From the Inside Out: Understanding Stress-Related Enamel Defects in Great Ape Canines

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Canines

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Dedication

This dissertation is dedicated to the late Dr. Dana Alan Cope, Associate Professor Emeritus at the College of Charleston, South Carolina.

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Abstract of Dissertation

From the Inside Out: Understanding Stress-Related Enamel Defects in Great Ape Canines

Developmental defects of enamel are commonly used to reconstruct aspects of the health, growth patterns, and life history of modern humans, hominins, and nonhuman primates. This dissertation focuses on hypoplastic defects that appear as grooves on the outer enamel surface and are often termed linear enamel hypoplasia, or LEH. LEH defects have been linked to stressors such as malnutrition, injury, and illness in clinical and experimental settings. LEH is common in great apes, but previous work reported that one species, Virunga mountain gorillas, have far fewer defects than other taxa. This dissertation characterizes LEH defect morphology on the outer enamel surface of great ape canines (Chapter 2). The results suggest that mountain gorilla defects are shallow compared to those in other taxa, which may have led to their underestimation in previous studies. Females in the pooled sample were found to have deeper defects than males. The deepest defect in the sample belongs to a western lowland gorilla that was captured as an infant. Based on the location and approximate developmental timing of this defect, it might correspond to her capture. In Chapter 3, I incorporate histological data to assess whether enamel growth variables, namely linear enamel thickness, enamel extension rates, and striae of Retzius angles, correspond to the documented variation in LEH defect depth described in Chapter 2. Inter- and intraspecific variation in enamel extension rates and striae of Retzius angles, and to a lesser extent linear enamel thickness, tracked the sex- and species-differences in defect depth. This suggests that enamel growth variation influences LEH defect morphology on the outer enamel surface. Enamel growth patterns
should therefore be carefully considered when reconstructing stress severity based on the appearance of defects on the outer enamel surface alone. In Chapter 4, I conduct a detailed histological analysis of four mountain gorilla individuals, three of which are of known age and sex. I found that all LEH defects from Chapter 2 co-occur with underlying disruptions to enamel matrix secretion in the form of accentuated lines. However, there are many more accentuated lines than there are LEH defects in this sample, and because accentuated lines occur throughout the height of the tooth crowns, they provide a more complete history of growth disruptions. One specimen, GP.075, demonstrates a major plane-form defect in the permanent third molar that corresponds to a relatively minor accentuated line in the concurrently forming canine. Three poaching-related snare removals by veterinarians were recorded as LEH defects with co-occurring accentuated lines in the canines of two individuals, providing rare data on defect etiology in wild primates. Future work will incorporate more diverse hominoid samples into these analyses, including more frugivorous Bwindi mountain gorillas and eastern lowland gorillas, to better understand the links between behavioral ecology and enamel growth in closely related taxa. Taken together, these results increase our understanding of inter- and intraspecific canine enamel growth variation in great apes. This work characterizes “normal” growth variation as well as growth disruptions in the form of LEH defects and accentuated lines. A key finding is that enamel geometry plays an important role in determining defect severity or depth; it is important to characterize defect morphology within and among species and sexes to understand whether a given defect represents an outlier at the population level. Particularly deep LEH defects might correspond to severe stressors, as in the case of the wild-captured apes in Chapter 2. Detailed growth histories
are best understood via histologic analyses, as Chapter 4 demonstrates. This work has relevance for researchers interested in reconstructing the growth histories of hominins and past populations of modern humans on the basis of enamel defects alone as they too exhibit variable enamel growth patterns, which might influence the morphology and interpretation of enamel defects.
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Chapter 1: Introduction

Dental tissues have long been used to reconstruct aspects of life history and development in extant and extinct hominoids (e.g., Smith, 1991; Robson & Wood, 2008). Enamel and dentine provide a permanent record of their development, including growth disruptions, which can be attributed to acute episodes of early life stress (Hillson, 2014). These disruptions appear as hypoplastic defects, often appearing as bands of reduced enamel thickness around the tooth crown with associated accentuated lines visible internally in thin sections (Hillson & Bond, 1997). The condition in which these defects occur is called linear enamel hypoplasia, or LEH.

Linear enamel hypoplasia is ubiquitous among great apes with over 75% of teeth having at least one defect (e.g., Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012). However, Virunga mountain gorillas do not exhibit the typical prevalence; instead, only 1 in 11 specimens showed a clear, macroscopic groove on their canines (Guatelli-Steinberg et al., 2012). The authors suggested that Virunga mountain gorillas might not experience the same level of physiological stress as other apes, given their almost entirely folivorous diet (Guatelli-Steinberg et al., 2012). Alternatively, they suggested that mountain gorillas might have shallower defects on the outer enamel surface that are more difficult to reliably identify using traditional methods (Guatelli-Steinberg et al., 2012). They proposed that shallow defect morphology may be a consequence of the enamel geometry of internal growth increments, which is shaped by variation in enamel growth patterns (Boyde, 1964; Shellis, 1984; Hillson & Bond, 1997; Guatelli-Steinberg et al., 2012). Thus, faster canine growth might be linked to shallower LEH defects in mountain gorillas (Guatelli-Steinberg et al., 2012).
This dissertation focuses on developmental disturbances that are evident in canine teeth, and aims to (a) develop a method to quantitatively assess whether Virunga mountain gorillas exhibit shallower defects on the outer enamel surface than other great apes, and whether males have shallower defects than females as a result of differences in enamel growth patterns related to sexual dimorphism; (b) assess the underlying enamel microstructure in great apes to test whether species- and sex-specific variation in enamel growth patterns influences defect depth on the outer enamel surface; and (c) examine mountain gorilla canine histologic sections to better understand the relationships among surface defects, accentuated lines visible internally, and variation in the timing, rate, and duration of enamel growth in this species.

**Enamel development**

Teeth permanently preserve the absolute chronology of growth from before birth through to the end of dental development (Hillson, 2014). The early stages of dental development are characterized by complex interactions between epithelium and mesenchyme tissues (Boyde, 1989; Ten Cate, 1994). During the histogenesis phase of crown development, enamel and dentine tissues develop along the enamel-dentine junction, or EDJ. Each ameloblast, or enamel-secreting cell, courses away from the EDJ until it achieves full enamel thickness in that crown region and reaches the outer enamel surface of the mature tooth (Boyde, 1976, 1989; Ten Cate 1994). Fully differentiated ameloblasts are organized in rows and secrete a protein matrix within which hydroxyapatite crystals grow (Boyde, 1976, 1989). Once the ameloblast reaches what will become the outer enamel surface, it helps to mineralize the enamel matrix by
removing organic components during the maturation phase (Boyde, 1989). New ameloblasts are recruited and this process continues from the first-formed enamel above the dentine horn until crown formation is completed at the cervix (Ten Cate, 1994).

Enamel is divided into bundles of crystallites (3-6 \( \mu \)m in diameter) called prisms. Prisms are separated by changes in the crystallite orientation, which are the result of the different orientations of the Tomes’ processes of ameloblasts (Boyde, 1976, 1989). In longitudinal histologic sections, individual prisms can be seen coursing from the EDJ to the outer enamel surface, but they do not always have a straight trajectory; in some crown regions, particularly under the cusp, they undulate in three dimensions in a phenomenon called “prism decussation” (Boyde, 1989; Ten Cate, 1994).

The incremental structure of enamel is what allows researchers to reconstruct the dental developmental history of individuals. Incremental growth layers, which are visible both within the enamel and on the outer enamel surface, reflect periodic variations in enamel matrix secretion as a result of regular biologic rhythms (Dean, 1989; Hillson, 2014). A daily rhythm is recorded as enamel cross striations, which appear via transmitted light microscopy as hatch marks along the long axis of enamel prisms (Boyde, 1976; Smith, 2006). Cross striations reflect a circadian rhythm of reduced ameloblast activity and are thus laid down once per day (Dean, 1987; FitzGerald, 1998). A second type of incremental growth line, called striae of Retzius, cut across the prisms, reflecting the location of the developing enamel front, or the position of secretory ameloblasts, at that moment (Retzius, 1837). Striae of Retzius first form over the dentine horn in the cuspal enamel, while within the imbricational enamel, they outcrop at the outer enamel surface in tile-like layers (Hillson, 2014). The brown color of striae of
Retzius when viewed using transmitted light microscopy is the result of the Rayleigh scattering effect and not due to staining (Boyde, 1976). They occur in a regular periodicity ranging from 6-12 days in extant great apes (Schwartz et al., 2001; McGrath et al., in prep). While periodicity can vary among members of the same species, it is constant within a single individual (Boyde, 1989; FitzGerald, 1998), but recent work in modern humans suggests that periodicity may vary between deciduous and permanent teeth within the same individual (Mahoney et al., 2016). Periodicity correlates positively with body mass in primates, and it has been hypothesized to relate to a centrally regulated growth rhythm (Bromage et al., 2009, 2012).

The surface manifestation of striae of Retzius are called perikymata, or “surrounding waves,” due to their appearance as small concentric ripples around the tooth crown (Preiswerk, 1895; Pickerill, 1913; Hillson, 2014). The morphology of perikymata differs down the height of tooth crowns, with marked variation in the spacing and overall shape of the grooves (Hillson, 2014). The angle at which striae of Retzius meet the outer enamel surface influences the spacing and prominence of perikymata. Towards the cusp tip, in the early-forming imbricational enamel, shallow angles produce broad, low perikymata grooves (Hillson & Bond, 1997). In the cervical region, they are tightly packed and difficult to visualize without high resolution microscopy (Hillson & Bond, 1997; Hassett, 2014). In modern humans, midcrown perikymata are ideal for macroscopic observation as they are moderately spaced. Perikymata reflect the same periodicity as striae of Retzius (Risnes, 1985), which can be determined by counting the number of cross striations between consecutive striae using traditional histological investigation, a confocal microscope (Boyde, 1990), or x-ray synchrotron microtomography (Tafforeau...
& Smith, 2008). Reid and Ferrell (2006) found higher perikymata counts on modern human teeth with lower striae of Retzius periodicities and vice versa. If this relationship holds true across other primate groups, it might help researchers estimate periodicity where histological sectioning or synchrotron microtomography are not an option.

In cases where histological sectioning of teeth is permitted, there are methodological barriers to the accurate reconstruction of growth history. Tooth wear eliminates the first growth increments, which are often crucial to any analysis. In well-preserved specimens, the ideal plane of section cuts through the dentine horn and cusp tip to expose these first growth increments (Hillson, 2014). The section is then ground down to an appropriate thickness for imaging and unambiguous visualization of internal structures. Dental wear, calculus, and specimen fragility are major problems in studies of surface defects. These, and other factors, place limits on sample size, and thus hinder the accurate reconstruction of developmental sequences.

**Growth disruptions**

Dental development is influenced by the genotype as well as the environmental influences such as diet and disease (Hillson, 2014). The majority of energetic resources go toward body maintenance. Growth requires a surplus, and if energetic sources are insufficient, growth stops, and then resumes when resources are available. Dental growth and development seems to be buffered from the environment compared to other skeletal elements (Hillson, 2014). However, evidence from studies comparing the growth of captive vs. wild primates suggests that the environment may influence growth rates, both
in terms of somatic growth (Altmann & Alberts, 2005) and to some extent, dental development (e.g., Phillips-Conroy & Jolly, 1988; Smith et al., 2010).

Acute disruptions to growth result in defects in developing enamel, which can appear internally as accentuated lines, and/or on the outer enamel surface as hypoplastic defects. Accentuated lines appear darker, broader, or more pronounced than nearby striae of Retzius (Hillson, 2014). They either occur between regular striae of Retzius, or if the timing lines up with the regular long-period rhythm, they appear as enhanced striae (Antoine et al., 2009). Accentuated lines are hypothesized to represent a “pathological intensification” of the slowing of enamel formation that occurs in the formation of striae of Retzius (Witzel et al., 2008). In this way, accentuated lines represent disruptions to all ameloblasts along the enamel-forming front at a particular point in time during enamel secretion (Witzel et al., 2008). Accentuated lines are the key to reconstructing the timing and sequence of crown development as they occur among the normal daily and near-weekly growth increments, and they can be matched to link the developmental timing of one tooth type to another (Hillson, 2014). The neonatal line, which is an accentuated line that occurs at birth, represents day 0 in the chronology of growth (Schour, 1936). The neonatal line is visible in all teeth forming at the time of birth, including the deciduous teeth, and in most cases in great apes and humans, the first permanent molar (Schour, 1936; Reid et al., 1998; Schwartz et al., 2006; Smith & Boesch, 2015). Accentuated lines in enamel can often be matched to corresponding and concurrently forming defects in dentine, such as accentuated lines, interglobular layers, or periradicular bands (Smith, 2008; Smith & Reid, 2009; Hillson, 2014). Accentuated lines in enamel and dentine are
often associated with deviations in shape of the enamel-dentine junction (EDJ) dividing the two tissues (Hillson, 2014).

Accentuated lines have been called Wilson bands when associated with hypoplastic defects on the outer enamel surface (Goodman & Rose, 1990). The extent to which the two defect types co-occur is not yet clear, though accentuated lines and hypoplastic defects are generally thought to represent internal and external manifestations of the same systemic growth disruption (Goodman & Rose, 1990; Hillson, 2014).

However, Kierdorf et al. (2000, 2004) and Witzel et al. (2006, 2008) proposed a threshold model at the cellular level to explain the variable appearance of developmental defects of enamel. They claim that minor hypoplastic defects can occur when the lowest threshold is passed without the formation of an accentuated line. They argue that the timing of the disruption is key, and only if all secretory ameloblasts are impaired will an accentuated line form, whereas a hypoplastic defect can occur when only late-stage ameloblasts are affected.

Hypoplastic defects on the tooth surface are reductions in enamel thickness, usually in the form of horizontal grooves (furrow-form defects, often termed linear enamel hypoplasia, or LEH), pits (pit-form defects), or entire planes of enamel may be missing (plane-form defects) (Berten, 1895; Hillson & Bond, 1997). Hypoplastic defects form during the secretory phase of enamel formation, or when the ameloblasts secrete protein matrix as discussed above. During this secretory phase, the enamel is only about 30% mineralized, and disruptions to ameloblast function at this time can lead to the reduction of enamel thickness, or hypoplasia (Ten Cate, 1994). Disruptions that occur
during the enamel maturation phase do not lead to the formation of hypoplasia, but instead to hypocalcifications, or areas of undermineralized enamel (Ten Cate, 1994).

LEH defects are the most common and best-understood type of hypoplastic defect. They are marked by an increase in the spacing between consecutive perikymata or among several perikymata grooves (Hillson & Bond, 1997). LEH defects range in appearance from macroscopic depressions to microscopic deviations in perikymata spacing (Hillson & Bond, 1997). They are formed when a wider portion of the Retzius planes is exposed than typically occurs in the formation of a “normal” perikyma due to the fact that a larger band of ameloblasts cease matrix formation at that time (Hillson, 1997). The result is wider spacing among associated perikymata in the occlusal wall of the defect (Hillson & Bond, 1997). Perikymata spacing returns to normal within the cervical wall of the defect, indicating the return to normal enamel secretion following the acute disruption (Hillson & Bond, 1997). LEH defects vary in width and depth, but the cuspal wall of the groove usually slopes more sharply than the cervical wall (Hillson, 2014). In plane-form defects, a single Retzius plane is widely exposed, while isolated pit-form defects are caused by the disruption of small groups of ameloblasts (Hillson & Bond, 1997). LEH defects are clearly associated with perikymata, and where preservation is sufficient, researchers are able to reconstruct the approximate timing and duration of growth disruptions on the basis of defect location and occlusal wall width, respectively (Dean & Reid, 2001).

Clinical and experimental studies in modern humans and other animals have linked hypoplastic defects to systemic physiological stressors (Goodman & Rose, 1990), including infections (Sarnat & Schour, 1941), malnutrition (Sweeney et al., 1971), and
parasitism (Suckling et al., 1986). Some researchers have hypothesized that defect depth reflects the severity of the stressor that interrupted growth (Skinner & Hopwood, 2004; Skinner & Skinner, 2017). Experimental studies have demonstrated some support for this claim using animal models (Suckling & Thurley, 1984; Kierdorf et al., 2004), but studies of a similar nature in primates are lacking.

Another important influence on defect morphology is the geometry of the underlying enamel growth increments. The angle at which striae of Retzius approach the outer enamel surface is related to the rate of enamel extension and secretion (Boyde, 1964; Shellis, 1984; Hillson & Bond, 1997; Guatelli-Steinberg et al., 2012, 2017; Kierdorf et al., 2015). In modern human teeth, crown regions with more acute striae of Retzius angles are associated with wider and shallower perikymata and defects compared to areas with more obtuse striae of Retzius angles (Hillson & Bond, 1997), and this is also true in great ape canines (Guatelli-Steinberg et al., 2012). Due to the normal variation in perikymata spacing down the crown, some crown regions show defects more prominently than others, with the midcrown region being the optimal location in modern humans (Hillson, 2014). Canines are the focus of this dissertation because more of their crowns are made up of midcrown-type perikymata (Hillson, 2014), especially in tall great ape canines. Canines also take the longest to form of any tooth type in great apes, and thus capture the longest window of development (Schwartz & Dean, 2001). Mandibular canines do not initiate before birth in great apes and humans, as does the first permanent molar, but an upper canine has been documented to have initiated before birth in a single orangutan (Winkler, 1995).
A large body of research exists on great ape dental development, including studies focused on hypoplastic defects (e.g., Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012; Skinner & Hopwood, 2004; Skinner et al., 2012; Skinner & Pruett, 2012; Skinner & Skinner, 2017). In general, great apes have more defects than monkeys and other primates (e.g., Guatelli-Steinberg et al., 1998; Newell, 1998). The exception to this pattern is Virunga mountain gorillas (Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012). Virunga mountain gorillas are the most folivorous of the great apes, and their comparative lack of access to seasonal fruit has been put forward as one potential explanation for their low defect prevalence (Guatelli-Steinberg et al., 2012). Another explanation focuses on the influence of enamel growth variation on defect depth, with Virunga mountain gorillas having faster growth and shallower defects that are difficult to reliably identify using traditional methods (Guatelli-Steinberg et al., 2012).

**Research goals**

This dissertation aims to characterize enamel defects in the canines of wild Virunga mountain gorillas (*Gorilla beringei beringei*), from both the outer enamel surface and from a histological perspective. This research incorporates teeth from other great ape species (*Gorilla gorilla gorilla, Pan troglodytes, Pongo* sp.) to provide a comparative context to better understand the unique dental development of Virunga mountain gorillas. Mountain gorilla skeletal specimens are primarily derived from the Mountain Gorilla Skeletal Project collection representing naturally-deceased individuals recovered from Volcanoes National Park in Rwanda (McFarlin et al., 2009; McFarlin et al., 2013). The majority of these specimens are known individuals with associated
behavioral, health, and climate records, providing an unparalleled context in which dental development can be studied.

The second chapter of this dissertation, “Quantifying linear enamel hypoplasia in wild mountain gorillas and other great apes,” aims to characterize the appropriate surface aspects of LEH defects and “normal” growth increments using optical profilometry. We test whether there are significant sex- and species-related differences in defect depth among great apes (Gorilla beringei beringei, Gorilla gorilla gorilla, Pan troglodytes, Pongo sp.). Depth was the focus of this chapter because linear enamel hypoplasia was originally described as a condition marked by localized reductions in enamel thickness (Berten, 1895). Differences in depth can be quantified in the absence of perfect surface preservation, which is not the case when analyzing the details of perikymata spacing. Some researchers have suggested that defect depth corresponds to the severity of the stressor that caused the defect to form (e.g., Skinner & Skinner, 2017), while others have proposed that enamel growth variation might influence depth (e.g., Guatelli-Steinberg et al., 2012). This chapter tests whether sex- and species-specific variation in depth exists, which might support the latter hypothesis. We predict that mountain gorillas will have shallower defects than the other taxa, and that males will have shallower defects than females overall.

The third chapter uses a comparative sample of histological thin sections from the same four species as Chapter 2. The goal of this chapter is to test whether there are sex- and species differences in enamel growth patterns, which might explain the differences in LEH defect depth on the outer enamel surface. The variables analyzed include linear enamel thickness, enamel extension rates, and striae of Retzius angles at the outer enamel
surface. We predict that mountain gorillas will have thinner enamel, faster extension rates, and shallower striae angles than the other taxa. We also predict that males will demonstrate the same pattern (thinner enamel, faster rates, shallower angles) compared to females.

The fourth chapter of this dissertation conducts a detailed histological study of three mountain gorilla specimens with particularly well-preserved dentitions. The main goal of this chapter is to assess the relationships among defect formation and the timing, rate, and duration of enamel growth in mountain gorillas. We test whether hypoplastic defects co-occur with accentuated lines, and assess the timing and frequency of both defect types down the tooth crown. We incorporate available health records to test correspondence between enamel defects and stress events in two individuals. We consider the results in light of the documented variation in enamel growth patterns between the sexes, and in the broader context of great ape canine development.

This dissertation uses novel approaches to tackle the topic of linear enamel hypoplasia from both the outer enamel surface and from a histological perspective. The innovative use of optical profilometry captures the outer enamel surface with a resolution that is appropriate for even the shallowest of defects. The careful histologic analyses contribute to our understanding of the way that the outer enamel surface is shaped by intrinsic growth factors, which will allow researchers to extract more information related to growth and development from the outer enamel surface alone. This work also contributes to the growing body of knowledge about the biology of mountain gorillas, a critically-endangered species that is poorly represented in museum collections. As this dissertation demonstrates, mountain gorillas can provide crucial data that increase our
understanding of great ape biology, and in turn influence our interpretations of the human fossil record.
Chapter 2: Quantifying linear enamel hypoplasia in Virunga mountain gorillas and other great apes

Publication Citation:

ABSTRACT

**Objective**

Linear enamel hypoplasia (LEH) is a condition marked by localized reductions in enamel thickness resulting from growth disruptions during dental development. We use quantitative criteria to characterize the depth of LEH defects and “normal” perikymata in great apes. We test the hypothesis that mountain gorillas have shallow defects compared to other taxa, which may have led to their underestimation in previous studies.

**Materials and Methods**

Previous attempts to characterize LEH morphology quantitatively have been limited in sample size and scope. We generated digital elevation models using optical profilometry (Sensofar PLu Neox) and extracted 2D coordinates using ImageJ to quantify depths in canines from three great ape genera (N=75 perikymata; 255 defects).

**Results**

All defect depths fall outside the distribution of perikymata depths. Mountain gorilla defects are significantly shallower than those of other great ape taxa examined, including
western lowland gorillas. Females have significantly deeper defects than males in all taxa. The deepest defect belongs to a wild-captured zoo gorilla. Virunga mountain gorilla specimens collected by Dian Fossey exhibit deeper defects than those collected recently.

**Discussion**

Shallow defect morphology in mountain gorillas may have led to an underestimation of LEH prevalence in past studies. Defect depth is used as a proxy for insult severity, but depth might be influenced by inter- and intra-specific variation in enamel growth. Future studies should test whether severe insults are associated with deeper defects, as might be the case with Haloko, a wild-captured gorilla. Ongoing histologic studies incorporating associated behavioral records will test possible factors that underlie differences in defect morphology.
INTRODUCTION

Linear enamel hypoplasia (LEH) is a condition marked by localized reductions in enamel thickness in all concurrently forming teeth (Berten, 1895; Hillson & Bond, 1997) (Fig. 1). LEH is known as a nonspecific indicator of physiological stress during dental development, with defects representing acute disruptions to enamel secretion (Goodman & Rose, 1990). The approximate developmental timing of LEH defects can be inferred because they occur within the sequence of “normal” enamel growth increments, or perikymata, on the outer enamel surface (Reid & Dean, 2000). Previous research on LEH in great apes has primarily focused on determining defect prevalence by scoring anterior teeth using light microscopy and/or scanning electron microscopy (e.g., Skinner & Hopwood, 2004; Guatelli-Steinberg et al., 2012). Defect severity, defined as the depth and/or width of a defect, has been hypothesized to reflect the magnitude of the insult that caused the defect (Skinner & Hopwood, 2004; Skinner & Skinner, 2017). However, variation in the internal geometry of underlying enamel growth increments may also influence defect depth (Guatelli-Steinberg et al., 2012). While histological analysis is the only way to obtain a complete understanding of how individual defects formed (Witzel et al., 2008), nondestructive imaging-based methods have been developed to quantitatively characterize defect morphology from the outer enamel surface (Bocaeg et al., 2010; Bocaeg & Hillson, 2016; Guatelli-Steinberg et al., 2004; Hasset, 2012, 2014; Henriquez & Oxenham, 2017; Hillson & Jones, 1989; Hillson, 2014; King et al., 2002, Le Cabec et al., 2015; Marchewka et al., 2014; Skinner & Pruetz, 2012; Skinner & Skinner, 2017; Temple et al., 2013). These methods have the potential to reduce interobserver error in the identification and characterization of defects, but fundamental questions remain about
the extent of inter- and intraspecific variation in defect morphology in primates. Virunga mountain gorillas have been characterized as having much lower LEH prevalence than other great ape taxa (Colyer, 1936; Moggi-Cecchi & Crovella, 1991; Guatelli-Steinberg, 1998; Newell, 1998; Guatelli-Steinberg, 2000; Guatelli-Steinberg, 2001; Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012). We hypothesize that this is due to mountain gorillas having shallower defects that are harder to reliably identify using qualitative methods. In this study, we use quantitative criteria to assess inter- and intraspecific variation in mandibular canine perikymata and LEH defect depth in four great ape taxa (Gorilla beringei beringei, Gorilla gorilla gorilla, Pan troglodytes, Pongo sp.). We also test whether there are differences in defect depth between Virunga mountain gorillas that developed their dentition during a period of high habitat destruction and poaching compared to those that lived under increased protection more recently. Our goals are to: (1) characterize perikymata and LEH defect morphology in great apes, (2) test whether inter- and intraspecific patterns of LEH defect expression are consistent with the expected influences of inter- and intraspecific enamel growth variation, and (3) evaluate the possibility that insult severity also affects the formation of LEH defects in great apes.

Dental development and LEH

Teeth are important for studies of health and development because they are made of tissues that provide a permanent and detailed record of their absolute chronology of growth. Enamel grows incrementally following two biological rhythms: one short and one long. Short-period growth increments, termed cross striations, record daily
fluctuations in enamel matrix secretion (Smith, 2006). They reflect the circadian rhythm of ameloblasts, the enamel matrix-secreting cells (Boyde, 1989). Under light microscopy, cross striations appear as hatch marks between successive long-period growth increments, or striae of Retzius (Fig. 2; Retzius, 1837). The periodicity of striae of Retzius can be determined by counting the number of cross striations between successive striae. This number ranges from 6-12 days in great apes (Schwartz et al., 2001). While periodicity varies among individuals within a species, it is consistent across the dentition of any single individual (FitzGerald, 1998). However, a recent study suggests that this rhythm may not be constant between deciduous and permanent teeth (Mahoney et al., 2016). Striae of Retzius periodicity correlates with body mass in primates, and it has been hypothesized to relate to a centrally-regulated growth rhythm (Bromage et al., 2009, 2012). Perikymata, which consist of one ridge and one trough on the enamel surface, are the external expression of striae of Retzius (Figs. 1&2; Preiswerk, 1895). Perikymata only occur in the lateral enamel, where striae of Retzius outcrop on the outer enamel surface. As perikymata are the external manifestation of underlying striae of Retzius, each perikyma also represents one long-period interval (Hillson & Bond, 1997). They can be accessed nondestructively to glean information about the timing and duration of enamel matrix development, and to calibrate disruptions to dental development.
Disruptions to enamel matrix secretion form localized reductions in enamel thickness, or hypoplasia, in all concurrently forming teeth (Berten, 1895; Hillson & Bond, 1997). Different types of enamel hypoplasia have been described, including furrow-form, plane-form, and pit-form (Hillson & Bond, 1997). Furrow-form defects are circumferential depressions across the enamel surface that occur within the sequence of “normal” perikymata, or long-period growth increments (Fig. 2) (Goodman & Rose, 1990). They vary in their appearance from microscopic lines to more pronounced furrows (Hillson & Bond, 1997). Plane-form defects have a stria of Retzius that is partially or totally exposed. These defects are suggested to represent a more major disruption to dental development of a shorter duration compared to other forms of LEH (Hillson & Bond, 1997). Pit-form defects usually appear as a band of pits around the tooth crown.
(Hillson & Bond, 1997). Internally, hypoplastic defects are often, but not always, associated with accentuated striae (Fig. 2) (Witzel et al., 2008). Furrow-form defects are the most common and best-understood kind of enamel hypoplasia (Berten, 1895; Hillson & Bond, 1997), and they are the focus of this study.

Figure 2. Photomontage of GP.033 permanent mandibular canine thin section.

**Known and hypothesized causes of LEH**

Linear enamel hypoplasia is known as a non-specific indicator of physiological perturbations during the secretory phase of amelogenesis (Goodman & Rose, 1990). LEH
has been studied extensively across extant great apes (e.g., Guatelli-Steinberg et al., 2012; Skinner & Hopwood, 2004; Skinner & Pruetz, 2012; Smith & Boesch, 2015; Skinner & Skinner, 2017), anatomically modern humans (e.g., Boyde, 1970; Goodman et al., 1980; King et al., 2005), and other primates (e.g., Newell 1998; Guatelli-Steinberg, 2000; Chollet & Teaford, 2010). Experimental and clinical studies in modern humans and other animals have established disease and/or malnutrition as causes of LEH (Goodman & Rose 1990). In nonhuman primates, there is also evidence supporting a relationship between LEH and malnutrition: LEH prevalence differed in Cayo Santiago macaques before and after the introduction of provisioning, with those that were provisioned having significantly fewer defects than those that were not provisioned (Guatelli-Steinberg & Benderlioglu, 2006). Other hypothesized causes of LEH include weaning (Goodman & Rose, 1990; Ogilvie et al., 1989; Skinner et al., 2012), seasonality (Skinner & Pruetz, 2012), and parasitism (Suckling, 1986). Skinner (1986) noted that great ape canines and incisors are marked with repeated defects with regular spacing, and suggested this rhythm reflects the influence of alternating dry and rainy seasons. Other studies have tentatively linked accentuated striae to seasonal cycles, reporting a negative correlation between defect timing and rainfall abundance in *Theropithecus oswaldi* and common chimpanzees (Macho et al., 1996; Smith & Boesch, 2015). The proximate factors suggested to explain the regular rhythm of hypoplastic defects include seasonal variation in fruit availability, heat or water stress during dry seasons, or diseases that cycle seasonally such as malaria or hookworm (Skinner & Hopwood, 2004; Skinner & Preutz, 2012).

Linear enamel hypoplasia is ubiquitous among great apes and LEH prevalence typically exceeds 75% of all individuals examined (e.g., Guatelli-Steinberg et al., 2012;
Skinner & Hopwood, 2004; Skinner & Pruetz, 2012; Smith & Boesch, 2015; Skinner & Skinner, 2017). However, Virunga mountain gorillas are an exception to this pattern, with prior studies reporting much lower prevalence (5-11%) (Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012). Published data support a relationship between LEH defect prevalence and degree of frugivory within Gorilla. More frugivorous western lowland gorillas have higher reported prevalence than eastern lowland gorillas, which represent a dietary intermediate along this continuum; eastern lowland gorillas in turn have higher prevalence than Virunga mountain gorillas (Watts, 1984; Yamagiwa et al., 1994; Guatelli-Steinberg et al., 2012). Mountain gorillas from the Virunga Massif live at the highest altitudes observed among great apes, and their habitats have little available fruit. Instead, they consume terrestrial herbaceous vegetation that is spatially and temporally abundant (Fossey & Harcourt, 1977; Watts, 1984; McNeilage, 2001). In contrast, the relationship between fruit consumption and defect prevalence is not consistent across Pan (Guatelli-Steinberg et al., 2012). The bonobo diet is reportedly more seasonally stable than that of chimpanzees (White, 1998), but over 95% of bonobo canines were found to have at least one defect, with a mean number of defects (4.7, N=23) well above that of chimpanzees (3.1, N=35) (Guatelli-Steinberg et al. 2012). The relationship between frugivory and LEH prevalence is complicated by the fact that more frugivorous taxa exhibit slower somatic development (e.g., Galbany et al., 2017; Leigh, 1994). If dental development is characterized by a similar dietary pattern, this might create a longer “window of vulnerability” to growth interruptions during development (Guatelli-Steinberg et al., 2012; Vrijenhoek, 2004). However, researchers have not yet
demonstrated a direct link between seasonal lows in fruit availability and defect formation in great apes.

Though Virunga mountain gorillas may not be subject to the same nutritional stressors as more frugivorous great apes, a variety of disease-related or psychosocial factors may alternatively influence LEH expression in this population. Mortality rates and respiratory infection, a leading cause of death in Virunga mountain gorillas, were reported to correlate with rainfall between 1967 and 1991 (Watts, 1998).

Thermoregulatory stress, which is expected to vary with rainfall, could also influence disease susceptibility (Watts, 1998), and thus LEH defect formation. Moisture cycles influence parasitism, which has been shown to initiate hypoplastic defects in developing mammalian teeth (Suckling et al., 1986). Schaller (1963) found probable hookworm eggs in over 50% of mountain gorilla fecal samples, and more recent work suggests high parasite loads in this population (Hassell et al., 2013). Social interaction and disturbance, as revealed by endocrine data from other species, are other potential sources of stress (Bahr et al., 1998; Muller & Wrangham, 2004). Human disturbance and changes in social dynamics have been found to correspond with accentuated lines in primate dentitions (Bowman, 1991; Dirks et al., 2002; Dirks et al., 2010; McFarlin et al., 2014; Schwartz et al., 2006). Schwartz et al. (2006) additionally found accentuated lines to correspond with injuries and hospital visits in a captive western lowland gorilla. Smith and Boesch (2015) found a possible social correlate between a mother’s aggressive behavior and a prominent hypoplastic defect in a wild chimpanzee, but it was impossible to decouple this from a co-occurring respiratory infection. In mountain gorillas, encounters between neighboring groups and solitary males involve the risk of losing females, lethal injury, and infanticide.
These interactions have become six times more common in recent years due to the near doubling of the Virunga mountain gorilla population following decades of habitat loss to humans (Gray et al., 2013; Caillaud et al., 2014). The Virunga population dwindled to an estimated 254 individuals by 1981, but it has recovered as a result of targeted conservation measures including veterinary care and close monitoring of individual gorillas (Robbins et al., 2011). While these measures have benefitted the size of the population, it is less clear what effect they may have on LEH expression through time. Higher rates of poaching during the early years of monitoring could be expected to influence defect formation, but so could veterinary interventions of recent years.

**Identification and characterization of LEH defects**

There are a number of microscopic and macroscopic approaches used to identify and characterize LEH defects, but there is no methodological consensus, and thresholds for diagnosis vary considerably from study to study (Hassett, 2012, 2014). The traditional procedure is to use the naked eye or a hand lens to find distinctive “grooves” or “lines” on the enamel surface (Buikstra & Ubelaker, 1994). LEH defect severity is commonly assessed either macroscopically or microscopically based on the width and/or depth of a given defect. It is assumed to reflect the duration and/or magnitude of the growth disruption (Duray, 1996; Guatelli-Steinberg et al., 2003; Guatelli-Steinberg, 2004; Skinner & Pruetz., 2012; Marchewka et al., 2014; Bocaige & Hillson, 2016; Skinner & Skinner, 2017; Henriquez et al., 2017). Researchers have used imaging techniques to measure the depth of individual defects using engineer’s measuring microscopes,
microCT (computed tomography) and digital microscopy in modern humans and chimpanzees (Skinner and Pruetz, 2012; Marchewka et al., 2014; Henriquez & Oxenham, 2017).

While most studies of modern humans and nonhuman primates define defects as macroscopic circumferential depressions on the crown surface (e.g. Goodman & Rose, 1990; Skinner & Hopwood, 2004; Guatelli-Steinberg et al., 2012; Smith & Boesch, 2015; Skinner & Skinner, 2017), some researchers define defects as having wider spacing between consecutive perikymata (e.g. Hillson & Bond 1997; King et al., 2002; Guatelli-Steinberg, 2003; King et al., 2005; Hassett, 2012; Temple et al., 2013; Bocaège & Hillson, 2016). Microscopic studies of perikymata spacing use focus variation microscopy, scanning electron microscopy (SEM), or engineer’s measuring microscopes to aid in the visualization of the outer enamel surface and quantitatively identify defects in modern human teeth (King et al., 2002, 2005; Bocaège et al. 2010; Temple et al., 2013; Bocaège & Hillson, 2016). While these methodological advances increase the replicability of defect identification among observers, the threshold for defect diagnosis remains arbitrarily defined. Very minor variations in perikymata morphology (such as changes in spacing) around the tooth crown are more easily flagged as defects using these quantitative approaches (Hassett, 2014; Hillson, 2014). Defect depth, rather than variation in perikymata spacing, might provide a more robust signal of defect presence when measured at sufficient resolution. Skinner & Skinner (2017) used optical profilometry to measure defect depths in Pongo abelii and Pongo pygmaeus canines. The imaging technique used in their study renders high resolution information about the outer enamel surface. Analyses of defect depth using optical profilometry are currently limited.
to those grooves that are macroscopically visible; combining this approach with detailed analyses of perikymata spacing might allow for the reliable identification of more minor defects, including accentuated perikymata. In this way, optical profilometry offers the promise of defining a quantitative threshold for defect diagnosis through 3D surface analysis of both perikymata spacing and depth in well-preserved samples, though this is beyond the scope of the current research.

**Biological significance of defect depth**

It is widely accepted that the timing (Reid & Dean, 2000; Skinner & Hopwood, 2004) and duration (Guatelli-Steinberg, 2004; Temple et al., 2013) of the disruption to enamel secretion can be estimated for specimens with well-preserved perikymata. In terms of defect severity, early work in modern humans suggested that narrow and wide grooves may reflect infection and malnutrition, respectively (Sarnat & Schour, 1941). The biological significance of defect depth is less clear, although it has been hypothesized to relate to the intensity of the stressor that interrupted growth (Skinner & Hopwood, 2004; Skinner & Skinner, 2017). In an experimental study of miniature pigs, increased doses of fluoride were associated with “larger” (i.e., deeper and wider) hypoplastic defects in molar teeth (Kierdorf et al., 2004), mirroring the same pattern previously found in sheep (Suckling & Thurley, 1984). However, experimental and/or observational studies of a similar nature in modern humans and nonhuman primates are lacking.

Enamel geometry might also influence the morphology of LEH defects on the outer enamel surface. The angle at which the striae of Retzius approach the outer enamel
surface is related to the rate of enamel extension and secretion (Shellis, 1984; Hillson & Bond, 1997; Guatelli-Steinberg et al., 2012; Guatelli-Steinberg et al., 2017; Kierdorf et al., 2015). In modern humans, crown regions with more acute striae angles as they approach the outer enamel surface are associated with wider and shallower surface perikymata and LEH defects. This makes perikymata and furrow-form defects harder to reliably identify using traditional methods (Hillson & Bond, 1997; Hassett, 2014). Preliminary histological data from McFarlin et al. (2014) suggest that mountain gorillas may develop their molars more quickly than wild western lowland gorillas (Beynon et al., 1991; Kelley & Schwartz, 2010). If borne out with continued study, this would be consistent with the hypothesis that mountain gorilla lateral enamel is characterized by more acute striae angles, and therefore more shallow surface features, than other great apes (Guatelli-Steinberg et al., 2012). Shallow defects are presumably more difficult to identify with qualitative techniques, necessitating the quantitative analysis presented here.

**Specific Aims**

We address two major aims. First, we use high-resolution optical profilometry of the outer enamel surface to quantify “normal” perikymata depths and LEH defect depths, and test whether there are significant sex and species differences among Virunga mountain gorillas and other great ape taxa. If differences in enamel growth influence variation in perikymata and LEH defect depth among great apes (Guatelli-Steinberg et al., 2012), we expect to find sex and species differences in accordance with what is known about variation in canine crown development. As Guatelli-Steinberg et al. (2012)
found no clear differences in striae angles among the canines of a small sample of *Gorilla gorilla*, *Pan troglodytes*, and *Pongo* sp., we expect to find no differences in defect depth among these taxa. However, if canine crown development is accelerated in Virunga mountain gorillas as is reported for other developmental life history characteristics (Galbany et al., 2017; McFarlin et al., 2013; Stoinski et al., 2013), we expect this to be reflected in significantly shallower LEH defects compared to *Gorilla gorilla*, *Pan troglodytes*, and *Pongo* sp. Great ape canines are sexually dimorphic, and this can be primarily attributed to differences in the duration of growth between the sexes (Schwartz & Dean, 2001). However, intraspecific differences in enamel thickness, as well as extension rates in lateral enamel, have also been noted (Schwartz & Dean, 2001; Schwartz et al., 2001). We therefore hypothesize that females have deeper defects than males, which might reflect slower extension rates, thicker enamel, and more obtuse striae of Retzius angles in female canines. We expect this difference to be most pronounced in *Gorilla* and *Pongo*, the more dimorphic genera, and less pronounced in *Pan*.

Second, we test whether there is a difference in LEH defect depth in Virunga mountain gorillas through time by comparing specimens recovered by Dian Fossey between 1968 and 1983 with those recovered in more recent decades. The earlier period was marked by increased poaching and human encroachment, while in the later period, the population recovered following the intensification of targeted conservation measures (Robbins et al., 2011).
MATERIALS AND METHODS

Sample

Anterior teeth are usually used for studies of LEH because more of their tooth crowns are occupied by lateral enamel, where defects are visible as grooves on the surface, compared to posterior teeth (Hillson & Bond, 1997). In great apes, canines are favored because their formation spans the majority of dental development and because they are subject to less occlusal wear than other teeth (Guatelli-Steinberg, 1998; Guatelli-Steinberg et al., 2012; Newell, 1998; Skinner, 1986). The sample utilized in this study is described in Table 1. The Virunga mountain gorilla (Gorilla beringei beringei) sample is primarily derived from the Mountain Gorilla Skeletal Project (MGSP) collection in Rwanda (McFarlin et al., 2009). Over 50% of the specimens in this collection have associated life history data as a result of long-term research and veterinary monitoring in Rwanda’s Volcanoes National Park by Dian Fossey Gorilla Fund International’s Karisoke Research Center (initiated in 1967), the Mountain Gorilla Veterinary Project, and the Rwanda Development Board’s Department of Tourism and Conservation. The MGSP collection includes specimens collected after 1996, and the birth years of known individuals included in this study range from 1968-2002. The remaining Virunga mountain gorilla specimens are from the Smithsonian’s National Museum of Natural History (USNM). These specimens were collected by Dian Fossey and colleagues during the late 1960s and 1970s and donated to the USNM between 1968 and 1983. Of the MGSP sample of 24 mountain gorillas, 8 have unknown or uncertain birth years, and only two individuals have the potential to overlap in the timing of their dental development with those from the USNM collection. The majority of the sample
representing *Gorilla gorilla gorilla*, *Pan troglodytes*, and *Pongo* sp., come from the USNM and were collected in the late nineteenth and early twentieth centuries. Additional specimens from these taxa were sourced from the Dirks-Dean-Reid Newcastle Collection, now housed at GW. Most of the sample represents animals that lived in the wild; five animals lived in captivity at the Smithsonian’s National Zoological Park (NZP), all of which were wild-born. Fourteen specimens are of unknown provenience, but based on their early museum accession dates, they were likely wild-shot.

**Table 1. Composition of replica sample**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sex</th>
<th>Source</th>
<th>Origin</th>
<th>N specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gorilla beringei</em></td>
<td>F</td>
<td>MGSP,</td>
<td>Rwanda</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>USNM</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td><em>Gorilla gorilla</em></td>
<td>F</td>
<td>USNM,</td>
<td>Cameroon, Congo, Equatorial Guinea, Gabon; NZP;</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>DDRN</td>
<td>unknown</td>
<td>12</td>
</tr>
<tr>
<td><em>Pan troglodytes</em></td>
<td>F</td>
<td>USNM,</td>
<td>Cameroon, Equatorial Guinea, Gabon, Liberia,</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>DDRN</td>
<td>Uganda; NZP; unknown</td>
<td>5</td>
</tr>
<tr>
<td><em>Pongo</em> sp.</td>
<td>F</td>
<td>USNM,</td>
<td>Sumatra and Borneo, Indonesia; NZP; unknown</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>DDRN</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>89</strong></td>
</tr>
</tbody>
</table>

MSGP: Mountain Gorilla Skeletal Project, Musanze, Rwanda  
USNM: Smithsonian’s National Museum of Natural History, Washington, D.C.  
DDRN: Dirks-Dean-Reid Newcastle Collection, The George Washington University, Washington D.C.

Sample sizes listed in Table 1 include the total number of mandibular canines analyzed. One canine was analyzed per individual. Due to variation in surface
preservation and defect abundance, between 1-10 (median=3) defects were measured per canine. Similarly, between 1-7 perikymata (median=3) were analyzed per canine. Right or left canines were selected based on crown completion and surface preservation. Only teeth with completely formed crowns and preserved midcrown perikymata were included, as dental calculus commonly obscures the cervical fifth, and use wear abrades the cuspal fifth, of great ape canines. The midcrown region of great ape canines is understood to be fairly consistent in its microanatomy and growth parameters. This is supported by a recent study that found LEH defects not to vary in depth across the canine crown (Skinner & Skinner, 2017). For these reasons, we analyzed LEH defects located in the middle 3/5 of the crown, as determined by measuring total crown height using plastic calipers.

**Specimen preparation**

In preparation for making high-resolution dental replicas, original teeth were gently brushed with a soft toothbrush, and diluted ethanol (70%) was swabbed across the tooth surface. Impressions were made using Coltene’s President Jet Light Body Dental Impression Material (Coltène-Whaledent). Positive replicas were made from these impressions using Loctite Hysol E-60NC epoxy.

**LEH defect identification methods**

First, defects were qualitatively identified. Like Skinner and Skinner (2017), we chose to focus on macroscopically visible LEH defects. While furrow-form defects are most common, it is likely that plane-form defects were also included in the sample as it is
often difficult to discriminate between the two, particularly in worn specimens (Kierdorf et al., 2012; Skinner & Skinner, 2017). Grooves with clear pits (i.e., pit-form defects) were excluded.

Dental replicas were viewed under oblique light using a 14x hand lens. Diagrams were drawn to note the locations of all LEH defects on each replica prior to imaging. Antimeres (i.e., the opposite side) were visually checked for matching LEH defects as this suggests that the disruption to enamel growth was systemic (Hillson & Bond, 1997), except when the antimere was lost or damaged (in 15% of the sample). In preparation for scanning, each replica was affixed on the microscope stage using clay, orthogonally in relation to the lens. LEH defects were imaged using a Sensofar PLu Neox white-light optical profiler (Sensofar, Spain), housed at the Paleoanthropology Imaging Laboratory, Eberhard Karls Universität Tübingen. All LEH defects included in this study were (1) clearly identifiable on both antimeres (except when missing or broken), 2) not obscured by calculus or wear, and 3) within the middle 3/5 of the crown. The areas of normal growth before the disruption to enamel secretion, and the resumption of normal growth following the disturbance, were also scanned. We used a 20x objective with a numerical aperture of 0.45 and vertical resolution of <20 nm. Auto-focus quick scanning of the surface was first used to confirm that the surface was relatively flat within a field of view of 4.07 x 3.49 mm² (Fig. 3B). Due to the curvature of the labial aspect of the canines in both the longitudinal and latitudinal directions, only the surface surrounding the target defect could be leveled without manipulation of the raw point cloud data. Therefore, for this study, independent surface scans were collected for each defect along a transect in the midline of the labial aspect of canines, and no data manipulation was performed.
Between two and five adjoining scans were automatically stitched together using Sensoscan software to sample the surface area surrounding each LEH defect at 8.14-20.35 x 3.49 mm² (Fig. 3B). This scanning process was repeated for all LEH defects (N=255) in the replica sample. Regions with well-preserved perikymata were also scanned following the same protocol to characterize the “normal” morphology of growth increments on the outer enamel surface (N=75). Perikymata have been shown to vary in their spacing along the long axis of the crown in humans (e.g., Bocaeghe & Hillson, 2016), most notably near the cusp tip and the cervix, i.e., the areas that are avoided in the current study.

**LEH defect and perikymata depth measurements**

Each 3D scan was analyzed using NIH-developed freeware ImageJ64. First, plugin “XYZ2DEM Importer” was used to convert the three-dimensional point clouds into digital elevation models (DEMs; Fig. 3C). Next, minor noise was eliminated using the “remove outliers” command with a radius of 10 pixels and a threshold of 15 pixels, which was the lowest threshold that effectively removed noise caused by oils and other debris. The straight line tool was used to draw a horizontal transect through the length of the scan (i.e., orthogonal to the defect of interest). A 2D profile of extracted points was then plotted and visualized within ImageJ. The “Find Peaks” plugin was used to identify the coordinates of peaks and valleys within the plot (Fig. 3D). To calculate the maximum depth, the z-coordinate of the deepest point was subtracted from that of the occlusal shoulder of the defect. The occlusal shoulder was selected to calculate depth because the
cervical shoulder is understood to reflect the recovery period (Hillson & Bond, 1997). This process was repeated for all LEH defects in the sample (N=255).

Perikymata depths were also measured in the midcrown region of canines. Only perikymata not associated with LEH defects were measured, defined as being at least 0.5mm away from the nearest defect. The process was the same as that described above for LEH defects. The relative influence of dental wear on perikymata depth is likely greater than in defects because they are absolutely shallower, so we were careful to select lightly worn or completely unworn teeth.

To assess the replicability of the initial identification of LEH defects, KM rescored a subsample of replicas (one female and one male from each taxon; N=8) one to two years after the initial scoring. KM identified the same number of defects per canine (100% intraobserver reliability). The replicability of depth measurements was assessed by measuring: 1) multiple parallel transects from the same scan (N=10 scans); 2) the same LEH defect on the antimere (N=6 defects); and 3) repeated scans of the same defect collected one year apart (N=2 defects). These measurements were collected six months to one year following initial data collection. In all cases, the difference in defect depth was minimal, and ranged from 0.0-1.6 µm in absolute depth, and from 0-6.2% difference (mean=2.5%). We also compared perikymata depth values collected from multiple parallel transects of the same scan (N=3 scans). The difference ranged from 0.1 to 0.2 µm in absolute depth, and from 0.2-7% difference (mean 3.9%).
Figure 3. A: Z-stacked photo of an epoxy replica made from female mountain gorilla canine USNM 545031. B: Photomontage rendered by the quick scan function of the Sensofar profiler. The boxes represent the area targeted for scanning. C: Resulting digital elevation model (DEM) with an LEH defect marked by the arrow. D: 2D profile extracted from the midline of the x-axis of the above DEM. The occlusal shoulder and maximum depth are marked with stars. The shading represents defect depth.
Statistical analyses

We first compared the distribution of LEH defect depths to those collected from regular perikymata. We then used separate linear mixed models (LMMs) to examine perikymata and LEH defect depths by species and sex, with the interaction of species and sex included as a fixed explanatory variable. The interaction between species and sex was not significant in either model, so it was dropped from the final models. As the number of perikymata analyzed per specimen ranged from 1-7, and the number of LEH defects ranged from 1-10, we included specimen ID as a random effect. Perikymata and defect depth values were natural log-transformed prior to analysis because they were right skewed. Assumptions of normality and homogeneity of variance were visually assessed using residual diagnostic plots. Although species designations were available for many individuals in the *Pongo* sp. sample, the results did not differ whether they were grouped by species or genus, so we chose to maintain the larger sample size of the latter. Differences between multiple levels of significant fixed effects were examined using Tukey’s post hoc tests.

A Cook’s distance test was used to assess the influence of outliers on the LEH defect depth model. Bollen et al. (1990) suggest that the equation $4/n$, or number of observations, should be used to determine the cut-off point for determining highly influential values. The three outliers that fell above that threshold were removed before the model was rerun. The results of both models are presented here.

Finally, within mountain gorillas, we compared LEH depths among naturally accumulated skeletons collected by Dian Fossey (Smithsonian’s USNM; 1968-1983) and
those collected recently (Mountain Gorilla Skeletal Project, Rwanda; post-1996) using a Wilcoxon rank sum test.

All statistical analyses were performed in R (Version 0.99.903, R Core Development Team, 2016) using package nlme for the mixed models (Pinheiro et al., 2017).

RESULTS

Perikymata depth

The distribution of perikymata depths does not overlap with that of LEH defect depths across all taxa (Fig. 4). Raw perikymata depths are shown in Table 2. There is a significant relationship between perikymata depths and species overall (F(3,17)=3.85, p=0.029), and post hoc tests reveal that Pongo sp. has significantly deeper perikymata than Pan troglodytes (p=0.016) (Fig. 5). No significant differences were found among the Gorilla taxa. Females were not found to have deeper perikymata than males in the combined sample (F(1,17)=0.64, p=0.436), however Figure 5 shows depths divided by species and sex to aid in visualization.
Figure 4. Logged perikymata (N=75) and LEH defect depths (N=255) for all taxa combined.
Figure 5. Perikymata depths by taxon and sex. Significance differences are indicated based on Tukey’s post hoc tests (*p<0.05). Boxes show interquartile range (IQR - 1\textsuperscript{st} quartile, median, and 3\textsuperscript{rd} quartile; whiskers show 1.5 x IQR; outliers extend beyond the range of the boxplot).

Table 2. Perikymata depths by species and sex

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sex</th>
<th>N perikymata</th>
<th>Median (µm)</th>
<th>Range (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. g. beringei</em> beringei</td>
<td>F</td>
<td>11</td>
<td>2.6</td>
<td>2.2-4.0</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>14</td>
<td>1.8</td>
<td>1.3-2.8</td>
</tr>
<tr>
<td><em>G. g. gorilla</em> gorilla</td>
<td>F</td>
<td>6</td>
<td>2.5</td>
<td>1.9-3.3</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>12</td>
<td>2.2</td>
<td>1.4-3.0</td>
</tr>
<tr>
<td><em>Pan troglodytes</em></td>
<td>F</td>
<td>6</td>
<td>1.2</td>
<td>1.0-1.8</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5</td>
<td>2.0</td>
<td>1.7-2.8</td>
</tr>
<tr>
<td><em>Pongo sp.</em></td>
<td>F</td>
<td>13</td>
<td>2.6</td>
<td>1.5-5.0</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>8</td>
<td>2.5</td>
<td>1.8-3.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>75</td>
<td>2.2</td>
<td>1.0-5.0</td>
</tr>
</tbody>
</table>
LEH defect depth

Mountain gorillas (*Gorilla beringei beringei*) exhibit significantly shallower LEH defects than *G. gorilla gorilla*, *Pan troglodytes*, and *Pongo* sp. (F(3,84)=19.63, p<0.0001) (Table 3, Fig. 6).

![Figure 6. LEH defect depth by taxon. Significance differences are indicated based on Tukey’s post hoc tests (***p<0.001). Boxes show interquartile range (IQR - 1st quartile, median, and 3rd quartile; whiskers show 1.5 x IQR; outliers extend beyond the range of the boxplot).](image-url)
Table 3. LEH defect depths by species and sex

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sex</th>
<th>N defects</th>
<th>Median (µm)</th>
<th>Range (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gorilla beringei beringei</em></td>
<td>F</td>
<td>33</td>
<td>23.6</td>
<td>10.5-44.7</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>63</td>
<td>15.6</td>
<td>6.2-45.8</td>
</tr>
<tr>
<td><em>Gorilla gorilla gorilla</em></td>
<td>F</td>
<td>17</td>
<td>47.2</td>
<td>12.4-276.0</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>33</td>
<td>35.6</td>
<td>10.8-135.1</td>
</tr>
<tr>
<td><em>Pan troglodytes</em></td>
<td>F</td>
<td>35</td>
<td>38.0</td>
<td>15.1-97.5</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>18</td>
<td>43.4</td>
<td>8.1-211.8</td>
</tr>
<tr>
<td><em>Pongo sp.</em></td>
<td>F</td>
<td>33</td>
<td>55.3</td>
<td>10.4-109.3</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>23</td>
<td>32.5</td>
<td>12.1-84.5</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td><strong>255</strong></td>
<td><strong>28.4</strong></td>
<td><strong>6.2-276.0</strong></td>
</tr>
</tbody>
</table>

Females exhibit significantly deeper defects than males in the combined sample (F(1,84)=6.07, p=0.016). There was no interaction between species and sex, but we include Figure 7 to allow visualization of defect depths between males and females by species. Within taxa, *Pan troglodytes* does not seem to follow this pattern; males have deeper defects on average than females, although sample sizes were small and did not provide sufficient power to test this statistically.
The deepest defects belong to a female western gorilla (Haloko, 276.0 µm) and a male chimpanzee (212.0 µm), both of whom are known or suspected to have been wild-born and captive-raised, respectively (Fig. 8).

The Cook’s distance test determined that three outliers were highly influential on the defect depth model results. Of the three outliers, two were LEH defects from female western lowland gorillas that are particularly deep (276.0 and 149.6 µm), and one is a shallow defect from a female orangutan (11.3 µm). The Cook’s distance test did not flag any male outliers as being highly influential on the model results. The three outliers were removed, and the LMM was rerun. The results remained largely the same, with mountain
gorillas having significantly shallower defects than all other taxa (F(3,82)=20.24, p<0.001), and females having significantly deeper defects than males (F(1,82)=4.55, p=0.036).

Figure 8. Left: Haloko’s (USNM 586541) permanent mandibular canine replica. Right: male chimpanzee (USNM 599172) permanent mandibular canine replica with major repeated defects.

In the comparison of LEH defect depth through time in the Virunga population of mountain gorillas, those specimens collected by Fossey (N=17 defects) tend to have deeper defects than those that lived more recently (N=79 defects; Wilcoxon rank sum test, p=0.052; Fig. 9). However, it should be noted that the sample sizes are uneven, and this result should be interpreted with appropriate caution.
DISCUSSION

Species and sex differences in perikymata depth

Perikymata depths do not overlap with the depth of macroscopically-visible LEH defects across all taxa. This suggests that the qualitatively-identified defects characterized in the current study represent true deficiencies in enamel thickness, and thus reflect disruptions to enamel secretion. *Pongo* sp. was found to have deeper perikymata than
*Pan troglodytes*. *Pongo* sp. has the thickest enamel and among the slowest canine growth of all taxa included in this study (Schwartz & Dean, 2001; Schwartz et al., 2001). Given our predictions about the relationship between slow growth and obtuse striae angles, it is not surprising that *Pongo* has deeper perikymata. It is surprising, however, that mountain gorillas were not found to have the shallowest perikymata, given their shallow LEH defect depths. Also contrary to expectations, females were not found to have significantly deeper perikymata across taxa. As no other perikymata depths are available for great apes, future studies incorporating larger sample sizes, especially for *Pan*, are needed to better characterize inter- and intraspecies variation in perikymata depth.

**Species differences in LEH defect depth**

Previous studies found that 5-11% of mountain gorilla canines had at least one LEH defect, compared to >75% of other great ape canines examined (Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012). Explanations proposed by Guatelli-Steinberg et al. (2012) for the low frequency of LEH in mountain gorillas include: 1) folivory buffers them from seasonal nutritional stress; and 2) shallow striae of Retzius angles lead to the formation of shallow LEH defects, which might be difficult to identify qualitatively. Dental wear, including abrasion, has also been raised as possibly confounding studies of LEH in folivorous species (Newell et al., 2006; Guatelli-Steinberg et al., 2012). However, a recent study has demonstrated that mountain gorillas have less age-related occlusal wear compared to the more frugivorous western lowland gorillas (Galbany et al., 2016), making dental wear an unlikely contributor to their shallow defect morphology. As predicted, mountain gorilla defects are significantly shallower than those
in other great ape species, including western lowland gorillas. We suggest that 1) differences in defect depth, 2) variation in interobserver diagnosis thresholds, and 3) limited access to adequate sample sizes may have led to the previous underestimation of LEH prevalence in mountain gorillas.

This finding has implications for LEH analyses in other primate taxa that might also exhibit shallow defect morphology. For example, most monkey species have been characterized as having lower LEH prevalence than great apes (Colyer, 1936; Moggi-Cecchi & Crovella, 1991; Guatelli-Steinberg, 1998; Newell, 1998; Guatelli-Steinberg, 2000; Guatelli-Steinberg, 2001; Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012), but it is possible that they too exhibit shallow defect morphology that is more challenging to identify qualitatively (Guatelli-Steinberg et al., 2012). Indeed, it has been noted that their striae angles at the outer enamel surface are often more acute than those of comparable crown regions in great apes (Guatelli-Steinberg et al., 2012). In enamel, slower growth rates create more obtuse striae angles at the outer enamel surface, and the formation of deeper LEH defects (Guatelli-Steinberg et al., 2012). From a dietary perspective, those taxa with deeper defects share a greater reliance on fruit compared to folivorous mountain gorillas (Delgado & van Schaik, 2000; Rogers et al., 2004; Stanford et al., 2003; Watts, 1984). This fits well with the Ecological Risk Aversion Hypothesis which predicts that natural selection may favor slower growth rates as a strategy to reduce starvation risk (Janson & van Schaik 1993). Prolonged development may be a consequence of reduced growth rates, and provide a longer window in which disruptions to growth can occur (Vrijenhoek, 2004). Guatelli-Steinberg et al. (2012) found no significant differences in striae angles between *Gorilla gorilla*, *Pan troglodytes*, and
Pongo sp., which corresponds to the lack of differences in defect depth among these taxa reported in the current study. Future studies should test whether mountain gorillas are characterized by shallower striae angles compared to other great apes. If so, this variable might provide important context for the interpretation of LEH defects in other primate taxa.

**Sex differences in LEH defect depth**

Females have significantly deeper defects than males in the pooled sample. We hypothesize that this relates to more obtuse striae angles as a result of differences in enamel extension rates, particularly in more sexually dimorphic taxa. There is little reason to suspect that behavioral differences contribute to this sex difference in defect depth as enamel forms before the development of pronounced male-male competition in these taxa. A number of studies have examined the extent to which differences in the ontogeny of canine growth contributes to their sexual dimorphism in great apes (Anemone et al., 1991, 1996; Beynon et al., 1991; Kuykendall, 1996; Reid et al., 1998; Schwartz & Dean, 2001), though mountain gorillas remain poorly studied in this respect. Schwartz and Dean (2001) found major intraspecific differences in the duration of canine growth between *Gorilla gorilla*, *Pan*, and *Pongo*, with males growing their canines for a longer period, especially in the more dimorphic *Gorilla gorilla* and *Pongo*. Differences in canine growth rates are most pronounced between taxa, with *Gorilla* and *Pongo* having faster extension rates than *Pan* (Schwartz & Dean, 2001). However, sex differences in lateral extension rates exist, and contribute to the documented variation in canine crown height, particularly in *Gorilla* and *Pongo* (Schwartz & Dean, 2001; Schwartz et al.,
These features are expected to correspond to more obtuse striae angles in the lateral enamel of these taxa, which might explain the significantly deeper LEH defects in females in the current study. However, striae are also noted to “diverge unpredictably as they course through… [the enamel]… from the EDJ towards the enamel surface” (Schwartz & Dean, 2001:274). Further study is needed to assess the relationship between enamel growth parameters and striae angles at the outer enamel surface, where LEH defects occur.

In the current study, *Pan troglodytes* does not seem to follow the pattern of females having deeper defects than males. This might relate to the relatively small sample size, or the fact that 30% of the male *Pan* sample is derived from an individual with many deep defects (Fig. 7). Alternatively, *Pan troglodytes* is the least sexually dimorphic in canine height and exhibits the least intraspecific variation in enamel growth parameters of these taxa (Schwartz & Dean, 2001; Schwartz et al., 2001), so any differences in defect or perikymata depth might be less pronounced.

**Quantitative thresholds for diagnosing LEH defects**

This study does not specify a quantitative threshold for diagnosing LEH defects. However, all qualitatively-identified LEH defect depths fall completely outside the distribution of perikymata depths (Fig. 4). New research addresses the problem of defining normal vs. abnormal surface morphology on the basis of perikymata spacing via measuring microscope, SEM, and focus variation microscopy (Bocaege et al. 2010; Bocaege & Hillson, 2016; King et al., 2002, 2005), and on the basis of depth using microCT scans (Marchewka et al., 2014), digital microscopy (Skinner and Pruett, 2012),
or a measuring microscope (Henriquez & Oxenham, 2017). A challenge with identifying wider-than-average perikymata is that even slight deviations from “normal” spacing are flagged as potential LEH defects (Bocaège & Hillson, 2016; Hassett 2014; Hillson, 2014). Moreover, the precise quantification of perikymata spacing requires near-perfect surface preservation, and sample size will be further limited to those specimens with no or very minimal wear. O’Hara (2017) recently demonstrated that standard perikymata profiles can be used to estimate the number of perikymata between defects with high reliability, increasing the utility of microscopic studies of specimens with incomplete surface preservation. The technique introduced by Henriquez & Oxenham (2017) also allows for the inclusion of specimens with discontinuous perikymata preservation. However, the threshold for defect diagnosis remains arbitrary, and the vertical resolution of measuring microscopes (>5-10 µm) is not sufficient to capture the morphology of shallow defects. A limitation of using microCT scans to measure LEH defects is that images are typically generated with a voxel size of 10-30 µm. As demonstrated here, many LEH defects, particularly in mountain gorillas, fall within or even below that threshold, so a microCT-based method may result in an underestimation of LEH prevalence.

Recent work by Skinner and Skinner (2017) measured LEH defect depth using an optical profiler (µ surf Mobile Plus) in Sumatran and Bornean orangutan canines. Depth measurements reported in the current study are deeper than those reported by Skinner and Skinner (2017) for Pongo sp., with our median depth measuring at 34.8 µm compared to their 18.6 µm. This discrepancy might reflect differences in our data collection techniques or a true difference in defect depth between the study populations. The
reported resolution of the two microscopes is comparable, so it might relate to differences in defect selection and measurement. It is possible that in the current study, only major defects were measured, whereas in Skinner and Skinner (2017), more minor furrows were included. While the landmarks that define LEH defects were the same between the two studies, the trigonometry performed by Skinner and Skinner (2017) might contribute to the differences in depth, as our protocol did not include any data manipulation of raw data. Skinner et al. (2012) reported higher defect depths in Pan canines based on micro-CT scans, with repetitive LEH defect depths ranging from 19.3-35.3 µm. They reported coronal waisting depths ranging 80-300 µm, which are defects spanning a larger portion of the canine crown as they are related to a slight decrement in dentinal crown volume. The depths presented here were highly consistent based on repeated measurements and error tests, including measurements taken from scans that were collected years apart. Direct comparisons between studies would be facilitated by standardizing defect selection criteria and analysis protocols.

Compared to microscopic approaches that measure deviations in perikymata spacing down the tooth crown, the method employed here allows for the inclusion of specimens with imperfect perikymata preservation and thus results in larger sample sizes. It assesses LEH defect morphology in terms of its original definition – a localized reduction in enamel thickness, in the form of grooves that run parallel to perikymata (Berten, 1895). We found no overlap between perikymata depths and LEH defect depths, but the question of where to place the threshold for LEH diagnosis in great apes still remains as defects appear on a continuum.
The significance of LEH defect depth

Skinner and Skinner (2017) found significant differences in LEH defect depth between Sumatran and Bornean orangutans, and suggested that the deeper repeated defects in Sumatran orangutans reflect more severe stress. This result is contrary to the authors’ prediction that Bornean orangutans would have deeper defects, as Sumatran orangutans live in a more productive habitat and thus have better access to food resources (Delgado et al., 2000; Knott, 1998). Unfortunately, we did not have an appropriate sample size to replicate this comparison in Pongo. However, the two deepest LEH defects belong to wild-born animals that are known or were likely to have been transferred to captivity. The deepest defect (276.0 µm) is consistent with the developmental timing of a female gorilla’s capture from the wild between two and three years of age (pers. comm. with Stephanie Eller, registrar). Named Haloko, she was born in approximately 1967 and came to the Philadelphia Zoo in 1970 (Fig. 8). The second deepest defect (212.0 µm) belongs to a male chimpanzee that was also wild-born and may have also lived in captivity (pers. comm. with Kristofer Helgen, former USNM curator) (Fig. 8). We do not have access to behavioral or health records with the resolution necessary to confirm whether the defects coincide with documented stressful events, nor do we have the capacity to histologically section their teeth to determine the absolute timing of these disruptions. However, the estimated timing of Haloko’s capture aligns with what information is available based on published estimates of western lowland gorilla canine crown formation (Schwartz & Dean, 2001). It is also worth noting that wild-born captive animals have a higher median defect depth of 47 µm compared to 37
µm for the rest of the sample, excluding mountain gorillas of which there are no captive representatives.

These findings underscore the importance of determining the extent to which the type or magnitude of an insult is reflected in defect depth. If defect depth is to be used as a proxy for stress experienced by extant or fossil primates without associated behavioral records, studies of defect etiology in individuals with well-documented life histories will be an important next step, as will ongoing analyses examining the influence of enamel thickness, striae angles, and enamel growth parameters on within- and among-species variation in LEH defect depth.

**LEH defect depth through time in Virunga mountain gorillas**

The trend towards deeper defects in Virunga mountain gorillas that were recovered by Dian Fossey compared to those that lived more recently mirrors a previous study that found a difference in brain size between these two collections (McFarlin et al., 2013). Given that we compared mountain gorilla defects in the same midcrown region, we effectively controlled for the potential effect of variation in striae angles on defect morphology among species and between sexes. This allows for changes in defect depth in the Virunga mountain gorilla population over time to be assessed more objectively than would be possible via prevalence data alone. As this relationship was not significant, and sample sizes were uneven, we can only speculate based on these findings; however, the smaller brain size and deeper defects of the earlier USNM-Fossey collection may point to more early life stress. Stress negatively effects brain development through the fetal hypothalamic-pituitary-adrenal axis (Lupien et al., 2009), and has been associated with
atrophy of the hippocampus (Sapolsky, 1996). This might relate to the high levels of habitat destruction and poaching that were associated with the population declines of the late 1960s-1970s (Robbins et al., 2011).

Conclusions and future directions

The inter- and intraspecific variation in LEH defect depths presented in the current study demonstrate the value of objective techniques for the identification and characterization of defect morphology. Defect width and depth have been used as proxies for the duration and intensity of the disruption to growth, respectively (e.g., Skinner & Skinner, 2017), but interspecific variation in enamel growth parameters likely influences defect morphology (Guatelli-Steinberg et al., 2012). While it was possible to assess defect depth through time in the Virunga mountain gorilla population, only by coupling individual life history records with dental histology will researchers be able to test whether deeper defects correspond to particular events.

The approach presented here combines the benefit of visualizing the outer enamel surface in 3D with the ability to extract precise information about localized reductions in enamel thickness. Classic techniques that measure surface profiles using a stylus can damage specimens and do not afford the control and precision of the 3D imaging methods used. Any optical profiler with submicron resolution can be used to collect comparable data. In contrast to detailed analyses of perikymata distribution, measuring defect depth allows the analysis of larger samples, and thus more easily allows for comparative analyses. Future work will be directed toward assessing the influence of population-level factors on defect formation in great apes, including altitude,
precipitation, range, and diet, in addition to analyses incorporating documented
individual life history records where possible. By understanding the mechanisms behind
LEH formation in a well-documented great ape population, researchers will be better
equipped to make inferences about the experiences of primate populations that lack
associated behavioral records, including those from fossil assemblages.

Ongoing histological work will also allow for the evaluation of underlying
microanatomical differences, including the influence of enamel thickness and striae
angles, on LEH defect expression in great apes. Enamel thickness likely constrains the
maximum depth of an LEH defect, and because canine enamel thickness differs among
species and sexes (Schwartz et al., 2001), the individual and combined influence of
microanatomical variables will need to be assessed to better understand the results
presented here. Analyses of perikymata spacing and depth could be combined to identify
more minor defects, like accentuated perikymata, using optical profilometry in the future.
As defect timing and duration become available for individual mountain gorillas via
histologic analyses, it will be possible to test whether they correspond with any observed
major life events (e.g., intergroup encounters, illness, or injury) recorded in associated
behavioral and veterinary records (McFarlin et al., 2014; Schwartz et al., 2006; Smith &
Boesch, 2015). These data hold out the prospect of improving our understanding of LEH
defect etiology in mountain gorillas and other great apes.
Chapter 3: Enamel growth variation corresponds to defect depth in great ape canines

ABSTRACT

Objective
To refine our understanding of what LEH defects may represent in terms of stress in non-human primates, it is necessary to understand how defect dimensions are influenced by intrinsic aspects of enamel growth. We previously documented inter- and intraspecific differences in LEH defect depth in great apes (McGrath et al., 2018), showing that mountain gorillas have shallower defects than other great apes, and females have deeper defects than males across taxa. Here, we assess the correspondence of differences in defect depth across species and between sexes to several intrinsic aspects of enamel growth: linear enamel thickness, enamel extension rates, and striae of Retzius angles.

Materials and Methods
The sample includes histological thin sections of great ape canines (N=41) from *Gorilla beringei beringei*, *Gorilla gorilla gorilla*, *Pan troglodytes*, and *Pongo* sp. Linear enamel thickness and the angle of intersection between striae of Retzius and the outer enamel surface were measured in the imbricational enamel. Enamel extension rates were calculated per deciles of enamel-dentine junction length.
Results

Mountain gorillas have thinner imbricational enamel than western lowland gorillas and orangutans, but not chimpanzees. Mountain gorillas also have faster enamel extension rates, and shallower striae angles, than the other taxa examined. Orangutan males exhibit higher periodicity (12 days) and longer canine crown formation times (10-11 years) than previously reported for extant great apes. In the combined-taxon sample, females have thicker imbricational enamel and slower extension rates than males. Females also exhibit more obtuse striae angles than males, except in the cuspal third of the crown.

Discussion

Canine enamel growth variation corresponds to documented inter- and intraspecific differences in LEH defect depth. Striae angles and enamel extension rates vary in a way that is consistent with defect depth differences among species. Female great apes exhibit thicker imbricational enamel, more obtuse striae angles, and slower enamel extension rates, and this is reflected in deeper LEH defects compared to males. Mountain gorillas have more acute striae angles and faster extension rates than other taxa, explaining their shallow LEH defect morphology and the underestimation of defect prevalence in previous studies. These results suggest that stressors of similar magnitude might produce defects of different depths in one species or sex vs. another, which has implications for our interpretations of stress histories in hominins with variable enamel growth patterns.
INTRODUCTION

Linear enamel hypoplasia (LEH) is known as a nonspecific indicator of physiological stress during dental development (Goodman & Rose, 1990). LEH defects occur on the outer enamel surface as localized reductions in enamel thickness, and as they are caused by a systemic stressor, they manifest in all concurrently forming teeth (Berten, 1895) (Fig. 2). Such defects are exceedingly common in great apes (e.g., Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg, 2012). Defect severity is hypothesized to reflect the magnitude of the stressor that temporarily disrupted growth and led to enamel defect formation (Skinner & Hopwood, 2004; Skinner & Skinner, 2017). Experimental studies in non-primate mammalian taxa demonstrate a link between higher doses of administered fluoride and deeper and wider surface defects (Kierdorf et al., 2004, Suckling & Thurley, 1984). However, comparable data on stress-related correlates of enamel depth variation do not exist in primate models.

Previous work indicates that variation in enamel growth patterns and geometry of internal growth increments may influence LEH defect depth (Hillson & Bond, 1997; Guatelli-Steinberg, 2004; Guatelli-Steinberg, 2008; Guatelli-Steinberg et al., 2012, 2017; McGrath et al., 2018). While stress severity may influence defect dimensions, the potential for variation in enamel growth patterns to shape and constrain defect dimensions complicates direct inference of stress severity from surface depths alone. This study was designed to assess the degree to which differences in canine enamel growth patterns predict LEH defect depth in great apes (Gorilla beringei beringei, Gorilla gorilla gorilla, Pan troglodytes, Pongo sp.). We measure growth variables that have been hypothesized to influence defect depth, including linear enamel thickness, enamel
extension rates, and the angle of intersection between striae of Retzius and the outer enamel surface. We test the relationships among these variables to assess how well they correspond to documented differences in LEH defect depths among species and sexes. If defect depth is correlated with enamel growth variables, researchers should be cautious in assuming that depth reflects stress severity alone.

**Canine development and LEH**

Teeth preserve a permanent record of their incremental growth in the form of short- and long-period incremental lines (Boyde, 1964). Short-period lines reflect daily fluctuations in enamel secretion. Termed cross-striations, they record the circadian rhythm of enamel-secreting cells or ameloblasts (Boyde, 1989) (Fig. 1). Long-period lines, or striae of Retzius (Retzius, 1837), record episodic disturbances in enamel formation that occur with a regular periodicity in all permanent teeth of an individual (Fitzgerald, 1998). Striae of Retzius periodicity ranges from 6-12 days in extant great apes (Schwartz et al., 2001; McGrath et al., *in prep*). The first striae are laid down over the cusp tip and do not reach the outer enamel surface (Beynon & Wood, 1987). Once maximum cusp height is attained, occupying roughly 5-20% of total crown height in ape canines (Schwartz & Dean, 2001), growth continues down the crown toward the cervix as ameloblasts differentiate along the enamel-dentine junction, or EDJ (Boyde, 1964; Shellis 1984) (Fig. 1). These layers are laid down in a tile-like manner in the lateral and cervical regions of the crown, or the imbricational enamel (Pickerill, 1913; Beynon & Dean, 1987). It is in the imbricational enamel that striae of Retzius reach the outer enamel surface and form perikymata (Preiswerk, 1895; Pickerill, 1913). LEH defects occur
among perikymata grooves, which allows for the calculation of the approximate developmental timing of growth disruptions (Reid & Dean, 2000).

The majority of research on developmental defects in great apes has focused on canines due to their long periods of growth and “windows of vulnerability” to stress (Vrijenhoek, 2004; Guatelli-Steinberg et al., 2012). Compared to postcanine teeth, a greater proportion of canine crowns is made up of imbricational enamel, where defects are visible on the enamel surface (Hillson & Bond, 1997). Permanent canines start to mineralize shortly after birth in great apes, and they, along with the third molars, are the last teeth to fully emerge into the mouth by around 11 years of age (Dean & Wood, 1981; Beynon et al., 1991; Schwartz & Dean, 2001; Reid et al., 1998). Early radiological studies did not report significant differences in the age at first mineralization, total period of crown formation, or age of gingival emergence in Pan, Gorilla, or Pongo (Dean & Wood, 1981). Later, Kuykendall (1996) found a difference in total crown formation time between the sexes in Pan, but no difference in dental emergence (Kuykendall et al., 1992). Schwartz and Dean (2001) provided the first study of the ontogeny of canine dimorphism in known-sex great ape samples. They found that within species, males achieve their tall canines primarily through extending the duration of crown formation compared to females. Differences between taxa can be explained by differences in both the duration and rate of growth (Schwartz & Dean, 2001), but it is not yet clear how enamel extension rates contribute to this variation. The current study provides the first histological data on canine development in Virunga mountain gorillas (Gorilla beringei beringei), a population that lives at an ecological extreme in terms of elevation, relies on an almost entirely folivorous diet, and demonstrates accelerated somatic growth and life
history compared to other great apes, including western lowland gorillas (Watts & Pusey, 1993; Taylor, 1997; Stoinski et al., 2013; Galbany et al., 2017).

**Linear enamel thickness**

The majority of enamel thickness research measures the volume of enamel relative to tooth size in postcanine teeth to address questions about diet and phylogenetic relationships among extinct and extant primates (e.g., Molnar & Gantt, 1977; Martin, 1985; Shellsis et al., 1998; Schwartz, 2000). Less research has focused on characterizing enamel thickness in anterior teeth, but it is an important variable to consider in this context because it places a constraint on the maximum potential depth of a surface defect (i.e., a defect cannot be deeper than the enamel thickness). It similarly shapes the gross morphology of the tooth crown (Beynon et al., 1991). Linear enamel thickness has been measured in the canines of a number of hominoid species, including western lowland gorillas, chimpanzees, and orangutans (Schwartz et al., 2001). Schwartz et al. (2001) found that females have significantly thicker enamel, particularly in the more sexually dimorphic *Gorilla* and *Pongo*, which they attributed to faster daily enamel secretion rates near the enamel-dentine junction. Interspecifically, they found that canines mirror postcanine teeth with *Pan* having thinner enamel than *Gorilla*, and *Gorilla* having thinner enamel than *Pongo*. These differences may relate to variation in daily secretion rates toward the outer enamel surface, especially in the cusp (Schwartz et al., 2001), as well as differences in the duration of the secretory period of ameloblasts in *Pongo* compared to the other taxa (Beynon et al., 1991). Enamel thickness values have not been reported for
mountain gorillas, but as dedicated folivores, they are expected to exhibit thin enamel, and the same sex differences reported for other sexually dimorphic great apes.

**Enamel extension rates**

Enamel extension rates refers to the rate at which ameloblasts differentiate at the enamel-dentine junction or EDJ (Shellis, 1984). Boyde (1964) first described the relationship between enamel extension rates and the angle of striae of Retzius in relation to the EDJ. Shellis (1984) developed a method for estimating enamel extension rates based on Boyde’s (1964) model of enamel growth by calculating the distance over which ameloblasts differentiate each day. He established in modern humans that extension rates are highest at the cusp tip, fall to a relatively constant level in the midcrown, and then the rates drop off even further at the cervix (Shellis, 1984). Like Boyde (1964) predicted, where the intersection of striae and the EDJ are more acute, a greater number of ameloblasts are differentiated throughout the course of the day, and thus extension rates are higher (Shellis, 1984). Some studies have categorized the pace of hominoid dental development as being “fast” or “slow” (e.g., Martin, 1985). However, it is important to recognize that there is no sharp distinction; instead there is a continuum of secretion rates, which vary down the tooth crown and across the thickness of enamel (Beynon et al., 1991). Recent studies have focused on assessing the relationship between crown formation times and enamel extension rates in modern humans and hominins (Dean, 2009; Guatelli-Steinberg et al., 2012b). They suggest that enamel extension rates and crown formation times can vary independently, emphasizing the need for detailed histologic studies of more hominoid taxa. Schwartz et al. (2001; Schwartz & Dean, 2001)
found some differences in canine growth rates between species, but they emphasized that differences in crown formation time explain canine sexual dimorphism in great apes. The current study is the first to assess the relationship between enamel extension rates and LEH defect depth in great apes. It is unclear to what degree the pattern of dental growth mirrors somatic trajectories in the development of sexual dimorphism in great apes, but the current sample provides data that might help to address this question in the future.

**Striae of Retzius angles**

Recent work suggests that mountain gorillas have shallower LEH defects than other great apes (McGrath et al., 2018), which may have led to underestimation of defect prevalence in previous studies (Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012). Guatelli-Steinberg et al., (2012) hypothesized that mountain gorillas might have fewer defects compared to other great apes as a result of their 1) dedicated folivory and reduced seasonal food-related stress, and/or 2) shallower defect morphology, which might cause defects to be overlooked using qualitative methods. They suggest that shallow defects might result from shallow striae of Retzius angles, or the angle at which long-period growth lines meet the outer enamel surface (Hillson & Bond, 1997; Guatelli-Steinberg et al., 2012) (Figs. 1-2). Guatelli-Steinberg et al. (2012) provided the first data on striae angles at the outer enamel surface in great apes, reporting that defects are most likely to occur in crown regions with higher striae angles. There were no significant differences in striae angles among *Gorilla gorilla, Pan*, and *Pongo*, which corresponds with nonsignificant differences in defect depth among these taxa found recently.
(McGrath et al., 2018). However, Guatelli-Steinberg et al. (2017) reported that Gorilla gorilla exhibits shallower angles at the EDJ (called EFF angles) than Pan or Pongo.

Other work has focused on measuring the angle at which striae of Retzius extend from the EDJ (Shellis, 1984, 1998; Witez et al., 2006; Hogg & Walker, 2011; Guatelli-Steinberg et al., 2017). These studies tested whether striae angles at the EDJ correlate with body size (Shellis, 1998), encephalization quotient (Hogg & Walker, 2011), degree of folivory, and periodicity (Guatelli-Steinberg et al., 2017). Boyde (1964) was the first to point out that extension rate is reflected by striae angles at the EDJ, with slow extension rates associated with larger striae angles, and rapid extension rates associated with shallower striae angles. The relationship between striae angles at the EDJ and the outer enamel surface has not been tested, but the current study focuses on angles at the outer enamel surface because they directly relate to LEH defects. Striae are known to diverge as they course through the thickness of imbrication enamel (Beynon et al., 1991), so it is unclear to what extent striae angles reflect enamel extension rates at the EDJ, or whether other variables like linear enamel thickness, periodicity and/or daily secretion rates might also influence striae angles and thus defect depths at the outer enamel surface.

**Specific objectives**

We use histological methods to assess the influence of inter- and intraspecific enamel growth variation on LEH defect morphology in four great ape taxa (Gorilla beringei beringei, Gorilla gorilla gorilla, Pan troglodytes, Pongo sp.). Our goals are to:
1) Test for differences in imbricational linear enamel thickness that might contribute to the documented variation in LEH defect depth. Enamel thickness provides a threshold for defect depth, and we therefore expect to find thicker imbricational enamel in females and in non-mountain gorilla taxa with deeper surface defects;

2) Assess whether shallower striae of Retzius angles at the outer enamel surface are associated with shallower defects, specifically in mountain gorillas and in males;

3) Test whether shallower striae angles and defects occur in faster-growing crowns, as determined by measuring enamel extension rates, or the rate at which enamel-secreting cells differentiate down the canine crown;

4) Assess the relationships among these variables and defect depth to determine which best tracks the documented differences in defect depth.

Figure 1. Left: Female mountain gorilla mandibular canine thin section (GP.038). Middle: Variables of interest, including linear enamel thickness, striae angles, and the enamel dentine junction, along which enamel extension rates are measured. Right: Daily cross striations between consecutive striae of Retzius.
Figure 2. Photomontages of the midcrown region of male mountain gorilla (left) and female western lowland gorilla (right) mandibular canine thin sections. Mountain gorillas have shallow surface defects (McGrath et al., 2018), and are expected to exhibit the traits shown on the left compared to other great apes as represented on the right. Females have deeper defects than males, and are thus expected to exhibit the thicker enamel, larger striae angles, and slower extension rates than males in the combined sample.
MATERIALS AND METHODS

Sample

Table 1. Composition of permanent mandibular canine histological thin section sample

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sex</th>
<th>Source</th>
<th>N (LET, SRA)</th>
<th>N (EER)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gorilla beringei</em></td>
<td>F</td>
<td>MGSP</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>beringei</em></td>
<td>M</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>Gorilla gorilla</em></td>
<td>F</td>
<td>RDDN, RCS, UZH</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td><em>gorilla</em></td>
<td>M</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><em>Pan troglodytes</em></td>
<td>F</td>
<td>RDDN, RCS, UZH</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><em>Pongo sp.</em></td>
<td>F</td>
<td>RDDN, RCS, UZH</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>F</td>
<td></td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

LET: Linear enamel thickness analysis  
SRA: Striae of Retzius angles analysis  
EER: Enamel extension rates analysis

MSGP: Mountain Gorilla Skeletal Project, Musanze, Rwanda  
RDDN: Reid-Dirks-Dean Newcastle Collection, The George Washington University, Washington D.C.  
RCS: The Royal College of Surgeons, London, United Kingdom  
UZH: Anthropological Institute and Museum, University of Zürich, Switzerland

The sample includes histological thin sections of 41 permanent mandibular canines of known sex (Table 1). The Virunga mountain gorilla (*Gorilla beringei beringei*) specimens are derived from the Mountain Gorilla Skeletal Project (MGSP) collection in Rwanda (McFarlin et al., 2009). Mountain gorilla canine thin sections were prepared in the Hard Tissue Biology Lab at The George Washington University following standard protocols using a Buehler Isomet 1000 Precision saw and Buehler Ecomet 4000 grinding and polishing machine. Canines were embedded in a methyl methacrylate resin and sectioned along the midline axial plane through the cusp tip and the entire buccal aspect of each crown, i.e., ideally capturing the first- to last-formed enamel. Sections were lapped to a final thickness of approximately 100 microns, as close
as possible to the tip of the dentine horn and the pulpal horn. They were then polished using 1-micron diamond suspension liquid on polishing cloths, mounted to stain-free plastic slides and cover slipped for imaging. Western lowland gorilla (*Gorilla gorilla gorilla*), chimpanzee (*Pan troglodytes*), and orangutan (*Pongo sp.*) specimens were sourced as prepared histological thin sections from the Dirks-Dean-Reid Newcastle Collection (DDRN), The Royal College of Surgeons (RCS), and the Anthropological Institute and Museum at the University of Zürich (UZH).

**Image collection and preparation**

Digital photomontages were created using 10x lenses on one of three microscope setups. Canines from the DDRN collection were imaged using a Leica EZ4 microscope, digital camera, and LAS EZ software (1 pixel=1.28 micron resolution). Specimens from the RCS and UZH were imaged using Motic BA310 microscope and Moticam2 digital camera/software (1 pixel=1.26 micron resolution). MGSP canines were imaged using Zeiss AxioImager microscope, Optronics MicroFire CCD camera, and StereoInvestigator software (1 pixel=0.74 micron resolution). In all cases, transmitted light was used to collect overlapping images along the labial side of canine crowns. With the first two setups, it was necessary to align adjacent image fields using Photoshop CC2015 software. Images were “photomerged” using the reposition function without the blend option selected. Photo pairs that did not successfully auto-merge in the software were aligned by hand. As the Virtual Slice tool in StereoInvestigator automatically creates photomontages, the mountain gorilla specimens did not require further image manipulation.
Full resolution photomontages were viewed and measured at 150-300% zoom in Adobe Photoshop CC2015. The best-preserved canines of those available were selected for this study. Some level of wear was unavoidable given the need to optimize sample size, but we were particularly careful to select the least worn specimens when calculating enamel extension rates, thus further reducing the sample size for that analysis. Enamel extension rates were calculated down the entire EDJ, whereas linear enamel thickness and striae angle measurements were only collected at three midcrown locations. Thus, small deviations in the location of the dentine horn would have a relatively smaller effect on the latter analyses. Crown height reconstructions were performed for incomplete specimens following Saunders et al. (2007) (Fig. 3): where the outer enamel surface was worn or chipped, lines were extended until they met, and an arc was drawn to estimate the location of the cusp tip. This method has been demonstrated to reliably reconstruct cusp tips with different degrees of wear (<2% error) (Saunders et al., 2007).

Photomontages of canine crowns were oriented horizontally and aligned along a horizontal reference axis between the dentine horn, or estimated location of the dentine horn in cases of slight section obliquity or wear, and the cervix. The linear distance between the dentine horn and cervix along this horizontal reference axis was then divided into three equally-spaced regions of the labial enamel: 1) cuspal third, 2) midcrown, and 3) cervical third. Within each of these crown regions, linear enamel thickness and striae angles were collected within 750 micron windows of crown height, situated to avoid major LEH defects and accentuated lines while constraining measurements to a relatively small area. Each window is located as close as possible to the midpoint of each crown region. Enamel extension rates were measured in deciles along the EDJ to track changes
in growth rates down the crown following Reid et al. (1998). Additional images of daily cross striations were opportunistically collected at higher magnification (40x) to assess striae of Retzius periodicity (Fig. 1).

*Figure 3.* From Guatelli-Steinberg et al. (2017) (left), and Saunders et al., 2007 (right). The canine cusp tips of incomplete specimens were reconstructed following Saunders et al. (2007) by extending the outer enamel surface toward the midline, and drawing an arc to mirror the estimated extent of the cusp tip.
Measurement protocol

Linear enamel thickness was calculated by dragging the measurement tool in Adobe Photoshop CC 2015 orthogonally from the enamel-dentine junction to the outer enamel surface, defined as the edge of enamel (i.e., not including calculus or other obscurities) (Fig. 1). This pathway roughly follows the secretory axes of ameloblasts, but we note that linear enamel thickness was not collected along the length of individual enamel prisms. Striae of Retzius angles were measured at the outer enamel surface using the angle tool. The first angle arm was aligned with the outer enamel surface, and then the second arm was aligned with the stria of Retzius being measured. The smaller of the two angles was measured. Striae are known to diverge as they pass from the EDJ to the outer enamel surface (Beynon et al., 1991), so the second arm was dragged to align with the course of an individual stria 350 microns into the enamel thickness to make measurements more easily repeatable. Three measurements were collected in each crown location and averaged for analysis: for linear enamel thickness, these were spaced evenly throughout the 750 micron strips, and for striae angles, separate striae were measured each time. We used Adobe Photoshop CC2015 to collect these measurements, but NIH-developed freeware ImageJ would be a suitable substitute.

Enamel extension rates, or the rate at which ameloblasts differentiate along the EDJ, were calculated in deciles of EDJ length. We first measured the curvilinear length of the EDJ from the dentine horn, or the first-formed enamel, to the last-formed enamel at the cervix (Fig. 1). The EDJ was then divided into 10 segments or deciles, and markers were placed on closely-associated striae of Retzius marking the boundaries between the deciles. In cases where decile divisions fell between successive striae of Retzius, the
nearest stria was selected as the boundary. The number of striae were counted within each decile and multiplied by the periodicity of the individual to determine the number of days represented in that segment of the imbricational crown. Periodicities were determined by counting the number of cross striations visible between successive striae of Retzius for all specimens (Fig. 1). Total crown formation times were calculated by adding the formation times of each decile together. Striae do not reach the outer enamel surface in the cuspal region, and due to decussation cross striations are often hard to visualize along an entire enamel prism, so this can hinder attempts to calculate cuspal formation time (Schwartz & Dean, 2001). Additionally, we lacked consistent high magnification images of the cusp in several Gorilla gorilla, Pongo, and Pan specimens. Therefore, in specimens with unclear cross striations, or partially reconstructed cusp tips (N=8), we measured the length of enamel prisms from the dentine horn to the outer enamel surface and divided by the average cuspal daily secretion rate for each species and sex as reported in the literature for western lowland gorillas, chimpanzees, and orangutans (Schwartz et al., 2001). While calculating crown formation times was not a major goal of this research, we report the data as their calculation was necessary to determine enamel extension rates for the first decile(s), or before striae reach the outer enamel surface in the imbricational enamel. We were able to directly calculate cuspal daily secretion rates for each mountain gorilla thin section by 1) counting individual cross striations, and/or 2) measuring the length of individual cross striations throughout the inner, middle, and outer cuspal enamel and multiplying by the curvilinear length of an individual prism coursing from the dentine horn to the cusp tip. Once the number of days of enamel growth was determined for each decile, we divided by the length of that
segment of the EDJ, or 1/10 total EDJ length, to determine the daily rate of enamel extension (Reid et al., 1998). This was done for a subsample of the best-preserved canine thin sections in the sample (Tables 1,5).

**Statistical analyses**

We used separate linear models to examine linear enamel thickness and striae angles by species and sex at each of the three crown locations, with the interaction of species and sex as a fixed explanatory variable. The interaction between species and sex was not significant, so it was dropped from the final models. Separate models were run for each of the three crown regions (i.e., cuspal third, midcrown, cervical third). Assumptions of normality and homogeneity of variance were visually assessed using residual diagnostic plots; linear enamel thickness and striae angles were normal, so no transformation was necessary. Although species designations were available for many (but not all) individuals in the *Pongo* sp. sample, the results did not differ whether they were grouped by species or genus, so we maintained the larger sample size of the latter. Differences between multiple levels of significant fixed effects were examined using Tukey’s post hoc tests.

No statistical tests were conducted to examine whether there are intra- or interspecific differences in enamel extension rates; the sample sizes were too small given that repeated measurements were collected for each individual in deciles of crown height. To assess the relationships between the three enamel growth variables (i.e., linear enamel thickness, enamel extension rates, striae of Retzius angles) and defect depth, we conducted a pairwise correlation analysis using the Pearson method. Data density differed
among each of the variables: linear enamel thickness and striae angles were calculated for the full sample of thin sections in this study (N=41), but enamel extension rates were only calculated for 16 individuals. Therefore, we used sex- and species-specific means of enamel extension rates in this analysis. We also included mean defect depths reported in McGrath et al., (2018). As defect depths were measured within the middle 3/5ths of the crown in that study, we averaged the enamel extension rates within the middle six deciles to make the data most comparable. Only midcrown values for linear enamel thickness and striae angles were included in this analysis for the same reason. All data were natural logged prior to analysis. All statistical analyses were performed in R (Version 0.99.903, R Core Development Team, 2016).

RESULTS

Sex and species differences in linear enamel thickness

Both species and sex are significant predictors of linear enamel thickness in the cuspal region (species: F_{3,36}=19.68, p<0.001; sex: F_{1,36}=6.49, p=0.15), the midcrown (species: F_{3,36}=21.94, p<0.001; sex: F_{1,36}=13.75, p<0.001), and the cervical region (species: F_{3,36}=12.87, p<0.001; sex: F_{1,36}=17.91, p<0.001). Inter- and intraspecific differences in linear enamel thickness are summarized in Table 2 and Figure 4. Mountain gorillas have significantly thinner enamel than western lowland gorillas, except in the cuspal region. Chimpanzees and mountain gorillas do not differ in linear enamel thickness, nor do chimpanzees differ from western lowland gorillas. Orangutans have significantly thicker enamel than all of the other taxa examined. In the combined taxon sample, females have significantly thicker enamel than males at all three crown locations.
Figure 5 shows linear enamel thickness broken down by species and sex at the midcrown region.

**Table 2. Summary of ANOVA results for linear enamel thickness by species and sex**

<table>
<thead>
<tr>
<th>Group comparison</th>
<th>cervical third</th>
<th>midcrown</th>
<th>cuspal third</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gorilla beringei beringei vs.</td>
<td>p=0.007</td>
<td>p=0.009</td>
<td>p=0.432</td>
</tr>
<tr>
<td>Gorilla gorilla gorilla</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gorilla beringei beringei vs.</td>
<td>p=0.072</td>
<td>p=0.155</td>
<td>p=.344</td>
</tr>
<tr>
<td>Pan troglodytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gorilla beringei beringei vs.</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Pongo sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gorilla gorilla gorilla vs.</td>
<td>p=0.241</td>
<td>p=0.107</td>
<td>p=0.765</td>
</tr>
<tr>
<td>Pan troglodytes</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gorilla gorilla gorilla vs.</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Pongo sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pan troglodytes vs. Pongo sp.</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Females vs. males</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.015</td>
</tr>
</tbody>
</table>

Sample sizes are listed in Table 1. Interspecific comparisons are based on Tukey’s posthoc tests with a significance threshold of p<0.05 (in boldface).
Figure 4. Summary plot showing species and sex means for linear enamel thickness at three crown locations. Measurements were collected within 750 micron windows noted in the above photomontage of a female Pongo canine thin section (specimen 1988 from UZH). The scale bar measures 500 microns.
Figure 5. Linear enamel thickness in the midcrown by species and sex. Using a linear model, we found significant differences (**p<0.01; ***p<0.001) between \textit{G. b. beringei} and \textit{G. g. gorilla} (p=0.009), and \textit{Pongo} sp. and all three other taxa (p<0.001). There is no difference between \textit{G. b. beringei} and \textit{P. troglodytes}, nor between \textit{G. g. gorilla} and \textit{P. troglodytes}. Females have significantly thicker enamel in the pooled sample (p<0.001).

\textbf{Sex and species differences in striae angles}

Both species and sex are significant predictors of striae angles in the cuspal region (species: $F_{3,36}=8.13$, p<0.001), the midcrown (species: $F_{3,36}=8.39$, p<0.001; sex: $F_{1,36}=14.58$, p<0.001), and the cervical region (species: $F_{3,36}=10.10$, p<0.001; sex: $F_{1,36}=24.51$, p<0.001), except in the cusp, where sex does not significantly predict striae angles (sex: $F_{1,36}=0.32$, p=0.57). Results are described in Table 3 and Figure 6. Mountain gorillas have significantly shallower striae angles than all other taxa examined at the three crown locations. Western lowland gorillas and chimpanzees differ significantly in
the midcrown, whereas chimpanzees and orangutans differ in the cervix and cusp. Western lowland gorillas have shallower angles than orangutans in the cervix and midcrown. Females in the combined sample have larger striae angles at the cervix and midcrown, but not in the cuspal region. Figure 7 shows striae angles by species and sex in the midcrown region.

Table 3. Summary of ANOVA results for OES striae angles by species and sex

<table>
<thead>
<tr>
<th>Group comparison</th>
<th>cervical third</th>
<th>midcrown</th>
<th>cuspal third</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gorilla beringei beringei vs. Gorilla gorilla gorilla</td>
<td>p=0.035</td>
<td>p=0.016</td>
<td>p=0.003</td>
</tr>
<tr>
<td>Gorilla beringei beringei vs. Pan troglodytes</td>
<td>p=0.006</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Gorilla beringei beringei vs. Pongo sp.</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Gorilla gorilla gorilla vs. Pan troglodytes</td>
<td>p=0.209</td>
<td>p=0.011</td>
<td>p=0.064</td>
</tr>
<tr>
<td>Gorilla gorilla gorilla vs. Pongo sp.</td>
<td>p&lt;0.001</td>
<td>p=0.002</td>
<td>p=0.056</td>
</tr>
<tr>
<td>Pan troglodytes vs. Pongo sp.</td>
<td>p=0.006</td>
<td>p=0.562</td>
<td>p=0.026</td>
</tr>
<tr>
<td>Females vs. males</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.573</td>
</tr>
</tbody>
</table>

Sample sizes are listed in Table 1. Interspecific comparisons are based on Tukey’s posthoc tests with a significance threshold of p<0.05 (in boldface).
Figure 6. Summary plot showing species and sex means outer enamel surface striae angles at three crown locations. Measurements were collected within 750 micron windows noted in the above photomontage of a female *Pongo* canine thin section (specimen 1988 from UZH). The scale bar measures 500 microns.
**Figure 7.** Striae angles measured at the outer enamel surface in the midcrown by species and sex. Using a linear model, *G. b. beringei* has significantly shallower striae angles (*p*<0.05; **p**<0.01; ***p***<0.001) than *G. g. gorilla* (*p*=0.016), *P. troglodytes* (*p*<0.001), and *Pongo sp.* (*p*<0.001). *G. g. gorilla* has shallower angles than *P. troglodytes* (*p*=0.011) and *Pongo sp.* (*p*=0.002). There are no differences between *P. troglodytes* and *Pongo sp.* Females have significantly larger striae angles than males in the pooled sample (*p*<0.001).

Table 4 provides a summary of the linear enamel thickness and striae angle measurements, including the range and mean values for the three crown regions evaluated. Orangutan females have the thickest enamel, falling outside the range of all other species, while mountain gorilla males have the thinnest enamel. In most species, enamel thickness increases from the cervix to the cusp. Again, female orangutans have the largest striae angles, while male mountain gorillas have the most acute striae angles. In most species, striae angles are the largest in the midcrown, except mountain gorillas, which show larger angles in the cusp.
### Table 4. Summary of linear enamel thickness (LET) and striae angle (SA) measurements

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>N</th>
<th>LET cervix (um)</th>
<th>LET midcrown (um)</th>
<th>LET cusp (um)</th>
<th>SA cervix (°)</th>
<th>SA midcrown (°)</th>
<th>SA cusp (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. b. beringei</td>
<td>F</td>
<td>2</td>
<td>500 (485-514)</td>
<td>565 (562-568)</td>
<td>679 (673-685)</td>
<td>21.0 (20.5-21.5)</td>
<td>24.0 (23.1-24.9)</td>
<td>20.2 (18.4-22.1)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2</td>
<td>368 (320-415)</td>
<td>423 (387-460)</td>
<td>491 (460-522)</td>
<td>15.4 (15.3-15.4)</td>
<td>18.5 (17.7-19.3)</td>
<td>21.1 (18.9-23.3)</td>
</tr>
<tr>
<td>G. g. gorilla</td>
<td>F</td>
<td>12</td>
<td>573 (474-652)</td>
<td>645 (565-785)</td>
<td>669 (557-839)</td>
<td>27.5 (19.3-33.1)</td>
<td>32.9 (25.8-38.6)</td>
<td>27.8 (24.2-34.4)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5</td>
<td>460 (323-541)</td>
<td>551 (481-591)</td>
<td>593 (530-650)</td>
<td>20.1 (15.1-27.0)</td>
<td>23.3 (19.5-28.1)</td>
<td>29.6 (25.3-33.3)</td>
</tr>
<tr>
<td>P. troglodytes</td>
<td>F</td>
<td>4</td>
<td>492 (419-568)</td>
<td>564 (521-608)</td>
<td>671 (596-803)</td>
<td>29.5 (24.9-31.9)</td>
<td>35.8 (34.1-38.4)</td>
<td>32.5 (27.5-37.7)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>6</td>
<td>488 (433-531)</td>
<td>536 (433-607)</td>
<td>617 (555-707)</td>
<td>23.8 (18.8-28.3)</td>
<td>32.0 (25.7-45.7)</td>
<td>31.7 (24.2-44.0)</td>
</tr>
<tr>
<td>Pongo sp.</td>
<td>F</td>
<td>5</td>
<td>660 (640-705)</td>
<td>817 (687-926)</td>
<td>996 (651-1258)</td>
<td>38.8 (30.2-49.6)</td>
<td>38.1 (30.5-45.5)</td>
<td>31.5 (26.1-38.9)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5</td>
<td>578 (513-619)</td>
<td>718 (597-812)</td>
<td>897 (744-1089)</td>
<td>27.9 (18.4-39.0)</td>
<td>32.9 (25.8-44.7)</td>
<td>32.5 (28.3-36.4)</td>
</tr>
</tbody>
</table>

Mean values are in bold and ranges are in parentheses.

Table 5 summarizes the canine growth variables assessed in this study. These include the curvilinear EDJ lengths; canine crown formation times; striae of Retzius periodicity; and the number of imbricalional striae. Male western lowland gorillas have the longest EDJ lengths and the highest number of imbricalional striae. Females of all species, especially mountain gorillas, chimpanzees, and orangutans have similar EDJ lengths, but chimpanzees have a higher number of imbricalional striae. Orangutan males have the longest crown formation times of 10-11 years, while female mountain gorillas have the shortest. Orangutans also show a higher periodicity (12 days) than is currently reported for extant great apes (Fig. 10).
Table 5. Summary of canine growth variables

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>N</th>
<th>EDJ Length (range in mm)</th>
<th>CFT (range in years)</th>
<th>Periodicity (days)</th>
<th>Number of imbricational striae</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. b. beringei</td>
<td>F</td>
<td>2</td>
<td>14.6-15.7</td>
<td>4.38-4.41</td>
<td>7-8</td>
<td>180-200</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2</td>
<td>23.4*-26.2</td>
<td>4.81*-6.04</td>
<td>6-7</td>
<td>270*-297</td>
</tr>
<tr>
<td>G. g. gorilla</td>
<td>F</td>
<td>2</td>
<td>16.0-18.2</td>
<td>5.90-6.47</td>
<td>8-9</td>
<td>246-251</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2</td>
<td>28.5-31.8</td>
<td>8.52-9.78</td>
<td>10</td>
<td>311-339</td>
</tr>
<tr>
<td>P. troglodytes</td>
<td>F</td>
<td>2</td>
<td>15.3-15.6</td>
<td>6.08-6.27</td>
<td>8</td>
<td>241-277</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2</td>
<td>19.1-22.5</td>
<td>7.10-7.19</td>
<td>6-8</td>
<td>304-398</td>
</tr>
<tr>
<td>Pongo sp.</td>
<td>F</td>
<td>2</td>
<td>14.2-15.5</td>
<td>6.11-6.17</td>
<td>9-10</td>
<td>194-217</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2</td>
<td>27.7-29.0</td>
<td>10.05-11.02</td>
<td>12</td>
<td>290-317</td>
</tr>
</tbody>
</table>

N.B. GP.075 male mountain gorilla died just before canine crown completion, so estimates marked with an asterisk (*) might be under representative of the final form.

Table 6 and Figures 8-9 show the mean and range of enamel extension rates in a subsample of especially well-preserved individuals included in this study (N=16). While the sample sizes were too small to conduct statistical tests, the range of values among mountain gorillas and other species do not overlap in the midcrown, or the middle six deciles, where LEH defects are most commonly visible. Mountain gorillas have faster mean enamel extension rates in all 10 deciles compared to the other taxa. Males have faster mean enamel extension rates overall compared to females in all taxa, with no overlap between males and females in mean extension rates in the midcrown among chimpanzees and mountain gorillas. However, male and female western lowland gorillas and orangutans overlap in mean enamel extension rates in a larger number of deciles compared to the other species. In contrast to the pattern that has been described for
modern humans, with enamel extension rates slowing down toward the end of enamel secretion, extension rates in these taxa either remain stable or increase in the last deciles. Male mountain gorillas are the only exception to this pattern, but it is possible that the last deciles of GP.075 do not reflect normal growth patterns as this individual died just before crown completion.

Table 6. Mean and range enamel extension rates (um per day) by decile (cusp [1] to cervix [10])

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
<th>D9</th>
<th>D10</th>
<th>AV</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. b. beringei</em> (N=4)</td>
<td>F</td>
<td>18.5</td>
<td>14.0</td>
<td>11.0</td>
<td>10.1</td>
<td>10.2</td>
<td>9.0</td>
<td>6.9</td>
<td>8.3</td>
<td>8.1</td>
<td>10.4</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>16.1</td>
<td>15.7</td>
<td>12.8</td>
<td>12.8</td>
<td>12.8</td>
<td>12.7</td>
<td>12.6</td>
<td>13.0</td>
<td>11.8</td>
<td>10.2</td>
<td>13.0</td>
</tr>
<tr>
<td><em>G. g. gorilla</em> (N=4)</td>
<td>F</td>
<td>15.2</td>
<td>10.4</td>
<td>7.2</td>
<td>8.0</td>
<td>6.5</td>
<td>6.1</td>
<td>6.9</td>
<td>8.3</td>
<td>8.1</td>
<td>10.1</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>23.0</td>
<td>7.4</td>
<td>6.6</td>
<td>7.8</td>
<td>8.1</td>
<td>9.0</td>
<td>9.3</td>
<td>8.8</td>
<td>9.5</td>
<td>11.0</td>
<td>10.4</td>
</tr>
<tr>
<td><em>P. troglodytes</em> (N=4)</td>
<td>F</td>
<td>14.0</td>
<td>9.8</td>
<td>5.3</td>
<td>5.9</td>
<td>5.4</td>
<td>5.8</td>
<td>5.9</td>
<td>6.1</td>
<td>8.3</td>
<td>10.1</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>11.1</td>
<td>9.7</td>
<td>6.3</td>
<td>6.4</td>
<td>6.9</td>
<td>7.3</td>
<td>7.9</td>
<td>8.3</td>
<td>10.1</td>
<td>11.9</td>
<td>8.6</td>
</tr>
<tr>
<td><em>Pongo sp.</em> (N=4)</td>
<td>F</td>
<td>10.0</td>
<td>7.3</td>
<td>5.5</td>
<td>6.1</td>
<td>6.0</td>
<td>5.7</td>
<td>6.7</td>
<td>5.9</td>
<td>6.2</td>
<td>7.2</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>18.7</td>
<td>8.0</td>
<td>6.2</td>
<td>6.9</td>
<td>7.2</td>
<td>7.4</td>
<td>7.0</td>
<td>6.1</td>
<td>6.9</td>
<td>8.4</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Mean and range of enamel extension rates (um/day) by decile of EDJ length, a proxy for crown height. The final AV column lists mean values across the crown.
Figure 8. Enamel extension rates in female great apes by decile of EDJ length. Mountain gorillas only overlap with the other taxa in the first and last two deciles (values listed in Table 6).
Figure 9. Enamel extension rates in male great apes by decile of EDJ length. Mountain gorillas only overlap with the other taxa in the first and last two deciles (values listed in Table 6).
Figure 10. Male *Pongo* (specimen 10791) mandibular canine thin section at midcrown, imaged using transmitted light microscopy. Both *Pongo* males included in this study have periodicities that exceed the published range for extant great apes. Here, 12 cross striations (red dots) can be counted between consecutive striae of Retzius.

**Relationships among enamel growth variables and defect depth**

We found that the three enamel growth variables (i.e., linear enamel thickness, enamel extension rates, and striae angles) are correlated with each other. Correlation coefficients and significance levels are reported in Table 7. There are also significant correlations among all pairs of enamel growth variables and defect depth, with a strong negative correlation between defect depth and enamel extension rate (Table 7). This indicates that higher extension rates are related to shallower defect depths on the outer enamel surface, as in mountain gorillas compared to other species, or males compared to females. A negative correlation was also observed between enamel extension rates and
striae angles. Though the effect size is not as large, both striae angles and linear enamel thickness are positively correlated with defect depth, meaning larger angles and thicker enamel are related to deeper defects on the outer enamel surface. However, it is also the case that a large proportion of the variation in defect depth is not explained by these variables as demonstrated by the $r^2$ values in Table 7. For example, only 66% of the variation in defect depth is explained by enamel extension rates despite the strong negative correlation between these variables.

Table 7. Pairwise correlation analysis of enamel growth variables and defect depth

<table>
<thead>
<tr>
<th>Variables</th>
<th>$r$ (Pearson correlation coefficient)</th>
<th>$r^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LET - SA</td>
<td>0.45</td>
<td>0.20</td>
<td>p=0.004</td>
</tr>
<tr>
<td>LET - EER</td>
<td>-0.55</td>
<td>0.30</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>SA - EER</td>
<td>-0.75</td>
<td>0.56</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>LET - Depth</td>
<td>0.53</td>
<td>0.28</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>SA - Depth</td>
<td>0.63</td>
<td>0.40</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>EER - Depth</td>
<td>-0.81</td>
<td>0.66</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

LET: linear enamel thickness; SA: striae of Retzius angle; EER: enamel extension rates.

**DISCUSSION**

The purpose of this study was to assess the extent to which species- and sex-related variation in canine enamel growth influences LEH defect morphology at the outer enamel surface among great apes. In particular, mountain gorillas have been characterized as having fewer defects than other great apes (e.g., Guatelli-Steinberg et al., 2012). However, recent work demonstrates that mountain gorillas have significantly shallower defects than other taxa, which might have led to their underestimation in previous studies (McGrath et al., 2018). Mountain gorilla canine enamel growth patterns
have not been described previously, and this study demonstrates they have thinner linear enamel, faster enamel extension rates, and shallower striae angles compared to other great apes, which contributes to their shallower LEH defects on the outer enamel surface. Our results suggest that striae angles at the outer enamel surface reflect enamel extension rates at the EDJ. This result builds upon the work of Boyde (1964) and Shellis (1984) which demonstrated the relationship between extension rates and striae angles at the EDJ. The pattern of enamel growth variation between mountain gorillas and other great apes is consistent with the sex differences observed across the sample, reflecting broader relationships between defect depth and linear enamel thickness, enamel extension rates, and striae angles.

**Species differences in canine growth**

Despite having used a different method to measure linear enamel thickness, our results fit well with what Schwartz et al. (2001) reported for great apes: *Pongo* sp. has the thickest enamel, followed by *Gorilla gorilla*, and *Pan troglodytes*. In the combined sex sample, mountain gorillas do not have thinner enamel than chimpanzees, but it is clear from the plots that male mountain gorillas in particular stand out as having the thinnest enamel in the sample (Fig. 4). Mountain gorillas have significantly thinner enamel than western lowland gorillas, except in the cusp. This might relate to differences in daily secretion rates (Schwartz et al., 2001), and/or differences in the secretory lifespan of ameloblasts in those areas (Beynon et al., 1991). Western lowland gorillas have longer total crown formation times than mountain gorillas (means of 7.7 vs. 7.1 years in the combined sex sample, respectively). They also have slower enamel extension rates than
mountain gorillas in the midcrown, where defects are most likely to be macroscopically visible. Our data suggest that mountain gorillas maintain a high enamel extension rate from the cuspal to cervical regions, rather than exhibiting a slowing of extension rate throughout the lateral and cervical regions like the other taxa. Overall, great apes do not follow the modern human pattern reported by Shellis (1984), characterized by high initial extension rates followed by a steady, intermediate rate before dropping off at the cervix. Instead, individuals in our sample often show an increase in extension rate towards the end of canine crown development.

Our results underscore earlier observations that striae angles are not constant (Shellis, 1984; Witzel et al., 2006; Guatelli-Steinberg et al., 2012); just like daily secretion and extension rates, they vary down the crown and through the thickness of enamel. In the majority of our sample, the angle at which striae of Retzius approach the outer enamel surface is shallowest toward the cervix, largest in the midcrown, and shallow again toward the cusp. Male gorillas of both species are the only exception to this pattern. Great ape striae angles vary in ways that are consistent with previously reported LEH defect depths, with mountain gorillas having the shallowest angles and the shallowest defects in the sample (for the latter, see McGrath et al., 2018). Striae angles also reflect enamel extension rates at the EDJ: faster extension rates correspond to more acute striae angles at the outer enamel surface in mountain gorillas, while slower rates correspond to more obtuse angles in the other taxa (Fig. 7). Western lowland gorillas, Pongo, and Pan have similarly slow extension rates, but Pongo has thicker enamel, and more obtuse striae angles (Figs. 5, 7). These results suggest that there is a close relationship between outer enamel surface striae angles and extension rates, but other
factors like enamel thickness and daily secretion rates might also affect striae angles where there are significant differences in the sample.

We were not able to statistically test for differences in enamel extension rates due to limitations in sample size, but the clearest relationship to emerge from this study is that between LEH defect depth and extension rates. The correlation between extension rates and defect depth is the highest of all pairwise comparisons (Table 7). Western lowland gorillas, chimpanzees, and orangutans do not appear to differ in enamel extension rates, especially in the middle six deciles of crown height. McGrath et al. (2018) measured defect depth in the middle 3/5ths of canine crowns of these same taxa and found no significant differences. Mountain gorillas, however, exhibited significantly shallower defects, mirrored by their higher extension rates found in the current study. If the relationship between surface defect depth and extension rates holds true among a broader comparative sample (e.g., Paranthropus vs. Australopithecus – Guatelli-Steinberg et al., 2004, 2008), median defect depths at the population-level might be used as a proxy to infer important growth information about fossil taxa, i.e., extension rates that can only currently be gleaned through histological analyses.

**Sex differences in canine growth**

Canine dimorphism is linked to several behavioral and ecological factors, including sexual selection, body size dimorphism, phylogeny, predation pressure, and more (reviewed in Plavcan & van Schaik, 1994). Mountain gorillas have a higher degree of body size dimorphism compared to western lowland gorillas (Taylor, 1997), but according to our EDJ lengths, male western lowland gorillas have larger canines than
male mountain gorillas. However, it is not yet clear to what extent dental development follows the trajectory of somatic growth. Comparative ontogenetic data on body size dimorphism suggest that different mechanisms underlie comparable levels of adult dimorphism in great apes (Leigh, 1995; Leigh & Shea, 1995; Shea, 1986). While males of some taxa might acquire a larger size through extending the duration of growth, others accomplish the same through increasing the rate (see Smith, 2016; Schwartz & Dean, 2001 for similar findings in dental development). Our data support this hypothesis in that similar-looking canines can grow differently (Guatelli-Steinberg et al., 2009), even in closely related taxa. Male western lowland gorillas have slower extension rates than male mountain gorillas, but they have taller canines, which they accomplish by growing their crowns for a longer time. Recent data from living mountain gorillas suggests that they similarly reach maximum body size at earlier ages than western lowland gorillas (Galbany et al., 2017).

Extension rates are calculated by multiplying periodicity by the number of striae of Retzius in each decile, information which at this time can only be gleaned from histological investigation or by using synchrotron computed microtomography. Our results suggest that the range of periodicity for extant great apes is broader than previously reported by Schwartz and Dean (2001) and Smith (2016), with Pongo males exhibiting periodicities of 12 (Fig. 10). However, fossil Pongo specimens have been previously reported to exhibit periodicities of 12 (Hu et al., 2012; Smith, 2016). Pongo enamel extension rates fit well with those of western lowland gorillas and chimpanzees, but Pongo males have comparatively prolonged crown formation times, more than one year beyond what has been previously reported for any extant ape (11.0 in this study vs.
The lifespan of individual ameloblasts is also expected to be longer in *Pongo* compared to other taxa as evidenced by their relatively thick enamel (Beynon et al., 1991). It is not particularly surprising that *Pongo* males exhibit extended canine development given that they have the slowest life histories of the great apes, including the longest interbirth intervals and a prolonged period of dependency (Knott, 1998; 2001). However, it is worth noting that female orangutans do not exhibit a similarly prolonged period of canine crown formation compared to other taxa. Female canine growth patterns are hypothesized to provide a “template” for species canine morphology, and thus better reflect the life history of the species, due to the fact that male canine growth is influenced by sexual selection (Zingeser & Phoenix, 1978; Guatelli-Steinberg et al., 2009). The longer the period of canine formation, the longer the “window of vulnerability” to stress, and potential for defect formation (Guatelli-Steinberg et al., 2012).

Females have thicker enamel in the combined sex sample with little overlap between males and females except in *Pan*, the least sexually dimorphic taxon in this study (Figs. 4-5). Female mountain gorilla linear enamel thickness measurements fall within the range of both sexes of western lowland and chimpanzee values, well above the range for male mountain gorillas. In order to create a canine of a similar crown height and intermediately-thick enamel compared to the other taxa, female mountain gorillas have accelerated enamel extension rates in their imbricational enamel. They also exhibit shallower striae angles throughout the crown compared to females of other species. It is not yet clear why females have thicker enamel than males, but thicker enamel is more resistant to wear, which might allow for longer use by females that have longer lifespans.
than males in many primate species (Clutton-Brock & Isvaran, 2007; Pampush et al., 2013; Austad & Fischer, 2016).

Schwartz and Dean (2001) attributed the intraspecific difference in crown height to differences in canine crown formation times. They found that *Gorilla gorilla* and *Pongo* males grew their canines for 3-4 years more than females of the same species, while *Pan* males only grow their canines for about one year longer than females, mirroring documented differences in canine crown height. Our results suggest that enamel extension rates also play an important role in determining canine height in great apes: female western lowland and mountain gorillas have similar crown heights, but different crown formation times and extension rates (Tables 4-5). Males of all taxa have higher mean extension rates than females overall, but not in every decile (Table 5). Larger sample sizes would allow statistical testing of the hypothesis that higher extension rates in males contributes to canine dimorphism in great apes, in addition to the documented difference in the length of time that they grow (Schwartz & Dean, 2001).

The significant sex differences in LEH defect depths found by McGrath et al. (2018) might reflect variation in a combination of factors including enamel thickness, extension rates, and possibly even daily secretion rates, striae spacing, and periodicity.

**Inter-individual variation in canine growth patterns**

Individuals of the same sex-class with lower periodicities have higher imbricational striae of Retzius counts than conspecific individuals with higher periodicities, ultimately producing crowns of similar height in roughly the same amount of time (as was described by Reid & Ferrell, 2006). This pattern holds true for all sex-
classes that demonstrate variation in periodicity in this sample except western lowland gorilla females; they showed the largest amount of variation in crown formation times, and the female with a higher periodicity grew her canine for a longer duration. In *Pongo*, one female has a periodicity of 10 and 194 imbricational striae, while another has a periodicity of nine and 217 imbricational striae, yet they formed their canine crowns in 6.1 and 6.2 years, respectively. The latter individual has a longer EDJ length, our proxy for canine crown height, of 15.5 mm vs. 14.2 in the other individual (Table 4). This same pattern is evident in *Pan* males, where one individual has a periodicity of six, an imbricational striae count of 398, and a canine crown formation time of 7.2 years, while a male with a periodicity of eight has only 304 imbricational striae, but formed its canine in 7.1 years. Again, the individual with a longer crown formation time has a longer EDJ length at 22.5 vs. 19.1 mm in the other individual. Larger sample sizes will allow for statistical testing of this relationship, and potentially offer insight into the evolution of these traits over time.

**Enamel growth variation and defect depth**

Enamel extension rates have a strong negative correlation with defect depth at the outer enamel surface (Table 7). This holds true despite the fact that enamel extension rates are measured at the EDJ, while defects are measured at the outer enamel surface. As described by Boyde (1964), variation in extension rates has a profound effect on the geometry of the crown. However, 34% of the variation in defect depth was not explained by enamel extension rates based on this model (Table 7). Striae angles are moderately correlated with enamel extension rates and defect depth, but again, a large proportion of
variation in depth is unexplained by this variable. Our results lend support to the idea that striae angles are tightly linked to extension rates, and perhaps share the same biological mechanism as both have a moderate to strong relationship with median defect depth. Linear enamel thickness is positively correlated with defect depth, but not as strongly as the other variables. Enamel thickness sets a threshold for defect depth, but it is influenced by variables other than enamel extension rate, such as daily secretion rate (Beynon et al., 1991). The extent to which defect depth is explained by all three variables together is not yet clear; larger sample sizes of measurements derived from the same individuals will allow further testing of these relationships in the future.

**CONCLUSIONS**

Our findings support the hypothesis that variation in microscopic enamel growth patterns influence the morphology of the outer enamel surface, including the appearance of LEH defects. The final shape of the crown is heavily influenced by ameloblast secretory activity (Beynon et al., 1991), and this variation should be taken into consideration when interpreting outer enamel surface morphology. The three variables investigated here, namely linear enamel thickness, the angle at which striae of Retzius approach the outer enamel surface, and enamel extension rates were all shown to contribute to the appearance of LEH defects at the population level. However, much variation in LEH defect depth remains unexplained by these factors. Stress severity warrants continued study as a possible explanatory factor for variation in defect depth (e.g., McGrath et al., 2018), as do other enamel growth variables not included in this study.
Regarding the influence of enamel growth on LEH, enamel extension rates best track the sex and species-specific patterns in defect depth, followed closely by striae angles, which are likely to reflect the same biological mechanism (Boyde, 1964). While we find sex differences in enamel extension rates, thicker linear enamel might also contribute to the deeper surface defects in females compared to males in great apes. If these relationships hold true among a larger and more diverse sample, some information about enamel growth could be gleaned on the basis of median surface defect depths using nondestructive imaging methods. Future work aims to incorporate a more diverse sample of extant great apes, including the more frugivorous Bwindi mountain gorillas and eastern lowland gorillas, with the goal of assessing the variation of these traits among closely related, yet differently-adapted, taxa with associated life history, climate, and health records. An implication of this work is that stressors of a similar magnitude might be expected to produce defects of different depths in different species or sexes due to variation in enamel growth. Given the documented variation in enamel growth among modern humans and extinct hominins, the relationships among these variables should be considered when interpreting the life histories of fossil or archaeological samples that cannot be sectioned.
Chapter 4: From the inside out: Histological analysis of enamel defects in Virunga mountain gorilla (*Gorilla beringei beringei*) canines

ABSTRACT

Objectives

We analyze the macro- and microstructural structure of developmental defects in mountain gorilla canines (*Gorilla beringei beringei*) to better understand their formation, focusing on LEH defects at the outer enamel surface and internal accentuated lines. We previously reported inter- and intraspecific differences in LEH defect depth in great apes (McGrath et al., 2018), which correspond to variation in enamel growth patterns (McGrath et al., *in prep*). Here, we assess the prevalence and timing of LEH defects and accentuated lines, assess co-occurrence between both defect types, and investigate their relationship to stressful events.

Materials and Methods

We use established histological methods to identify the timing and crown locations of abnormal enamel microstructure in wild Virunga mountain gorillas from Rwanda (*Gorilla beringei beringei*) (N=4). We incorporate LEH defect depth measurements collected using optical profilometry to assess the response by secretory ameloblasts at times of stress from both a surface and histological perspective.

Results

Our results suggest that LEH defects co-occur with internal accentuated lines, but accentuated lines occur more frequently and without corresponding LEH defects.
Accentuated lines occur most often in the cervical region in this sample, which is a notoriously difficult and unreliable location for LEH defect identification using qualitative techniques. Deeper LEH defects correspond to more pronounced accentuated lines. Mountain gorillas initiate and complete their canine crowns at earlier ages compared to published estimates for western lowland gorillas and other great apes. One specimen exhibits many accentuated lines, and while it has no marked surface defects on the canine, it does have a large plane-form defect on the third molar. Two specimens with known snare injuries demonstrate both LEH defects and accentuated lines at the timing of snare removals.

Discussion

These results fit in well with what is currently known about Virunga mountain gorilla development: they are characterized by accelerated life histories, and this study suggests that canine formation is no exception. Within this sample, LEH defects are reliable indicators of underlying accentuated lines, both of which represent disruptions to normal enamel formation. While macroscopic LEH defects are most likely to occur in the midcrown region of canines, accentuated lines occur throughout the height of the crowns. Major stressors, here represented by the removal of snares, are represented as LEH defects with associated accentuated lines, providing rare data on defect etiology in wild primates. At present it is necessary to conduct histological investigation to assess the full growth histories of individuals. However, future work will address whether accentuated lines are associated with microscopic surface defects, also known as accentuated perikymata, which can be analyzed nondestructively in well-preserved specimens.
INTRODUCTION

Enamel does not undergo remodeling or repair, so the microstructure of mature enamel preserves a permanent record of both normal and abnormal growth processes (Boyde, 1989; Dean & Beynon, 1991). Enamel grows incrementally following two biological rhythms producing regular incremental markings: prism cross striations and striae of Retzius (Boyde, 1989; Retzius, 1837). Cross striations record daily fluctuations in enamel matrix secretion and are governed by the circadian rhythm (Boyde, 1989; Smith, 2006). Under light microscopy, cross striations appear as hatch marks between successive long-period growth increments, or striae of Retzius (Retzius, 1837). The periodicity of striae of Retzius can be determined by counting the number of cross striations between successive striae. This number, which ranges from 6-12 days in great apes (Schwartz et al., 2001; McGrath et al., *in prep*), varies among individuals but it is consistent across a single permanent dentition (FitzGerald, 1998). Striae of Retzius periodicity has been found to positively correlate with body mass in primates, and it has been hypothesized to relate to a centrally-regulated growth rhythm (Bromage et al., 2009, 2012). Analogous long-period lines also occur in dentine, which suggests that the rhythm affects the formation of more than just enamel (Reid & Ferrell, 2006).

Perikymata, which are the external expression of striae of Retzius (Figs. 1&2; Preiswerk, 1895; Pickerill, 1913), consist of one ridge and one trough, the latter having an occlusal and a cervical wall on the outer enamel surface. Perikymata only occur in the imbricational enamel, where striae of Retzius outcrop on the outer enamel surface (Pickerill, 1913); each perikyma represents one long-period interval (Hillson & Bond, 1997). Unlike striae of Retzius, perikymata can be visualized nondestructively to glean
information about the timing and duration of enamel matrix development, and to calibrate disruptions to dental development (e.g., Dean & Reid, 2001).

Great ape canines frequently have marked horizontal grooves and/or pits in the surface enamel (e.g., Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012). These hypoplastic defects record acute disruptions to enamel secretion due to episodes of nonspecific stress during dental development (Goodman & Rose, 1990). Hypoplastic defects often (McGrath et al., 2018), but not always (Witzel et al., 2006), co-occur with accentuated lines within the enamel. The approximate developmental timing of the most common type of hypoplastic defect, termed linear enamel hypoplasia (LEH), can be inferred because they occur within the sequence of “normal” enamel long-period growth increments, or perikymata, on the outer enamel surface (Reid & Dean, 2000). Internally, the timing and duration of accentuated lines can be reconstructed with a higher level of accuracy given that they occur among both long-period and short-period growth increments (Witzel et al., 2006).

LEH defects are often referred to as furrow-form defects (e.g., Hillson & Bond, 1997), which range in appearance from a slight deviation in the “normal” spacing of one or two perikymata to major, macroscopically-visible grooves spanning many weeks of development (Hillson, 2014). Several furrows will commonly occur in rapid succession creating a “washboard” effect on the tooth crown (Hillson, 2014). Berten (1895) also described two other kinds of hypoplastic defects, called plane-form and pit-form. Plane-form defects are lesions in which a much larger portion of a single stria is exposed (Hillson & Bond, 1997). Pit-form defects are scattered around the crown in a discontinuous band (Hillson & Bond, 1997).
LEH defect morphology has been linked to the duration of stress events, the number of affected ameloblasts, and the position of affected ameloblasts along the enamel formation front (Berten, 1895; Hillson & Bond, 1997; Witzel et al., 2006). Defect severity, which has been operationalized as the depth and/or width of a defect on the outer enamel surface, has been hypothesized to reflect the magnitude of the insult that caused the defect (Skinner & Hopwood, 2004; Kierdorf et al., 2004; Skinner & Skinner, 2017). However, sex- and species-specific enamel growth variation may also influence defect depth (Hillson & Bond, 1997; Witzel et al., 2006, 2008; Guatelli-Steinberg et al., 2012; McGrath et al., in prep).

LEH is most evident on the anterior teeth of hominoids as most of their crowns are made up of imbricational enamel, in which striae of Retzius reach the outer enamel surface, or outcrop (Hillson, 2014). Defects are most pronounced on the labial surface of teeth and in the midcrown region (Hillson & Bond, 1997; Hillson, 2014). Furrows are formed through variation in perikymata spacing, which is a proposed consequence of a larger number of ameloblasts than usual slowing down or ceasing enamel secretion at the affected perikymata grooves (Hillson & Bond, 1997). In effect, the perikymata in LEH defects are more widely spaced and exhibit more sharply-defined Tomes’ pit processes than usual (Hillson & Bond, 1997). However, Witzel et al. (2008) reported that perikymata spacing can be reduced rather than increased in association with furrow-form defects. Witzel et al. (2008) thus urges caution for those researchers interested in reconstructing growth history from the surface morphology alone.

When analyzing teeth using histological methods, enamel microstructure can be used to reconstruct the history of growth disruptions with great accuracy (e.g., Witzel et
Disruptions to enamel secretion create accentuated lines, which have also been called accentuated or pathological striae, Wilson bands, among other names (Wilson & Shroff, 1970; Goodman & Rose, 1990; Witzel et al., 2008). FitzGerald & Rose (2000) argued that Wilson bands are distinct from regular striae of Retzius as they appear broader, more accentuated, and they show an irregular prism structure. Wilson bands course more deeply into the thickness of the enamel (at least ¾ of its length). In nonhuman primates, stressors like illness and injury have been linked to accentuated lines in individuals of known life history (Schwartz et al., 2006; Smith & Boesch, 2015), but comparatively little is known about the etiology of enamel defects in wild primates vs. modern humans.

Many researchers have assessed whether accentuated lines, as they will be called here, co-occur with enamel hypoplasia, and if so, under what conditions (e.g., Goodman & Rose, 1990; Wright, 1990; Hillson & Bond, 1997; Kierdorf & Kierdorf, 1997; Kierdorf et al., 2000, 2004; FitzGerald & Saunders, 2005; Witzel et al., 2006; Smith & Boesch, 2015). Goodman and Rose (1990) proposed a threshold model to explain what they described as minor hypoplastic defects that lack co-occurring microscopic abnormalities in the tissue below, and thus may not represent physiological stress. Witzel et al. (2008) set out to test whether it was possible to reconstruct the reaction pattern of secretory ameloblasts from both a histologic and surface perspective in modern human teeth. They found marked variation along the enamel forming front, even within the same defect, with some ameloblasts showing signs of recovery after stress events, while other cells did not recover (Witzel et al., 2008). Where LEH defects occurred on the outer enamel surface, a higher number than usual ameloblasts had ceased enamel matrix secretion,
increasing the spacing of perikymata on the surface (Witzel et al., 2008). Where LEH defects are shallow, the authors suggest that only the final secretory cycle of ameloblasts is skipped, leading to abnormal microstructure only in the outermost enamel, and therefore not forming an accentuated line. Witzel et al. (2008) also found accentuated lines that did not correspond to hypoplastic defects on the surface. They consider these very brief disruptions to enamel secretion from which ameloblasts recovered before forming surface defects.

While histological analysis is the only way to obtain a complete understanding of how individual defects formed (Witzel et al., 2008), nondestructive imaging-based methods have been developed to quantitatively characterize defect morphology from the outer enamel surface (e.g., Bocaege & Hillson, 2016; Skinner & Skinner, 2017; McGrath et al., 2018). Virunga mountain gorillas have been characterized as having fewer LEH defects than other great ape taxa (Hannibal & Guatelli-Steinberg, 2005; Guatelli-Steinberg et al., 2012). However, recent work suggests that mountain gorillas have shallower defects that are harder to reliably identify using qualitative methods (McGrath et al., 2018). Evidence suggests that sex- and species-specific enamel growth variation contributes to LEH defect morphology (McGrath et al., in prep), but this has yet to be linked to the reaction pattern of secretory ameloblasts leading to such defects (Witzel et al., 2008).

**Specific Aims**

In this study, we use histological methods to assess the nature and etiology of hypoplastic defects in Virunga mountain gorillas (Gorilla beringei beringei), including
(1) the co-occurrence of LEH defects with internal accentuated lines hypothesized to represent the same disruption to enamel secretion; (2) the absolute and relative timing of defects; and (3) what factors, including enamel geometry and stressors recorded in associated life history records, might influence whether one type of defect or both are formed in response to stress events. We report the first data on permanent mandibular canine initiation and formation times for mountain gorillas, and we discuss how our results fit in with the existing literature on great ape canine formation.

Figure 1. Female mountain gorilla (specimen ID GP.038) permanent mandibular canine thin section (left), and close-ups of enamel (middle and right) with major features and landmarks are labeled.

MATERIALS AND METHODS

The sample is derived from the Mountain Gorilla Skeletal Project (MGSP) collection in Rwanda (McFarlin et al., 2009) (Table 1). Over 50% of the mountain gorilla (Gorilla beringei beringei) specimens in this collection have associated life history data as a result of long-term research and veterinary monitoring in Rwanda’s Volcanoes National Park by Dian Fossey Gorilla Fund International’s Karisoke Research Center
(initiated in 1967), the Mountain Gorilla Veterinary Project, and the Rwanda Development Board’s Department of Tourism and Conservation. The MGSP collection specimens included in this study were collected after 1996.

Permanent mandibular canines were selected for this study because (1) they have the longest crown formation times of all tooth positions; (2) a large proportion of their height is imbricalational enamel where surface defects are manifest; and (3) the majority of research on enamel defects in great apes has been conducted on canines. The four canines in the current study were either unworn (GP.075 canine) or minimally-worn (all others), with well-preserved cusp tips (Table 1, see ages at death).

Table 1. Mountain gorilla individuals included in this study

<table>
<thead>
<tr>
<th>Specimen ID and name</th>
<th>Sex</th>
<th>Birth and death dates</th>
<th>Age at death (years)</th>
<th>Striae of Retzius periodicity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP.038 Mpanga</td>
<td>F</td>
<td>08/30/1991-05/09/2002</td>
<td>10.69</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Birth error=none]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP.030 Unknown</td>
<td>F</td>
<td>Unknown</td>
<td>Juvenile – M2 dental stage</td>
<td>7</td>
</tr>
<tr>
<td>GP.075 Arusha</td>
<td>M</td>
<td>08/15/1993-09/13/1999</td>
<td>6.08</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Birth error=1, Williamson &amp; Gerard-Steklis, 2001]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP.033 Nyarusizi</td>
<td>M</td>
<td>12/20/1991-09/05/2004</td>
<td>12.71</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[MGVP]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GP=Gorilla Project (Mountain Gorilla Skeletal Project)
Figure 2. GP.038 (female) canine, epoxy replica, and digital elevation models (DEMs) of surface defects. Black scale bars are 1 cm. DEM depth values are in microns and were reported in McGrath et al. (2018). The star roughly corresponds to two major life events.

Figure 3. GP.075 (male) canine. The individual died just before crown completion so there is no visible root formation. Three furrow-form defects with associated depth information are labeled. Additional defects are visible, as well as the pencil-drawn plane of section. Black scale bar is 1 cm.
Specimen preparation and imaging

Three of the four individuals included in this study were of known sex and age (Table 1). Right or left canines were selected based on crown completion and preservation. LEH defects were analyzed using epoxy replicas created for McGrath et al. (2018). The same specimens, as well as associated first permanent molars for two individuals (GP.038 and GP.075), were histologically prepared following standard protocols in the Hard Tissue Biology Lab at The George Washington University using a Buehler Isomet 1000 Precision saw and Buehler Ecomet 4000 grinding and polishing machine. Canines were embedded in a methyl methacrylate resin before sectioning along the midline axial plane. Sections were lapped to a thickness of approximately 100 microns, polished using 1-micron suspension liquid on polishing cloths, mounted to stain-free plastic slides, cover-slipped and imaged using transmitted light microscopy. Digital photomontages were created using a Zeiss AxioImager microscope, Optronics MicroFire CCD camera, and StereoInvestigator software (1 pixel=0.74 micron resolution). One third permanent molar (GP.075) is also briefly discussed; it was prepared following the same protocols in the HTBL at GW for another ongoing study on molar development.

Quantification

Macroscopic inspection of the tooth surface was conducted by KM for a previous study (McGrath et al., 2018). Once identified, LEH defects were scanned using optical profilometry, and maximum defect depths were obtained using NIH-developed ImageJ software (McGrath et al., 2018). Defect depths did not overlap with normal perikymata
depths, and were thus considered reliable indicators of disruptions to enamel growth (McGrath et al., 2018).

All histological analyses were conducted using Adobe Photoshop CC2015. Canine crowns were divided into deciles of crown height by measuring the curvilinear distance of the EDJ from the dentine horn to the cervix. The EDJ was then divided into 10 segments or deciles of equal length. Markers were placed on closely-associated striae of Retzius to designate the boundaries between the deciles. The crown locations of LEH defects were identified by measuring the distance from the cervix to the defect. That distance was then measured in the corresponding thin section to visually assess whether accentuated lines are present in the same locations. In some cases, the furrows are clearly visible in section. In others, particularly when working with mountain gorillas and their shallow defects, furrows are slight or even imperceptible in section. The ability to reliably reconstruct the correct crown location using this method was verified in cases where defects were clearly visible in section.

The selection criteria used to assess accentuated line presence/absence are: accentuated lines must be darker and/or wider than nearby striae of Retzius, and they must course visibly deeper (i.e., closer to the EDJ) into the section thickness than regular striae (reviewed in Witzel et al., 2008). The presence/absence of accentuated lines was independently assessed by both KM and DJR with >95% agreement; where there was disagreement, KM’s scores were used. Accentuated lines were identified blind to the locations of LEH defects included in this study.

Canine initiation times were calculated by registering the canine to the first permanent molar for two specimens: GP.038 and GP.075 (Fig. 4). To do this, accentuated
lines were examined for a discernable pattern so that they could be registered from the first molar to later-initiating teeth, or in this case, the canine. Once the pattern of accentuated lines was matched (Fig. 4), it was necessary to calculate the age at initiation of the canine. First, the neonatal line, which typically forms at birth (day 0) in the cuspal enamel of great ape and human first molars, was identified. This feature was used to register the timing of tooth development to absolute age. Then, the first discernable accentuated line in the M1 enamel was identified. Age at formation of this first accentuated line was calculated by counting the daily cross striations in the first molar from the neonatal line, along a single prism, to this first accentuated line (Reid et al., 1998). Cross striations were then counted in the canine from the EDJ to the same landmark (Fig. 4). The matching pattern of identified accentuated lines in the M1 and canine is understood to represent synchronous disruptions to enamel growth, and support for this comes from being able to count the same number of daily cross striations between concurrent accentuated lines (Fig. 4). The difference between the cross striation counts in the M1 and the canine provides the age of initiation of the canine, or the “missing” time between birth and when mineralization of the canine crown began.

Once initiation timing is established, cuspal and imbricalional formation times can be calculated. In this study, canine cuspal formation times were calculated by counting individual cross striations along a single prism from the EDJ to the first imbricalional stria. These values were compared to estimates of cuspal formation time calculated by measuring the curvilinear distance along the same prism and multiplying by the mean daily enamel secretion rates in the inner and outer cusp regions (Dean, 1998). The latter estimate was very similar to the direct calculation of cuspal formation time in
both specimens, with only a few days of variation between the methods. Imbricational enamel formation times were calculated by counting the number of imbricational striae of Retzius (i.e., those that meet the outer enamel surface), and multiplying by the striae of Retzius periodicity of that individual (Reid et al., 1998). Striae of Retzius periodicity was calculated by counting the number of cross striations between consecutive striae (Fig. 1) in a minimum of three different areas of the crown. The timing of defects was readily determined by their relative location among imbricational striae for which we established chronological ages at formation. For GP.033, the first molar was not available for analysis, so the canine initiation time was estimated by averaging the values of the other two specimens to provide an approximate chronology of enamel growth disruptions (Table 2). Striae of Retzius periodicity, initiation times, and cuspal and imbricational formation times were independently collected by KM, SCM, and DJR with >95% agreement. All calculations were conducted without access to associated data about these specimens; health records were evaluated following all other analyses.
Figure 4. Mesiobuccal cusp of the first permanent molar (top row), and canine (bottom row), of GP.038 (female). The neonatal line (NNL), representing day 0 or birth, is preserved in the cuspal enamel of the M1. A series of accentuated lines were identified in the first molar and registered in the canine to calibrate the chronology of canine development in the absence of the NNL.
RESULTS

Previous macroscopic inspection of the canine tooth surfaces revealed the presence of several LEH defects in this sample (McGrath et al., 2018). Histological examination of the same crowns showed that all defects included (i.e., those with associated defect depth data from McGrath et al., 2018) were associated with accentuated lines. The internal border of the accentuated line was continuous with the portion of the enamel surface that forms the occlusal wall of the defects. This line delineates the position of the enamel forming front at the time when enamel matrix secretion was disrupted (Fig. 5).

Figure 5. GP.033 (male) canine with a furrow-form hypoplastic defect in the midcrown region. This is the deepest defect that has been measured in male mountain gorillas (McGrath et al., 2018). The accentuated line is continuous with the occlusal wall of the defect. The occlusal aspect of the cusp is to the right.

Tables 2-4 and Figures 2-6 show the results of the study. Table 2 reports canine initiation times of 85-89 days after birth. Cuspal formation times range from 124-208
days, with both males having shorter cuspal crown formation times than both females in this sample. When comparing the three complete crowns (i.e., excluding GP.075), it is clear that males have prolonged crown formation times compared to females, as a consequence of longer durations of imbricational enamel formation.

Table 2. Canine enamel growth variables

<table>
<thead>
<tr>
<th>Specimen ID and sex</th>
<th>Enamel initiation (days)</th>
<th>Cuspal formation time (days)</th>
<th>Enamel crown formation time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP.038 (F)</td>
<td>85 days after birth</td>
<td>161 days</td>
<td>182 imbricational striae x 8 = 1,456 days + 161 days of cuspal = 1,617 days (4.43 years)</td>
</tr>
<tr>
<td>GP.030 (F)</td>
<td>M1 not available; estimate = 85+89/2 = 87 days (estimate)</td>
<td>208 days</td>
<td>200 imbricational striae x 7 = 1,400 days + 208 days of cuspal = 1,608 days (4.41 years)</td>
</tr>
<tr>
<td>GP.075 (M)</td>
<td>89 days after birth</td>
<td>132 days</td>
<td>270 imbricational striae x 6 = 1,620 days + 132 days of cuspal = 1,752 days* (4.81 years)</td>
</tr>
<tr>
<td>GP.033 (M)</td>
<td>M1 not available; estimate = 85+89/2 = 87 days (estimate)</td>
<td>124 days</td>
<td>297 imbricational striae x 7 = 2,079 days + 124 days of cuspal = 2,203 days (6.04 years)</td>
</tr>
</tbody>
</table>

*N.B. GP.075 died just before canine crown completion; the last-formed enamel was lost post-depositionally, so the age here does not match the age at death of the individual.
Table 3 shows co-occurrence of hypoplastic defects with accentuated lines. Every LEH defect with associated depth measurements (from McGrath et al., 2018) was found to co-occur with an accentuated line. In one case (defect A), there are two additional accentuated lines in the deepest part of the defect (Fig. 5). Larger sample sizes are needed to test whether hypoplastic defects occur seasonally or with other discernable regularity. However, the months of December and January appear to be overrepresented in the sample with 6 of 17 defects occurring during these months which fall during the lesser dry season in Rwanda. All LEH defects reported here occur in the midcrown, or the middle 3/5ths of crown height, where defects are most likely to be macroscopically visible (Hillson & Bond, 1997). Deeper surface defects were found to correspond with darker and broader accentuated lines (e.g., Figs. 4, 6). This was true in all three specimens with associated depth data (GP.038, GP.075, GP.033).

Two specimens (GP.038, GP.033) were known to have experienced snare injuries during early life. The dates of snare removal by the Mountain Gorilla Veterinary Project are known and correspond to LEH defects with associated accentuated lines. Though the corresponding LEH defects in GP.038 did not have associated depth information, they were identified on the surface prior to analysis, and they are marked with a star in Figure 2. GP.038 experienced two snare removals, one on day 1,396 (at 3.81 years of age) and the other on day 1,530 (at 4.19 years of age). Both events correspond to the day with accentuated lines that are visible in the section (Fig. 8). GP.033 experienced one snare removal on day 1,461 of development, and that event corresponds to an accentuated line and hypoplastic defect K (Table 3), estimated to have occurred on day 1,463.
Figure 6. GP.075 (male) canine thin section showing the defects pictured in Fig. 3. All three hypoplastic defects correspond to accentuated lines in thin section. Deeper defects are associated with darker and broader accentuated lines that course more deeply into the section thickness than shallower defects within individuals. Depth values were measured using surface scans collected using optical profilometry. Scale bar is 250 microns.
### Table 3. Hypoplastic defect timing and descriptions

<table>
<thead>
<tr>
<th>Specimen ID and timing of enamel crown formation</th>
<th>Hypoplastic defect type(s) and locations (by decile of crown height)</th>
<th>Timing of associated accentuated line(s) (chronological in days)</th>
<th>Defect depth (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GP.038 (F)</strong>&lt;br&gt; (4.43 years of crown formation; from age 0.23 to 4.66 years)</td>
<td>(A) Furrow and accentuated line (Day 808); subsequent accentuated lines in D6 (Fig. 7)</td>
<td>Day 808 (11/15/1993; 2.21 years) Day 824 (12/01/1993; 2.26 years) Day 832 (12/09/1993; 2.28 years)</td>
<td>17.33µm</td>
</tr>
<tr>
<td></td>
<td>(B) Furrow and accentuated line in D8 (Fig. 8)</td>
<td>Day 1096 (08/30/1994; 3.00 years)</td>
<td>23.59µm</td>
</tr>
<tr>
<td><strong>GP.075 (M)</strong>&lt;br&gt; (4.81 years of crown formation; from age 0.24 to 5.05 years)</td>
<td>(C) Furrow and accentuated line in D7 (Fig. 6)</td>
<td>Day 1210 (12/07/1996; 3.32 years)</td>
<td>18.15µm</td>
</tr>
<tr>
<td></td>
<td>(D) Furrow and accentuated line in D8 (Fig. 6)</td>
<td>Day 1346 (04/22/1997; 3.69 years)</td>
<td>14.29µm</td>
</tr>
<tr>
<td></td>
<td>(E) Furrow and accentuated line in D9 (Fig. 6)</td>
<td>Day 1536 (10/29/1997; 4.21 years)</td>
<td>27.10µm</td>
</tr>
<tr>
<td><strong>GP.033 (M)</strong>&lt;br&gt; (6.04 years of crown formation; no M1 so initiation time estimated at 87 days)</td>
<td>(F) Furrow and accentuated line in D2</td>
<td>Day 378 (01/01/1993; 1.04 years)</td>
<td>16.67µm</td>
</tr>
<tr>
<td></td>
<td>(G) Furrow and accentuated line in D3</td>
<td>Day 637 (09/17/1993; 1.75 years)</td>
<td>12.92µm</td>
</tr>
<tr>
<td></td>
<td>(H) Furrow and accentuated line in D5 (Fig. 5)</td>
<td>Day 943 (07/20/1994; 2.57 years)</td>
<td>27.35µm</td>
</tr>
<tr>
<td></td>
<td>(I) Furrow and accentuated line in D6</td>
<td>Day 1155 (02/17/1995; 3.16 years)</td>
<td>25.48µm</td>
</tr>
<tr>
<td></td>
<td>(J) Furrow and accentuated line in D6</td>
<td>Day 1197 (03/31/1995; 3.28 years)</td>
<td>10.99µm</td>
</tr>
<tr>
<td></td>
<td>(K) Furrow and accentuated line in D7</td>
<td>Day 1463 (12/22/1995; 4.01 years)</td>
<td>11.61µm</td>
</tr>
<tr>
<td></td>
<td>(L) Furrow and accentuated line in D7</td>
<td>Day 1491 (01/19/1996; 4.08 years)</td>
<td>6.16µm</td>
</tr>
<tr>
<td></td>
<td>(M) Furrow and accentuated line in D7</td>
<td>Day 1505 (02/02/1996; 4.12 years)</td>
<td>7.32µm</td>
</tr>
<tr>
<td></td>
<td>(N) Furrow and accentuated line in D8</td>
<td>Day 1554 (03/22/1996; 4.26 years)</td>
<td>12.99µm</td>
</tr>
<tr>
<td></td>
<td>(O) Furrow and accentuated line in D10</td>
<td>Day 2072 (08/22/1997; 5.68 years)</td>
<td>16.66µm</td>
</tr>
</tbody>
</table>

**N.B.** GP.030 (F) is not included in this table because it lacks associated defect depth data.
Figure 7. GP.038 (female) canine thin section at defect A in the midcrown and the associated digital elevation model. Perikymata on the surface can be matched to underlying striae of Retzius, including three accentuated lines. Each line is labeled with a number that represents the age of the individual in days when ameloblast secretory activity was intermittently impaired. Scale bar is 250 microns.

Figure 8. GP.038 (female) canine thin section at the cervical end. Two stressful events – snare removals – correspond to hypoplastic defects (more clearly visible in snare removal #1) and accentuated lines in enamel and dentine. Scale bar is 250 microns.
Table 4 shows the total number of accentuated lines in these specimens and their developmental timing. Accentuated lines were counted by decile, starting with the cusp (D1) and ending with the cervix (D10). Here a pattern emerges: accentuated lines occur most frequently in the cervix, or the last-formed enamel. While all specimens had accentuated lines in every decile, GP.075 showed the most extreme condition with 101 accentuated lines in total. This individual died just before canine crown completion. It is not clear whether this and the other specimens are representative of Virunga mountain gorillas as a population, and whether this level of disruption is a common occurrence among great apes.
*Table 4. Total accentuated lines in canine thin sections*

<table>
<thead>
<tr>
<th>Specimen ID and timing of enamel crown formation</th>
<th>Number of accentuated lines by decile of crown height</th>
<th>Timing of deciles (chronological)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Years</td>
</tr>
<tr>
<td>GP.038 (F) (4.43 years of crown formation – from age 0.23 to 4.66 years)</td>
<td>D1 – 2, D2 – 4, D3 – 4, D4 – 4, D5 – 3, D6 – 4, D7 – 2, D8 – 5, D9 – 7, D10 – 8</td>
<td>85 – 144, 145 – 248, 249 – 384, 385 – 568, 568 – 720, 721 – 880, 881 – 1096, 1096 – 1280, 1281 – 1480, 1481 – 1702</td>
</tr>
<tr>
<td>GP.030 (F) (4.41 years of crown formation – no initiation time available, but estimated at 87 days)</td>
<td>D1 – 4, D2 – 3, D3 – 2, D4 – 4, D5 – 5, D6 – 3, D7 – 5, D8 – 7, D9 – 9, D10 – 7</td>
<td>87 – 224, 225 – 343, 344 – 476, 477 – 602, 603 – 749, 750 – 931, 932 – 1155, 1156 – 1337, 1338 – 1519, 1520 – 1695</td>
</tr>
<tr>
<td>GP.075 (M)* (4.81 years of crown formation – from age 0.24 to 5.05 years)</td>
<td>D1 – 6, D2 – 5, D3 – 4, D4 – 7, D5 – 10, D6 – 9, D7 – 8, D8 – 11, D9 – 21, D10 – 20</td>
<td>89 – 252, 253 – 420, 421 – 624, 625 – 786, 787 – 972, 973 – 1134, 1135 – 1320, 1321 – 1482, 1483 – 1656, 1657 – 1842</td>
</tr>
</tbody>
</table>

*The positioning of these deciles in GP.075 is impacted by loss of cervical enamel/incomplete crown formation and thus may not be fully equivalent to deciles in the complete specimens.*
Figure 9. GP.075 (male) third permanent molar distolinguinal cusp. A plane-form defect and associated accentuated line is indicated by the blue arrowheads. An accentuated line internal to this defect is also evident, indicated by orange arrows. The timing of this major plane-form defect (calculated for another ongoing study in our lab) coincides with the middle of canine crown formation. The canine lacks a similar plane-form defect, while this molar is missing more than half of its cuspal growth increments.

Figure 9 shows that there were several growth disruptions that manifest as accentuated lines in the earliest part of M3 cusp formation in GP.075, followed by the line marked by the pink arrow, which had a profound effect on the contour of the striae, visible as a peak between the two pink arrows. The plane marked by the outer blue arrows is where growth totally stopped across the cusp (Fig. 8). A matching accentuated line formed at this time in all concurrently forming teeth, including the canine, but none of the other teeth exhibit plane-form defects of this nature.
DISCUSSION

This study provides the first direct assessment of the co-occurrence of LEH defects and accentuated lines in gorillas. The results suggest that where there are macroscopically-visible LEH defects, there are underlying accentuated lines. This mirrors the co-occurrence reported by Suckling and Thurley (1984) in sheep, as well as by Condon (1981) in modern humans. However, the reverse is not always true as there were many more accentuated lines than LEH defects in all four sections. Deeper LEH defects were associated with darker and broader accentuated lines that course more deeply into the enamel thickness. These results differ from those reported by Witzel et al., (2008), in which one specimen clearly had a hypoplastic defect that was not accompanied by an accentuated line in section. There are remarkably few analyses that incorporate both a surface and a histological component in any species, and more studies are needed to further explore the mechanism(s) behind enamel defect formation.

We found that two individuals with known snare injuries (GP.038, GP.033) exhibit accentuated lines that co-occur with the removal of snares based on veterinary records. This corresponds to what was reported by McFarlin et al. (2014) – an infant gorilla was captured, treated, reintroduced to another social group, injured again and eventually died, and all events corresponded to accentuated lines in the developing molars. While the accentuated lines are clearly identifiable, the associated hypoplastic defects are highly variable. The second snare removal for GP.038 occurred in the 10th decile and corresponds to what looks like a very shallow defect that is yet to be quantified in depth, and was thus not included in Table 3, while the first snare removal in D9 is clearly associated with a hypoplastic groove, also yet to be quantified (Fig. 8). The
associated defect in GP.033 is macroscopically visible and has been measured at 11.61 microns in depth (K, Table 3). This is not particularly deep and actually sits at the low end for male mountain gorillas (McGrath et al., 2018). There are two deeper defects (H and I, Table 3) that formed well before the treatment by veterinarians, and might be attributed to other stressors yet to be investigated.

The specimen with the highest number of accentuated lines is GP.075, a male mountain gorilla that died just before canine crown completion at the age of 6.08 years. Smith and Boesch (2015) conducted a similar analysis in wild chimpanzees. Of their three study individuals, the one with the highest number of accentuated lines was a juvenile that showed slow skeletal growth and prolonged maternal dependence (Smith & Boesch, 2015). They were able to match some, but not all, accentuated lines to injuries and disease outbreaks in the Taï forest community (Smith & Boesch, 2015). They also found a significant negative correlation between accentuated line frequency and rainfall, but no relationship with fruit availability nor significant annual trends. The dry months of December and January are overrepresented in the timed LEH defect sample reported here (Table 3), mirroring what was reported by Smith & Boesch (2015) for accentuated lines. The time period in which our study animals developed their dentitions unfortunately does not have associated rainfall or temperature data, but we plan to assess the correspondence of health and life history events with enamel defects in the future. Even if we are unable to link defects to life history events, this study demonstrates that the timing of the disruption to enamel is a crucial factor determining the morphology of the resulting defect. As this one individual shows, a disturbance that manifests as a simple accentuated
line in the midcrown of the canine, one among >100 others, instead manifests as a major plane-form defect in the corresponding cuspal M3 (Fig. 9).

Hillson & Bond (1997) suggested that plane-form defects in which all of the cuspal enamel layers above the affected plane are totally missing are rare events. The corresponding region of the canine crown in GP.075 is marked by a faint to moderate accentuated line, and the midcrown region in general is occupied by several shallow hypoplastic defects, but none stand out as representing major disruptions to enamel matrix secretion (Fig. 3). If abnormally-spaced perikymata represent growth disruptions, it is often assumed that the extreme phenotype of this kind of plane-form defect represents a large, long-lasting, or severe disruption (Hillson & Bond, 1997). But the example provided here complicates that notion. GP.075’s canine does not exhibit any particularly deep defects for male mountain gorillas, thus the M3 defect morphology is likely due to the timing of the growth disruption rather than the severity or duration of the insult alone. Indeed, it has been argued that the width of the exposed stria has no relationship with the duration of the disruption in cuspal enamel, as it does in imbricational enamel (Hillson & Bond, 1997).

Accentuated lines occur more frequently overall and across all deciles, thus providing a more comprehensive history of developmental disturbances than provided by LEH defects (Witzel et al., 2008). They also occur most often in the cervix. However, the question remains – is this because the mountain gorilla individuals examined here were pathological, as evidenced by their early deaths, or is this typical and related to either the packing of striae in that crown region, or to the influence of some yet-to-be-identified stressor? Hassett (2014) demonstrated that where perikymata are more densely packed,
such as in the cervical region of modern human anterior tooth crowns, surface defects are often “missed” using traditional qualitative techniques. However, in orangutans, O’Hara (2017) found that while there is some variation in the number of perikymata per decile of crown height, they lack the characteristically tightly-packed perikymata pattern typical of the cervical region in modern humans. O’Hara’s results are supported by our recent work (McGrath et al., in prep), which suggests that some great ape species exhibit an increase in enamel extension rates at the cervix. High enamel extension rates are understood to relate to more spaced out perikymata, while slower rates are reflected in more tightly packed perikymata (Hillson & Bond, 1997).

The canine initiation times reported here are early compared to those for chimpanzees (Reid et al., 1998) and a captive gorilla (Schwartz et al., 2006). Our estimates range from 85-89 days after birth compared to >120-40 days in the other species. Virunga mountain gorillas also exhibit faster rates of enamel secretion across all deciles of crown height and earlier canine crown completion times compared to other taxa (McGrath et al., in prep). This fast canine crown formation, in both sexes but especially in males, is linked to the shallow defect morphology that inspired this work. The shallow defects in mountain gorillas are much harder to reliably identify using qualitative techniques, and this has likely resulted in their being overlooked in previous studies. When comparing complete crowns (i.e., excluding GP.075), it is clear that males have prolonged crown formation times compared to females, with longer durations of imbricational enamel formation as has been previously documented in other great apes (e.g., Schwartz & Dean, 2001; McGrath et al., in prep). When comparing defect counts, it is also important to consider them in relation to the period of vulnerability to accentuated
lines, which is shorter in females compared to males; it is even shorter when only considering hypoplastic defects, which don’t occur in the cuspal enamel (McGrath et al., in prep).

Just as studies of enamel hypoplasia must grapple with poor surface preservation, histological analyses are subject to their own sampling biases, including inconsistent visibility throughout the section, cracks, degradation, as well as imperfections from the sectioning and preparation process. Although the methods used for the identification of accentuated lines might sound straightforward, in practice, it is difficult to establish a clear and consistent threshold to determine what is and what is not an accentuated line. When characterizing the outer enamel surface, McGrath et al. (2018) defined a defect as a groove that was deeper than sex- and species-typical perikymata depths. Unless a new technique is developed to identify variation in mineralization among “normal” stria and “pathological” accentuated lines, the definition remains somewhat subjective as the ability to visualize the structure of growth increments changes throughout the thickness and the length of the section. There is no evidence of missing striae associated with defects; daily prism cross striations and periodicity continue unabated within the walls of defects. As was described by Hillson (2014), not all accentuated lines are clearly associated with a particular day or days outside the long-period rhythm; disruptions may coincide with regular periodicity in some cases. Hillson (2014) argues that this fits with what we know about the cause of developmental defects of enamel, namely illness, malnutrition, and social stress (Goodman & Rose, 1990), which might cause a change in the amplitude of the long-period rhythm. Deeper defects are associated with darker and more pronounced accentuated lines that course deeply into the enamel thickness.
compared to shallower defects in the same individual (Figs. 5 & 6). What depth means in
terms of stress severity, however, is not fully understood. McGrath et al., (2018) found
that the deepest defect in the comparative great ape sample occurred in a female western
lowland gorilla that was captured from the wild as an infant, and the developmental
timing of the defect likely corresponds to this event. In mountain gorillas, the maximum
defect depth appears to be constrained by factors like enamel thickness, enamel extension
rates, and geometry of growth increments (McGrath et al., in prep). The current study
demonstrates that major stressors, like injury and veterinary intervention, do correspond
to development defects of enamel as has been reported in the literature for great apes
(Schwartz et al., 2006; McFarlin et al., 2014; Smith & Boesch, 2015), but that the
relationship between the visual or quantitative “severity” of the defect may not be a
reliable indicator of stress severity. These relationships should continue to be tested in
larger samples and ideally incorporating associated life history, health, and climate
records.

Fitzgerald and Saunders (2005) hypothesized that when disruptions to enamel
matrix secretion coincide with the normal long period rhythm, the combined effect would
produce a Wilson band, or accentuated line with associated surface defect. They argued
that if the disruption instead occurs between beats of the long-period rhythm, there would
be a less pronounced effect on the outer enamel surface. Our results do not support this
hypothesis, as the appearance of hypoplastic defects in the imbricational enamel does not
seem to correspond to the timing of the disruption in relation to the long-period rhythm.
For example, Figure 5 shows the deepest furrow-form defect in the male mountain gorilla
sample, which does not coincide with the long-period rhythm, but falls in-between two
consecutive striae. It does not appear that these “extra” lines form additional perikymata on the outer enamel surface, but further work should test that relationship.

Our results can be compared by the threshold model proposed by Kierdorf et al., (2000, 2004) and Witzel et al. (2006), building upon the work of Goodman and Rose (1990) at the cellular level. Witzel et al. (2008) proposed that the timing of the disruption to enamel matrix secretion is a crucial factor in determining the reaction of secretory ameloblasts. When the lowest threshold is crossed, matrix secretion is reduced, but only slightly, leading to a reduction in the width between increments (i.e., perikymata or striae of Retzius), but retaining normal, prismatic enamel structure. The second threshold involves the loss of the distal portion of the Tomes’ process by the ameloblasts, impairing secretory function and creating aprismatic enamel. When the final threshold is passed, the ameloblast ceases enamel secretion completely, and this cessation may be fleeting or permanent. In their study of modern human teeth, Witzel et al. (2008) found marked variation in how ameloblasts along the enamel forming front responded to the same stress events. They reported an instance of a hypoplastic defect without an associated accentuated line, and attributed this to a stress event in which only late secretory ameloblasts were affected due to their higher susceptibility to impairment. We did not find the same phenomenon here, but given that we only evaluated defect co-occurrence in cases where defect depths were available, and in the case of specific snare injuries, it is possible that we missed shallower hypoplastic defects that would have been difficult to identify qualitatively, and might demonstrate this same pattern.

Our results otherwise fit well with what has been proposed by Witzel et al. (2008): we find many accentuated lines without clearly associated hypoplastic defects,
representing homogenous reductions in matrix section of all secretory ameloblasts. We also found several LEH defects, which occur when late secretory ameloblasts cease enamel matrix secretion following a stress event. However, given the disproportionate number of accentuated lines vs. LEH defects, it is difficult to assess the hypothesis of Rose et al. (1985) that the formation of a hypoplastic defect might represent a more severe disruption to enamel matrix secretion than the formation of an accentuated line. An important next step will be to test whether the “extra” accentuated lines that we found in this study correspond to microscopic defects on the outer enamel surface, sometimes called “accentuated perikymata.” If so, the question of which defect type represents a more “severe” disruption to enamel matrix secretion will become even more complicated.

CONCLUSIONS

The vast majority of studies on developmental defects of enamel have focused on the outer enamel surface, limiting the amount of information that can be gleaned about enamel formation. LEH defects co-occur with accentuated lines in all cases in this study, and deeper defects correspond to darker and more pronounced accentuated lines. Major stress events of snare removals correspond to LEH defects with accentuated lines in two specimens, though corresponding LEH defect depths have yet to be measured in GP.038. We found many “extra” accentuated lines throughout all deciles of crown height in the sample. Accentuated lines were most likely to occur in the last deciles, or at the end of enamel crown formation. It is not clear whether this relates to the specimens in this study representing pathological individuals, as they all died at early ages for their species, and two of the individuals are known to have experienced snare injuries and amputations.
during life. One individual, GP.075, demonstrated the highest frequency of disturbances to enamel secretion, with 101 accentuated lines throughout the crown. Future work will test whether these defects, particularly those that exhibit both surface and internal manifestations, correspond with other known events other than snare removals in associated records of these individuals.

Virunga mountain gorillas exhibit earlier canine initiation times than any other great apes described in the literature (Reid et al., 1998; Schwartz et al., 2006). This fits in with what is known about mountain gorilla life history and biology – that they reach their large adult size through accelerated body growth, and that they exhibit earlier ages at weaning and/or and shorter interbirth intervals compared to other great apes, including more frugivorous western lowland gorillas and Bwindi mountain gorillas (Watts et al., 1993; Taylor, 1997; Robbins et al., 2006; Robbins et al., 2009; Stoinski et al., 2013; Galbany et al., 2017).

Our results support the claim by Witzel et al., (2006, 2008) that histological investigation is a crucial step in order to be able to fully assess the enamel growth histories of individuals. In GP.075, an accentuated line in the midcrown of the canine corresponds to a major plane form defect in the third molar. This anecdote provides an important caveat to researchers interested in inferring stress experiences from the outer enamel surface alone as the full story is hidden below the surface.

In addition to incorporating life history records to analyses of individual great apes, future work will test whether “extra” accentuated lines are associated with microscopic surface defects, defined as having abnormal perikymata spacing for that crown region. Abnormal perikymata are typically more widely spaced in humans (e.g.,
Bocaenge & Hillson, 2016), but this relationship has yet to be systematically tested in nonhuman primates. We will also expand this work to include other closely related taxa, including the Bwindi population of mountain gorillas and eastern lowland gorillas.
Chapter 5: Conclusions

Dental development is one of several traits used to infer life history characteristics of hominins (Robson & Wood, 2008). Mountain gorillas are an important species to consider in relation to questions about the rates and patterns of growth and development in hominoids as they represent an ecological and dietary extreme, living at higher elevations, consuming a more folivorous diet, and exhibiting faster body growth compared to other great apes (Fossey & Harcourt, 1977; Watts et al., 1993; Taylor, 1997; McNeilage, 2001; Stoinski et al., 2013; Watts, 1984; Galbany et al., 2017). Comparative studies of the rates and patterns of dental development in extant great apes, particularly those with associated life history, health, and climate data, form a crucial basis upon which inferences can be made about fossil hominins (Smith, 1991). While there have been several foundational studies on canine enamel growth patterns (e.g., Reid et al., 1998; Schwartz et al., 2001; Schwartz & Dean, 2001) and LEH defect depth (Skinner & Pruetz, 2013; Skinner & Skinner, 2017) among great apes, this dissertation addresses aspects of enamel growth that remain poorly understood. Gorillas, which until now have only been represented by western lowland gorilla specimens in histological studies, have long been understood to grow their canines quickly compared to other great apes (Schwartz & Dean, 2001; Schwartz et al., 2001). The data provided in this dissertation suggest that mountain gorillas exhibit accelerated canine growth compared to other great apes, and that faster growth has many downstream effects on the final tooth form, including enamel defect morphology.

This dissertation focuses on linear enamel hypoplasia, a condition marked by linear grooves on the outer tooth surface (Berten, 1895). Linear enamel hypoplasia was
thought to be rare in mountain gorillas while being exceedingly common among all other great apes (Guatelli-Steinberg et al., 2012), but this dissertation suggests otherwise. In Chapter 2, we show that mountain gorillas have significantly shallower defects compared to other taxa, which suggests that they were likely overlooked using traditional methods. We also found that males have significantly shallower defects than females in the combined great ape sample. These results support the proposal by Hillson and Bond (1997) and Guatelli-Steinberg et al. (2012) which suggests that enamel growth variation plays an important role in defect morphology on the outer enamel surface. However, we also found that the deepest defect in the sample was observed in a western lowland gorilla, and the timing of the defect likely corresponds to her capture from the wild as an infant. This suggests that stress severity might also contribute to defect depth within the confines of local enamel geometry. This chapter demonstrates that while a large amount of information can be gleaned from the outer enamel surface, it is necessary to analyze associated histological thin sections to fully understand the role that enamel growth variation plays in defect formation.

In Chapter 3, we assess the relationship between sex- and species-specific variation in enamel growth and defect depth in great apes. We found that mountain gorillas exhibit significantly thinner enamel (particularly in males), faster enamel extension rates, and shallower striae of Retzius angles compared to other taxa. Like the second chapter, males also follow this pattern in the combined sample, having thinner enamel, faster rates, and shallower angles, as a result of sexual dimorphism in growth. We also tested the relationships among these enamel growth variables to determine which best tracks differences in defect depth. We found that enamel extension rates, followed
by striae angles, are most strongly correlated with defect depth, although at least 34% of the variation in defect depth remains unexplained in our models. While previous studies primarily attributed canine dimorphism to the extended duration of canine crown formation in males (Schwartz et al., 2001; Schwartz & Dean, 2001), this study underscores that sex- and species-specific rate differences are also present among great apes, and these differences contribute to documented variation in LEH defect depth (McGrath et al., 2018). This study demonstrates that substantial enamel growth variation can exist in macroscopically similar teeth among closely related taxa. It also expands the documented striae of Retzius periodicities in extant great apes by showing that extant *Pongo* specimens exhibit high periodicities of 12, only previously reported in fossil *Pongo* specimens (Smith, 2016). Most importantly, this study shows that while enamel growth variation contributes to LEH defect morphology, other factors, yet to be explained, also shape defect depth on the outer enamel surface. Stress severity likely plays a role, as suggested by other authors (e.g., Suckling & Thurley, 1984; Kierdorf et al., 2004; Skinner & Hopwood, 2004; Skinner & Pruetz, 2009; Skinner & Skinner, 2017), as may other growth variables not included in this study.

In Chapter 4, we conduct a detailed analysis of three mountain gorilla specimens of known sex and age, and another of unknown age. We test whether LEH defects co-occur with underlying accentuated lines visible in thin section, and found that they do in all cases. We also found that there are many “extra” accentuated lines throughout the canine crowns, and that they occur throughout all deciles of crown height. These results fit with what has been reported by some authors for modern humans and sheep (Condon, 1981; Suckling & Thurley, 1984), but not the partial correspondence that has reported by
other authors focusing on modern human samples (Goodman & Rose, 1990; Witzel et al., 2008). In one specimen, a rare plane-form defect occurred during cusp formation in the third permanent molar, which is missing more than half of the cuspal growth increments. The same defect looks ordinary in the matching canine, represented by a simple accentuated line. This implies that defect timing, as it relates to local geometry, can be a crucial factor in the formation and the appearance of defects. We found that deeper LEH defects correspond to more pronounced accentuated lines in specimens with associated depths. We also found that the removal of poaching-related snares corresponds with accentuated lines and co-occurring LEH defects in two mountain gorilla individuals.

More research incorporating larger sample sizes will increase our understanding of the mechanisms behind defect formation, including their cellular formation, as well as the etiology of individual defects.

This dissertation takes advantage of a special skeletal collection to address questions that are not only of interest to those researchers who study mountain gorillas, but others who use teeth as a proxy for life history or development. We expanded the range of canine crown formation time in great apes to as little as four years in female mountain gorillas to as high as 11 years in male orangutans. We developed new methods to provide the first perikymata depths in nonhuman primates; defect depths in several great ape species; enamel extension rates in great apes; canine initiation and formation times, among other results. We demonstrate that enamel growth plays an important, but not determinate, role in shaping LEH defect morphology in great apes by testing the hypotheses proposed over the last several decades in a diverse sample of great apes. Much is still yet to be learned about these relationships, especially the role that stress
severity might play in shaping LEH defect morphology. However, the methods and results in this dissertation can be used to help researchers to extract useful information from other mammalian samples. Crucially, and thankfully, this work builds on decades of careful histology and microscopy by generations of researchers who are also enamored by these seemingly simple, yet woefully complex enamel surface features.
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