 1980, 1984; Goodman & Armelagos, 1985; Hillson, 1992; Sognnaes, 1956; Mellanby, 1929).

Enamel defects come in a variety of shapes and sizes and each type can be caused by different factors, making differential diagnosis in ancient samples difficult. In contrast, clinical studies have linked enamel hypoplasia to a variety of specific conditions and disturbances (Aine et al., 2000; Croft et al., 1965; Eliot et al., 1934; Gaul et al., 2015; Grahnen & Selander, 1954; Nikiforuk & Fraser, 1979, 1981; Pisanty et al., 1977; Purvis et al., 1973; Radu & Soficaru, 2016; Seow et al., 1984; Stimmler et al., 1973; Wright et al., 1993). Most studies of archaeological and other ancient, or non-human, samples, since they do not have patient records, can only conclude that an individual had a 'non-specific stress' if they display enamel hypoplasia, or that a population was more/less stressed than other samples depending on the prevalence of a particular type of hypoplastic defect. Typically, 'stress' in this context refers to illness or malnutrition. In this study all types of macroscopically visible enamel hypoplasia are recorded in three extant primate species and three fossil hominin species. We hypothesize that dental characteristics (morphology, developmental timing and enamel structure), and specific genetic/environmental factors heavily influence enamel hypoplasia

prevalence, with different defects typically having diverse aetiologies. If this is the case, there should be substantial variation in the types of defects that species' display, and different types of enamel hypoplasia should typically not be associated with one another.

2. Materials and Methods

The samples studied include specimens assigned to *Homo naledi*, *Paranthropus robustus*, *Australopithicus africanus*, gorillas, chimpanzees and baboons (Table 1). Specimen numbers and species classifications are detailed in the Appendix. Some data presented has been published in Towle and Irish (2019), with additional data added in the present study, notably localised hypoplasia prevalence's. The hominin samples are curated at The Ditsong National Museum of Natural History and the University of the Witwatersrand. The extant primate samples are curated at the Powell-Cotton Museum, and comprise common chimpanzees (*Pan troglodytes*), western lowland gorillas (*Gorilla gorilla gorilla*), and olive baboons (*Papio anubis*). They were killed in their natural habitats (Dean & Jones, 1992; Guatelli-Steinberg & Skinner, 2000; Lukacs, 2001).

Table 1. Number of teeth for each sample, split by observable and not observable.

Species	Teeth observable	Not observable	Total teeth
Early *Homo*	47	19	66
Australopithecus sediba	10	1	11
Paranthropus robustus	304	127	431
Homo naledi	142	14	156
Australopithecus africanus	360	122	482
Gorilla gorilla gorilla	1693	392	2085
Pan troglodytes	1837	677	2514
Papio anubis	774	92	866

Teeth were held under a lamp and rotated allowing light to hit the surface at different angles. The smallest discernible macroscopic defect was recorded, with a hand lens only used to rule out postmortem damage. Methods for recording LEH follow Goodman & Rose (1990), Guatelli-Steinberg (2003), Lukacs (1989), and Miszkiewicz (2015). Localised hypoplasia was recorded following Skinner et al. (2016). PEH was recorded if there was multiple circular/oval

enamel defects on a tooth crown. If pitting was present within a LEH band then it was recorded as LEH not PEH, but the pitting was noted. Plane-form enamel hypoplasia was recorded following Towle et al. (2017). If defects on a tooth didn't fit into one of these four categories it was described and recorded separately, and not included in analysis.

To record defects each tooth was assigned a number. 0 was used to signify that there were no visible defects. Teeth where it would not be possible to tell if a defect was present due to post-mortem damage were marked as 8. Numbers 1, 2, 3 and 4 represent LEH, localised, PEH and plane-form defects respectively. Examples of each type of defect are displayed in Figure 1. Defects were photographed using a Dino-Lite® camera (Dino-Lite AM2111 handheld microscope).

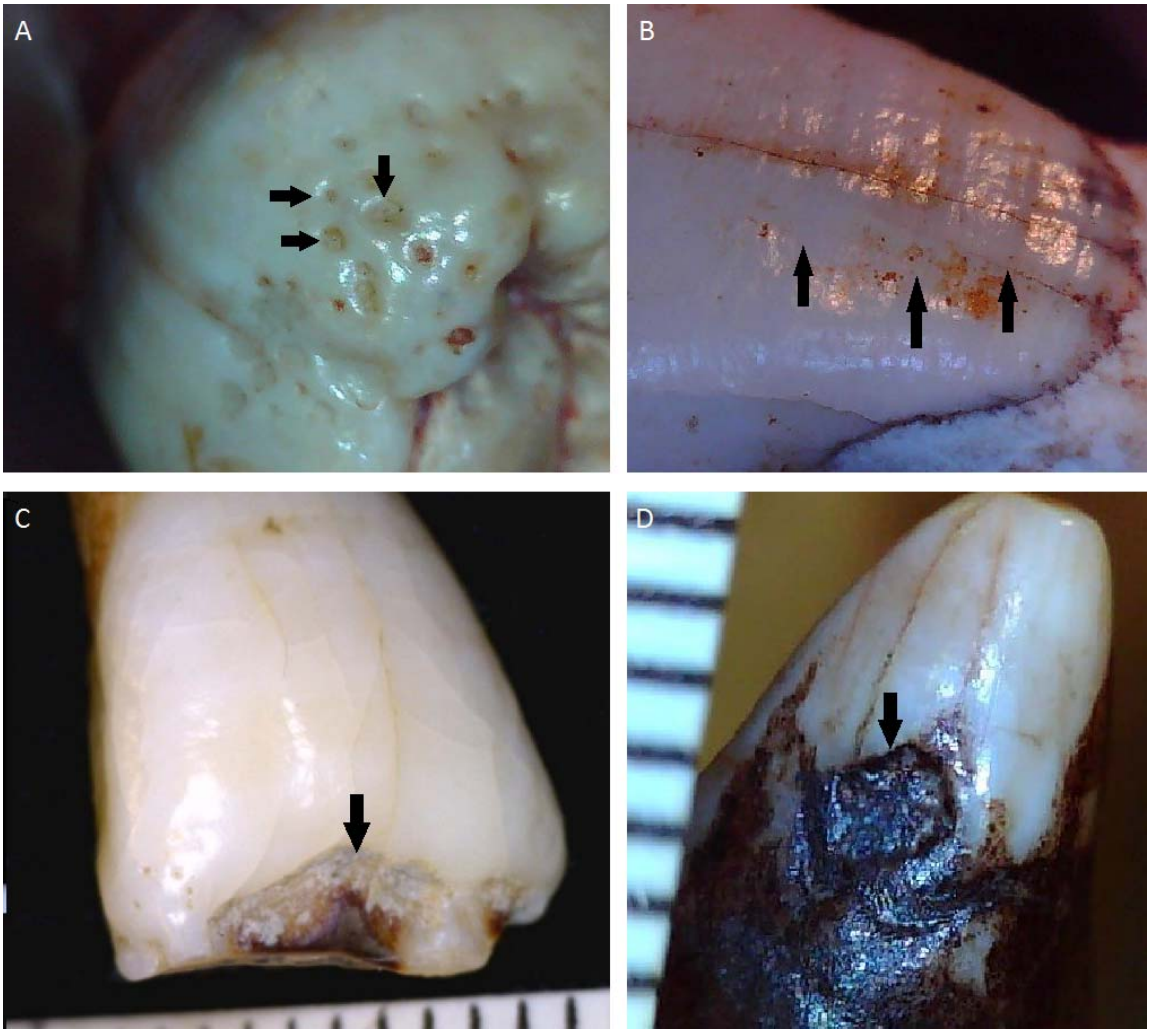

Figure 1. Enamel hypoplasia types. A) pitting enamel hypoplasia (*Australopithecus africanus*, SK 9); B) linear enamel hypoplasia (*Homo naledi*, UW 101-38). C) plane-form enamel hypoplasia (*Homo sapiens*, Towle et al., 2017); D) localised hypoplasia (*Gorilla gorilla gorilla*, M 667).

Due to how defects are displayed on tooth crowns, Hassett (2012) concluded that enamel hypoplasia prevalence based solely on macroscopic observation could be misleading, and create biases in comparing populations. To add to this debate, it has also been suggested that microscopic techniques likely miss defects too, with micro-CT imaging showing enamel abnormalities that do not show up in SEM or light microscopy (Marchewka et al., 2014; Xing et al., 2015). However, there are advantages to macroscopic observation; it is quick, inexpensive, non-destructive and allows large collections to be studied. It can therefore give a good overview of health, disease and genetic conditions on a population level.

With increasing wear, all else being equal, fewer macroscopic enamel defects should be visible on a crown. Instead of rejecting teeth worn past a certain point, all teeth with remnant enamel, and not broken due to post-mortem damage, are included. This approach will clearly lead to teeth being included that have had enamel defects worn away. However, the alternative of excluding such teeth will also lead to bias, since an entire sample would consist of individuals that died young. This methodology is also justified by the presence of PEH and localized defects on severely worn teeth. There is variation in wear patterning between the samples studied, however overall the average wear severity is similar between species meaning wear is unlikely to have had a significant effect on overall enamel hypoplasia differences (Towle and Irish, 2019; Towle, 2019). Data are presented by tooth count rather than individual, with the number of hypoplastic teeth displayed as a percentage of the total number of observable teeth. To compare certain groups a chi-square test of homogeneity was used, with significance set at the 0.05 alpha level.

3. Results

Table 2 displays the prevalence for the different types of enamel hypoplasia in permanent and deciduous teeth of each species. The hominin samples have higher rates of LEH than the extant great apes, with baboons having the lowest frequency. Localised hypoplasia is not found on any of the hominin deciduous tooth samples. This is in contrast with the extant primate sample in which it is common. PEH is rare in all deciduous samples except *P. robustus*, in which over 40% of teeth have defects (Towle and Irish, 2019; Figure 2A). In specimens with pitting LEH, typically multiple rows of these defects are present on the

crown surface (Figure 2B). No plane-form defects were recorded in any of the hominin samples. A specific example of plane-form hypoplasia was found in the chimpanzee sample and has been published as a case study (Towle et al., 2018). Figures 3 and 4 highlight two examples of enamel defects that were difficult to categorise as one of the four enamel hypoplasia types.

Table 2. Per tooth prevalence (%) of linear enamel hypoplasia (LEH) pitting enamel hypoplasia (PEH) and localised hypoplasia for permanent and deciduous teeth.

Species	**Permanent teeth**			**Deciduous teeth**	
	PEH (# teeth)	**LEH (# teeth)**	**Localised**	**PEH (# teeth)**	**Localised**
Pan troglodytes	0.65 (12/1837)	8.06 (148/1837)	0.98	4.23 (25/591)	5.08
Gorilla gorilla gorilla	2.89 (49/1693)	4.25 (72/1693)	0.95	1.39 (6/433)	12.93
Papio anubis	0.00 (0/774)	2.07 (16/774)	1.68	0.00 (0/107)	3.74
Homo naledi	0.70 (1/142)	14.79 (21/142)	0.70	0.00 (0/16)	0.00
Australopithecus africanus	5.03 (18/358)	15.08 (54/358)	0.28	5.00 (2/19)	0.00
Paranthropus robustus	14.75 (41/278)	11.51 (32/278)	1.08	41.30 (19/46)	0.00

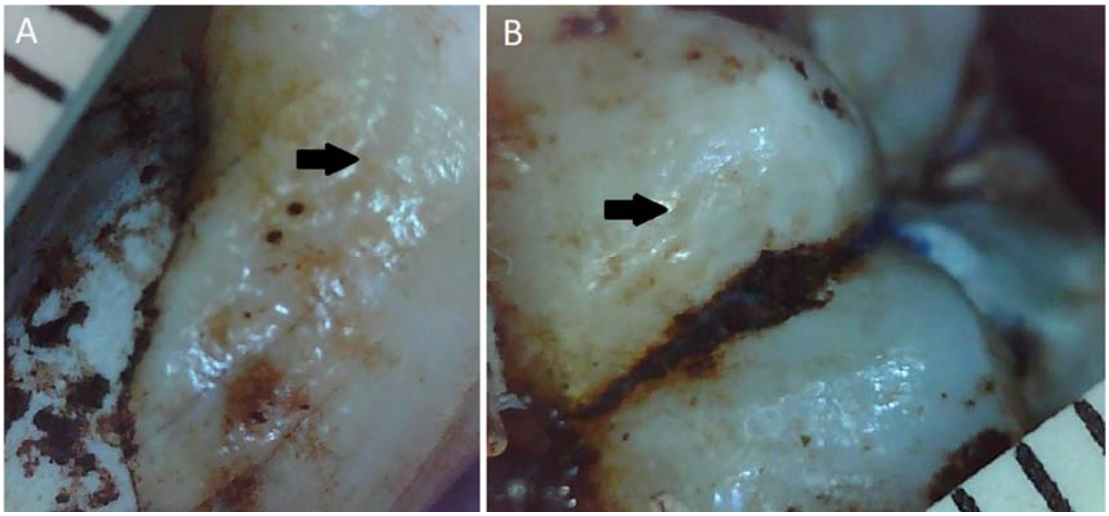

Figure 3. Abnormal enamel in *H. naledi*. Black arrow highlights vertical 'wavy' grooves. A) Buccal surface of UW 501 (canine); B) Buccal surface of UW 377 and 1014 (second molar).

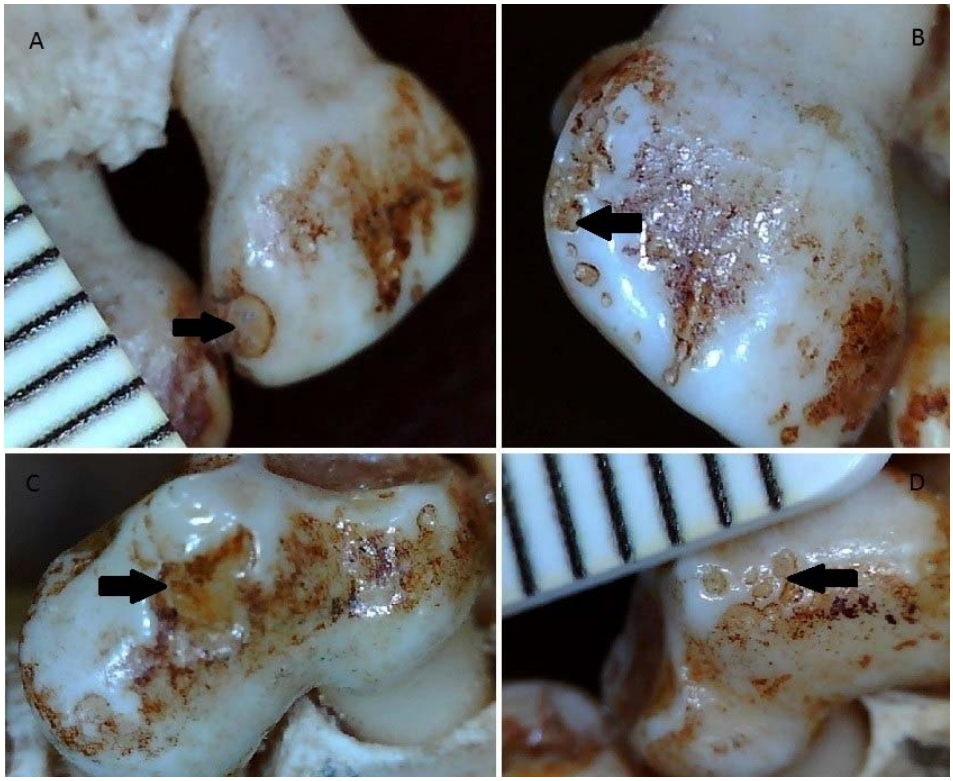

Figure 4. Male chimpanzee displaying non-symmetric localised/pitting hypoplasia on multiple deciduous teeth (M 475). A) Upper left lateral incisor; B) Upper right lateral incisor; C) Lower left first molar; D) Lower right first molar. All buccal view. Black arrows indicate defects.

When individuals with and without localised hypoplasia are analysed separately, there is more PEH in the group with no localised enamel lesions for both gorillas and chimpanzees. For chimpanzees, in individuals with at least one localised defect, 1.2% of teeth have PEH, whereas for individuals with no localised defects 5.42% of their teeth have PEH. For gorillas, the figures are 0% and 5.77% respectively. For both species this is a statistically significant difference (gorillas: X^2= 12.533, 1 df, p= 0.0004; chimpanzees: X^2= 4.416, 1 df, p= 0.0356).

4. Discussion

People with amelogenesis imperfecta that display groove/linear enamel defects typically also show other enamel abnormalities, and all, or most, teeth are typically affected to some degree (Sundell and Koch, 1984, Crawford et al., 2007; Wright, 1985, Aldred et al., 2003, Chamarthi et al., 2012, Schuurs, 2012; Wright et al., 1993, Mehta et al., 2013). Additionally, animal and clinical studies have extensively shown that malnutrition and disease can cause LEH (Goodman & Rose 1990; Guatelli-Steinberg & Benderlioglu, 2006). Therefore, it is justifiable to use LEH as a basis for health during tooth development, although if accompanied

by other enamel abnormalities (e.g., PEH, reduced enamel thickness, hypomineralisation), a genetic aetiology should also be considered. The results of the present study suggest it is common for PEH to have a different aetiology than LEH. The PEH in *P. robustus* is likely genetic in origin (Towle and Irish, 2019), and the clearest example of PEH in the chimpanzee sample was also caused by amelogenesis imperfecta (Towle at al., 2018). Similarly, specific genetic conditions and illnesses are associated with specific types of PEH in humans (Crawford et al., 2007; Lauc et al., 2015). Lastly, bands of pits (pitting LEH), often show numerous bands on different parts of a single tooth suggesting it may not simply be a consequence of crown position that leads to these defects. These observations add support to the suggestion that pitting defects in a sample may commonly have a different aetiology to LEH.

There is compelling evidence that many types of localised defects are caused by crypt fenestration (Suckling, 1980; Skinner and Skinner, 2017; Suckling et al., 1983; Suckling et al., 1986; Skinner et al. 2014; Skinner et al., 2016; Skinner, 1986; Skinner & Newell, 2003). It is suggested the overarching reason may be linked to deficient growth in infancy of the mandible and maxilla (Lukacs, 1999; Skinner et al., 2016). This theory is supported by studies that highlight a link between general ill health and an increase in localised enamel hypoplasia (Koch, 1999; Scheutzel & Ritter, 1989; Silberman et al., 1991; Skinner, 1986; Skinner & Hung, 1989). Studies on primates, rats and pigs, have also shown such a link, however these are not based on wild populations and the animals involved were subject to severe starvation and malnutrition (Dressino & Pucciarelli, 1997; Garat et al., 2006; McCance et al., 1961; Tonge & McCance, 1973). Skinner et al. (2016) suggest there is a relationship between malnutrition and dental overcrowding in humans, although the only significant relationship is in mouth-breathing adolescents (Thomaz et al., 2010).

The results of the present study find individuals with localised defects on deciduous canines do not show higher rates of other forms of hypoplasia. Therefore, certain species/populations may be predisposed to certain types of localised enamel hypoplasia, in certain teeth, due to cranial/dental morphology and therefore many of these defects may be more linked to phylogeny than to the individual's health. Specific genetic and environmental factors may also be important to consider (Skinner, 1996; Hart et al., 2003). In Figure 4, a juvenile male chimpanzee with a full deciduous dentition has defects on the maxillary canines, lateral incisors, and right first molar, as well as all mandibular teeth except the deciduous

second molars. If found in isolation, some teeth would likely be recorded as PEH or plane-form defects, and the rest localised hypoplasia. The fact an antimere is not affected, and the pattern of the defects are different on each tooth, suggests these defects may be best described as localised enamel hypoplasia. This case highlights an issue in studying enamel hypoplasia in fragmented collections, i.e., these defects all likely share a common aetiology, but if individual teeth were found isolated several types of defect and/or aetiologies may have been suggested. Even LEH on isolated teeth may be associated with other enamel abnormalities. Therefore, in ancient samples it is crucial to record all teeth available, and all types of enamel hypoplasia (and other enamel abnormalities if possible), to be able to help rule out genetic and non-systemic factors.

Other unusual defects that don't fit into any of the four categories (e.g., 'wavey' or 'vertical' defects) are uncommon, although systematic recording of prevalence's in different samples is rare. These macroscopic defects likely have a variety of aetiologies, but factors may include tooth properties (e.g., underlying morphology or epithelium folding during development), dentine defects, specific dietary/environmental factors (e.g., fluorosis), or genetic conditions (Braunn et al., 2014; Xing et al., 2015; Musale et al., 2019). Therefore, these abnormalities are not necessarily a form of enamel hypoplasia, making recording difficult in ancient samples. Unusual enamel abnormalities such as these were rare in the sample's studies, except in *H. naledi* in which 'wavey' and 'vertical' enamel abnormalities were recorded (Figure 4). Tobias (1967) notes similar defects in a *P. boisei* specimen, but otherwise such abnormalities are rarely recorded in fossil hominin samples. Other types of enamel defects that are relatively common in people today and in some recent archaeological samples (e.g., molar incisor hypomineralisation and plane form defects) are rare or absent in earlier populations, such as in the present study, suggesting modern lifestyle (e.g., medicines, environment, diet and disease) has had a significant impact on the types and prevalence of enamel defects (Gualdi-Russo et al., 2017; Kühnisch et al., 2016; Ioannou et al., 2016; Ogden, 2007; Pramanik and Saha, 2017). Therefore, depending on the age of the sample, it may be important to consider other types of enamel defects. When comparing these different types of defects, histological, microscopic and micro-CT scan analysis, may offer a more complete understanding of how an abnormality formed, and therefore potentially further insight into timing and aetiology of specific abnormalities (Witzel et al., 2008; Hassett, 2014).

Genetic and environmental differences on a population level are also important to consider. For example, populations that have recently undergone intensive selection in relation to an enamel property (e.g., thickness or structure), may be predisposed to specific types of enamel defects, due to loss of stability in specific genes or through pleiotropy effects (Pavličev and Cheverud, 2015, Fiddes et al., 2018, Hlusko et al., 2018). For example, the ENAM gene shows signs of strong positive selection in certain species, likely relating to enamel thickness (Kelley and Swanson, 2008; Horvath et al., 2014), and mutations in this gene are also associated with many types of amelogenesis imperfecta (Crawford et al., 2007, Kelley and Swanson, 2008, Wang et al., 2015). Therefore, species that have recently evolved a substantial increase/decrease in enamel thickness or tooth size, may be more prone to certain types of enamel abnormalities (Towle et al., 2019). Other genetic factors will heavily influence enamel abnormality prevalence on a population level, including founder effects, and the complex, and not well understood, interaction between genotype and environmental and epigenetic factors (Wang et al., 2016; Pramanik and Saha, 2017; Vieira et al., 2005; Russell, 1962; Musale et al., 2019).

Tooth properties (e.g., morphology, size and enamel structure), will also affect the likelihood of enamel hypoplasia being visible on a macroscopic level, and influence the shape and shape of defects (Guatelli-Steinberg et al., 2012; Braunn et al., 2014; McGrath et al., 2018). For example, the angle at which striae of Retzius reach the outer enamel surface will affect the depth and size of LEH defects, meaning different teeth, and surfaces, are more/less likely to show macroscopic defects (Guatelli-Steinberg et al., 2012, 2017; Hillson & Bond, 1997; Kierdorf, Witzel, Kierdorf, Skinner, & Skinner, 2015; Hassett, 2014). Other enamel properties also affect the expression of defects, including, perikymta spacing and the age of ameloblasts (Hillson and Bond, 1997; Witzel et al., 2006; Witzel et al., 2008; Guatelli-Steinberg et al., 2012; Hassett, 2012, 2014). It is well known that tooth development (e.g., speed and total time) with also influence enamel hypoplasia prevalence's, with the results of the present study supporting literature that finds higher rates of LEH in great apes than other primates (e.g., Guatelli-Steinberg, 2001; Moggi-Cecchi & Crovella, 1991). This likely relates at least partly to extended tooth formation, with great apes living longer through disease, nutritional deficiencies and seasonal disturbances (Zihlman et al., 2007). In sum, there are a variety of ways in which phylogeny influences enamel hypoplasia prevalence's, even before behaviour,

health and diet is considered. This is especially important considering hominin groups, and primates more generally, differ substantially in terms of these dental properties.

5. Conclusions

The results of this study highlight how proportions of different kinds of enamel hypoplasia varies substantially between samples. Tooth properties along with environmental and genetic factors likely heavily influence frequencies. Therefore, studies that include only one form of enamel hypoplasia to compare the 'stress' between populations may miss crucial information. Instead, it may be more beneficial to display and described different types of defects separately and attempt to understand the aetiology on an individual and population bases. Incorporating tooth property and phylogeny information into analysis may also allow more robust conclusions.

Acknowledgements

The authors thank L. Berger and B. Zipfel from the University of the Witwatersrand, I. Livne from the Powell-Cotton Museum, and S. Potze from the Ditsong Museum of South Africa for access to their collections. Data was collected thanks to a studentship from Liverpool John Moores University.

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