THE PREVALENCE AND TIMING OF ENAMEL HYPOPLASIA IN THE BONOBO, *Pan paniscus* [Coolidge, 1933]

by

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TITLE OF THESIS/PROJECT/EXTENDED ESSAY

The Prevalence and Timing of Enamel Hypoplasia in the Bonobo, *Pan paniscus* [Coolidge, 1933]

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ABSTRACT

Systematic analysis of prevalence and timing of developmental stress in the bonobo, *Pan paniscus*, is accomplished through examination of enamel hypoplasia (EH), a non-specific stress-induced defect permanently recorded in the developing dentition.

Bonobo crania (n=182) housed at the Royal Museum for Central Africa in Tervuren, Belgium, are grossly examined under natural and artificial oblique lighting for presence of EH. Three methods are employed to determine the timing of linear enamel hypoplasia (LEH). (1) Imbricational enamel formation schedules are created for bonobo teeth (n=6) by counting the number of perikymata from the cemento-enamel junction to the occlusal surface. (2) Caliper measured defects are calculated as a proportion of crown height and multiplied against crown formation times for bonobo and chimpanzee to establish age at which defects form (n=490 teeth). (3) Using scanning electron microscopy, tooth crown surfaces (n=22) are examined and perikymata counted within and between enamel defects.

Bonobos are very stressed; 100% of adults (n=58) are affected with LEH – the highest prevalence reported for any ape. Bonobos also yield a high prevalence of pitting and localized hypoplasia of the primary canine but not for Darcy's Defect, indicating these primates are more impacted by or susceptible to physiological stressors than other apes. Distribution, however, is consistent with other apes, i.e., anterior teeth demonstrate a higher degree of EH; LEH and pits are common in permanent and deciduous dentitions, respectively. No significant differences exist between sexes.

Imbricational enamel formation times for the bonobo are 2.9 and 3.4 years for the maxillary central (n=3) and lateral incisors (n=1), respectively, 3.3 years for the mandibular lateral incisor (n=1), and 5.2 years for the mandibular canine (n=1). Onset of LEH occurs around 2 to 3 years of age and continues throughout crown formation. LEH is found to be episodic, averaging 6-month intervals, and stress lasts 6 to 8 weeks. The

periodic nature of LEH is attributed to seasonal rainfall and disease cycles (e.g., parasitic infection) rather than nutritional stress. Teeth picked up milder microscopic episodes of stress suggesting a chronic problem exacerbated at various times, including seasonal moisture cycles.

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CHAPTER 1: INTRODUCTION

Teeth, according to Stanley M. Garn, are "a rich storehouse of individual historical events" (Larsen 1994:5 citing Garn 1976:454). Enamel hypoplasia is a sensitive but non-specific marker of physiological or metabolic stress; nevertheless "there is a deep-seated desire to obtain a definitive diagnosis: to determine the exact cause of the observed lesion and to discourse on the effects which this disease must have had on the individual" (Waldron 1994:5). While not a disease, the dental enamel defect known as enamel hypoplasia is one such "lesion" that has been the focus of much speculation as to its exact cause in both animals and humans.

As one of the more common dental enamel defects (El-Najjar *et al.* 1978:185; Hillson 1986:129; Newell 1998:27 citing Nikiforuk and Fraser 1981), enamel hypoplasia is also "one of the most commonly recorded dental features" (Hillson 1994:107). Diagnostically, enamel hypoplasias are macroscopic enamel defects on the tooth crown surface. They are clinically defined as a "deficiency of enamel thickness, disrupting the contour of the crown surface, initiated during enamel matrix secretion" (Hillson 1996:165). While the term includes all defects in enamel thickness, all of these hypoplasias are thought to result from among three different conditions: heredity, localized trauma, or systemic metabolic stress (Goodman and Rose 1991:281, 1990:64). The latter stress has been the subject of hundreds of clinical and epidemiological studies in humans and a few experimental studies in animals.

Until recently, published research concerning the study of enamel hypoplasias in non-human primates was sporadic and atheoretical. Even with the proliferation of research involving a large portion of the Primate Order at the turn of the 21st century, the bonobo, or pygmy chimpanzee (*Pan paniscus*) has not been examined thus far.

MOTIVATION AND RESEARCH OBJECTIVES

Why should enamel hypoplasia (EH) in the bonobo be studied?

- The bonobo has been ignored in EH studies involving non-human primates. This neglect may be attributed to the fact that the Royal African Museum in Belgium houses the only extensive collection of bonobo skeletons, and access to the museum may be limited by travel constraints.
- A new dimension will be added to the small knowledge base for this primate by an examination of EH in the bonobo. As de Waal and Lanting (1997:xiv) state, "a single cardboard box will do for a complete collection of literature on bonobos."
- Standard frequencies of intra/inter-tooth distributions between sexes, deciduous and permanent dentitions, and type of EH may be established for comparative purposes within the Primate Order.
- The presence and distribution of EH in the bonobo may be compared to EH in the other *Pan* species and its subspecies. Differences may be attributed to a variety of potential explanations (e.g., climate, geography, availability of resources, etc.).
- The manifestation of EH in the smaller dentition of this chimpanzee species can be observed.
- The age at which the bonobo experiences stress may be compared with the common chimpanzee. Ageing of EH may be elucidated from the counting of incremental crown surface structures known as perikymata.
- The counting of these perikymata may lead to the estimation of crown formation times. Differences in interspecies crown formation rates may account for differences in the manifestations of EH.
- By studying the association of perikymata with EH in the bonobo, contributions will be made to the "comparative body of evidence on enamel microstructures in living primates, especially the great apes" (Mann *et al.* 1990:131).
- Because EH may be considered a timed physiological stress, this enamel defect imparts information about the ecology of this animal and its adaptation to its habitat. This raises questions concerning the conservation of this species.

By examining enamel hypoplasia in the bonobo, a number of contributions will be made to the interdisciplinary fields of physical anthropology, dental anthropology, primatology, paleopathology, and zoology. The objectives of this proposed research, therefore, are two-fold: (1) to conduct a systematic distribution of the frequency of enamel hypoplasias in the bonobo, and (2) to correlate the timing of defects to potential stressors (e.g., moisture and/or disease cycles) as gleaned from perikymata counts from tooth crown surfaces.

SPECIFIC RESEARCH QUESTIONS

Schultz and colleagues (1998:298) identify three standard methods for the morphological investigation of enamel hypoplasias: macroscopic, microscopic, and histological examinations. All of these methods are employed in this study of enamel hypoplasia in the bonobo.

From the macroscopic examination of the bonobo teeth, the following research questions will be addressed:

1) Does the bonobo demonstrate a high frequency of EH within the Primate Order?
2) Are there sex differences in the prevalence of EH?
3) Do certain tooth classes preferentially express EH?
4) Do primary and secondary dentition waves differentially express pitting and linear enamel hypoplasia (LEH)?
5) At what age(s) does the bonobo express EH?

With the use of scanning electron microscopy (SEM), additional questions will be explored:

6) At what age does the bonobo express EH?
7) Is the presence of EH episodic in nature?
8) Are the same number of perikymata expressed between enamel hypoplasias?
9) What is the duration within and between stress intervals?

Lastly, in order to age appropriately the enamel hypoplasias using perikymata counts, the periodicity of this microstructure needs to be established for the bonobo. This can be accomplished through the histological examination of cross-striations, circadian micro-increments, which lie between striae of Retzius which are, in turn, manifested as perikymata on the tooth crown surface. Thus the final question raised is the following:

10) Are the number of cross-striations between the striae of Retzius and hence the perikymata indeed representative of weekly time intervals?

EXPECTED RESULTS

From the descriptive analysis, it is expected that the prevalence and timing of EH within the bonobos' dentition, will parallel that of common chimpanzees. The frequency

of EH will be consistent with those reported for other large apes; the anterior and mandibular teeth will demonstrate a higher degree of EH; and pitting defects will be more common in the deciduous dentition. However, there will not be as marked a difference in the degree of EH among the sexes as there is with common chimpanzees due the differences in bonobo and common chimpanzee social organization. Also, if semi-annual and annual rains play a role in cyclical stress (as suggested by Skinner 1986b, Skinner *et al.* 1995, Skinner 2000, Skinner and Hopwood 2000, Skinner and Hopwood 2003), then those bonobos closer to the equator will experience a lower frequency of EH because the marked difference in rain cycles decreases as one nears the equator. It also is anticipated that the timing of these enamel defects from the perikymata counts is related to semi-annual and annual meterological events that may be further linked to disease and nutrition (i.e., the availability of resources).

ORGANIZATION OF THIS THESIS

The literature review, Chapter 2, follows this introductory chapter. Chapter 2 contains a general overview of dental anatomy, histology, and enamel hypoplasias. Enamel hypoplastic studies involving non-human primates are elaborated upon, with emphasis on chimpanzee studies. Techniques involving the ageing of enamel defects are briefly discussed, and the use of scanning electron microscopy to aid in determining the timing of enamel hypoplasia is synthesised.

Chapter 3 summarizes the materials and methodologies employed in this study. The skeletal collection, macroscopic and microscopic data collection procedures, and statistical analyses are also described in this chapter.

Results are presented in two chapters. The prevalence inventory, (i.e., macroscopic analyses), is presented in Chapter 4, whereas the timing and periodicity study results, (i.e., the microscopic analyses), are presented in Chapter 5.

The final chapter, Chapter 6, contains a summary of the bonobo findings and their implications, as well as recommendations for further investigation.

CHAPTER 2: LITERATURE REVIEW

Researchers in archaeological and anthropological studies record enamel defects in teeth so that disease, health, and nutritional status of individuals can be inferred (Capasso and Goodman 1992:11; Clarkson 1989:96; Dobney and Ervynck 1998:263). The appearance and distribution of enamel defects vary throughout the dentition (Suckling and Thurley 1984:357). The most common groups of enamel defects are enamel hypoplasias (El-Najjar *et al.* 1978:185; Hillson 1986:129; Newell 1998:27 citing Nikiforuk and Fraser 1981). According to Hillson (1994:107), they are "one of the most commonly recorded dental features." In general, enamel hypoplasias are used to infer non-specific systemic stress in an individual. The ageing of these enamel defects is also possible through a number of means. By determining the timing of enamel hypoplasias, the age at which the individual experienced systemic stresses may be inferred. Knowing the age permits the researcher to develop hypotheses concerning the life history of the individual by examining various developmental, behavioral, and environmental conditions.

This chapter contains a review of investigations of enamel hypoplasias among non-human primates. General dental morphology and histology are reviewed, with a focus on enamel microstructures. Classes, scoring methods, and etiologies of enamel hypoplasia are examined and studies involving non-human primates are briefly described. Methods used to age enamel defects are outlined and the use of scanning electron microscopy to study the timing of enamel hypoplasia is then reviewed.

DENTAL ANATOMY AND HISTOLOGY

According to Skinner and Goodman (1992:153), "the finer points of tooth formation and the appearance of histological structures are not readily expressed nor is their meaning agreed upon." Furthermore, our understanding of developmental defects of

enamel and their application to anthropological problems is inhibited by uncertainties in specifics of tooth morphology, formation, and variation within populations (Mann *et al.* 1990:113). That being said, general tooth formation is briefly reviewed here in order to contextualize enamel hypoplasia and the methods used to study this enamel defect.

Gross Morphology

The two main components of the tooth are the crown and the root (Hillson 1996:8, 1986:9). The crown projects into the oral cavity and is coated with enamel, a largely inorganic tissue. Anchored in the jaw is the root, which is coated with a thin layer of cementum, a calcified collagen. The core of the tooth is comprised of a tissue known as dentine which surrounds the pulp chamber that contains the blood and nerve supply to the tooth (Hillson 1996:8, 1986:9).

Three types of "boundaries" are created where these tissues meet (Hillson 1996:8). The cemento-enamel junction (CEJ) divides the tissues of the crown and root. The cement-dentine junction (CDJ) and the enamel-dentine junction (EDJ) are created at their respective interfaces.

Histology

Enamel is a non-cellular, inorganic, highly mineralized tissue. The cells responsible for enamel production are the ameloblasts and the process of enamel formation, known as amelogenesis, occurs in two stages (Hillson 1996:148, 1986:113; Skinner and Goodman 1992:153). The first stage is matrix production in which the ameloblasts secrete an organic matrix containing amelogenin, an enamel matrix protein (Hillson 1986:114). The second stage, maturation, involves the replacement of the amelogenin with apatite to form the mature enamel (Hillson 1986:114). What makes dental enamel unique is its highly inorganic phase of hydroxyapatite crystals, $Ca_{10}(PO_4)_6(OH)_2$, that comprise 96 - 97 % of the mature enamel by weight (Hillson 1986:113).

These hydroxyapatite crystals are further organized into bundles called prisms (Hillson 1996:149, 1986:115). The prisms vary in orientation and form three different

packing patterns that are classified as pattern 1, pattern 2, and pattern 3 enamels (Boyde and Martin 1984a:344; Hillson 1996:149, 1986:115; Martin *et al.* 1988:1506). These different patterns are attributed to the arrangement of Tomes process pits of the ameloblast (Martin and Boyde 1984a:347). Boyde and Martin (1984a), Boyde *et al.* (1988), Levalle *et al.* (1977), and Martin *et al.* (1988) review the investigations of prism packing patterns of non-human primates.

Incremental Structures of Enamel

From thin sections of teeth, two normal types of developmental markings can be identified in dental enamel: cross-striations and striae of Retzius (Bromage and Dean 1985:535; Dean 1987:158; Dean 1989:163; Dean and Wood 1981:122-123; FitzGerald and Rose 2000:167; Lavelle *et al.* 1977:199). Cross striations are thought to represent daily growth increments that are situated 3 to 5 μm along each prism in human teeth (Boyde *et al.* 1988:1485; Lavelle *et al.* 1977:199).

The striae of Retzius, on the other hand, represent roughly a weekly interval of time and occur at larger intervals of 30 μm each in humans (Lavelle *et al.* 1977:199). Under the microscope, these striae appear as "exaggerated incremental lines" spaced at fairly regular intervals (Boyde 1990:239). Thus, "each of the striae represent the position and shape of the developing enamel front at a point in time" (Dean and Wood 1981:123). In humans, 7 to 8 cross-striations have been observed between these intervals, hence the inference of a weekly time period (Boyde 1990:239; Boyde *et.al.* 1988:1485; Hillson and Jones 1989:97). The periodicity of these lines, however, is representative of an "unknown cycling phenomenon" (Boyde 1990:245; Skinner and Goodman 1992:154).

When the striae of Retzius approach the external surface of the crown, they overlap to give a "stepped" appearance (Lavelle *et al.* 1977:199). This overlapping causes the surface topography of the crown to be arranged in a series of incremental rings known as perikymata (Hillson and Jones 1989:96; Lavelle *et al.* 1977:200; Risnes 1985:185). That is, the striae of Retzius are expressed as perikymata on the external surface of the crown (Boyde 1990:230; Boyde *et al.* 1988:1485; Bromage and Dean

1985:525; FitzGerald and Rose 2000:169; and Martin *et al.* 1988:1511), and constitute imbricational enamel.

Under the scanning electron microscope, the incremental arrangement of rings appears to be uniform and these rings emerge as alternating bands of smooth ridges and grooves (Lavelle *et al.* 1977:200; Risnes 1985:185). The grooves are comprised of "the remains of pits in the ends of prisms once occupied by the Tomes processes of the ameloblasts" whereas the smooth areas are "prismless" (Lavelle *et al.* 1977:200). Risnes (1985) examined the circumferential configuration of the perikymata and determined that the ridges and grooves formed closed circles and were not aligned in a continuous spiral.

The "wave-like undulations" of the perikymata (Boyde 1990:239; Hillson 1996:163; Risnes 1985:185) are shallow at the occlusal third of the crown and become more sharply defined as they near the cervix of the tooth (Hillson 1996:163, 1989:100). Not all striae of Retzius can be manifested as perikymata at the cusp tip (Mann *et al.* 1990:119). During the initial period of mineralisation, a variable amount of cuspal enamel forms and "never appears on the surface of a fully grown tooth" (Reid and Dean 2000:135). Thus striae in this cusp tip are referred to as hidden striae of Retzius and characterize cuspal appositional enamel also known as "buried enamel" (Dean 1987:162; Mann *et al.* 1990:119). In contrast, surface enamel, which is characterized by pronounced perikymata, is often referred to as imbricational enamel (Mann *et al.* 1990:119). Permanent central incisors and permanent canines "express a greater proportion of growth increments on the surface as perikymata than do any other teeth" (Dean 1987:162), i.e., molariform teeth typically have more buried enamel.

Because of these marked differences in visibility of perikymata, Hillson (1996:161-163) with Bond (1997:91 citing Boyde 1971) define three types of perikymata based on the regions in which they are found. The occlusal type is demonstrated by "broad, shallow waves" and Hillson and Bond (1997:91) identify this type as the classic perikymata form. The mid-crown type retains the wave-like form; however, the spacing between the grooves is closer. The cervical type is identified as the "classic imbrication line form," which displays sharper grooves and ridges (Hillson and Bond 1997:91). Beynon and Reid (1987:889) suggest the "increased density" of perikymata in the

cervical region is a result of a "slowing of crown formation with an increase in crown height."

Scanning electron micrographs of Tomes' process pits, striae of Retzius, and perikymata are presented in Figure 2-1, and the relationship of some these structures to the tooth crown surface is shown in Figure 2-2.

ENAMEL HYPOPLASIA

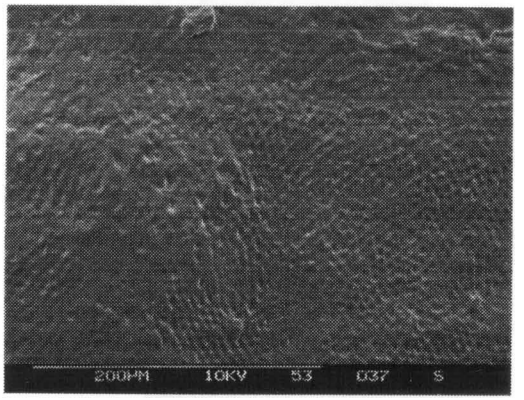
Enamel hypoplasias are macroscopic enamel defects on the tooth crown surface. Clinically, they are defined by Hillson (1996:165) as a "deficiency of enamel thickness, disrupting the contour of the crown surface, initiated during enamel matrix secretion."

Sarnat and Schour (1941) cite Otto Zsigmondy (1893) as the first to define enamel hypoplasias. They were first defined as "deficiencies in enamel thickness resulting from physiological stress" (Hillson 1986:129 citing Sarnat and Schour 1941; Newell 1998:1 citing Sarnat and Schour 1941).

The majority of these enamel hypoplasias follow the perikymata and are usually oriented in a "band" around the tooth's circumference (Hillson 1996:165). Because enamel hypoplasia is a consequence of the halting or slowing of the ameloblast secretory process, the surface morphology of developing enamel is still fully mineralized (Boyde and Martin 1984a:352). Episodic disruptions in crown development, therefore, are recorded.

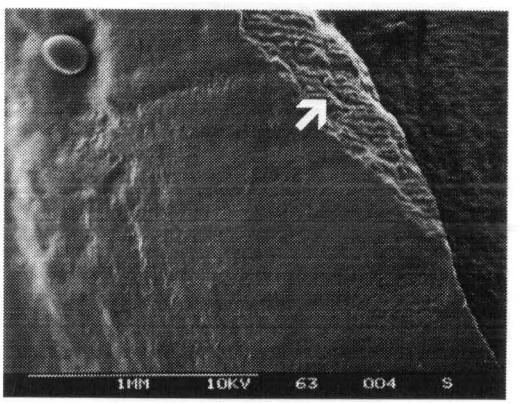
Enamel hypoplasias are thought to result from three different conditions: heredity, localized trauma, or systemic metabolic stress (Goodman and Rose 1991:281, 1990:64). This latter factor has been the subject of a few experimental studies in animals and of hundreds of clinical and epidemiological studies in humans. The latter have been the focus of numerous studies, largely in part in the "hopes of finding clinical significance" of this enamel defect (Eckhardt 1992:294).

Figure 2-1: Examples of Dental Enamel Microstructures from Casts of Bonobo Teeth



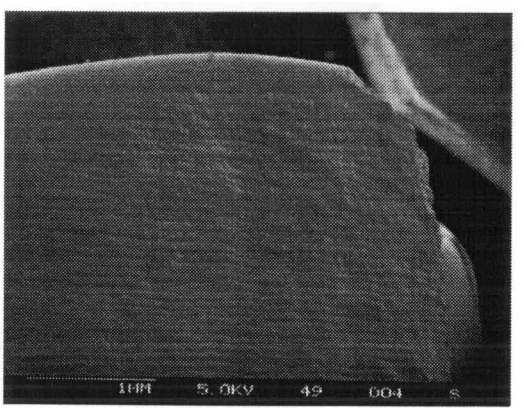
Top Left:
Tomes' process pits

[RG 22908, SEM 53-1 (10)]



Centre Left:
Exposed striae of Retzius
(Noted with arrow)

[RG 9338 , SEM 63-3 (4)]

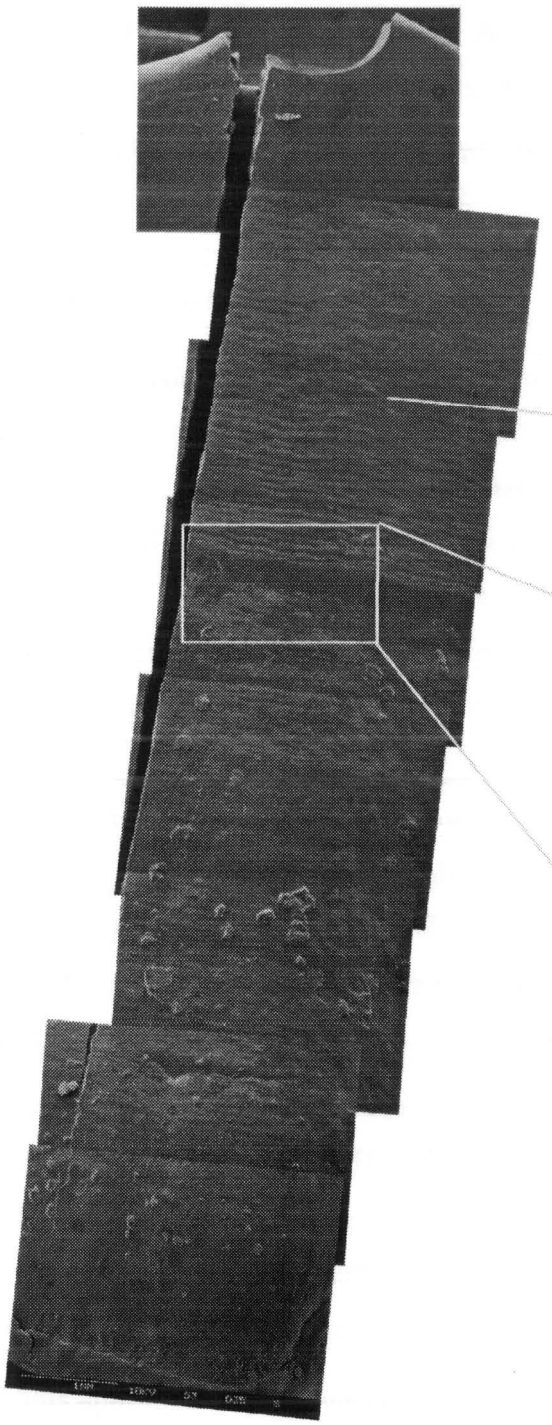


Bottom Left:
Wave-like undulations of perikymata

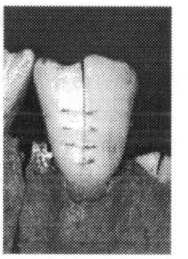
[RG 29053, SEM 49-1 (1)]

SEM IMAGING: Suyoko Tsukamoto, University of British Columbia, June/July 2000

Figure 2-2: Relationship of Microstructures to the Tooth Crown Surface



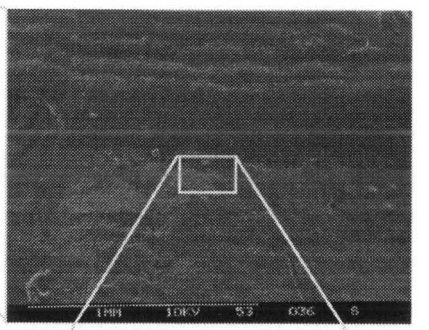
SEM montage of RG 22908

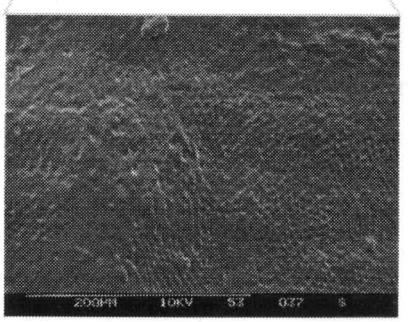


Photograph of tooth crown
(Pencil Marked)

Wave-like undulations of
perikymata grooves

Close-up of enamel hypoplastic groove





Tomes' process pits on tooth crown
surface

SEM Imaging: Suyoko Tsukamoto, University of British Columbia, June/July, 2000

Classification

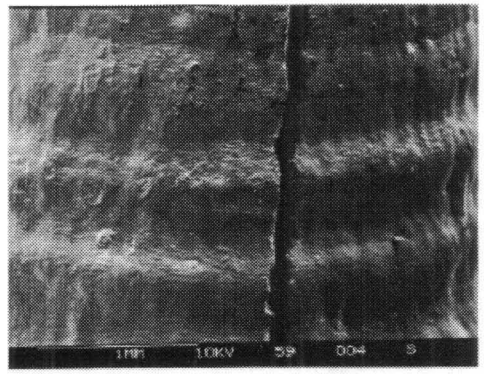
The term enamel hypoplasia encompasses all defects in enamel thickness (Goodman and Rose 1991:281, 1990:64). According to Hillson (1996:166), Berten's (1895) original classification of hypoplastic defects continues as the main types typically identified. The three basic forms of enamel hypoplasia are furrow-type, pit-type, and plane-type. However, according to Hillson and Bond (1997:89), the minimum level of recordable hypoplastic defects has never been defined. The common types of hypoplasia described below may be found in Figure 2-3.

The most common hypoplasia is the furrow-type. It is usually referred to as linear enamel hypoplasia (LEH) (Hillson 1996:167; Skinner and Goodman 1992:157), but the term transverse enamel hypoplasia (TEH) also is used (Eckhardt 1992, with Protsch von Zieten 1991). This enamel defect is "distinguished by a marked horizontal or nearly horizontal area of decreased enamel thickness" (Goodman and Rose 1990:65-66). Because these defects are distributed throughout an individual's teeth in a clearly chronological fashion, their appearance is attributed to systemic metabolic affecting teeth of differing degrees of formation (Goodman and Rose 1991:281, 1990:65). Skinner (1986b, with colleagues 1995, with Hopwood 2003) has also noted the episodic nature of LEH. Guatelli-Steinberg and Skinner (2000) refer to LEH which appear at regular intervals as regularly-repeated LEH (rrLEH). In her dissertation, Guatelli-Steinberg (1998:68, 74) establishes, within a species, three or more defects within 0.7mm of each other as the criteria for rrLEH.

Vertical enamel hypoplasia (VEH) and amelogenesis imperfecta (AI) are rare forms of enamel hypoplasia. VEH is considered a furrow type defect, but "is not well defined and its relationship to the development of hypoplasia is obscure" (Hillson 1996:174). AI, which is an inherited enamel defect, is often manifested as both opacities and hypoplasia and often affects the whole tooth (Hillson 1996:165).

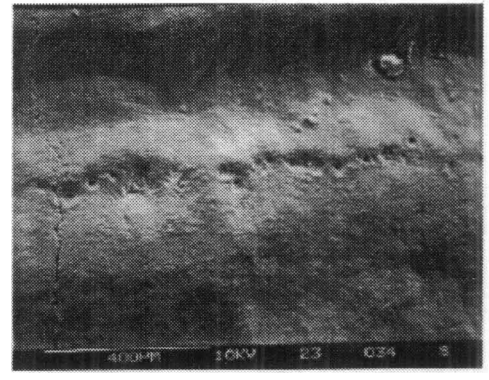
Pits appear as a "pronounced hollow" on the crown tooth surface (Puech and Albertini 1981:450). Depending on the number of ameloblasts affected, pits vary in size and the "floors" of these defects "represent the exposed plane of a brown stria, and are often marked by Tomes' process pits" (Hillson 1996:167).

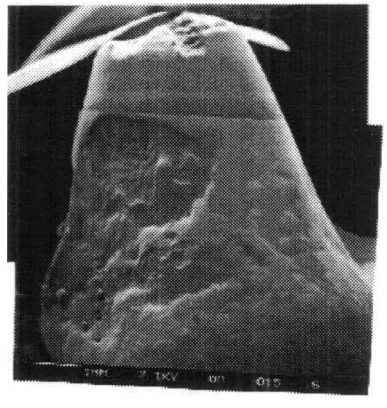
Figure 2-3: Examples of Enamel Hypoplastic Defects



Top Left:
Linear Enamel Hypoplasia (LEH)
(Also an example of rLEH)
[RG 28712, SEM 59c-3 (4)]

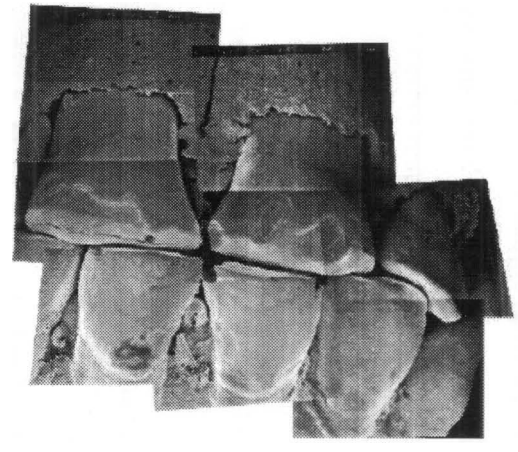
Top Right:
Discrete Pits (below a LEH groove)
[RG 29055, SEM 123a-2 (89X)





Bottom Left:
Localized Hypoplasia of the
Primary Canine (LHPC)
[RG 27011, SEM 57a (1,2)

Bottom Right:
Plane-Type Hypoplasia of the
Deciduous Dentition
[RG 22336, SEM 113a (A-F)]



SEM Imaging: Suyako Tsukamoto, University of British Columbia, June/July 2000

Large areas of stria are also exposed in plane-type defects (Hillson 1996:167), and Lukacs (1999:354) classifies localized hypoplasia of the primary canine (LHPC) under this type of defect. LHPC was first identified in humans and described in the mid-1980s. It is characterized as "a roughly circular hypoplastic defect restricted to the labial surface of the deciduous canine tooth" (Skinner 1986a:59). According to Lukacs (1999a:351), LHPC is the "most common form of enamel hypoplasia in deciduous ape teeth."

Lukacs (1999b) elaborates upon another hypoplastic defect, interproximal contact hypoplasias (IPCH), first identified by Skinner (1996) on single canine from a Middle Paleothic site. Lukacs (1999b), however, found a high frequency of hypoplasias on the interproximal crown surfaces of primary teeth in a prehistoric population in western India. The suspected etiology of this defect is thought to involve the "mesial compaction of developing teeth due to slow longitudinal growth of the jaws [in which] episodic bone remodeling results in ephemeral fenestrae in the mesial and distal walls of the dental crypt permitting tooth–tooth contact and disruption of amelogenesis" (Lukacs 1999b:718).

The latest hypoplastic defect to be identified is the diagonal defects of the maxillary lateral incisor (DDMLI), identified by Hannibal (2003, 2000b) in her study of the great ape collection at the Smithsonian National Museum of Natural History. Hannibal (2000b:8-9) described DDMLI as "only exhibited on the maxillary incisors, restricted to the cervical and mesial quarter of the crown, oriented diagonally starting on the labial surface and extending mesio-cervically. [With the defect ranging] from mild to severe, exhibiting a range from shallow depression to deep depression with little to no enamel."

A table [Table A-1] listing the different descriptive categories of enamel hypoplasias identified by different governing bodies and commonly cited researchers may be found in the appendices.

Methods for Scoring Enamel Hypoplasias

In 1977, the Fédération Dentaire Internationale (FDI) Commission on Oral Health, Research and Epidemiology was established to standardize a dental enamel defect index (Hillson 1996:172; Skinner and Goodman 1992:157). As a result, the

Developmental Defects of Enamel (DDE) Index was created in 1982 (Hillson 1996:172; Skinner and Goodman 1992:157).

For dental anthropologists, this classification is not adequately detailed to embrace all defects and their severity (Clarkson 1989:96; Donat and Rose 1991:69; Hillson 1996:172; Skinner and Goodman 1992:157). In 1988, the Dental Anthropological Association requested a re-classification of a hypoplastic index (Donat and Rose 1991:69).

A modified form of the DDE index for classifying enamel defects was suggested by Clarkson (1989:96) as a "general method" for "international use." Descriptive categories would be utilized since the etiologies of enamel defects are unknown and thus cannot be recorded. The use of a descriptive category, while not identifying the exact cause of the defect, would be appropriate for the "retrospective evaluation" of the etiology (Clarkson 1989:96).

Hindle (1998) reviews the methodological problems incurred in the analysis of enamel hypoplasia. Classification, severity gradients, inter-observer error, instrumental error, variable lighting conditions, teeth used, ageing criteria and standards, and general assumptions (the systemic nature of enamel hypoplasia) are discussed in her paper.

Etiologies of Enamel Hypopolasia

Over a hundred etiological factors have been linked to enamel hypoplasias (Neiburger 1991:464; Suckling and Thurley 1984:357). Many researchers (Clarkson 1996:96; Neiburger 1991:464; Rose *et al.* 1985:284; and Suckling and Thurley 1984:357) believe it is not possible to determine the exact cause of many enamel defects. Whereas this may be true in the case of an individual, Goodman (1991:462) believes hypoplasias can be predicted with reliability within a population, particularly if illnesses and nutritional histories are known.

According to Suckling and Thurley (1984:357), in order to determine the etiology of enamel defects, it is essential to decide the age span during which the disturbance in amelogenesis took place and relate this to a retrospective medical or dental history. The ability to make such an association, however, is impeded by the lack of knowledge

pertaining to the time of the defect formation and its associated ameloblastic phase and the age of the subject during the stage of enamel development at the time of insult (Suckling and Thurley 1984:357-358). Various methods used by researchers to age such enamel defects are reviewed later in the chapter.

At the general level, enamel hypoplasias may be considered non-specific indicators of systemic stress (Goodman and Rose 1990; Hodges and Wilkinson 1990:553; Katzenberg *et al.* 1996:186; Skinner 1986:290). Cerebral disorders, rickets, measles, whooping cough, pneumonia, gastrointestinal disease, hormonal imbalances, low serum calcium levels, and vitamin A deficiencies are some of the many systemic influences thought to induce enamel hypoplasias (Skinner 1986b:290).

Enamel hypoplasias have been induced experimentally in dogs, rats, and sheep through varying diet, disease, and infection, respectively. Lady Mellanby (1929) conducted the first experimental animal model of enamel hypoplasia in beagles by demonstrating that vitamin A and D deficiencies could cause hypoplasias. Kreshover (1942) purposely infected rats with tuberculosis and related enamel hypoplasias to the time of insult. Lastly, Suckling with various colleagues induced enamel hypoplasia in sheep (Suckling, Elliott, Thurley 1983, 1986, Suckling and Thurley 1984, Purdell-Lewis, Suckling, Triller, and Jongebloed 1987, Suckling 1989). High doses of parasites common to sheep (i.e., *Trichostrongylus vitrinus*. and *Ostertagia circumcincta*) caused severe disturbances to ameloblastic activity creating pit and plane-type hypoplasias. Purdell-Lewis and colleagues (1987) later proposed that the "severity rather than the cause of the insult to the ameloblasts determine[d] the degree of hypoplasia."

ENAMEL HYPOPLASIAS IN NON-HUMAN PRIMATES

When compared to the hundreds of enamel hypoplasia studies in human populations, reports of enamel defects in non-human primates have been few (Eckhardt 1992:293; Lukacs 1999a:353; Moggi-Cecchi and Crovella 1991:106). In 1922, Carter conducted the first systematic survey of primate enamel (Boyde and Martin 1984a:345). The first interspecies comparison of enamel hypoplasias was conducted by Colyer in

1936, and he was also the first to demonstrate that chimpanzees possessed more hypoplastic defects when compared to other primates (Newell 1998:43).

A decade after Colyer's (1936,1947) studies, Schuman and Sognnaes (1956) conducted the first major histological study of enamel hypoplasia in non-human primates (Newell 1998:44). Using shadow-cast replicas and ground sections, they examined gross and histological structures of chimpanzee (N=78), gorilla (N=3), gibbon (N=3), orangutan (N=2), and rhesus monkey (N=39) teeth (Schuman and Sognnaes 1956:207). Again the chimpanzee was shown to possess more hypoplastic defects. Two decades would pass before Molnar and Ward (1975) conducted another histological study of enamel defects.

In 1975, Molnar and Ward suggested a "broad comparative study" of the prevalence of enamel hypoplasias in non-human primates be conducted. Twenty-five years later, two doctoral students submitted extensive dissertations concerning the prevalence of enamel hypoplasias in all non-human primate taxa. Newell(1998:70) examined 3,375 non-human primates specimens from 117 taxa for linear and pit-type hypoplasias while Guatelli-Steinberg (1998:64) examined LEH patterns in 17 primate genera.

The first dissertation involved a broad comparative study of enamel hypoplasia in the non-human primates by Newell (1998). Newell's primary goal was to "rectify methodological and comparative inconsistencies and provide an accurate account of enamel hypoplasia among non-human primates" through the macroscopic evaluation of skeletal museum specimens. Four American museum collections were used. She employed a modified version of the DDE scoring index with all the primate taxa. Two classifications of hypoplasias were identified: linear and pitting. Linear hypoplasias were subdivided into mild, moderate, and severe forms, whereas pitting was differentiated by number (multiple or single) and size (small or large). Frequencies of enamel hypoplasia were compared among taxa, sexes, tooth type, class of hypoplasia, and between deciduous and permanent dentition. Statistical methods included chi square, least means square regressions, correlation coefficients, co-efficient of determination, and analysis of variance (ANOVA).

Guatelli-Steinberg (1998) also examined LEH variation across the non-human primate order. In her dissertation, she studied the relationship of LEH among the taxa, with crown height and crown formation, as well as inter-tooth patterns. In addition, she specifically examined "correlations between defect formation and physiologically stressful life history conditions" in monkeys (Guatelli-Steinberg 1998:3). Five museum collections were examined in this study. Only LEH was examined in her interspecies comparisons and the defects were rated as mild, moderate, or severe. Statistical analyses included logistic regression, Poisson regression, ANOVA, t-tests, and chi-square tests.

Since these major studies, the examination of enamel hypoplasias in non-human primates has been conducted by several researchers and their students: R.B. Eckhardt (1992, 2000, and colleagues 1992, with Protsch von Zieten 1993), Debra Guatelli-Steinberg (1998a, 1998b, 2000, 2001, with Dirks 2001, with Lukacs 1998, 1999, 2000, with Newell (2000), with Skinner 2000), Darcy Hannibal (2000a,b), John Lukacs (1999, 2000, 2001), Jacopo Moggi-Cecchi (1989, 2000, with Crovella 1991,1992, with Crovella and Turoni 1988, with Tobias and Beynon 1998); Elizabeth Newell (1998, 2000, 2003), and Mark Skinner (1986b, 2000, and colleagues 1995, and Hopwood 2003, and Newell 2003).

In the year 2000, many of these aforementioned researchers assembled for a symposium on "Patterns of Physiological Stress in Primates: The Dental Evidence" at the American Association of Physical Anthropologists 69th Annual Meeting in San Antonio, Texas. This symposium was created to "explore the use of development dental defects as stress indicators in primates." Topics included "the relationship between enamel defects and sources of physiological stress," the "influence of sex, maturation length, and taxonomic status on the manifestation of enamel defects," new studies of modern fossil primates, and "new methods for accurately aging defects."

From this body of work, a number of observations have been made with respect to the prevalence and distribution of enamel hypoplasia among non-human primates:

- Hominoidea display a greater overall frequency of enamel defects when compared to Ceboidea and Cercopithecoidea (Guatelli-Steinberg 1998; Molnar and Ward 1975:9; Moggi-Cecchi and Crovella 1991:109; Newell 1998:253; Skinner 1986b; Vitzthum and Wikander 1988:284).

- Enamel hypoplasia in prosimians is rare (Newell 1998, Guatelli-Steinberg 1998).
- The canine tooth is the most affected (Coyler 1936 cited by Schuman and Sognnaes 1956:205; Hannibal 2000b, Moggi-Cecchi and Crovella 1991:109; Protsch van Zieten and Eckhardt 1991:146; Schuman and Sognnaes 1956:196; Skinner 1986b).
- The anterior teeth are more affected than the posterior teeth (Hannibal 2000b, Moggi-Cecchi and Crovella 1991:109; Newell 1998:253; Skinner 1986b).
- The middle third of the tooth is most commonly affected (Moggi-Cecchi and Crovella 1991:109, Skinner and Hopwood 2003).
- Hominoidea demonstrate a higher frequency of defects in the cervical third of the tooth when compared with Ceboidea and Cercopithecoidea (Moggi-Cecchi and Crovella 1991:109).
- Ceboidea demonstrate a greater frequency of pits than grooves or lines (Moggi-Cecchi and Crovella 1991:106).
- Cercopithecoidea display an equal frequency of pits, grooves, and lines (Moggi-Cecchi and Crovella 1991:106).
- Hominoidea show a greater frequency of grooves and lines than pits (Moggi-Cecchi and Crovella 1991:106).
- The permanent dentition demonstrates a greater frequency of LEH than pitting whereas the reverse is true in the deciduous dentition. Differences between the maxillary and mandibular teeth were also noted (Eckhardt 1992, Eckhardt and Protsch von Zieten 1993, Lukacs 1999a, Newell 1998:254, Newell 2003).
- Significant differences are observed between males and females (Guatelli-Steinberg 1998:109; Newell 1998:253).
- Certain teeth express specific hypoplasias: deciduous canines, LHPC (Lukacs 1999a, Skinner 1986a, Skinner and Newell 2003), maxillary lateral incisors, DDMLI or Darcy's Defect (Hannibal 2000b, 2003), and sectorial premolars (Guatelli-Steinberg and Lukacs 1998).

Chimpanzees and Enamel Hypoplasia

A number of chimpanzee skeletal collections have been the focus of EH studies. Both the deciduous *and* permanent dentitions have been examined in the Powell-Cotton Museum at Quex Park (Birchington, Kent, England); the Hamann-Todd Chimpanzee Collection at the Cleveland Museum of Natural History; the Great Ape Collection housed at the Smithsonian Institution's National Museum of Natural History; and the Liberian Chimpanzee Collection from the Frankfurt Anthropological Institute at the Johann Wolfgang Goethe Universität.

The Powell-Cotton Museum

Deciduous Dentition. From this collection, Lukacs (2001) examined 42 subadult chimpanzee crania (n=17 female, n=25 male) that retained their deciduous canine teeth. He found that the defects were primarily of the pit and plane-form type and their prevalence was generally low except for the canine, of which the mandible was most commonly affected. LEH was found to be absent. Overall, 47.6% of chimpanzees had one or more canines exhibiting enamel hypoplasia with 52.9% and 44.0% of the females and males affected, respectively. The sex differences, however, were not statistically significant. The cervical region/zone of deciduous canines was most commonly affected (Lukacs 2001:203) which could be reflective of "birth trauma or postnatal environmental challenges to the physiological homeostasis of the neonate" (Lukacs 2001:207).

Permanent Dentition. As part of his study of enamel hypoplasia in sympantric chimpanzees and gorillas, Skinner (1986) examined 110 chimpanzees from this collection (n=75 females, n=35 males). Overall, 58% of the chimpanzees expressed enamel hypoplasia, with 66% and 55% of the males and females affected, respectively. Skinner also found that side, sex, body size, or pathology did not appear to affect the incidence of enamel hypoplasia. He did find, however, that there appareaed to be a "marked regularity of hypoplastic grooving" which was thought to "reflect a semi-annual cycle of seasonal stress tentatively identified as the major and minor rainy seasons" (Skinner 1986:309).

The Hamann-Todd Chimpanzee Collection

Deciduous Dentition. As part of his study, Lukacs (1999) examined localized hypoplasia of the primary canine (LHPC) in the deciduous teeth of 40 infant and juvenile chimpanzee crania from this collection (n=15 females, n=3 males). Lukacs (1999) combined his observations from this collection with observations procured from the Smithsonian collection. Although this enamel defect was the most common in the deciduous dentition, *Pan* species demonstrated the lowest frequency (22.0%) when compared to the *Gorilla* (88.7%) and *Pongo* (88.0%). Furthermore, the expression of the LHPC varies significantly with the mandible, reflecting a frequency of 24.2% whereas a

frequency of 5.0% was calculated for the maxilla. Left and right sides of the dental arcade did not appear to display any significant differences in frequency.

Lukacs (1999:360) suggests the differences observed in these primates may be the result of "differential levels of developmental stress." Factors that were observed to affect humans include ancestral background and nutritional status; also levels of sun-light and vitamin A appear to be factors (Skinner *et al.* 1994). In non-human primates, Lukacs (1999:361-362) proposes morphologic (crown size, crypt size, shape of jaw), developmental (period of calcification), and other variables (genetics, behavior, and nutrition) play a role in the prevalence of EH.

Permanent Dentition. Stottlemire's (1998) work complements Lukacs' (1999) study by reporting on EH of the adult primate dentition from the same skeletal collection. Between the two, a "taxonomic reversal" of EH frequencies was observed (Lukacs 1999a:362). Stottlemire reported that in the permanent dentition, chimpanzees were dramatically affected (80.6%) when compared to gorillas (27.5%). Lukacs (1999:362) proposes different developmental stages and stress experiences, separate etiologies for LEH and LHPC, and inter-observer differences to explain these reversals in LEH/LHPC frequencies.

Smithsonian National Museum of Natural History

Deciduous Dentition. Lukacs (1999) examined localized hypoplasia of the primary canine (LHPC) in the deciduous teeth from 11 infant and juvenile apes (n=2 females, n=3 males) from this collection housed in the Department of Mammals. Lukacs (1999) combined his observations from this collection with observations procured from the Hamann-Todd Collection. (The results of this study were described in the Hamann-Todd section.)

Permanent Dentition. Hannibal (2000b, 2003) studied chimpanzees (n=25) in this collection. Sixty-eight percent (68%) of the chimpanzees expressed LEH and 35.7% displayed a plane-type defect known as "diagonal defect of the maxillary lateral incisors" or DDMLI, which was first described by Hannibal (2000b) in her thesis. Hannibal also found that mandibular and anterior teeth demonstrated a higher frequency of LEH than

the maxillary and posterior dentition. Hannibal (2000b:16) suggested biological and environmental factors influence the high frequency of LEH in chimpanzees. As for DDMLI, Hannibal (2003) suggests physiological stress as the cause of this bilateral defect, not systemic and genetic factors.

The Liberian Chimpanzee Collection - Frankfurt

Deciduous Dentition. Eckhardt and Protsch von Zieten (1993) observed pitting in the deciduous dentition of the Liberian chimpanzee. Using scoring methods established by Goodman and Rose (1990), the researchers examined 70 specimens possessing deciduous dentition. Frequencies of EH, however, were noted by an individual specimen count method rather than by a tooth count prevalence. Of the five infants examined, pits were observed in four. In juveniles, 1 out of 23 specimens displayed pits in the maxillary dentition while 6 out of 15 specimens displayed pitting in the mandibular dentition. Of the 31 subadult specimens available, none displayed pits.

Permanent Dentition. Eckhardt (1992:297) claims to have surveyed "the largest number of specimens sampled from a natural population of a single hominoid species using the two teeth (maxillary central incisors and mandibular canines) that have been identified...as incorporating the greatest density of information on the incidence of dental enamel hypoplasia." LEH was scored as either present or absent in specimens containing maxillary central incisors (N=70) and mandibular canines (N=59). Frequencies were based on the presence of LEH in specimens and whereas 70 and 59 specimens had incisors and canines, respectively, not all were suitable for examination. The observations made in this study are in the table below [Table 2-1].

From their studies, Eckhardt (1992), also with Protsch von Zieten (1993), observed pitting in the dentition of infants and juveniles, with a greater frequency of pits

Table 2-1 Eckhardt's (1992) Chimpanzee Results

Dentition	Incisor			Canine		
	+	N	%	+	N	%
Deciduous	0	12	0	0	20	0
Permanent	21	45	46.7	23	33	69.7

in the mandibular dentition. Pitting was not observed in subadults. LEH, on the other hand, was prevalent in the permanent dentition, particularly in the mandibular canine but it was not observed in the deciduous dentition. It should be noted that the Liberian Chimpanzee Skeletal Collection contains 280 skulls; thus the frequencies of EH in the entire sample will be different if this value is considered.

Pits, lines, and grooves have been noted in chimpanzees and other non-human primates. Vertical enamel hypoplasias (VEH), however, have only been reported by one set of researchers in non-human primates. Eckhardt, Protsch, and Protsch von Zieten (1992) found two male chimpanzees displaying VEH in their maxillary canines in the Frankfurt Liberian Chimpanzee Collection. The frequency of this enamel defect ranges from 0.7 – 5.4%, depending on the number of specimens (N=2/280) or the number of specimens completely retaining their canines (N=2/37) (Eckhardt *et al.* 1992:111). These researcher suggest that this low frequency of VEH is often related to genetic trait thresholds.

Other Studies

In 1956, Schuman and Sognnaes were the first to examine a large sample of chimpanzee teeth (N=78 specimens, N=179 teeth). Again, the Liberian chimpanzee was examined; however, this collection was housed at Harvard's Peabody Museum. Surface (N=179) and histological (N=78) examinations were conducted and while ground sections and replicas were typically made from M1 and M3, the prominence of LEH in the incisors and canines was noted (pg.196). They cite Colyer's (1936) suggestion of food shortages to explain this enamel defect.

Skinner (1986b, 2000, with colleagues 1995, with Hopwood n.d.) is one of two researchers in non-human primate EH studies who has moved beyond the descriptive work of EH frequency studies and attempted to understand the cause and *timing* of this enamel defect. While the timing of EH has been determined in human skeletal samples, such investigations have not been conducted on non-human primates. In 1986, the onset of LEH in West African chimpanzees was determined through comparisons with canine crown formation times from radiographs of the chimpanzee skeletal material examined

from the Powell-Cotton Museum in Kent, England. The distribution of LEH was determined to be episodic in nature and the timing between these episodes was calculated to be 6 months on average. This time interval coincides with the major and minor rainy season in the subtropics. These rainy periods were later paralleled with the seasonal prevalence of malaria throughout Africa (Skinner *et al.* 1995). Skinner's work with Hopwood (2000, n.d.) is described in the next section.

Other chimpanzee EH studies involved small sample sizes from unspecified collections, selective examination of teeth, and vague descriptions of the enamel defect, and they employed a variety of experimental methodologies and techniques. A chronological outline of the chimpanzee studies involving enamel hypoplasia may be found in Table A-2 of the appendices.

EH and Perikymata in Non-Human Primates

Qualitative observations of perikymata with non-human primates EH studies have been made by Schuman and Sognnaes (1956) and Molnar and Ward (1975). "Irregular perikymata patterns" were observed in replicas of chimpanzee molars (Schuman and Sognnaes 1956:198). Irregular perikymata were also observed in orangutans and gorillas but not in gibbons or rhesus monkeys. Molnar and Ward (1975) cite Pedersen and Scott's (1951) observation "perikymata grooving in chimpanzee enamel is comparable in frequency of occurrence to modern human populations."

In their examination of EH within the dentition of Cayo Santiago rhesus macaque skeletal collection, Guatelli-Steinberg and Lukacs (1998) noted the preferential distribution of LEH on the sectorial premolar. Rather than recommending developmental factors (crown height, timing of crown development, and duration of crown formation) as likely explanations for the distribution of EH, they propose enamel thickness, perikymata spacing, and prism orientation (Guatelli-Steinberg and Lukacs 1998:179). The authors advise a comparative investigation of perikymata groove spacing of the mandibular canine and 3rd premolars to study this phenomenon.

Recently, Skinner (2000, with Hopwood 2003) has studied the "periodicity of repetitive linear enamel hypoplasia in Asian and African apes." For orangutans, scanning

electron micrographs of tooth crown replicas were made and from these images the number of perikymata between episodes of LEH were counted. From these counts, a semi-annual pattern was discerned. It is attributed to a relationship between seasonal rain cycles' and with parasite ecology.

Etiology of EH in Non-Human Primates

A number of etiological factors for the presence of enamel hypoplasias in non-human primates has been proposed. Colyer first proposed vitamin deficiencies and rickets, as well as captivity, as potential causative agents of enamel hypoplasia in the chimpanzee (Colyer 1936, Miles and Grigson 1990). Molnar and Ward (1975) also proposed captivity as a potential stressor although an examination of their chimpanzee samples shows that wild chimpanzees had more hypoplasias than their captive counterparts. Cohen and Bowen (1972:646) suggest nutritional agents, severe infections, ingestion of certain chemicals, and local trauma as causes which induce EH.

Hypoplasias are also thought to occur during the period of weaning (Guatelli-Steinberg and Dirks 2001, Skinner 1986b). Cohen and Bowen (1972:646) state that "it is not surprising that hypoplasia of the deciduous dentition is rarely seen" because "in cases of severe maternal nutritional insufficiency, the enamel of the infant may be free of outward blemishes...[and]...the developing tooth appears to have first call on resources of maternal foetal calcium even when severe calcium deprivation exists" (Cohen and Bowen 1972:646). Current research (Eckhardt and Protsch von Zieten 1993, Lukacs 1999a, 2000, 2001, Skinner and Newell 2003) into enamel hypoplasias of the deciduous dentition of non-human primates shows that is not the case – particularly for LHPC. LHPC is one of the hypoplasias thought to result from localized trauma; specifically it is thought to reflect osteopenia of the facial bone in infants creating a fenestration through which ameloblasts are mechanically affected creating LHPC.

Skinner (1986, 2000, with colleagues 1995, with Hopwood 2003) observed that linear enamel hypoplasias are often repetitive or cyclical in nature, and he attributes this periodicity to seasonal and parasitic cycles such as hookworm and malaria.

There is one documented case of an enamel hypoplastic event. Schwartz, Dean, Reid, and Zilhman (2001a) studied the permanent dentition of a captive, juvenile, female, western lowland gorilla (*Gorilla gorilla gorilla*) of known-age of death. Shortly after birth, the female was attacked by another gorilla in the compound causing severe trauma to its eye that required a number of trips to the veterinarian for surgery (Schwartz *et al.* 2001b). Comparing the histological thin sections to hospital records showed that observed stress lines in the tooth corresponded to the trips to hospital and that the one hypoplastic event on the tooth crowns corresponded to the time of the eye injury. This method of correlating medical records to stress lines in histological thin sections of teeth has also been employed by Skinner and Anderson (1991) in a forensic context.

All these etiological factors, however, may be lumped under three general conditions: genetic and developmental, behavioral, and environmental. Newell (1998) adapted such a model from Goodman and Rose (1990) to explain the prevalence of enamel hypoplasias across the Primate Order.

AGEING OF ENAMEL DEFECTS

With respect to age and maturation, enamel has been the prime focus in most anthropological studies (Mann *et al.* 1990:112). There are three main ways of measuring and recording dental development: the observation of tooth emergence/eruption, calcification intervals, and the counting of incremental structures from histological thin sections (Dean 1989:161). The combination of all these methods best leads to "reliable conclusions" in comparative dental assessments (Dean 1989:161).

There are also three popular methods to age enamel defects: the comparison of defects to the aforementioned growth schedules, the measurement of the CEJ/enamel defect distance, and the counting of perikymata on the crown surface. This latter method may be further used to match perikymata and hypoplastic sequences between adjacent tooth crowns (Hillson 1996:177, Reid and Dean 2000). Regardless of the method used, "the most important factor in determination of the chronology of enamel hypoplasia relates to the choice of developmental standard and how it is interpreted and employed"

(Skinner and Goodman 1992:165 citing Goodman and Rose 1991:287). Goodman and Song (1999) review the sources of variation in approximated ages of linear enamel hypoplasia formation.

Growth Crown Schedules

Sarnat and Schour (1941) are credited with first recognizing the "chronometric potential of enamel hypoplasias" (Goodman and Rose 1991:285). From the defect's position on the tooth's crown, the age at which LEH is formed may be estimated (Berti and Mahaney 1992:19; Hillson and Bond 1997:91; Hodges and Wilkinson 1990:553; Newell 1998:31). The time of defect formation is elucidated from tooth development schedules because formation and maturation of teeth are assumed to occur at predictable rates (Hillson 1996:175; Hillson and Bond 1997:91; Lovell 1999:70).

Three types of "enamelization schedules" are commonly used: the species-wide chart method, the sample specific method, and the tooth specific method (Berti and Mahaney 1992:19-20). Questions regarding the precision and accuracy of these schedules have been raised (Hillson 1996:173; Hillson and Bond 1997:91; Lovell 1999:70). However, an estimation of the "relative timing" of physiological stress during tooth development may still be obtained from the position of the enamel defect (Lovell 1999:72). Massler and colleagues' (1941) schedules for humans and Dean and Wood's (1981) schedules for pongids are commonly employed in estimations of age.

Non-Human Primate Schedules.

Prior to Dean and Wood's (1981) radiographic study of developing pongid dentition, no such comprehensive study had been conducted (Dean and Wood 1981:112). From radiographs of mandibular teeth from animals of unknown age, chronological calcification times, eruption times, and developing dentition charts were created for *Gorilla, Pan*, and *Pongo* by Dean and Wood (1981). It was found that dental development patterns among these species do not differ but there is a difference between pongid and human dentition. When compared to humans, pongid teeth are larger, yet require a shorter period of time to develop. Reasons attributed for these differences in crown formation times include a condensed growth period, reduced enamel thickness, a

larger pulp chamber, and shorter root formation times. The two main criticisms of this work, however, are the researchers did not differentiate by sex and they used a skeletal collection of unknown individual age (Mann *et al.* 1991:125). Since then, calcification sequences have been determined from live captive chimpanzees by Anemone and colleagues (1991) and Kuykendall (1996, with Bozic 2001).

Cemento-Enamel Junction (CEJ) Measurements

In addition to using growth charts, CEJ measurements may be used. This technique was implemented in 1966 by Swärdstedt, who converted these measurements to ages after calculating mean crown heights of affected teeth and comparing them with growth schedules (Goodman and Rose 1990:82; Hillson 1996:172).

Usually to estimate the age of insult, caliper measurements of the dental defect are made from the CEJ to the occlusal or cervical margins of the tooth and then compared to a development table (Dobney and Ervynck 1998:267; Hillson and Bond 1997:91; Hodges and Wilkinson 1988:223; Lovell 1999:72). According to Lovell (1999:73), the better estimate of age is obtained by distances measured between the occlusal surface and the defect. However, measurements made from the CEJ to the "most occlusal aspect of the defect" are considered "more practical" due to the wear of the occlusal surfaces (Lovell 1999:73 citing Buikstra and Ubelaker 1994). The following formula from Goodman and Rose (1990:289) may be used to calculate the timing of the enamel defect:

$$\text{age at formation} = \text{age at crown completion} - \frac{[\text{years of formation X defect height (from CEJ)}]}{\text{crown height}}$$

Incremental Markers of Non-Human Primate Teeth

The periodic nature of the incremental structures makes it an appealing tool in the estimation of skeletal age and the ageing of enamel defects. Studies involving the microincremental structures of ape enamel are lacking. Mann and colleagues (1990:126) identify preliminary work by Beynon and Dean (1989) and Dean (1989) as the only "quantified data on incremental markers in apes." Since the publication of that article, however, Beynon, Dean, and Reid (1991), Bromage (1991), Dean (1998), Dirks (1998), Guatelli-Steinberg and Dirks (2001), Reid and Dirks (1997), Reid, Hillson, and Dean

(2000), Reid *et al.* (1998), Schwartz and Dean (2000), and Schwartz, Dean, Reid, Zihlman (2001a) have examined incremental markers in a variety of primates. Bonobo (*Pan paniscus*) teeth were taken from the Royal Museum in Central Africa for this study for the purposes of thin-sectioning and cross-striation counting (see Chapter 3).

It appears, however, that a couple of Japanese researchers were studying dental incremental markings in non-human primates prior to World War II. Apparently Mimura (1939) conducted the earliest work. Fukuhara (1959:322) reviews this early work in cross-striation studies and states Mimura used sodium fluoride to mark daily growth in monkeys. An examination of Mimura's (1939) article reveals, however, that he used pigs, rabbits, and dogs. Okada (1943) used sodium fluoride and lead acetate to mark at "irregular but known intervals to demonstrate that cross striations were daily increments" in monkeys and other animals (FitzGerald and Rose 2000:168). In his paper, Dean (1987:160) identifies Fukuhara (1959) as having counted cross striations between striae of Retzius in *Pongo pygmaeus, Macaca mulatta, Macaca fuscata, Macaca cyclopis,* and *Hylobates lar.* More recently Aimi and Nogami (1991) examined cross-striations in Japanese macaques, *Macaca fuscata.*

It was Bromage (1991:211), however, who confirmed the assumption that "enamel matrix formed in daily increments, reflected in enamel cross-striations." He was able to demonstrate this enamel incremental periodicity from two developing permanent first molars of *Macaca nemestrina* which had been labeled with polychorome fluorescent dyes. These animals had been sequentially administered fluorescent dyes at recorded intervals. From ground thin sections of their teeth, cross-striation counts were made between the fluorescent labels and the daily periodicity established.

Perikymata and Crown Formation Times.

Bromage and Dean (1985) first proposed perikymata counts as a "non-destructive means of determining early hominid enamel formation" (Bromage 1989:197, FitzGerald and Rose 2000:164). This recent connection between perikymata counts and their role in dental crown development times is the subject of debate (Capasso and Goodman 1992:11; FitzGerald and Rose 2000:164; Mann *et al.* 1991, 1990). But, according to

FitzGerald and Rose (2000:164) although "there are still a few who disagree, the time dependency of dental microstructures is no longer seriously questioned."

Bromage and Dean (1985:525) proposed the periodicity of these enamel incremental features could be used to determine the crown formation times for fossil hominids since 7 to 8 cross-striations were "consistently" observed between the striae of Retzius that were, in turn, manifested as perikymata on the external crown tooth surface. These crown formation times were then compared with root growth data, relative rates of crown formation, and radiographs of human and primate populations.

Such analyses were conducted through the use of silicone based impressions and epoxy resin replicas which were viewed with a SEM (Bromage and Dean 1985:526). Perikymata counts were made from montage photograph records of the dental replicas. All of the perikymata on the dental crown were counted. Bromage and Dean interpreted the differences in perikymata counts between modern humans and fossil hominids to mean that they experienced different crown formation times (Mann *et al.* 1991:176).

This replica method for the estimation of crown formation times from perikymata has been used by a number of researchers in the study of fossil hominids (Beynon and Dean 1988, Bromage and Dean 1985, Dean 1987, Dean *et al.* 1986, Mann *et al.*1991, and Moggi-Cecchi *et al.* 1998). Hillson (1992b:65) cites the replica method as the "most convenient way to study perikymata."

Hillson and Jones (1989) reviewed two instrumental procedures to aid in the detection and counting of perikymata that could be used to reconstruct rates and patterns of crown and defect formations, and estimations of age-at-death in juveniles. Hillson (1992a) then combined these two methods to study perikymata and enamel hypoplasias in Egyptian, Roman, and Medieval populations.

Criticisms. Much of the debate lies in the fact that cross-striations are "assumed" to represent daily increments of enamel growth and that the body of evidence surrounding this assumption is largely "circumstantial" (Boyde 1990:236, Mann *et al.* 1990:119). As previously stated, not all striae of Retzius reach the crown surface and thus cannot be manifested as perikymata (Goodman and Rose 1991:289; Mann *et al.* 1991:121). Furthermore, perikymata are subject to wear (Hillson 1992a:71; Mann *et al.*

1991:121) and they are not clearly visible on deciduous tooth crowns (Hillson 1992a:72). Because the perikymata are not visible on the surface tips of the cusps, the tooth must be sectioned to most accurately determine the age (Boyde 1990:244). If sectioning is not possible, certain limitations must be considered when using the non-destructive perikymata/SEM technique. Dean (1987:170) identifies these as follows:

- "age estimates of an individual are limited to early stages of the growth period when crowns are forming"
- "the short period between birth and the onset of calcification must be estimated"
- " a further estimate must also be made for the time it takes to form increments of enamel that are hidden beneath the cuspal region of incisors and which are not therefore expressed as perikymata."

In the face of all these criticisms, Hillson (1997:178) states "some of these objections can be answered directly by further research, but the main argument in favour of the method is that, where perikymata counts can be tested, it works."

In his review of the utilization of periodic markings in enamel, Shellis (1998) identifies the use of perikymata counts to estimate the crown formation time for intact teeth if the time interval between successive striae is known. However, in order to estimate the *total* crown formation time, it is necessary to have an estimate of appositional enamel formation times in addition to the imbricational enamel formation times (Shellis 1998:390). The differential modes of cuspal and lateral enamel formation, are referred to as "appositional" and "imbricational" enamel, respectively (Shellis 1998: 390). Because the cuspal enamel does not manifest striae on the outer surface of the tooth, Shellis recommends creating a sub-sample to determine appositional enamel times from sectioned or broken teeth. These estimates can then be added to the imbricational enamel counts to obtain the total crown formation time. That is, because striae are manifested on imbricational/lateral enamel, the formation times for this enamel can be determined non-destructively and then added to appositional/cuspal references to create estimates of tooth crown formation.

Dean and Reid (2001) used perikymata counts to estimate enamel formation times in fractions of crown height. The researchers sputter-coated anterior tooth replicas with gold to facilitate examination under a binocular microscope. The teeth were divided into

10 equal divisions or fractions of buccal crown height and the total perikymata count in each division were counted. The mean number of perikymata per millimeter was also calculated for each division. This method allowed the researchers to compare the distribution and spacing of perikymata among the anterior teeth of fossil hominins. Dean and Reid calculated the crown formation times for each tooth by adding cuspal and lateral enamel formation times. Reid and Dean (2000) had recommended, in another article published the year before, that determining which tooth crown fraction a hypoplastic defect was observed could be a "more reliable way" of estimating the timing of such defects since anterior crown formation is a non-linear process that slows towards the cervix in all teeth.

The Ageing of Enamel Hypoplasias in Non-Human Primates

At the time Guatelli-Steinberg (1998:39) cites Skinner (1986b, with colleagues 1995) as the only researcher who has attempted to age the onset of enamel hypoplasia formation in non-human primates. In their work, enamel hypoplasias were timed in the chimpanzee, gorilla, and Miocene fossil primate *Dryopithecus*.

In his examination of sympatric chimpanzees and gorillas, Skinner (1986b) took 100 radiographs of immature left mandibles of these species from the skeletal collection under study. A detailed examination of canine crown formation was conducted to establish which stress events would not be recorded on this tooth. Comparisons with other teeth indicated 1% of the stress episodes would not be recorded on the canine. These radiographs were then used to determine the time of enamel defect formation. Nissen and Riesen's (1964) normative standards were used to assign an age to the radiographed specimens. The onset of significant stress was observed to occur around 2.5 years and enamel hypoplasias were observed to occur at an average of every 6 months throughout crown formation. The timing of this episodic LEH was linked to the major and minor rain seasons in West Africa.

Episodic LEH was also observed in the Miocene ape fossil *Dryopithecus* by Skinner, Dupras and Moyà-Solà (1995). The timing of these enamel defects was compared to measurements from radiographs of 6 immature captive chimpanzees of

known age. This episodic nature of LEH is attributed to stress affiliated with seasonal rain cycles and the associated periodicity of malarial infections.

Recently Skinner (2000, with Hopwood n.d.) examined the timing of LEH in *Pan troglodytes*, *Gorilla gorilla*, and *Pongo pygmaeus*. Two different ageing methods were employed in this study. For the chimpanzee and gorilla, the distance of the first and last episode of LEH from the CEJ as well as crown heights, were measured and expressed as absolute measures and ratios of crown heights. For the orangutans, the number of perikymata between and within episodes of LEH were counted from composite scanning electron micrographs. It was found these apes express a semi-annual pattern of stress in their dentitions. This phenomena is attributed to a combination of seasonal moisture and fruiting cycles in concert with specific parasitic stressors.

Through the position and size of LHPC, Lukacs (2001) examined the developmental timing of deciduous enamel defects in large ape species. Lukacs hypothesized that tooth crown fractions could be indicative of prenatal or postnatal stress depending on a primate's tooth calcification schedule. He calculated hypoplastic defect prevalence for fractions of canine tooth crowns.

To determine which tooth fraction displayed a hypoplastic defect, Lukacs (2001:201) drew the defect on a dental outline chart noting the size, shape, and location of the defect on the tooth crown and also took caliper measurements of the height and width of the defect as well as its location from the CEJ. An acetate drawing of the labial surface of the canine crown, which was divided into three enamel zones (apical, middle, cervical), was superimposed on data sheets and the presence of EH noted for each zone. If a defect spanned more than fraction, both were scored EH positive.

SCANNING ELECTRON MICROSCOPY (SEM)

The first biological tissue to be examined with the scanning electron microscope was mature dental enamel (Boyde *et al.* 1988:1479). Since its induction in enamel studies in 1959 (Boyde *et al.* 1988:1479), the SEM has been used extensively to examine developing and mature enamel in clinical, anthropological, and archaeological settings.

In dental anthropology/archaeology, the SEM has been used to study the following:

- Dental microstructure and development (i.e., prism packing patterns) for the purposes of taxonomic analyses (Boyde and Martin 1984a; Boyde *et al.* 1988; Lavelle *et al.* 1977; and Martin *et al.* 1988) and perikymata, for the estimation of crown formation times (Beynon and Dean 1988, Bromage and Dean 1985, Dean 1987, Dean *et al.* 1986, Mann *et al.*1991, Moggi-Cecchi *et al.* 1998; Yuan *et al.* 1998).
- Methods and techniques, such as cleaning, acid etching, grinding, polishing, and thin-sectioning, replicating required for a SEM sample (i.e., enamel) preparation (Beynon 1987, Boyde and Martin 1984a, 1984b, Hillson 1992a, Martin *et al.* 1988).
- Micro wear patterns (Boyde 1990:253; Boyde and Martin 1984a; Capasso and Goodman 1992:11; Teaford and Oyen 1989; Walker and Teaford 1989).
- The gross surface examination of hypoplastic enamel (Hillson 1992a,b, Hillson and Bond 1997, Moggi-Cecchi 1989, Puech and Albertini 1981, Yanagisawa *e .al.* 1984).
- To validate enamel defect typologies, including EH, initially constructed from light microscopic examination (Marks 1988, 1992, Rose 1979).

Advantages

The advantages of using a SEM to examine the gross morphology of the enamel surface are numerous. The SEM possesses a "relatively enormous" depth of field and samples may be examined using a wide range of magnification from 15 to 100,000 X (Hillson 1996:313, 1986:174). With these features, "surfaces of high relief may be examined with ease" (Hillson 1986:174). Lastly, the specimen under review is not destroyed. (Hillson 1986:174). This last point is of particular importance when museum collections are examined.

Replicas of Crown Surfaces

As Boyde and Martin (1984a:352) state, "persons responsible for valuable museum specimens are naturally apprehensive about the changes which may be introduced by the cutting, grinding, and etching procedures used in preparing mature teeth for SEM." These destructive methods are necessary, however, to "extract the greatest amount of information pertaining to minor afflictions and disturbances" within

the tooth (Boyde 1990:229). Fortunately, both perikymata and enamel hypoplasias may be conveniently studied from impressions and replicas of tooth crown surfaces under the SEM, thus providing a non-destructive examination of museum and site collections (Hillson 1989:97).

Wolf is credited with inventing the replica technique to study the surface of enamel (Boyde 1990:253). The microscopic study of perikymata is related to Scott and Wyckoff's (1947) replica technique of intact tooth surfaces (Boyde 1990:253). The materials used to produce replicas for analysis are crucial when the reproductive quality of the tooth surface is considered (Mann *et al.* 1991:171). Mann, Monge, and Lampl (1991:171) found that the number of replicated perikymata differed with the brand of resin, thus affecting the counts from specimens. The replica method, however, is the "most convenient way" to study perikymata and associated enamel hypoplasias (Hillson 1992b:65).

Replicas are usually constructed from tooth crowns by covering the tooth's surface with latex or a silicone rubber (Hillson 1986:171). Because they are capable of picking up microscopic surface detail, silicone based impression materials, such Coltène President and 3M Express, are the most commonly used impression materials (Goodman and Rose 1990:94; Hillson 1996:299). The impression that is thus created may be directly examined using a low power microscope, or it may be further used as a mould to create a replica cast (Hillson 1996:299,1986:171). Replicas are made from epoxy resins like Epo-tek 301 or Araldite (Hillson 1996:299, 1986:171). The replica, in turn, is coated with a conduction material and examined in the SEM (Hillson 1986:171). The impression technique was devised by Beynon (1987) for the study of fossil enamel microstructure. Hillson (1992a) elaborates on Beynon's methodology by describing the various materials that can be used in replication techniques, what specimens should be selected and how they should be prepared.

SEM, Perikymata, and Enamel Hypoplasia: A New Approach

Boyde is credited with "introducing" the SEM to the examination of enamel development and structure (Boyde 1990:254) and he examined human enamel hypoplasia

with a SEM in 1970 (Boyde and Martin 1984a:351). Because Boyde (1970) had been able to observe ameloblastic pits in the base of hypoplastic grooves, Boyde and Martin (1984a:351) attempted to examine the "essential features of enamel development" on the tooth crown surface of fossil samples. The researchers found that enamel developmental features were destroyed in developing teeth subjected to drying processes and other postmortem changes but were preserved in hypoplastic grooves due to continued mineralization after ameloblastic cessation.

The relationship between perikymata and enamel hypoplasia morphologies was noted by Puech and Albertini in 1981. These researchers examined 200,000-year-old hominoid teeth recovered from the 1953 and 1958 excavations of the Lazaret site near Nice, France. A deciduous upper left incisor and a permanent lower right canine were examined using a SEM at 50X, 200X, 800X, and 2000X magnification. Pedersen and Scott's (1951) "Replica Method" was used, in which a replica was cast in resin and the model gold coated for SEM analysis. This method was the only one known to the authors that permits the "microscopic analysis of the dental surface when the tooth is to be placed in vacuum" (Pedersen and Scott 1951:449). Minor hypoplasias (macro/micro pits, craters, linear grooves) were evident over the entire crown surface and were thought to be the result of accentuated perikymata. Pits were formed from the converging striae of Retzius whereas micro-pits were thought to correspond to enamel prism extremities. The authors concluded that the boundaries between normal and pathological characteristics of enamel surfaces could not be distinguished microscopically.

Moggi-Cecchi (1989) also examined the typical morphology of pits, lines, and grooves under a SEM (Moggi-Cecchi and Crovella 1991:106). This analysis of enamel microdefects included the study of non-human primates.

Bermúdez de Castro and Pérez (1995:308) included SEM images to provide a visualization of the various classes of enamel hypoplasias (pits, grooves, and lines) described in their article. Although they identified these varieties of hypoplasias in their sample of Middle Pleistocene Hominids from the Sima de los Huesos site in Sierra de Atapuerca, Spain, they did not actually incorporate a SEM into their methodology when examining the frequencies of EH within these hominids.

Dental anthropologist Simon Hillson of the University College, London, is largely responsible for what little research has been conducted in the area of SEM analysis and the study of perikymata associated with enamel hypoplasias. Hillson (1989) was the first to propose that growth disruption times (i.e., enamel hypopolasias) could be determined from the counting of perikymata on the surface of tooth crowns. Two instruments, a stylus profiler and a measuring microscope, connected to a computer system were used in the counting of perikymata (Hillson 1992a:70; 1989:97). Although the profiler experienced difficulties in recording because of different degrees of firmness in replica surfaces (Hillson 1989:101), the measuring microscope proved to be a most useful instrument in the recording of perikymata counts (Hillson 1989:104).

Hillson (1992b) later utilized this measuring microscope to estimate the onset of enamel hypoplasias in Egyptian, Roman, Medieval and Post-Medieval materials from a number of collections in England. Using Beynon's (1987) method, Hillson (1992b:463) made silicone-based impressions and high resolution resin replicas of specimens selected specifically for prominent hypoplastic defects. To aid in the counting of perikymata, a modified engineer's microscope created perikymata profiles of the external enamel surfaces. Enamel defects and the perikymata profiles were then matched with the teeth of individual specimens to construct perikymata groove sequences. These sequences identified systemic disturbances, a consistent growth rhythm (i.e., periodicity) and were used to estimate crown formation times of different teeth within an individual.

Five years later, Hillson and Bond (1997:102) focused their research upon the perikymata grooves within enamel hypoplasias (i.e., the perikymata counts in enamel hypoplasias were used to determine the relative timing and sequence of these enamel defects). Furrows, also known as linear enamel hypoplasias, were observed to be "a part of a continuum of defects which extend down into a microscopic scale that would otherwise be ignored in a clinical setting" (Hillson and Bond 1997:89). Furthermore, it was observed that the width of these furrows can provide a "misleading impression of the duration of the disturbance" because in reality, the growth disturbance is restricted to the occlusal wall not the floor or cervical wall of this hypoplasia (Hillson and Bond 1997:98).

Lastly, Skinner (2000, with Hopwood n.d.) used the SEM perikymata methodology to closely examine the timing of enamel hypoplasias in non-human primates. Preliminary perikymata counts between hypoplastic defects in orangutans show that the average timing of LEH occurs every six months, supporting the earlier hypothesis that LEH is cyclical in nature (Skinner *et al.* 1995).

SUMMARY

The periodicity of growth layers is of interest because they "usually reflect a rhythmic metabolism which is synchronized with the environment" (Dean 1987:157). These incremental layers including cross-striations, striae of Retzius, and perikymata have been used to extrapolate growth curves of crown tooth surfaces and subsequent timings in the onset of defects in enamel, particularly in enamel hypoplasias. These enamel defects, which serve as non-specific indicators of systemic stress, have been used to infer the quality of life in both human and animal populations. Such scenarios are frequent in hominid studies and numerous within human populations (both archaeological and contemporary) as well in the case of certain non-human primates, particularly the chimpanzee, gorilla, and macaque. While the chimpanzee has been the focus of many EH studies, its counterpart, the bonobo (*Pan paniscus*) has been ignored.

CHAPTER 3: MATERIALS AND METHODS

The investigation of enamel hypoplasias in the bonobo was conducted at both the macroscopic and microscopic level. A gross examination of the teeth was performed to determine the overall prevalence of the defect in this ape, and a scanning electron microscope was used to examine the perikymata on the tooth crown surface to elucidate the timing and periodicity of this defect. Lastly, attempts were made to determine crown formation chronologies for bonobo incisors and canines from SEM perikymata counts and thin sections.

The Skeletal Sample

All dental material examined in this study is from the Pygmy Chimpanzee Zoological Collection housed at the Royal Museum for Central Africa in Tervuren, Belgium. A data collection form was created to record the dental information for each specimen (see Appendix C).

The museum was established by King Leopold II in 1898 as "la Musée du Congo." Its establishment was the result of public interest generated by his Central African exhibition of animal, plant, and ethnographic materials from the Congo (African Museum 1998). Since the 1960s, the museum has been called la Musée Royale de l'Afrique Centrale. It is regarded as "one of the major centres for the scientific study of Africa in the world" (African Museum 1998).

The Royal Museum holds the only sizeable collection of *Pan paniscus* skeletal material (Susman 1984:xviii).[1] To date, 206 specimens have been catalogued. The majority are crania (N=189) supplemented by a small number of skeletons, skins, and individuals preserved in alcohol. The collection is catalogued by the museum according to sex, age, last erupted tooth, locality, collector, year of procurement, and completeness of skeleton.[2]

Dental developmental ages for bonobos are not yet known; therefore the age at death was determined from dental eruption and cranial development (the fusion of the basi-occipito-sphenoid suture) criteria compiled by Lovell and colleagues (2000) for the common chimpanzee [Table 3-1].

From the 189 bonobo crania, one specimen comprised a cranial vault and could not be assigned a dental age (RG 29002). The remainder of the specimens could be assigned to a dental developmental category. They are represented in Table 3-2. The distribution of these age categories and a breakdown by sex are shown in Figure 3-1.

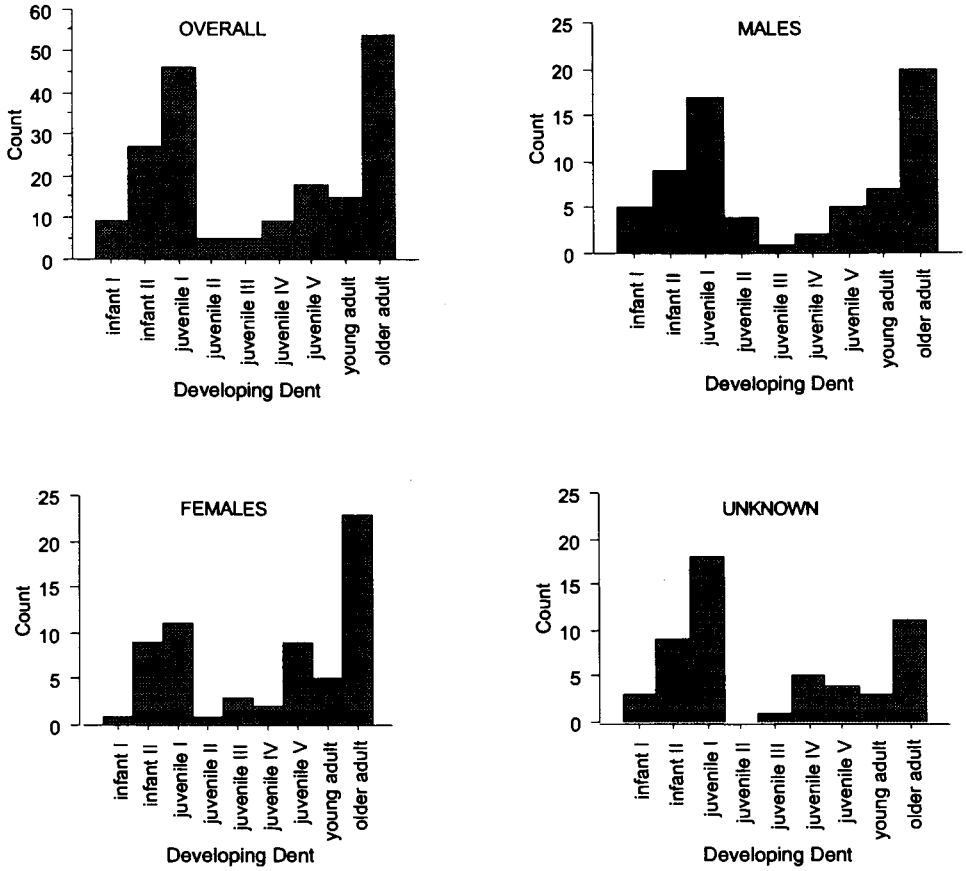
Table 3-1: Dental Developmental Stages and Chronological Age Estimates for Common Chimpanzees

Developmental Category	Criteria	Chronological Age (Years)
Infant I	Incomplete deciduous dentition	< 1
Infant II	Complete deciduous dentition	~ 1
Juvenile I	Deciduous teeth & permanent 1st molar	~ 3
Juvenile II	Deciduous teeth & permanent 1st molar & incisors	5 - 6
Juvenile III	Very mixed dentition	6 - 8
Juvenile IV	Deciduous canines & all permanent teeth except M3	8 - 9
Juvenile V	All permanent teeth except M3; no deciduous teeth	9 - 10
Young adult	All permanent teeth; basilar suture open	10 - 15
Older adult	All permanent teeth; basilar suture closed	> 15

Table 3-2: Total Number of Bonobo Crania at Tervuren

Developmental Category	Female	Male	Unknown	Total
Infant I	1	5	3	9
Infant II	9	9	9	27
Juvenile I	11	17	18	46
Juvenile II	1	4	0	5
Juvenile III	3	1	1	5
Juvenile IV	2	2	5	9
Juvenile V	9	5	4	18
Young adult	5	7	3	15
Older adult	23	20	11	54
Totals	64	70	54	188

Figure 3-1: AGE AND SEX DEMOGRAPHY OF TERVUREN BONOBO COLLECTION



MACROSCOPIC DATA COLLECTION

Enamel Hypoplasia Inventory

The Royal Museum's entire bonobo cranial collection was examined for the presence of enamel hypoplasia. The observations were made on an individual-by-individual and a tooth-by-tooth basis.

Observation/Selection Criteria

All deciduous, mixed, and permanent dentitions were grossly examined for the presence of enamel hypoplasia (EH) at the level of the individual. Analysis first was conducted at this level to determine the presence or absence of this enamel defect in specimens of all ages. Past methodologies have excluded individuals with excessive dental attrition, but in doing so, individuals in older age categories have been excluded. Their inclusion here is justified by arguing EH is manifested during the years of dental development. It is this period of development in which stress is examined. Despite the fact that dental crowns are worn, EH in the cervical third of the tooth in these aged individuals can still be detected. By acknowledging EH in these older individuals, another facet in the life histories of the population may thus be inferred.

Single teeth with excessive dental attrition were excluded. Exclusion was necessary during the examination for multiple defects whose presence would not have been evident if the tooth crown was worn. Also excluded at this level were teeth with chipped or missing enamel. Only completely erupted teeth were selected for further examination; however, incomplete dentitions due to postmortem loss were accepted.

The labial (cheek-side) surfaces of these teeth then were examined under natural and artificial oblique lighting for the presence of EH. A Bausch and Lomb 5 –20 X pocket magnifier and Bausch and Lomb 0.7 – 3 X stereomicroscope also were used to confirm identification of the hypoplasia. This method of observation is the recommended standard put forth by Goodman and Rose (1990:92) and has been followed by a number of researchers involved in studying EH in non-human primates (Ekhardt 1992, Guatelli-Steinberg 1998, Hannibal 2001b, Lukacs 1999a, Moggi-Cecchi and Crovella 1991, Newell 1998, Skinner 1986b, Skinner and Hopwood 2003).

For the macroscopic analysis, specimens were not cleaned. Cleaning all the teeth for examination would have been an enormous task and might have been detrimental to the preservation of the collection. For example, attempts appear to have been made previously by an unknown researcher to remove dental calculus from tooth crowns in this collection, resulting in the removal of the enamel surface, particularly from the molars. Although Goodman and Rose (1990:91) recommend cleaning and Newell (1998:57)

removed "excessive dirt, glue, and residue," Skinner (1986b:294) felt tartar stains within grooves enhanced defect definition.

Scoring Criteria

The enamel hypoplasias scored in this study of bonobo teeth were linear enamel hypoplasia (LEH), pits, plane-type, and localized hypoplasias of the primary canine (LHPC). LEH was scored at threshold values of absent, mild, moderate, and severe, based on their comparative prominence. Examples of these thresholds are found in Figures 3-2 to 3-4 and are listed in Table 3-3. Pitting hypoplasias were recorded as single or multiple pits, and plane-type and LHPC were recorded as present or absent.

At the museum a cursory examination of the collection was first made and examples of enamel hypoplasia thresholds were selected and set aside for reference. These specimens were subsequently photographed and impressions made for casting purposes. Replicas were cast and images of these teeth were captured with a scanning electron microscope (SEM). Thus, a permanent record of enamel hypoplasia thresholds is available from resin replicas and SEM images for future consultation and comparison.

Table 3-3: Royal Museum Bonobo LEH Threshold Reference Specimens

Museum Specimen No.	SFU Mould No.	EH Severity
RG 26993	55c	Mild
RG 23509	115a, 116a, 117a	Mild
RG 29060	124a	Moderate
RG 28712	59c	Moderate
88041 M8	128a, 129a	Moderate
RG 21742	112a	Severe
RG 22908	53c, 114a	Severe
RG 29040	61c, 121a, 122a	Severe

Measurements

Three methods are commonly used to age LEH: the comparison of defects to growth schedules, the measurement of the cemento-enamel junction (CEJ) to enamel defect distance, and the counting of perikymata on the tooth crown surface. Regardless

Examples of LEH Thresholds from the Royal Museum for Central Africa *Pan paniscus* Collection

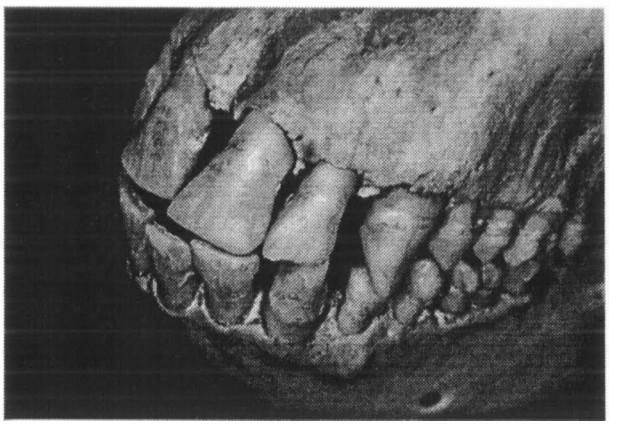


Figure 3-2:
Mild Enamel Hypoplasia

[RG 21697, CP3-26]

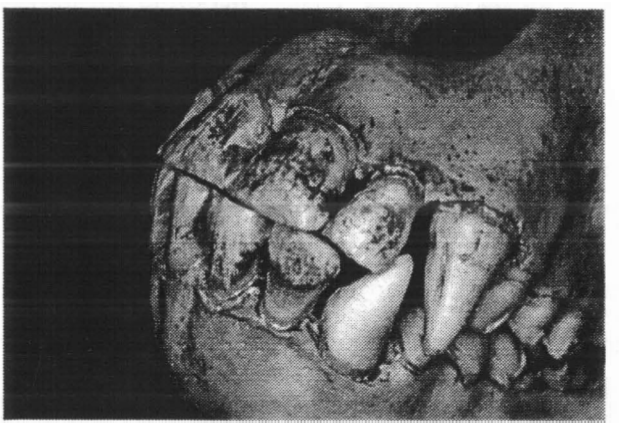


Figure 3-3:
Moderate Enamel Hypoplasia

[RG 26971, CP3-14]

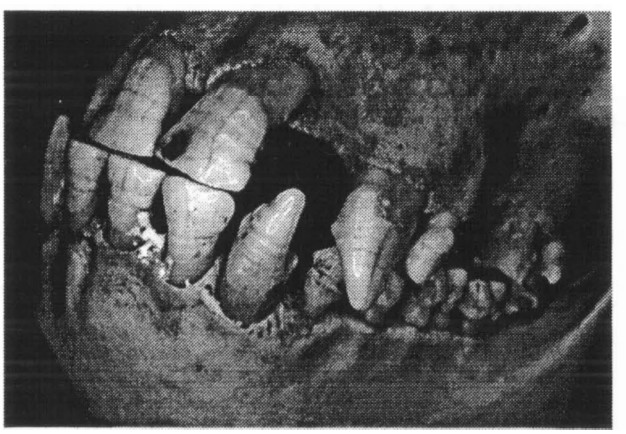


Figure 3-4:
Severe Enamel Hypoplasia

[RG 29040, CP3-1]

PHOTOGRAPHS: Joe Y. Tsukamoto, Belgium, May 2000.

of the method employed, according to Skinner and Goodman (1992:165 citing Goodman and Rose 1991:287) "the most important factor in determining the chronology of enamel hypoplasias relates to the choice of developmental standard and how it is interpreted and employed."

CEJ measurements were made by taking caliper measurements from the furthermost CEJ to the midpoint of the enamel defect, using Mituyo Digital Pointed Jaw Calipers (Model NTD12-6" C, Code 573-221-10). An exception to procedure was made if the hypoplasia displayed more widely separated margins, in which case both margins were measured. Measurements were recorded on the data collection form, and an outline of the tooth was sketched and marked with the corresponding hypoplasia. These measurements were converted to ages after calculating the relative position of the defect on the crown of the affected teeth and comparing them with known crown formation schedules for the chimpanzee.

With crown growth schedules, the timing of defect formation is extrapolated from tooth development schedules because formation and maturation of teeth are assumed to occur at predictable rates (Hillson 1996:175; Hillson and Bond 1997:91; Lovell 1999:70). In the case of the bonobo, crown growth schedules do not exist; thus dental crown calcification schedules for the common chimpanzee determined though histological means by Reid and colleagues (1998), Reid, Hillson, and Dean (unpublished data), and Schwartz and Dean (2001) were used for purposes of comparison.

Because the size of the bonobos' dentition is less than that of the common chimpanzee (Kinzey 1984, Johanson 1974a,b), crown formation times are also expected to differ between the species. As part of this study, imbricational crown formation schedules were created for a few bonobo teeth and also used in calculating the timing of defects. As a result, it is predicted that calculations based on bonobo crown formation times will be a more accurate reflection of the timing of the defect when compared to calculations involving available data for the other species of chimpanzee.

MICROSCOPIC DATA COLLECTION

Perikymata and SEM Imaging

The advantages of using a SEM to examine the gross morphology of the enamel surface are numerous. The SEM possesses a "relatively enormous" depth of field, and samples may be examined using a wide range of magnification 15 to 100,000 X (Hillson 1996:313, 1986:174). With these features of SEM, "surfaces of high relief may be examined with ease" (Hillson 1986:174). Furthermore, the specimen under review is not destroyed (Hillson 1986:174). This last point is of particular importance when museum collections are examined. As Boyde and Martin (1984a:352) state, "persons responsible for valuable museum specimens are naturally apprehensive about the changes which may be introduced by the cutting, grinding, and etching procedures used in preparing mature teeth for SEM." Fortunately, both perikymata and enamel hypoplasias may be conveniently studied from impressions and replicas of tooth crown surfaces under the SEM, thus sparing the destruction of museum and site collections (Hillson and Jones 1989:97).

Selection Criteria

For the microincremental analysis, the teeth which qualified for SEM examination had to display perikymata prominently in association with two or more LEH. From the collection of 189 crania at the Royal Museum for Central Africa, 24 crania had teeth that met the criteria. From these individuals, 51 teeth were impressed and casts made. From these teeth, 37 composite images were made and 22 images were used for further analyses. The number of individual bonobos ultimately chosen for inclusion in this study is 17 [see Table 3-4].

Impression/Replica Procedure

The impression/replica method devised by Beynon and Wood (1987) and Hillson (1992a,b) was used in this study. The impression technique was developed by Beynon and Wood (1987) for the study of fossil enamel microstructure. Hillson (1992a, 1992b,

Table 3-4: Sample of Bonobo Teeth for SEM Imaging and Perikymata Counts

Museum Specimen #	SFU Mould #	Sex	F.D.I.* Tooth #
RG 9338	49c	female	1-2
RG 13201	51c	female	3-2
RG 22908	53c	unknown	1-1
RG 26993	55c	female	1-3
RG 27698	58c	female	3-3
RG 28712	59c	male	3-3
RG 29040	60c	female	4-3
RG 29040	61c	female	1-1
RG 29040	61c	female	3-1
RG 9957	109a	unknown	2-1
RG 9997	110a	unknown	1-2
RG 21742	112a	female	2-2
RG 21742	112a	female	2-3
RG 29030	119a	female	2-1
RG 29031	120a	female	1-2
RG 29040	121a	female	2-4
RG 29040	122a	female	3-4
RG 29055	123a	female	1-2
RG 29060	124a	female	2-1
88041 M8	128a	unknown	2-1
88041 M8	129a	unknown	3-2
88041 M8	129a	unknown	3-1

* Fédération Dentaire Internationale (FDI)

1996) elaborates on their methodology by describing the various materials that can be used in replication techniques, the selection of specimens and their preparation.

The selected tooth, or group of teeth, was first cleaned with acetone (nail polish remover) and gaps were plugged with dental wax. Aluminium foil was smoothed over the cleaned tooth crown surface and Coltène President lab putty was pressed over the region to create a supporting mould. (Because they are capable of replicating microscopic surface detail, silicone based impression materials, such as Coltène President and 3M Express, are the most commonly used impression materials (Goodman and Rose 1990:94; Hillson 1996:299).

Once the putty had set, it was pulled away from the dentition and polyvinylsiloxane was dispensed with a syringe into the mould. The aluminium foil then

was removed and the tooth impressed into the silicone-based material. After the impression material had set, it was carefully pulled away from the tooth, labelled on the putty surface, and stored in a sterile bag [see Figures 3-6 to 3-9]. The impression that is thus created may be directly examined using a low power microscope or the mould may be further used to create a replica cast (Hillson 1996:299, 1986:171).

Replicas of these teeth later were made using Araldite (MY753) resin. A ratio of 100 milliliters of Araldite to 38 milliliters of hardener (XD716) was used. These casts were left to set for a week in a fume hood. The replicas then were bagged in Fisherbrand 3-inch by 7-inch sterile sample bags to prevent dust contamination.

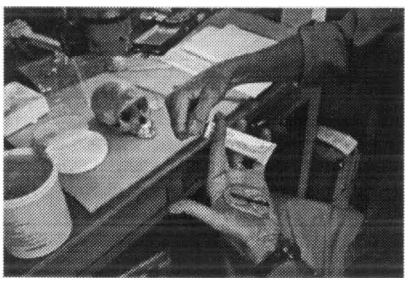
SEM Procedure

The casts were examined with a Cambridge 250T SEM at the University of British Columbia's Electron Microscopy Lab in Vancouver, British Columbia. The specimens were placed on aluminium specimen stubs, and Pelco colloidal silver paste was used if they did not remain mounted with adhesive tabs. A Nanotech SEM Prep II sputter coater was used to coat the teeth with gold. Sputter coat runs were 3.5 minutes in duration. Specimens were coated a second time if there was electronic charging of the specimen in the SEM. Specimens were stored in stub boxes, and final storage is to be in a container with desiccant.

Once in the SEM, the specimen was bombarded with electrons at 5 to 10 kilovolt (kV) and magnified between 20X to 30X. A portion of the tooth crown surface from its occlusal surface to the CEJ was captured on Polaroid 4-inch by 5-inch land film type 55 positive/negative. These SEM Polaroid photos of the teeth could then be overlapped (i.e., montaged) with the aid of computer software.

Adobe Photoshop Procedure

Photo montaging was done on a Macintosh PowerPC G4 500 MHz dual microprocessor loaded with 510 MB of RAM at Simon Fraser University's Department of Archaeology Photo Lab.



CREATING DENTAL IMPRESSIONS FOR CASTING and SEM ANALYSIS

Figure 3-5 (left): **STEP 1**

Clean dentition and plug gaps with dental wax Cover dentition with aluminium foil and mix putty.

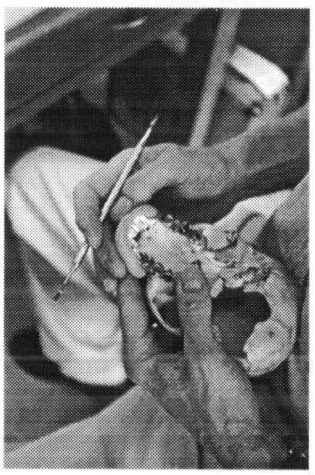


Figure 3-6 (left): **STEP 2**

To create a support mould, press dentition into putty and allow to set.

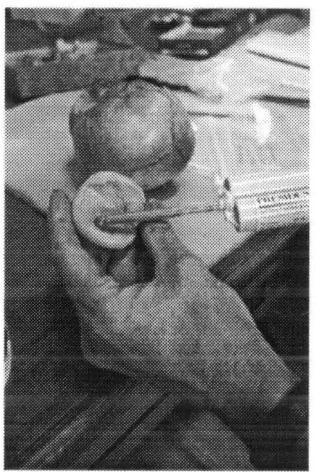


Figure 3-7 (right): **STEP 3**

Gently remove putty from dentition and fill mould with polyvinylsiloxane.

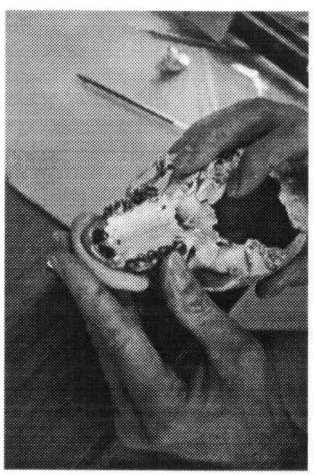


Figure 3-8 (left): **STEP 4**

Remove aluminium foil and impress dentition into the polyvinylsiloxane. Allow to set.

Figure 3-9 (right): **STEP 5**

Gently remove final mould from dentition. Immediately place in sterile bag to prevent dust contamination.

PHOTOGRAPHS: Suyoko Tsukamoto, Belgium, May 2000

The black and white SEM polaroids were scanned into Adobe Photoshop 6.0.2 with a UMAX Vista Scanner at 300 dpi as a black and white picture. They were saved as TIFF images and montaged in this graphics programme. Each photo was saved as a separate layer, and the final product was left uncompressed so that future analysis (additions, changes, observations) can be done. An example of a composite SEM image may be found in Figure 3-10.

Measurements

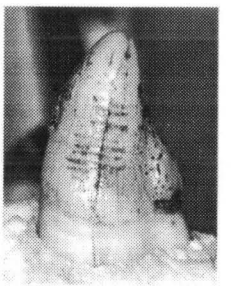
The enamel hypoplasias visible in the montage were checked against photographs taken in the field of macroscopically identified LEH, pencil marked for clarity, so that the same defects could be identified micro- and macroscopically. With these identified, further episodes were sometimes distinguishable in the SEM. In all cases of two or more LEH, the number of perikymata between episodes (interval) and the number of perikymata within grooves were counted. The perikymata were traced by magnifying the image and using the Adobe 6.0.2 line shape tool to mark their position. These lines can be labelled and saved as a separate layer on the image.

Cross-Striation Counts

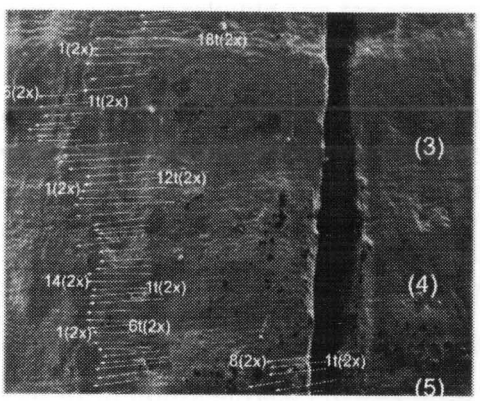
From the museum, two bonobo teeth were obtained for the express purpose of determining cross-striation counts between striae of Retzius from histological thin sections. Dr. Wim van Neer of the Royal Museum for Central Africa supplied the teeth with the proviso that replicas be made to replace the original teeth in the jaws and that the histological sections and remaining tooth sections be returned to the museum. Destructive analysis is permitted only on bonobos that died in captivity (i.e., zoo specimens).

One canine and one incisor were obtained from specimens RG 5374 and RG 11528, respectively. The sex for RG 5374 is not known although the size of the canines suggests it may be male. Drawing upon the developmental categories compiled by Lovell *et al.* (2000), this specimen is a juvenile whose chronological age is between 9 and 10 years, (i.e., all its permanent teeth are present except M3). This animal was collected in Ibembo, Belgium Congo, for the Jardin Zoologique d' Anvers (Antwerp) in 1921.

Figure 3-10: Example of ADOBE® Photoshop Composite SEM Image



Photograph of tooth crown
(LEH marked with pencil)



Close-up of traced perikymata and their counts

SEM montage of RG 28712

SEM Imaging: Suyoko Tsukamoto, University of British Columbia, June/July, 2000

Little is known about RG 11528. Based on its very mixed dentition, the chronological age for this female is between 6 and 8 years. This bonobo was collected in the Belgium Congo, but the exact locale is not known. It died at the Jardin Zoologique d' Anvers in 1932. Very likely these animals formed the majority of their enamel in the wild.

These teeth were sent to Dr. Don Reid at University of Newcastle's Department of Oral Biology in the School of Dentistry for histological sectioning. Preliminary results indicate that perikymata are representative of weekly growth, with the incisor and canine displaying six and seven cross-striations, respectively, between striae of Retzius (Dr. D.J. Reid, personal communication December 5, 2000).

Crown Formation Spans

Reid and Dean (2000) assert that tooth crown formation is not a linear process, thus by establishing the crown formation times for respective *fractions* of a tooth crown, from perikymata counts of imbricational enamel, a more reliable age for enamel hypoplastic defects can be acquired. From the histological examination of primate tooth crowns, Reid, Hillson, and Dean (2000) found there were "clear differences in the rates at which anterior crown heights grow among primates." They, therefore, devised a methodology for charting age intervals for anterior tooth formation in a number of primates to aid in the estimation of timing enamel hypoplastic defects on the tooth crown surface. To accomplish this they considered the mean initiation age for each tooth, cuspal enamel formation times, and the time to form imbricational enamel from histological thin sections.

Initiation times were determined by "matching accentuated striae of Retzius across the dentition with reference to the neonatal line of the first permanent molar" (Reid, Hillson and Dean 2000). Cuspal enamel formation was calculated by measuring the cuspal enamel thickness, multiplying by a constant, and dividing by mean cross-striation measurements. Lastly, the imbricational enamel of the tooth crown was divided into tenths. Striae, which reached the enamel surface, were counted in each fraction and multiplied by the cross-striation counts to determine the time in days to form imbricational enamel.

A modified version of the above methodology was employed in this study to determine the crown formation spans, i.e., imbricational enamel formation span, from imbricational enamel for the various fractions of bonobo tooth crowns. In this study, the imbricational enamel formation times were not determined from thin sections or from the examination of tooth crown surfaces with light microscopy but from the SEM montages which showed perikymata almost ranging from the occlusal surface to the cervical margin of the tooth crown surface.

Thus, from the permanent bonobo tooth sample, tooth crowns were divided into tenths and the frequency of LEH in each fraction was calculated. By determining which region or fraction of the tooth crown LEH was found, estimates could be made about the time of defect formation by comparing them to the bonobo imbricational enamel formation times for those particular zones. The museum specimens used for this analysis are listed in Table 3-5.

Table 3-5: Sample of Bonobo Teeth on which Perikymata Counts were Taken to Determine Imbricational Enamel Formation Rates

Museum #	Cast #	F.D.I. Tooth #	Sex
RG 9338	49c	2-1	Female
RG 22908	53c	1-1	Unknown
RG 29040	61c	1-1	Female
RG 9997	110a	1-1	Unknown
RG 29029	118a	1-1	Unknown
RG 29030	119a	2-1	Female
RG 29060	124a	2-1	Female
84036 M1	126a	3-2	Female
RG 29031	129a	1-2	Female
RG 29053	63c	2-3	Male

STATISTICAL ANALYSIS

Data analyses were divided into two parts: (1) a descriptive analysis of the prevalence of enamel hypoplasia within the bonobo, and (2) the further examination of the timing and the periodicity of LEH.

At the macroscopic level, enamel hypoplasias were compared with a number of variables such as presence and absence, multiple defects, severity, age (juvenile vs. adult), sex (male vs. female), tooth class (incisor, canine, etc.), and dentition (mandibular vs. maxillary, permanent vs. deciduous, left vs. right). Frequencies using these variables were generated and then tested for significance (α=0.05) using chi-square (χ^2) tests and ANOVA. Whenever a χ^2-table had one degree of freedom and the expected value was less than 5, a correction for continuity, also known as the Yate's correction, was calculated to improve the accuracy of the χ^2-test. Examples of testable null hypotheses include the propositions that there are no differences between the sexes, there are no differences between tooth class, and so forth.

The timing and periodicity of enamel hypoplasia were examined at both the macroscopic and microscopic level. In order to study this periodicity, bonobo teeth were selected for further study if they displayed two or more episodes of LEH. Based on caliper measurements, the timing of the first episode of LEH, and the intervals between stress episodes, as well as the duration of stress, can be calculated from comparisons with crown height growth schedules for the common chimpanzee. For the microscopic observations of perikymata, the numbers of perikymata between enamel defects and within enamel defects were determined using means and medians to seek any evidence of episodic stress.

With these statistical analyses, it should be noted that the Royal Museum Pygmy Chimpanzee Collection is an opportunistic sample, i.e., over a span of 60 years, the specimens in the collection were obtained by different researchers for different purposes. For a sample to be truly representative of the (target) population, however, specimens should be chosen at random from the population. From Figure 3-1, it is apparent that there was collection bias – young and old bonobos were typically captured thereby creating a hunting mortality cohort. Because the number of males and females per age category was limited in the collection, all individuals were included rather than taking a random stratified sample.

CHAPTER 3 NOTES

1. It was from the collection at Tervuren, in 1929, that Dr. Ernst Schwarz identified a new chimpanzee, *Pan satyrus paniscus*, the first bonobo type specimen (#RG9338) (van den Audenaerde 1984:5, Schwarz 1929), which Coolidge (1933) elevated to species status, *Pan paniscus*, after a complete examination of its skeletal material.
2. The bonobo collection at the museum in Tervuren is described in the first chapter of *The Pygmy Chimpanzee: Evolutionary Biology and Behaviour* (1984). In the book van den Audenaerde (1984:11) states that the bulk of the bonobo skeletal material was collected by Belgium embryologist and zoologist George Vandebroek. Vandebroek lead an expedition, supported by the Belgian Science Foundation, to the Congo in 1955 for the express purpose of collecting bonobo specimens for the Royal African Museum (van den Audenaerde 1984:7). In Kinzey's (1984:66) study of bonobo dentitions, the author also credits Vandebroek with procuring the bulk of the collection but goes on to explain that in addition to some of the specimens being wild-shot, "many were from animals used in medical laboratories there."

   According to van den Audenaerde (1984:11), "little chimpanzee material has been received in the past 20 years and collections have remained what they were following Vandebroek's expedition." While it is true that in the museum's catalogue Vandebroek is credited with collecting 77 bonobos (mostly crania) in 1955, another individual, Ghislain Courtois, is credited with collecting 49 crania. No dates are listed for his period of collection; however, Edward Hooper (1999), discusses work conducted by Courtois in the former Belgian Congo.

   A senior Belgian colonial physician, Courtois was head of the Laboratoire Médical de Stanleyville in the 1950s. He assisted with the establishment of Lindi camp with Hilary Koprowski, a virologist working on oral polio vaccine (OPV) tests in Africa (Hooper 1999:xiii). It was at this camp that captured *Pan paniscus* and *Pan troglodytes* were used in developing polio vaccine (*ibid*, p.542). The bonobos are thought to have died from severe stress and emotional trauma within two weeks of their capture (*ibid.*, p.544). After the animals died, they were "trepanned" and the brain removed (*ibid.*, p.578). Indeed a number of the crania in the Tervuren bonobo collection show evidence of this procedure. Courtois apparently had shipped 79 chimpanzee skulls to the museum prior to 1957 (*ibid.*, p.721): 49 bonobos and 30 common chimpanzees (*ibid.*, p.998n70). Although the museum catalogue reflects these numbers, Hooper believes that these animals possibly were mislabelled at source and the actual totals should be 47 *paniscus* and 32 *troglodytes* (*ibid.*, p.998n70).

   Of further interest is the fact that the chimpanzees used in the Lindi experiments from 1956 to 1958 were captured by Monsieur Gilbert Rollais, Stanleyville Zoo manager and expert in primate capture (*ibid*, p.579). However, Rollais' first job on coming to Stanleyville in 1955 was to collect *P. paniscus* skeletons for Professor Vandebroek (*ibid.*, p.582). His job was to capture bonobos and kill them, after which "they were placed in large petrol drums, and then boiled to remove the flesh" (*ibid.*, p.582). This behaviour contrasts somewhat with van den Audenaerde's (1984:7) account of Vandebroek's expedition in 1955. Vandebroek is said to have "encouraged local Africans and Europeans to preserve skulls and skeletons for him. In one place [he] left a petroleum drum with a small quantity of formaldehyde. When

he returned 3 months later, he found the petrol drum filled with more than 20 decomposing chimpanzee skulls."

Regardless of this discrepancy in reports of collection methods, Hooper's account of the bonobo autopsies and Vandebroek's employment of Rollais for hunting and subsequent processing of the pygmy chimpanzees are the only available references regarding the "preparation" of these museum specimens in the field. Furthermore, based on Rollais' account, his collection of *P. paniscus* for Courtois at Lindi Camp was made *after* the Vandebroek expedition, thus suggesting "collections have [not] remained what they were following Vandebroek's expedition." From Hooper's recounts, it appears then, Gilbert Rollais was responsible for the collection of the bulk of the Tervuren pygmy chimpanzee collection.

CHAPTER 4: RESULTS: PREVALENCE OF TYPES OF ENAMEL HYPOPLASIA

The analysis of enamel hypoplasia in the bonobo is divided into two parts. For this chapter, a general inventory of this dental enamel defect is conducted to determine its prevalence. For the next chapter, the timing and periodicity of enamel hypoplasia are determined through the caliper measurements and perikymata counts from the tooth crown surface.

For the prevalence inventory, the units of study involved two levels of analyses: individual-by-individual and tooth-by-tooth. For the individual analysis, enamel hypoplasias were examined in the various age classes and compared between sexes and dentitions and for severity of defect expression. For the tooth-by-tooth analysis, the occurrence of hypoplasias in the different tooth classes (incisors, canine, premolars, molars) was tabulated and comparisons made between side, jaw, severity, and number of defects.

Where possible, LEH, LHPC, pit, and plane hypoplasias were analyzed and compared with other chimpanzee studies. For the level of the individual adults, studies by Hannibal (2000b), Skinner (1986), Guatelli-Steinberg (1998), and Newell (1998) were used. The studies by the latter two researchers were used further for inter-tooth comparisons because they examined both maxillary and mandibular dentitions. Where possible, studies by Lukacs (1999), Newell (1998), and Skinner and Newell (2003) were used as comparisons for individual infants.

LINEAR ENAMEL HYPOPLASIA (LEH)

BY INDIVIDUAL – Frequencies of LEH

Of the 189 bonobo crania in the Royal Museum collection, 182 were analyzed at the level of the individual. One captive specimen was excluded and the other six crania

were excluded from the overall sample because they were missing both their maxillary and mandibular dentitions. LEH was scored as present if an individual expressed LEH on any tooth (regardless of whether the dentition was completely present).

To facilitate comparisons with other studies, the developmental age categories described in Chapter 3 were collapsed into infant, juvenile, adult, and senile categories [Table 4-1]. Infants I and II were reclassified as "infants;" "juveniles" were previously termed juvenile categories I to IV; "adults" were considered individuals which contain only permanent teeth and include juvenile V, young adults, and older adults. Individuals with extreme dental attrition were separated from the older individual age category and reassigned a "senile" age category. Mixed dentitions (i.e., the juvenile age categories) often have been excluded from enamel hypoplasia studies. (Also excluded from these prior studies have been individuals with extreme dental attrition (for reasons previously explained in Chapter 3). For this study, therefore, these individuals were classified as senile individuals.

The prevalence of LEH in the entire Tervuren collection is 54.4% with 99 individuals from all dental age categories scoring positive for LEH. The frequencies of LEH expression in male and female bonobos of varying age categories are displayed in Table 4-1.

Table 4-1: Prevalence of LEH in Bonobo Skulls of Varying Age Categories

Developmental	Females			Males			Unknown		
Age Category	+	N	%	+	N	%	+	N	%
Infant	1	10	10	0	14	0	1	11	9
Juvenile	5	17	29	3	24	13	7	23	30
Adult	28	28	100	22	22	100	8	8	100
Senile	8	9	89	9	9	100	6	6	100

Test of Significance Between Age Categories and Sexes

To test whether there is significance in the relative occurrence of LEH between the sexes in all the age classes, a χ^2-test was calculated. The null hypothesis is that there are no significant differences in the occurrence of LEH between male and female

bonobos of all categories. From this test, it was established that there are indeed no significant differences between the sexes (Table 4-2).

Table 4-2: Observed χ^2-Values for LEH between Male and Female Bonobos of Different Age Classes

Age	df	χ^2	*p*-value	Yates χ^2	Yates *p*
Infant (n=24)	1	1.461	0.2268	0.030	0.8629
Juvenile (n=41)	1	1.812	0.1782	0.914	0.3391
Adult (n=50)*	-	-	-	-	-
Senile (n=18)	1	1.059	0.3035	-	-

* 100% affected

Test of Significance Between Adult and Infant Dentitions

To determine if there is a significant difference in the occurrence of LEH between the adult and infant dentitions, a continuity correction χ^2-test was calculated [Table 4-3]. The null hypothesis is there are no significant differences in the occurrence of LEH between the adult and infant dental development categories. From this test, it was determined that there is a significant difference in the occurrence of this enamel defect (χ^2=65.124, df=1, p<0.0001). The null hypothesis is rejected.

Table 4-3: χ^2-Test for Significance of LEH between Adult and Infant Dentitions

	DENTITIONS		
LEH	Infant	Adult	Totals
Present	1	50	51
Absent	23	0	23
Totals	24	50	74

Yates χ^2=65.124, df = 1, p<0.0001

Frequency in Severity of LEH

The severity of LEH in all age classes was examined [Table 4-4]. All individuals were scored as expressing none, mild, moderate, or severe LEH, based on the most severe episode of LEH present in the mouth. One individual (RG 29051) was not scored for severity and was thus excluded from this analysis.

From the individuals that are affected with LEH (n=98), the majority (22%) are moderately affected, 17.6% are mildly affected, and 14.0% are severely affected.

Table 4-4: Severity of LEH in Varying Age Classes

	Absent		Mild		Moderate		Severe	
Age Classes	+	%	+	%	+	%	+	%
Infant (n=35)	33	94.3	1	2.9	1	2.9	0	0.0
Juvenile (n=65)	50	76.9	6	9.2	3	4.6	6	9.2
Adult (n=58)	0	0.0	18	31.0	27	46.6	13	22.4
Senile (n=24)	1	4.2	7	29.2	9	37.5	7	29.2
Total (N=182)	84	46.2	32	17.6	40	22.0	26	14.3

Test for Association between Severity of LEH and Sex

To determine if there is significance in the severity of LEH expression between the sexes, a χ^2-test was calculated. (Table 4-5). The null hypothesis is there is no significant difference in the severity of LEH expressed between the sexes. From this test, it was determined that there is not a significant difference in the severity of LEH expressed (χ^2=7.279, df=3, p=0.0635).

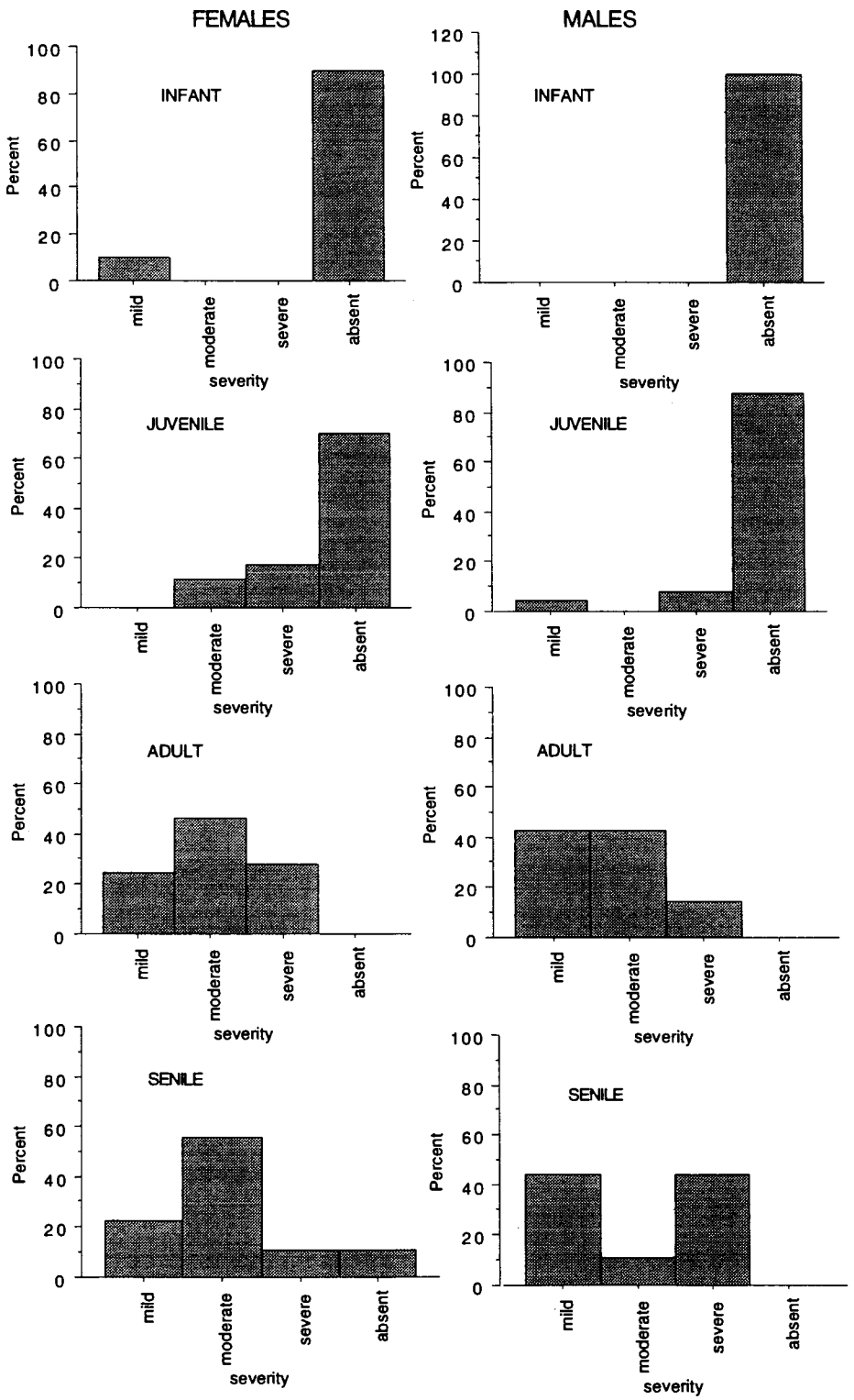
An examination of severity of LEH between the different age categories of the sexes also showed no significant differences between infants (expected value 0), juveniles (χ^2=4.593, df=3, p=0.204), adults (expected value 0), and senile individuals (χ^2=6.133, df=3, p=0.1053). The distribution in the frequency of severity between the sexes for the different age categories is presented in Figure 4-1.

Table 4-5: Test for Association between Severity of LEH and Sex

	Severity				
Sex	Absent	Mild	Moderate	Severe	Totals
Female	22	10	20	12	64
Male	35	14	10	9	68
Totals	57	24	30	21	132

χ^2 = 7.279, df=3, p=0.0635

Figure 4-1: DISTRIBUTION OF SCORES OF EH SEVERITY AMONG AGE AND SEX CLASSES OF BONOBO SAMPLE



Comparisons with other Adult Chimpanzees

Bonobos were predicted to manifest an equivalent LEH prevalence when compared with other chimpanzees. To determine the average prevalence of LEH in chimpanzees, results for chimpanzees by Guatelli-Steinberg (1998), Hannibal (2000b), Newell (1998), and Skinner (1986) were combined [see Table 4-6].

Table 4-6: Proportion of Adult Chimpanzee Samples with LEH

Researcher	LEH		Percent
	+	N	
Guatelli-Steinberg (1998:69)	23	28	82.1
Hannibal (2000b:30)	8	25	32.0
Newell (1998:72)	40	77	51.9
Skinner (1986:295)	65	110	59.1
Totals	136	240	56.7

To determine if there is a significant difference in the prevalence of enamel hypoplasia between the two chimpanzee species, a χ^2-test was calculated [Table 4-7]. The null hypothesis is that there is no significance in the comparative prevalence of LEH between the two species. From this test, it was determined that there is a significant difference (χ^2=38.607, df =1, p<0.0001) in the prevalence of LEH between the two primates and the null hypothesis was rejected.

Table 4-7: χ^2-Test for Significance of LEH Prevalence between Chimpanzee Species (Adults Only)

Chimpanzee	LEH		Totals
	Present	Absent	
Bonobo	58	0	58
Common Chimpanzee	136	104	240
Totals	194	104	298

χ^2=38.607, df = 1, p<0.0001

Different scoring thresholds among researchers may be a factor in this large discrepancy. The 18 adult bonobos, which were previously scored in this study as expressing mild LEH, will now be treated as individuals lacking LEH to see if overall proportions are then comparable to the common chimpanzee populations. As a

consequence, the overall number of bonobos with LEH is 40, or 69% of the adult population, which is still a larger value than the average proportion of chimpanzees with LEH. A second χ^2-test was then calculated [Table 4-8], and from this test, it was determined that there is a not a significant difference (χ^2=2.922, df =1, p=0.0874) in the prevalence of LEH between the two primates when the mild threshold for the bonobos is readjusted and the null hypothesis was accepted.

Table 4-8: χ^2-Test for Significance of LEH Prevalence between Chimpanzee Species

	LEH		
Chimpanzee	Present	Absent	Totals
Bonobo (corrected values)	40	18	58
Common Chimpanzee	136	104	240
Totals	176	122	298

χ^2=2.922, df = 1, p=0.0874

To determine if there is a significant difference with the occurrence of LEH between the sexes of bonobo and chimpanzee populations, additional χ^2-tests were calculated. Results from Skinner (1986:29) and Newell (1998:107) were pooled [Table 4-9] to determine average prevalence of LEH in male and female chimpanzees.

Table 4-9: Proportion of Individual Chimpanzees with LEH

Researcher	Male			Female		
	+	N	%	+	N	%
Newell (1998)	18	30	60	22	47	52
Skinner (1986)	23	35	66	41	75	58
Totals	41	65	63	63	121	52

The null hypothesis is that there is no difference in the occurrence of LEH between the sexes of the chimpanzee species, e.g., there is no difference in the occurrence of LEH between the males of the bonobo and common chimpanzee species. An example of this χ^2-test is found in Table 4-10.

Table 4-10: χ^2-Test for Significance of LEH between Chimpanzee Species of the Same Sex - Males

	LEH		
Chimpanzee (Male)	Present	Absent	Totals
Bonobo	28	0	28
Common Chimpanzee	41	24	65
Totals	69	24	93

χ^2=13.934, df = 1, p=0.0002

From these tests, it was determined that there is a significant difference in the prevalence of LEH between male bonobos and common chimpanzees (χ^2=13.934, df =1, p=0.0002) and female bonobos and common chimpanzees (χ^2=17.741, df =1, p<0.0001), thus the null hypothesis was rejected.

Because there is the concern for differential scoring thresholds, once again the bonobos, which were previously scored in this study as expressing mild LEH, will now be treated as individuals lacking LEH to see if overall proportions are then comparable to the common chimpanzee populations. The number of male bonobos (n=22) scoring positive for LEH is 12 and the number of females (n=28) scoring positive for LEH is 21. The null hypothesis is that there are no differences in the adjusted occurrence of LEH between the sexes of the chimpanzee species.

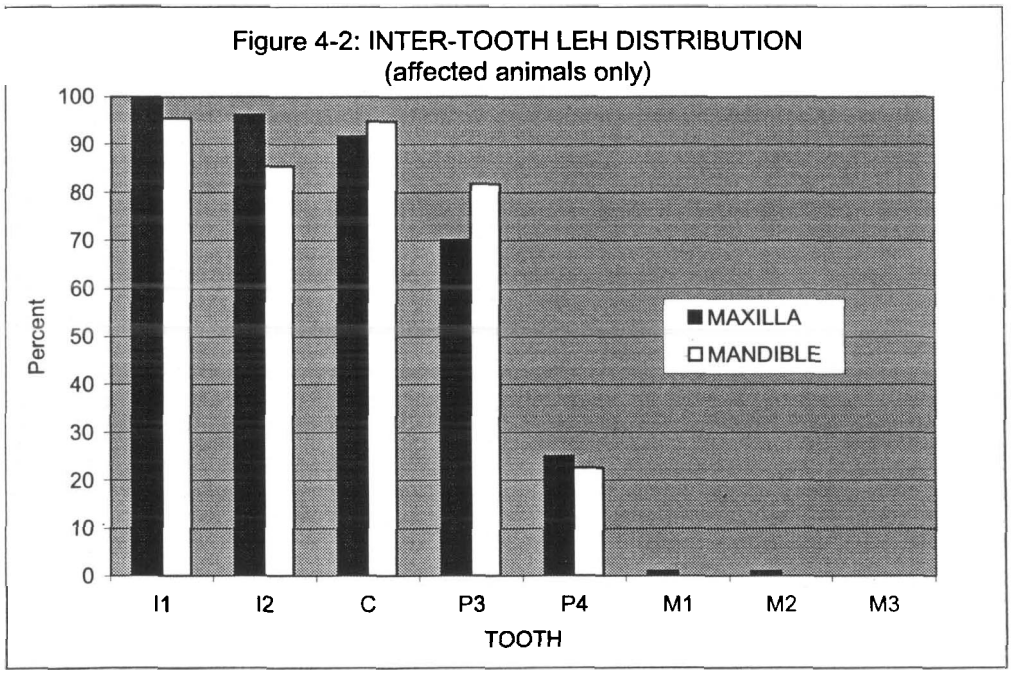
From these tests, it was determined that there is not a significant difference in the prevalence of LEH between male bonobos and common chimpanzees (χ^2=0.503, df =1, p=0.503) and but there is between female bonobos and common chimpanzees (χ^2=4.863, df =1, p=0.0274).

BY TOOTH: Inter-tooth Patterns of the Permanent Dentition

Inter-tooth comparisons were made on the 181 bonobos that scored positive for LEH. For their tooth-by-tooth analysis, both Guatelli-Steinberg (1998:138) and Newell (1998:136) calculated the frequency of the enamel hypoplastic defects by dividing the number of affected teeth by the total number of teeth in the *affected* individuals sample, i.e, individuals scoring positive for hypoplasia. An alternative method is to divide the total number of affected teeth by the total number of teeth in the sample of affected and

non-affected animals. However, to be consistent with previous research, the frequency of LEH was calculated by dividing the number of affected teeth by the total number of teeth from *affected* individuals. The total number of permanent teeth involved in this inter-tooth study of LEH from affected individuals is thus 1,431.

The breakdown of the LEH observations by tooth and the percentage of teeth affected for the maxilla and mandible may be found in Tables 4-11 and 4-12, respectively. Figure 4-2 is a histogram of the overall prevalence of LEH in the tooth. These results were obtained by pooling the maxillary and mandibular dentitions because there was no significant difference in expression between side and jaw (discussed below).



By Antimere and Isomere – χ^2 -Test for Independence

To determine if there is a significant difference in the inter-tooth occurrence of LEH in the isomeres (upper and lower jaws) as well as their antimeres (i.e., left and right sides), χ^2-tests were calculated for each tooth class (i.e., I1, I2, C, P3, P4, M2). The sample for these particular analyses contained only tooth classes, which share antimeric or isomeric pairs and in which one or both of the pair are affected by LEH. Thus, the first and third molars were excluded because there were no observations of LEH.

Table 4-11: Distribution of Linear Enamel Hypoplasia Among Permanent Tooth Types – Maxilla

Type	N[1]	%	Number of Events per Tooth Type (LEH ≥ 0)												#LEH /tooth	#LEH /affected tooth
			0	1	2	3	4	5	6	7	8	9	10	11		
I1 max R	40/40	100.0	0	1	9	13	10	2	4	1					3.5	3.5
I2 max R	40/41	97.6	1	5	9	14	6	5	1						2.9	3.0
C max R	28/29	96.6	1	1	2	7	8	3	1	3	1	1	0	1	4.4	4.6
P3 max R	35/50	70.0	15	17	15	3									1.1	1.6
P4 max R	8/40	20.0	32	5	3										0.3	1.4
M1 max R	0/60	0	60												0	0
M2 max R	1/55	1.8	54	1											0.02	1
M3 max R	0/30	0	30												0	0
I1 max L	45/45	100	0	3	6	13	14	7	2						3.5	3.5
I2 max L	39/41	95.1	2	5	13	10	6	4	1						2.7	2.8
C max L	27/31	87.1	4	1	2	4	8	8	1	0	1	0	1	1	4.0	4.6
P3 max L	40/55	72.7	15	20	16	4									1.2	1.6
P4 max L	12/42	28.6	30	10	1	1									0.1	0.3
M1 max L	1/54	1.9	53	1											0.0	1.0
M2 max L	0/62	0.0	62													
M3 max L	0/28	0.0	28													
Total	316/ 703	50.0	387	70	76	69	52	29	10	4	2	1	0	1	0.6[2]	2.9[3]

[1] Based upon number of teeth from bonobos scoring positive for LEH
[2] 915 defects / 1431 teeth from affected individuals = 0.7
[3] 915 defects / 316 affected teeth = 2.9

Table 4-12: Distribution of Linear Enamel Hypoplasia Among Permanent Tooth Types - Mandible

Type	N[1]	%	Number of Events per Tooth Type (LEH ≥ 0)												# LEH / tooth	# LEH / affected tooth
			0	1	2	3	4	5	6	7	8	9	10	11		
I1 man L	43/44	97.7	1	8	15	10	5	4	0	1					2.6	2.7
I2 man L	40/46	87.0	6	7	9	14	7	3							2.4	2.8
C man L	39/41	95.1	2	1	1	12	9	4	5	0	4	2	1	-	4.5	4.7
P3 man L	43/50	86.0	7	25	12	4	2								1.4	1.6
P4 man L	10/52	19.2	42	6	3	1									0.3	1.5
M1 man L	0/52	0	52	0											0	1.0
M2 man L	0/52	0	52	0											0	0
M3 man L	0/32	0	32	0											0	0
I1 man R	40/43	93.0	3	5	18	6	6	5							2.5	2.7
I2 man R	37/45	82.2	8	7	7	16	5	2							2.2	2.7
C man R	36/38	94.7	2	1	5	6	11	3	6	0	1	2	0	1	4.2	4.5
P3 man R	44/56	78.6	12	31	8	4	1								1.1	1.4
P4 man R	10/49	20.4	39	7	3										0.3	1.3
M1 man R	0/49	0	49												0	0
M2 man R	0/49	0	49												0	0
M3 man R	0/30	0	30												0	0
Total	342/728	47.0	386	98	81	73	46	21	11	1	5	4	1	1	0.7[2]	2.7[3]

[1] Based upon number of teeth from bonobos scoring positive for LEH
[2] 939 defects / 1431 teeth from affected individuals = 0.7
[3] 939 defects / 342 affected teeth = 2.7

The first null hypothesis is there is no difference in the occurrence of LEH of the permanent dentition and its antimeres. Overall, there is no significant difference in the inter-tooth occurrence of LEH and the antimere (χ^2=1.113, df=5, p=0.9530).

For the difference between jaws, the null hypothesis is there is no difference in the occurrence of LEH of the permanent dentition and its isomeres. Overall, there is no significant difference in the inter-tooth occurrence of LEH and the jaws (χ^2=3.386, df=5, p=0.6407).

Severity of LEH

From the sample of permanent teeth, the severity of LEH in each tooth was examined. The tooth was scored as expressing none, mild, moderate, or severe LEH, based on the most severe episode of LEH present on the tooth. The counts for the antimeres were pooled and percentages were calculated [Table 4-13].

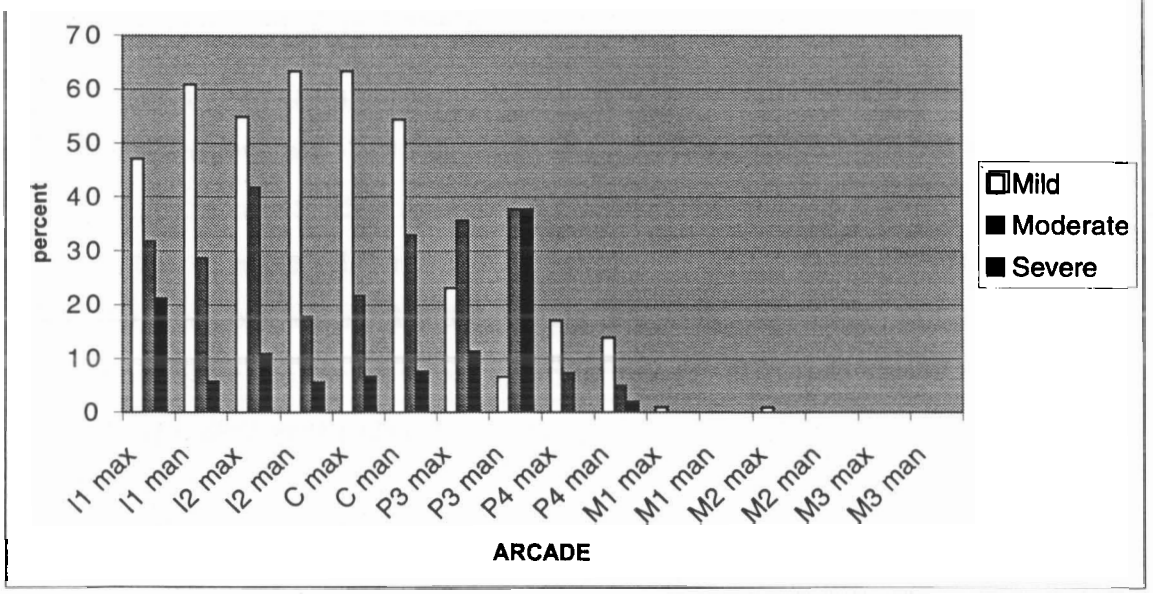
Table 4-13: Severity of LEH by Tooth

TOOTH		Severity of LEH							
		Absent		Mild		Moderate		Severe	
	N	+	%	+	%	+	%	+	%
I1 max	85	0	0.0	40	47.1	27	31.8	18	21.2
I2 max	82	3	3.7	45	54.9	25	41.7	9	11.0
C max	60	5	8.3	38	63.3	13	21.7	4	6.7
P3 max	105	32	30.5	24	23.1	37	35.6	12	11.5
P4 max	82	62	75.6	14	17.1	6	7.3	0	0.0
M1 max	114	113	99.1	1	0.9	0	0.0	0	0.0
M2 max	117	116	99.1	1	0.9	0	0.0	0	0.0
M3 max	58	58	100.0	0	0.0	0	0.0	0	0.0
Totals	703	389	55.3	163	23.2	108	15.4	43	6.1
I1 man	87	4	4.6	53	60.9	25	28.7	5	5.7
I2 man	91	13	14.3	57	63.3	16	17.8	5	5.6
C man	79	4	5.1	43	54.4	26	32.9	6	7.6
P3 man	106	19	17.9	7	6.6	40	37.7	40	37.7
P4 man	101	80	79.2	14	13.9	5	5.0	2	2.0
M1 man	101	101	100.0	0	0.0	0	0.0	0	0.0
M2 man	101	101	100.0	0	0.0	0	0.0	0	0.0
M3 man	62	62	100.0	0	0.0	0	0.0	0	0.0
Totals	728	384	52.7	174	23.9	112	15.4	58	8.0

The results are shown in Figure 4-3. It can be seen that there is a mesial to distal gradient in the presence of LEH per tooth class in the dental arcade, i.e., the anterior teeth have more defects than the posterior teeth.

Ranging from 1% to 63%, mild defects are common in most teeth. The third mandibular premolar shows many teeth with severe LEH scores (38%), followed by maxillary central and lateral incisors (21% and 11%, respectively).

Figure 4-3: SEVERITY OF LEH BY TOOTH
(does not include percentage where LEH absent)



Test of Significance Between Severity and Tooth Type

To determine if there is a significant difference in the inter-tooth severity of LEH, χ^2-tests were calculated for each tooth class (i.e., I1, I2, C, P3, P4, M1, M2). The third molars were excluded because there were not enough observations for LEH. The null hypothesis is there is no significant difference in the severity of LEH between the different tooth classes. Overall, there is a significant difference in the severity of LEH and tooth classes (χ^2=102.630, df=12, p<0.0001) when affected teeth (n=658, i.e., only mild, moderate, and severe defects included) are tested.

By Antimere and Isomere – χ^2-Test for Independence

A χ^2- test was calculated to determine if there is a significant difference in the severity of LEH per tooth type between the antimeres and isomeres. Once again, the sample for these particular analyses contained only tooth classes, which share antimeric or isomeric pairs and in which one or both of the pair are affected by LEH.

The null hypothesis is there are no significant differences in the severity of defects between antimeres. From this test, it was found that there is no difference in the expressions of severity in the teeth between antimeres when jaws are combined (χ^2=0.475, df=2, p=0.7885) or separately, maxillae (χ^2=0.925, df=2, p=0.6297) and mandible (χ^2=1.073, df=2, p=0.5847).

To test if there is a significant difference in the severity of expression between teeth of separate jaws, a χ^2- test was used. The null hypothesis for this test is there are no differences in the inter-tooth severity of LEH between the maxilla and the mandible. It was found that there is no difference in the severity of LEH between the maxillary and mandibular dentitions when left and right sides are pooled (χ^2=3.983, df=2, p=0.1365), or when antimeres are considered separately, right side (χ^2=2.254, df=2, p=0.3240) and left side (χ^2=3.313, df=2, p=0.1908).

Number of Defects Per Tooth

To determine whether teeth exhibit differences in the number of defects, the null hypothesis was tested: there are no significant differences in the number of LEH episodes between teeth. A χ^2-test shows that tooth differences are highly significant (χ^2=1161.965, df=60, p<0.0001). The distribution of the number of LEH episodes among the permanent tooth types are presented in Tables 4-11 and 4-12 for the maxilla and mandible, respectively.

Severity and the Number of LEH per Tooth Crown

It has been observed with rLEH on the canines of African apes that these teeth exhibit two or four grooves much more commonly than three (Skinner 1986, Skinner and

Hopwood 2003). Because Skinner and Hopwood (2003) were examining rLEH, teeth with 2 or more defects were used in their analyses; therefore, 410 bonobo incisors and canines expressing 2 or more defects were analyzed for this section.

The examination of bonobo teeth that express even- or odd-numbered LEH episodes reveals that bonobos do not share the pattern demonstrated in other the apes. There is not a trend of 4, 6, and 8 LEH being more common than 3, 5, or 7. The number of teeth expressing even-numbered episodes of LEH (i.e., teeth with either 2, 4, 6, etc., defects) is slight compared to the number of teeth with odd-numbered defects (i.e., teeth with either 3, 5, or 7, etc., defects) and is not found to be statistically significant (χ^2 = 0.009, df=2, p=0.9237) [see Table 4-14].

The ratio of bonobo teeth with even- to odd-numbered defects is 1.2. Skinner and Hopwood (2003) hypothesize if there is annual cyclical stressor, the ratio of even- to odd-numbered defects will be equal. The ratio increases if there are a number of cyclical stressors of equal magnitude but if the stressors are not equal in magnitude then the ratio will become closer to equality as not all defects may be expressed. The bonobos, therefore, are either exposed to cyclical stressors not of equal magnitude or a more likely affected by moisture cycles that are very evenly timed throughout the year (see Chapter 6 discussion).

Skinner and Hopwood (2003) also found more severe scores on canines from African apes expressing even numbers of LEH. The researchers attributed the pattern of severity in even numbered defects to semi-annual moisture cycles. In bonobos, an examination of the hypoplastic episodes themselves show once again that bonobos do not follow similar patterns with other apes [see Table 4-15]. There is not a trend for even-

Table 4-14 Number of Teeth with Even or Odd Numbered LEH Episodes per Tooth Crown

Number of Teeth	Incisor	Canine	Total
Odd	130	57	187
Even	156	67	223
Totals	286	184	410
Ratio (Even:Odd)	1.2	1.2	1.2

χ^2 = 0.009, df=2, p=0.9237

numbered hypoplastic episodes to be more severe in nature compared to odd-numbered LEH and the distribution of severity amongst even-odd LEH was not found to be statistically significant ($\chi^2 = 3.834$, df=2, p=0.470).

Table 4-15 Relationship between Severity and LEH based on Even- or Odd-Numbered Events (incisors & canines combined)

Number of LEH	Mild	Moderate	Severe	Total
Odd	307	93	35	435
Even	499	113	46	658
Totals	806	206	81	1093

$\chi^2 = 3.834$, df=2, p=0.470

The Canine – Severity and Number of LEH

For this study, it is theorized that the occurrence and degree of severity of LEH within the bonobo should not differ between the sexes. A χ^2- test was calculated to determine if there is a significant difference in the presence of LEH on the canine between the sexes. From this test, it was found that there is a slight difference in the presence of LEH between the sexes on this tooth (χ^2=3.932, df=1, p=0.0474) [see Table 4-16].

χ^2- tests are also calculated to determine if there is a significant difference in the number of LEH per canine between the antimeres and isomeres for the sexes. The canines for these particular analyses have shared antimeric or isomeric pairs. From these tests, it was found that there is no difference in the number of LEH expressed on the teeth between antimeres when jaws are combined (χ^2=0.088, df=1, p=.7668) [see Table 4-17]

Table 4-16: χ^2-Test for Significance for Prevalence of LEH for the Canine between Sexes

	Female	Male	Totals
Present	66	51	117
Absent	2	7	9
Totals	68	58	126

$\chi^2 = 3.932$, df=1, p=0.0474

or separately, maxillae (χ^2=0.048, df=1, p=0.8271) and mandible (χ^2=0.018, df=1, p=0.8928).

For the jaws, the null hypothesis is there are no significant differences in the number of defects between isomeres for the sexes. From this test, it was found that there is no difference in the number of LEH episodes for the jaws between the sexes when sides are combined (χ^2=0.108, df=1, p=0.7423) [see Table 4-18] or separately, left (χ^2=0.054, df=1, p=0.8163) and right (χ^2=0.038, df=1, p=0.8452).

Because there is no statistical significance between the isomeres, the maxillary right quadrant is used to test the significance between the number of LEH counts between the sexes. A χ^2-test on the number of LEH episodes between the sexes reveal no significant differences, χ^2=9.821, df=9, p=0.3651.

To test the degree of severity between the sexes, a χ^2 – test is calculated between the jaws (using right antimere only). There is no significant difference between severity between the sexes within the maxilla and mandible (χ^2 =4.036, df=2, p=0.1329). These results complement earlier findings in this chapter wherein the bonobo does not follow the significant trend that the other African apes species do for their even numbered hypoplastic defects to have more severe scores (whether all sides and jaws are combined χ^2 = 0.377, df=2, p=0.8281 or on a quadrant basis χ^2 =3.344, df=2, p=0.1878).

Table 4-17: χ^2-Test for Significance for Number of LEH on the Canine between Sexes and Antimeres

	Left	Right	Totals
Female	26	28	54
Male	21	20	41
Totals	47	48	95

χ^2 = 0.088, df=1, p=0.7668

Table 4-18: χ^2-Test for Significance for Number of LEH on the Canine between Sexes and Isomeres

	Female	Male	Totals
Upper	22	15	37
Lower	24	14	38
Totals	46	29	75

χ^2 = 0.108, df=1, p=0.7423

Comparisons with Other Chimpanzees. Past researchers have found that whereas LEH prevalence is not differentially expressed between the sexes, defect counts are sexually dimorphic (Guatelli-Steinberg 1998:110, Skinner 1986). Guatelli-Steinberg (1998:110) outlines Moggi-Cecchi's raw (unpublished) data of ranges in LEH episodes for a number of chimpanzee subspecies. Included in the table are observations Moggi-Cecchi made for the bonobo which show a slight difference in the number of LEH episodes expressed between the sexes. In his study, the range for male bonobos (n=16) is 1-8 episodes and the range for females (n=18) is 1-6 episodes of LEH. The discrepancy in bonobo observations between Moggi-Cecchi's study and this study may (both males and females displayed ranges from 1-11), again, be attributed to scoring thresholds as well as sample size. Furthermore, it is now apparent that the bonobo does not follow the significant trend that the other African apes species do for their even hypoplastic defects to have more severe scores.

PIT TYPE HYPOPLASIAS

BY INDIVIDUAL – Frequency of Pitting

The 182 specimens from the Royal Museum collection also were analyzed for pit-type hypoplasia at the individual level (recall captive specimens and specimens missing both maxillary and mandibular dentitions were excluded but individuals with worn and mixed dentitions were considered). The sample is organized into a number of dental age categories for purposes of future comparisons [see Table 4-19].

Pits were scored as present if a bonobo expressed pits on any tooth. LHPC was not considered a pit-type hypoplasia and was classified separately. The prevalence of pits

Table 4-19: Prevalence of Pits in Bonobos of Varying Age Categories

Age	Females			Males			Unknown		
Categories	+	N	%	+	N	%	+	N	%
Infant	8	10	80.0	10	14	71.4	8	11	72.7
Juvenile	15	17	88.2	18	24	75.0	16	23	69.6
Adult	7	28	25.0	4	22	18.2	2	9	22.2
Senile	0	9	0.0	1	9	11.1	0	6	0.0

in the entire Tervuren collection is 48.9 % with 89 individuals from all dental age categories scoring present for pits. An overall examination of the entire collection shows that there is no difference in the expression of pits between males and females (χ^2=0.012, df=1, p=0.9126).

There are 83 bonobos with only permanent teeth (including senile individuals). Of these individuals, the prevalence of pits is low at 16.9 %. When bonobos with excessive dental attrition are removed (i.e., 24 senile individuals), this frequency increases to 22.0 %. From the 64 individuals with mixed dentitions, 49, or 76.4%, express pit-type hypoplasias. From the individuals within the infant dental developmental category, 26 out of 35, or 74.3 %, expressed pitting.

Test of Significance Between Age Categories and Sexes

To test whether there is significance in the expression of pits between the sexes in all the age classes, a χ^2-test was calculated. The null hypothesis is there are no significant differences in the expression of pits between male and female bonobos of all categories. From this test, it was established that there are indeed no significant differences between the sexes (Table 4-20).

Table 4-20: Observed χ^2-Values for Pits between Male and Female Bonobos of Different Age Classes

Age	Observed χ^2	Degrees of Freedom	p-Value
Infant (n=24)	0.229	1	0.6326
Juvenile (n=41)	1.110	1	0.2931
Adult (n=50)	0.334	1	0.5635
Senile (n=18)	1.059	1	0.3035

Test of Significance Between Adult and Infant Individuals

To determine the significance in the expression of pits between adults and infants, a χ^2-test was calculated [see Table 4-21]. The null hypothesis is there is no significant difference in the expression of pits between the adult and infant dentitions. From this test, it was determined that there is a significant difference in the expression of this enamel defect (χ^2=24.707, df=1, p<0.0001). The null hypothesis is rejected.

Table 4-21: χ^2-Test for Significance of Pits between Individual Bonobo Dentitions

	DENTITIONS		
Pits	Adult	Infant	Totals
Present	13	26	39
Absent	46	9	55
Totals	59	35	94

χ^2=24.707, df = 1, p<0.0001

This result is comparable to other studies, in which pits are expressed differentially in the adult and infant developmental categories, with the infants displaying a higher frequency of pits.

Comparisons with other Chimpanzee Studies

In her study of enamel hypoplasias in non-human primates, Newell (1998:188) examined the prevalence of pits in the adult dentition. She observed that 11.4% of chimpanzees expressed pit-type hypoplasias. After conducting a χ^2-test to test to determine the significance between adult bonobo and chimpanzee populations, it was found that there is a slight but statistically non-significant difference between these two species [Table 4-22].

Newell (1998:196) also examined the prevalence of pits in the deciduous dentition. She found that 15.4%, 6 out of 39 of infants expressed pits compared to 26 out of 35 in this study. Her results contrasts dramatically with the percentage of pits in the bonobo, 74.3% (χ^2=26.074, df=1, p<0.0001).

Table 4-22: χ^2-Test for Pitting between Chimpanzee Species (Adults Only)

	Pitting		
Primate	Present	Absent	Totals
Bonobo	13	46	59
Chimpanzee	9	70	79
Totals	22	116	138

(χ^2=2.854, df = 1, p=0.0911)

BY TOOTH – Frequency of Pitting

Inter-tooth comparisons were made on the 182 bonobos that scored positive for pits. Teeth were examined from individuals in all dental developmental categories. Again, to be consistent with the LEH frequency analysis, the frequency of pit-type hypoplasias is calculated by dividing the number of affected teeth by the total number of teeth from the individuals expressing pit-type defects.

Permanent Dentition

The total number of permanent teeth from the Tervuren collection used in this inter-tooth analysis was 1,679. The number of teeth from individuals scoring positive for pits in the collection was 534. The breakdown of pits observed in the adult teeth may be found in Tables 4-23 and 4-24 for respective jaws. From the sample, 5.6% of adult teeth, 30 out of 534, express pits.

From Figure 4-4, it can be seen that the right lateral maxillary incisor manifests the most pits i.e., 40%, followed by the central incisors and premolars. Pit-type hypoplasias were not observed on the canines, maxillary M2s, or maxillary and mandibular M3s.

Figure 4-4: Distribution of Pits in Adult Teeth

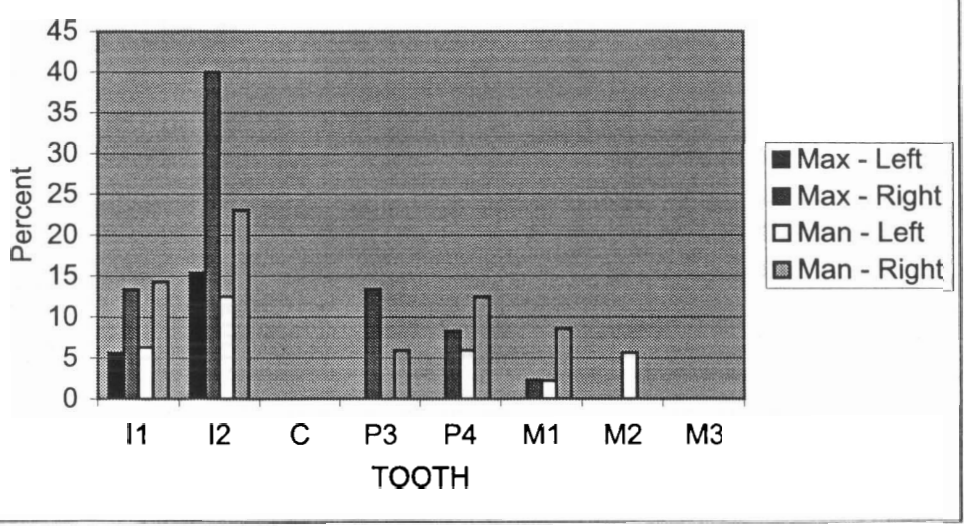


Table 4-23: Distribution of Pit Hypoplasias among Permanent Tooth Types - Maxilla

Type	N[1]	%	Number of Events per Tooth Type (pits ≥ 0)											#pits / tooth	# pits / affected tooth
			0	1	2	3	4	5	6	7	8	9	10		
I1 max L	1/18	5.6	17	1										0.06	1.0
I2 max L	2/13	15.4	11	2										0.15	1.0
C max L	0/8	0.0	8											0	0
P3 max L	0/18	0.0	18											0	0
P4 max L	0/12	0.0	12											0	0
M1 max L	0/12	0.0	12											0	0
M2 max L	0/18	0.0	18											0	0
M3 max L	0/14	0.0	14											0	0
I1 max R	2/15	13.3	13	0	1	0	0	1						0.47	3.5
I2 max R	4/10	40.0	6	2	1	-	-	-	-	-	1			1.20	3.0
C max R	0/8	0.0	8											0	0
P3 max R	2/15	13.3	13	1	0	1								0.27	2.0
P4 max R	1/12	8.3	11	-	-	-	-	-	-	-	1			0.67	8.0
M1 max R	1/43	2.3	42	1										0.02	1.0
M2 max R	0/17	0.0	17											0	0
M3 max R	0/16	0.0	16											0	0
Total	13/ 249	5.2	236	7	2	1		1			2			0.07[2]	2.7[3]

[1] Based upon number of teeth from bonobos scoring positive for pits on a particular tooth
[2] 35 pits / 534 teeth from affected individuals = 0.07
[3] 35 pits / 13 affected teeth = 2.7

Table 4-24: Distribution of Pit Hypoplasias among Permanent Tooth Types - Mandible

Type	N[1]	%	Number of Events per Tooth Type (pits ≥ 0)											# pits / tooth	# pits / affected tooth
			0	1	2	3	4	5	6	7	8	9	10		
I1 man L	1/16	6.3	15	0	0	0	1							0.25	4.0
I2 man L	2/16	12.5	14	2										0.13	1.0
C man L	0/11	0.0	11											0	0
P3 man L	0/16	0.0	16											0	0
P4 man L	1/17	5.9	16	1										0.06	1.0
M1 man L	1/45	2.2	44	0	0	1								0.07	3.0
M2 man L	1/18	5.6	17	1										0.06	1.0
M3 man L	0/15	0.0	15											0	0
I1 man R	2/14	14.3	12	2										0.14	1.0
I2 man R	3/13	23.1	10	3										0.23	1.0
C man R	0/10	0.0	10											0	0
P3 man R	1/17	5.9	16	0	1									0.12	2.0
P4 man R	2/16	12.5	14	0	2									0.25	2.0
M1 man R	3/35	8.6	32	1	2									0.14	1.7
M2 man R	0/17	0.0	17											0	0
M3 man R	0/9	0.0	9											0	0
Total	17/ 285	6.0	268	10	5	1	1							0.05[2]	1.6[3]

[1] Based upon number of individuals scoring positive for pits on a particular tooth
[2] 27 pits / 534 teeth from affected individuals = 0.05
[3] 27 pits / 17 affected teeth = 1.6

By Antimere and Isomere - χ^2-Test for Independence

To determine if there is a significant difference in the inter-tooth expression of pits in the upper and lower jaws as well as their antimeres (i.e., left and right sides), χ^2-tests were calculated for each permanent tooth class (i.e., I1, I2, P3, P4, M1, M2). The sample for these particular analyses contained only tooth classes, which share antimeric pairs and in which one or both of the pair are affected by pits, thus the canine and third molar were excluded because there were no observations for pits.

The first null hypothesis is there is no difference between the number of pits in the permanent dentition for the tooth type and its antimeres. Overall, there is not a significant difference in the inter-tooth occurrence of pits and the antimere (χ^2=4.074, df=5, p=0.5388). For the difference between jaws, the null hypothesis is there is no difference in the number of pits expressed in the permanent tooth classes (i.e., I1, I2, P3, P4, M1, M2) and their isomeres. The sample for these particular analyses contained only tooth classes, which share isomeric pairs and in which one or both of the pair are affected by pits, thus the canine and third molar are not included in the analysis.

Once again, there is not a significant difference in the inter-tooth expression of pits and the jaws (χ^2=9.259, df=5, p<0.0992). More pits are occur in the maxillary lateral incisor (I2) and third and fourth premolars (P3, P4) while central incisors (I1) and first and second molar express more pits in the mandible although these observations are based upon a few teeth expressing a number of pits.

Number of Pits per Permanent Tooth

To determine whether permanent teeth exhibit differences in the number of pits, the null hypothesis was tested: there is no significant difference in the number of pits between teeth.

χ^2-tests show that the number of pits expressed between the tooth types is not significant (χ^2=18.442, df=5, p=0.5583). No sex differences are observed in the expression in the number of pits (χ^2=2.000, df=5, p=0.7358) and no significant

differences are also observed between number of pits and antimere (χ^2=5.595, df=4, p<0.0992) or isomere (χ^2=5.057, df=4, p<0.2815). The mean number of pits on each permanent tooth is shown in Table 4-23 and Table 4-24 for the maxilla and mandible, respectively.

Deciduous Dentition

For the deciduous teeth, the total number included from the collection used for analysis was 1,203. The number of teeth from *affected* specimens in the sample was 996, and 20.0% of this sample had pits. The distribution of pits of the respective tooth classes is represented in Tables 4-25 and 4-26 for the maxilla and mandible, respectively.

From Figure 4-5, it can be seen that the maxillary teeth express more pits in the lateral incisors, whereas the mandibular teeth preferentially express multiple pits in the deciduous molars. The canine displays the least amount of pitting, but this could be attributed to the prevalence of LHPC that is predominantly manifested in the mandibular canines.

Figure 4-5: Distribution of Pits in Deciduous Teeth

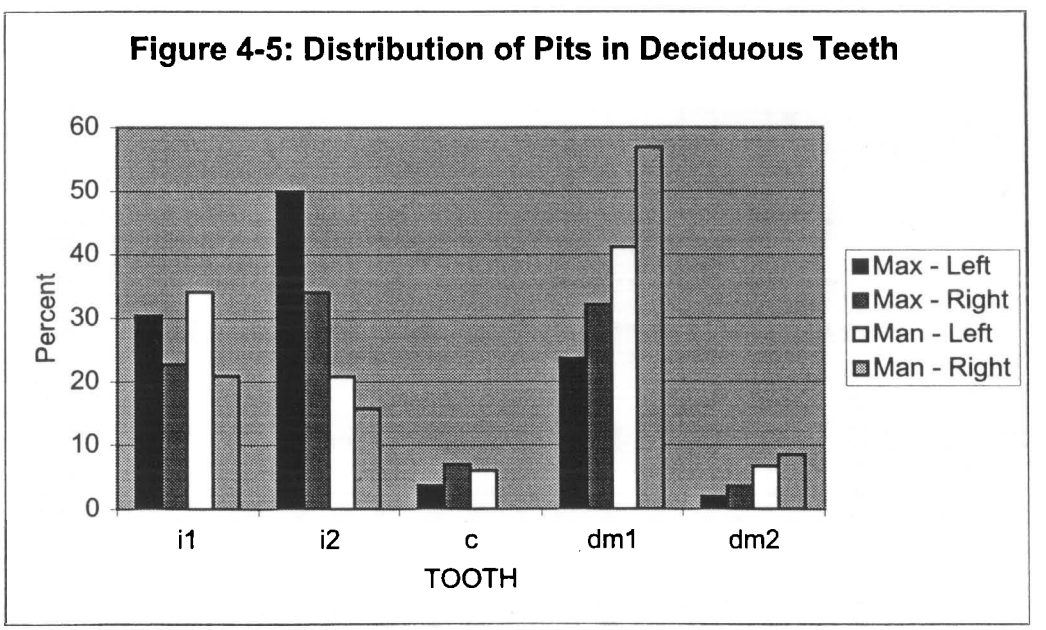


Table 4-25: Distribution of Pit Hypoplasias among Deciduous Tooth Types - Maxilla

Type	N[1]	%	Number of Events per Tooth Type													# pits / tooth	# pits / affected tooth
			0	1	2	3	4	5	6	7	8	9	10	11	12		
il max L	14/46	30.4	32	8	4	2										0.48	1.6
i2 max L	23/46	50.0	23	10	4	2	0	2	1	1	0	1	0	1	1	1.67	3.3
c max L	2/54	3.7	52	0	2											0.07	2.0
dm1 max L	13/55	23.6	42	8	3	0	1	1								0.42	1.8
dm2 max L	1/51	2.0	50	1												0.02	1.0
il max R	10/44	22.7	34	3	4	1	0	0	2							0.59	2.6
i2 max R	15/44	34.1	29	7	2	1	2	1	1	1						0.91	2.7
c max R	4/57	7.0	53	2	2											0.11	1.5
dm1 max R	18/56	32.1	38	7	5	3	0	1	1	1						0.79	2.4
dm2 max R	2/56	3.6	54	1	0	1										0.07	2.0
Total	102/ 509	20.0	407	47	26	10	3	5	5	3	0	1	0	1	1	0.25[2]	2.4[3]

[1] Based upon number of individuals scoring positive for pits on a particular tooth
[2] 247pits / 996 teeth from affected individuals = 0.2
[3] 247 pits / 102 affected teeth = 2.4

Table 4-26: Distribution of Pit Hypoplasias among Deciduous Tooth Types - Mandible

Type	N[1]	%	Number of Events per Tooth Type											# pits / tooth	# pits / affected tooth
			0	1	2	3	4	5	6	7	8	9	10		
il man L	15/44	34.1	29	9	3	2	1							0.57	1.7
i2 man L	10/48	20.8	38	7	2	1								0.29	1.4
c man L	3/50	6.0	47	3										0.06	1.0
dm1 man L	23/56	41.1	33	11	8	1	1	1	1					1.00	2.0
dm2 man L	3/45	6.7	42	1	2									0.11	0.0
il man R	9/43	20.9	34	6	1	2								0.33	1.6
i2 man R	8/51	15.7	43	7	0	1								0.20	1.3
c man R	0/52	0.0	52											0	0
dm1 man R	29/51	56.9	29	6	6	6	0	3	0	1				1.14	2.0
dm2 man R	4/47	8.5	43	2	2									0.17	2.0
Total	97/ 487	19.9	390	52	24	13	2	4	1	1				0.18[2]	1.9[3]

[1] Based upon number of individuals scoring positive for pits on a particular tooth
[2] 182 pits / 996 teeth from affected individuals = 0.2
[3] 182 pits / 97 affected teeth = 1.9

By Antimere and Jaw - χ^2-Test for Independence

To determine if there is a significant difference in the inter-tooth expression of pits in the upper and lower jaws as well as their antimeres (i.e., left and right sides), χ^2-tests were calculated for each deciduous tooth class (i.e., i1, i2, c, dm1, dm2). The sample for these particular analyses contained only tooth classes, which share antimeric pairs and in which one or both of the pair are affected by pits.

The first null hypothesis is there is no difference between the number of pits in the deciduous dentition for the tooth type and its antimeres. Overall, there is not a significant difference in the inter-tooth occurrence of pits and the antimere (χ^2=3.292, df=4, p=0.5102).

For the difference between jaws, the null hypothesis is there is no difference in the number of pits expressed in the deciduous tooth classes (i.e., i1, i2, c, dm1, dm2) and their isomeres. The sample for these particular analyses contained only tooth classes, which share isomeric pairs and in which one or both of the pair are affected by pits.

Overall, there is a significant difference in the inter-tooth expression of pits and the jaws (χ^2=15.226, df=4, p=0.0093). More pits are occur in the maxillary lateral incisor (i2) and the mandibular deciduous first molar. However, the number of pits expressed between the jaws themselves was not found to be significant (χ^2=3.270, df=4, p=0.5134). The greater frequency of pitting in the maxillary i2 and mandibular dm1 may been seen in Figure 4-6.

Number of Pits Per Deciduous Tooth

The mean number of pits per tooth in the sample of deciduous teeth with one or more pit-type hypoplasias may be found in Tables 4-25 and 4-26 for the maxilla and mandible, respectively. To determine whether deciduous teeth exhibit differences in the number of defects expressed between the tooth types, the null hypothesis was tested: there are no significant differences in the distribution of defects between teeth.

χ^2-tests show that tooth differences are not significant (χ^2=17.451, df=16, p=0.3570). χ^2-tests did not show sex differences in the number of pits per tooth

(χ^2=8.697, df=4, p=0.0691). Significant differences were not observed in the isomeres (χ^2=4.217, df=4, p=0.3775) nor for the jaws (χ^2=4.998, df=4, p=0.2875).

LOCALIZED HYPOPLASIA OF THE PRIMARY CANINE (LHPC)

BY INDIVIDUAL – Frequency of LHPC

By definition, LHPC is manifested in the deciduous canines. Individuals were scored as present or absent for LHPC whether or not the antimere or isomere of a pair was missing. From the 101 individuals possessing any deciduous canines, 53.5% express LHPC. From this sample, 42.9% of females, 61.5% of males, and 52.9% of unknown sex manifest LHPC [Table 4-27].

Table 4-27: Prevalence of LHPC in Bonobos with Deciduous Canines

Age	Female			Male			Unknown		
	+	N	%	+	N	%	+	N	%
Juvenile	8	19	42.1	17	25	68.0	12	22	54.5
Infant	4	9	44.4	7	14	50.0	6	12	50.0
Totals	12	28	42.9	24	39	61.5	18	34	52.9

Test of Significance Between Sexes

To test whether there is significance in the expression of LHPC between the sexes, a χ^2-test was calculated [Table 4-28]. The null hypothesis is there is no significant difference in the expression of LHPC between the sexes. From this test, it was established that there is not a difference in expression, (χ^2=2.288, df=1, p=0.1304) between the sexes.

Table 4-28: χ^2-Test for Significance of LHPC between Sexes

	SEXES		
LHPC	Female	Male	Totals
Present	12	24	36
Absent	16	15	31
Totals	28	39	67

χ^2=2.288, df = 1, p =0.1304

Comparisons with Other Chimpanzees

Lukacs (1999) conducted a study of enamel hypoplasia, namely LHPC, in the deciduous dentition of the great apes. He examined "any specimen that possessed one or more deciduous teeth," i.e., antimeric and isomeric pairs were not examined (Lukacs 1999a:354). For chimpanzees, he found a 22% prevalence for LHPC. The bonobos in this study have an overall prevalence of 53.5% in individuals with deciduous canines [see Table 4-29 for a comparison of results]. A χ^2-test calculated for the different totals in the chimpanzee species yielded a significant difference (χ^2=13.506, df=1, p=0.0002).

Skinner and Newell (2003) also studied LHPC in bonobos from the Royal Museum for Central Africa. Thirty-nine bonobos were examined and 61.5% had LHPC. Differences in LHPC expression between the sexes also were not observed.

Table 4-29: Individual Chimpanzee Skulls with LHPC

Species	Female			Male			Total		
	+	N	%	+	N	%	+	N	%
*P. troglodytes**	4	17	23.5	1	6	16.7	11	50	22
P. paniscus	12	28	42.9	24	39	61.5	54	101	53.5

*Lukacs (1999)

BY CANINE – Frequency of LHPC

On a tooth-by-tooth level of analysis, Lukacs (1999:355) calculated the tooth count prevalence of LHPC as "the percentage of deciduous canine teeth with hypoplastic lesions as a percentage of all deciduous canine teeth observed." Using Lukacs (1999) tooth count method, the results for bonobo canines are presented in Table 4-30 for purposes of comparison.

Table 4-30: Prevalence of LHPC in Bonobo Canines by Tooth Count

Side	Maxilla			Mandible		
	+	N	%	+	N	%
Left	3	71	4.2	46	63	73.0
Right	2	69	2.9	45	63	71.4
Totals	5	140	3.6	91	126	72.2

Because previous calculations for LEH and pits in this study were made on the number of teeth in *affected* individuals, LHPC also was calculated in this manner [Table 4-31]. Almost 50% (96/194 scorable canine teeth from 54 animals) showed LHPC.

Table 4-31: Prevalence of LHPC in Teeth from Affected Individuals

Side	Maxilla			Mandible		
	+	N	%	+	N	%
Left	3	46	6.5	46	51	90.2
Right	2	48	4.2	45	49	91.8
Totals	5	94	5.3	91	100	91.0

Number of Individuals = 54, number of canines from affected individuals = 194

To determine the significance of LHPC in the canines on the basis of Lukacs' (1999) tooth count method, χ^2-tests were used to test the null hypothesis: there is no difference in LHPC expression between jaw [Table 4-32] or side [Table 4-33]. It was found that there is a significant difference between jaw (χ^2=135.504, df=1, p<0.0001) but not side (χ^2=0.027, df=1, p=0.8704).

Table 4-32: χ^2-Test for Significance of LHPC and Jaw (Tooth Count Method)

	JAW		
LHPC	Maxilla	Mandible	Totals
Present	5	91	96
Absent	135	35	170
Totals	140	126	266

χ^2=135.504, df = 1, p<0.0001

Table 4-33: χ^2-Test for Significance of LHPC and Side (Tooth Count Method)

	SIDE		
LHPC	Left	Right	
Present	49	47	96
Absent	85	85	168
Totals	134	132	266

χ^2=0.027, df = 1, p=0.8704

Comparisons with Other Chimpanzees

Compared to Lukacs' study [Table 4-34], bonobos and chimpanzees do not demonstrate a significant difference in the expression of LHPC in their maxillary canines (χ^2=0.265, df=1, p=0.6068). There is, however, a significant difference in mandibular expression, (χ^2=41.377, df=1, p<0.0001). From the results below, it appears that the mandibular canine in the bonobo is three times as likely as the common chimpanzee to have LHPC.

Skinner and Newell (2003) also observed the lower jaw to be typically affected by LHPC in the bonobo. For the maxilla, percentages ranged from 3.3% to 9.1% for left and right canines, respectively. For the mandible, percentages ranged from 65.6% and 66.7% for the right and left antimeres.

Table 4-34: Prevalence of LHPC in *Pan* Canines by Tooth Count

Jaw	Common Chimpanzee*			Bonobo		
	+	N	%	+	N	%
Maxilla	4	80	5.0	5	140	3.6
Mandible	16	66	24.2	91	126	72.2
Totals	20	146	13.6	96	266	36.1

* from Lukacs (1999)

PLANE - TYPE ENAMEL HYPOPLASIA (PEH)

BY INDIVIDUAL – Frequencies of PEH

The 182 bonobo crania from the Royal Museum also were examined for plane-type hypoplasias at the level of the individual. PEH was scored as present if a bonobo expressed this defect on any tooth. Whereas Hillson (1996) classifies LHPC as a plane-type defect, LHPC was considered for separate analysis in this study.

The prevalence of PEH in the entire Tervuren collection is 20.9% with 38 individuals from all dental age categories scoring positive for plane-type hypoplastic defects. The frequencies of PEH expression in male and female bonobos of varying age categories are showed in Table 4-35. An overall examination of the entire collection

shown that there is no significant difference in the expression of PEH between the sexes (χ^2=0.739, df=1, p=0.3900).

Table 4-35: Prevalence of PEH in Bonobo Skulls by Age Category

Developmental	Females			Males			Unknown		
Age Category	+	N	%	+	N	%	+	N	%
Infant	4	10	40.0	5	14	35.7	3	11	27.3
Juvenile	7	17	41.2	9	24	37.5	6	23	23.1
Adult	1	28	3.6	2	22	9.1	1	9	11.1
Senile	0	9	0.0	0	9	0.0	0	6	0.0

From the 83 individuals whose permanent teeth have erupted, 3 out of 83, or 3.6%, have PEH. When the senile individuals are excluded, the percentage increases to 6.8%. With the individuals with mixed dentitions, 22 out of 64, or 34.4%, PEH. For the individuals in the infant age category, 12 out of 35, or 34.3%, demonstrated plane-type defects.

Test of Significance Between Age Categories and Sexes

To test whether there is significant difference in the expression of PEH between the sexes in all the age classes, a χ^2-test was calculated. The null hypothesis is there are no significant differences in the expression of PEH between male and female bonobos of all age categories. From this test, it was established that there are indeed no significant differences between the sexes (Table 4-36).

Table 4-36: Observed χ^2-Values for PEH between Male and Female Bonobos of Different Age Classes

Age	df	χ^2	p-Value	Yates χ^2	Yates *p*
Infant (n=24)	1	0.046	0.8307	0.000	>0.9999
Juvenile (n=41)	1	0.057	0.8121	0.000	>0.9999
Adult (n=50)	1	2.652	0.1035	0.813	0.3674
Senile (n=18)	-	-	-		-

Test of Significance Between Adult and Infant Dentitions

To determine if there is significant difference between the occurrence of PEH in adults and infants, a χ^2-test was calculated [Table 4-37]. The results from this test show a significant difference (χ^2=11.768, df = 1, p=0.0006) in the occurrence of plane-type defects between adults and infants, with infants expressing a higher proportion of PEH. Calculating the continuity correction χ^2, i.e., Yates, also shows a significant difference between the dentitions (Yates χ^2=9.901, df = 1, Yates p=0.0017).

Table 4-37: χ^2-Test for Significance of PEH between Adult and Infant Bonobos

	DENTITIONS		
PEH	Adult	Infant	Totals
Present	4	12	16
Absent	55	23	78
Totals	59	35	94

χ^2=11.768, df = 1, p=0.0006; Yates χ^2=9.901, df = 1, Yates p=0.0017

BY TOOTH – Frequency of PEH

Inter-tooth comparisons were conducted on the 38 out of the 182 bonobos that scored positive for PEH. Teeth were examined from individuals in all dental developmental categories. The frequency of PEH was calculated by dividing the number of affected teeth by the total number of teeth in the *affected* animals sample.

Permanent Dentition

Four permanent teeth from 3 adults displayed PEH. The maxillary left and right lateral incisors of RG 29044, the right mandibular canine of RG 29053, and the right maxillary 3rd premolar of RG29066 had PEH.

Deciduous Dentition

The total number of deciduous teeth examined from affected individuals was 481. From these teeth, 68, or 14.1% expressed PEH. Overall, 25.8 % central incisors (23/89), 12.8% lateral incisors (12/94), 3.1% canines (3/98), 21.5% deciduous first molars

(23/107), and 1.1% deciduous second molars (1/93) had PEH. The distribution of PEH in the various tooth classes is shown in Figure 4-6 and Table 4-38.

Figure 4-6: Distribution of PEH in the Deciduous Dentition

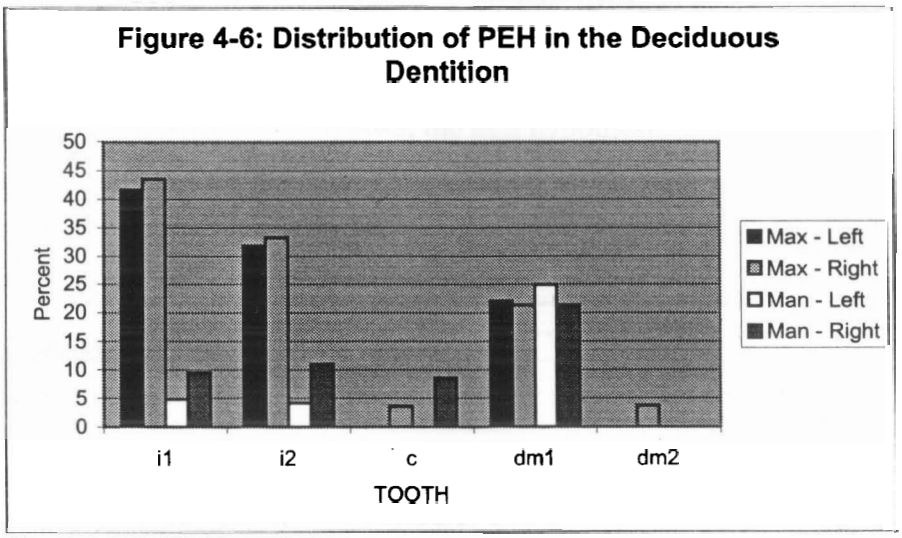


Figure 4-38: Inter-tooth Distribution of PEH in the Bonobo

Tooth	MAXILLA						MANDIBLE					
	Left			Right			Left			Right		
	+	N	%	+	N	%	+	N	%	+	N	%
i1	10	24	41.7	10	23	43.5	1	21	4.8	2	21	9.5
i2	7	22	31.8	7	21	33.3	1	24	4.2	3	27	11.1
c	0	26	0.0	1	27	3.7	0	22	0.0	2	23	8.7
dm1	6	27	22.2	6	28	21.4	6	24	25.0	6	28	21.4
dm2	0	25	0.0	1	26	3.8	0	19	0.0	0	23	0.0

By Antimere and Jaw - χ^2-Test for Independence

To determine if there is a significant difference in the inter-tooth expression of PEH in the upper and lower jaws as well as their antimeres (i.e., left and right sides), χ^2 - tests and continuity correction χ^2 –tests (i.e., Yates) were calculated. The sample for these particular analyses contained only tooth classes, which share respective antimeric or isomeric pairs and in which one or both of the pair are affected by plane-type hypoplasias (not including LHPC).

The first null hypothesis is there is no difference between the presence of PEH in the deciduous dentition for antimeric pairs. Overall, there is no significant difference in the occurrence of PEH and the antimeres (χ^2=0.102, df=1, p=0.7489; Yates χ^2=0.032, df=1, p=0.8579).

For the difference between jaws, the null hypothesis is there is no difference in the presence of PEH in the deciduous isomeric pairs. Overall, a highly significant difference was found in the expression of PEH and the jaws (χ^2=13.234, df=1, p=0.0003; Yates χ^2=12.247, df=1, p=0.0005). The greatest number of plane-type defects is found in the maxillary incisors.

VERTICAL ENAMEL HYPOPLASIA (VEH)

BY INDIVIDUAL AND TOOTH– Frequency of VEH

Of the 182 bonobo crania in the Royal Museum collection, 3 individuals or 1.6%, potentially exhibited VEH (see Table 4-39). The VEH phenomenon was only observed in maxillary incisors. In two individuals, RG 27013 and RG 29031, the VEH was manifested as a vertical string of pits whereas RG 22336 exhibited the more familiar vertical zone of enamel hypoplasia.

Table 4-39: VEH Observed in *Pan paniscus*

Museum Number	F.D.I. Tooth #	Sex
RG 22336	6-2	Unknown
RG 27013	5-1	Male
RG 29031	2-2	Female

Comparisons with Other Chimpanzees

In her study of the great ape collection at the Smithsonian National Museum of Natural History, Hannibal (2000b:9) did not observe VEH. Eckhardt with colleagues (1992) did observe VEH in 2 males from the Liberian Chimpanzee Collection (n=280) in Frankfurt. These researchers, however, observed "deep longitudinal grooves along the

vertical axis of the tooth" in both maxillary canines in one specimen, and the right maxillary canine of another.

The low incidence of VEH in bonobos, if correctly classified, is consistent with observations made for the common chimpanzee. That is, approximately 1% of chimpanzees may express VEH. It is of interest to note that in the non-human primate literature, only chimpanzees – both bonobo and common types – have been observed with VEH.

DARCY'S DEFECT (or DDMLI)

BY INDIVIDUAL – Frequency of DDMLI

In 2000, Darcy Hannibal identified a new enamel hypoplastic defect, which she termed "Diagonal Defects of the Maxillary Lateral Incisor (DDMLI)". Hannibal (2000b:8-9) first described DDMLI (referred to as Darcy's Defect here on in) as "only exhibited on the maxillary lateral incisors, restricted to the cervical and mesial quarter of the crown, oriented diagonally starting on the labial surface and extending mesio-cervically. [With the defect ranging] from mild to severe, exhibiting a range from shallow depression to deep depression with little to no enamel."

From the Royal Museum Collection, at least one individual (RG 29044) or 0.5%, demonstrated Darcy's Defect. One other potential specimen, RG 29031 female, demonstrated a hypoplastic zone on its right maxillary incisor. However, this defect was located along the distal portion of the tooth nearing the occlusal margin. Because of its size and location RG 29031's defect could alternatively be classified as a depressed area of reduced enamel thickness and not as Darcy's Defect. The teeth from both these individuals also manifested LEH.

Comparisons with Other Chimpanzees

Darcy's Defect was the second most common type of enamel hypolastic defect observed in the great ape collection housed at the Smithsonian, with 16.91% of the

individuals affected (Hannibal 2000b:1). From these great apes, 35.71 % of the chimpanzees were affected, making them one of the more affected species with gorillas being the least affected at 5.88% (Hannibal 2000b:12).

A χ^2-test was calculated to determine if there was a statistical significance between these two collection – the null hypothesis being that there was no statistical significance between bonobo and common chimpanzees.

From this calculation, it was a statistical significance as determined ($\chi^2 = 19.369$, df=1, $p<0.0001$; Yates $\chi^2 = 16.305$, df=1, Yates $p<0.0001$) and the null hypothesis was rejected. Clearly this value supports Hannibal's (2000b:13; 2003) observation that while all great apes are capable of manifesting Darcy's Defect, there are obvious taxonomic differences.

Because Darcy's defect is predominantly bilateral, Hannibal (2000b, 2003) believes the defect is caused by physiological stress but the defect "does not tend to occur in association with LEH...on either the same tooth or in the same individual." In contrast with this latter observation, the bonobos thought to manifest Darcy's defect also demonstrated LEH. Hannibal (2003) does not provide a statistic regarding the association of DDMLI with LEH in her study of the large body apes housed at the Smithsonian, providing further question into whether this defect is actually present in bonobos. An examination of life history variables and perikymata spacing was recommended (Hannibal 2000b:13).

SUMMARY OF RESULTS

Linear Enamel Hypoplasia (LEH)

Of the 182 individual bonobo crania analyzed at the Royal Museum for Central Africa, 54.4% of the collection expressed LEH. Of the adults, 100% expressed LEH, followed by juveniles, 23%, and infants, 0.5%. While LEH is expected to be the dominant hypoplasia in adult individuals ($p<0.0001$), the extremely high incidence in bonobos is significant when compared to the other chimpanzee species ($p<0.0001$) and is

the highest reported for large apes. (Different scoring thresholds were also considered and when mild defects observed in the bonobo were reclassified as absent and comparisons were again made to *P.troglodytes*, no significant difference was found between the species, p=0.0874.) No statistical significance was found between the sexes of the bonobos in any of the age categories. From the bonobos affected with LEH (n=98), the majority were moderately affected, 22%, 17.2% were mildly affected, and 14% were severely affected. No statistical significance was found between defect severity and sex (p=0.0625).

At the level of the dentition for the bonobo, anterior teeth demonstrated a greater prevalence than posterior teeth. No statistical significance was found between antimeres (p=0.8823) nor was one found between the jaws (p=0.6407). There was a significant difference in the severity of LEH and tooth classes (p<0.0001) but no differences were found between severity and the antimeres and isomeres. The sectorial premolar was the tooth with greatest severity of defect at 38% followed by the maxillary incisors, 11 to 21% for lateral and central incisors, respectively. There was a statistical significance between the number of defects per tooth type (p<0.0001), with the canine expressing the largest number of defects. No significant difference was found in the presence or number of LEH between the sexes on the canine teeth or between the number of defects on the isomeres and antimeres. The average numbers of LEH on the *affected* teeth of the maxilla and mandible are 2.9 and 2.7, respectively.

Pit Hypoplasias

Of the 182 individual bonobo crania analyzed at the Royal Museum for Central Africa, 48.9% of the collection expressed pits. Of the adults, 22% expressed pits, followed by juveniles, 76.6%, and infants, 74.2%. While pit-type hypoplasias are expected to be the dominant hypoplasia in younger individuals, once again the high incidence in bonobos with deciduous dentitions is significant when compared to the other chimpanzee species (p<0.0001). No statistical significance was found between the sexes of the bonobos in any of the age categories.

From the 534 permanent teeth from *affected* individuals, 5.8% had pits. The right permanent maxillary lateral incisor expressed the greatest number of pits at 40%. The high prevalence of pits in this particular tooth may be reflective of another form of hypoplasia, i.e., Darcy's Defect, which is specific to this tooth type. Darcy's Defect is a diagonal defect of the maxillary lateral incisor and differential eruption schedules between the two species of Pan may affect the manifestation of this defect. The lateral incisors in the bonobo have been reported by Kinzey (1984) to erupt later, thus contact with this tooth may be reduced in turn reducing the prevalence of Darcy's Defect that is instead being captured as hypoplastic pits in the bonobo.

From the deciduous dentition (n=996), 20% of the teeth expressed pits with the maxillary incisors expressing the greatest number of defects, 30-50%. A statistical significance was not found between antimeres (p=0.5388) or jaws (p=0.0992) in the permanent dentition. Nor was a statistical significance found between antimeres in the deciduous dentition (p=0.5102) but one was found between the jaws (p=0.0043). From χ^2 calculations there was no statistical significance between the number of pits per tooth type in the permanent (p=0.5583) or deciduous (p=0.3570) dentitions. The average numbers of pits on the *affected* permanent teeth of the maxilla and mandible are 2.7 and 1.6, respectively. The average numbers of pits on the *affected* deciduous dentition are 2.4 and 1.9, respectively for the maxilla and mandible.

Localized Hypoplasia of the Primary Canine (LHPC)

From the 101 individual bonobos which possessed their deciduous canines, 53.5% expressed LHPC. No significant difference was found in the expression of LHPC between the sexes (p=0.1304). The expression of LHPC was found to be significant between bonobos and common chimpanzees (p=0.0002)

Using Lukacs (1999) tooth count method for the examination of LHPC, a significant difference was not found between antimeres (p=0.8704) but a significant difference was found between the jaws (p<0.0001), with 72.2% of the mandibular canines expressing LHPC compared to 3.6% in the maxilla. When compared to the common chimpanzee, the bonobo is 3 times as likely to have LHPC.

Lukacs (1999:360) hypothesizes anatomical factors contribute to the prevalence of this defect, specifically an abnormal contact between tissues of the developing canine crown and an unusually constricted crypt wall. Recently Skinner and Newell (2003) examined LHPC in the bonobo and they suggest crypt fenestration results in the exposure of the developing crown to "mild physical trauma from an external agent" (Skinner and Newell 2003:68). In their study, the researchers also found a lack of significance in the expression of LHPC between the sexes and between antimeres, and found a greater prevalence of the defect in the lower dental arcade. The differences in prevalence between their study (61.5%) and this one (53.5%) is the researchers examined juvenile individuals while for this study Lukacs' method of examining any individual with a deciduous canine was employed.

Plane-Type Enamel Hypoplasas

Of the 182 individual bonobo crania analyzed at the Royal Museum for Central Africa, 20.9% of the collection expressed plane zones of enamel hypoplasia. Of the adults, 6.8% expressed PEH, followed by juveniles, 37.5%, and infants, 34.3%. There was a statistical significance in the expression of plane type defects and the permanent and deciduous dentitions ($p=0.0006$). No statistical significance was found between the sexes of the bonobos in any of the age categories.

Four teeth in 3 individuals with permanent dentitions expressed plane-type defects. From the deciduous dentition ($n=68$), 14.1% of the teeth expressed plane hypoplasias with the incisors expressing the greatest number of defects, 12.8 to 25.8%, followed by the deciduous first molars, 21.5%. No statistical significance was found between antimeres in the deciduous dentition ($p=0.8579$). Statistical significance was found between the jaws in the deciduous dentitions ($p=0.0005$), with the greatest number of plane defects being expressed in the maxillary incisors.

The presence and high prevalence of plane-type hypoplasias in the bonobos' deciduous dentitions is problematic for comparative purposes. It is not clear from the literature whether plane-type defects were not scored, alternatively scored as large pits, or simply not manifested in other apes' deciduous dentitions. The prevalence of the defect

in all deciduous molars followed by the maxillary incisors may be reflective of differential eruption schedules and susceptibility to postnatal stress for this primate.

There are other subcategories of plane-type defects: LHPC and DDMLI. LHPC is thought to result from trauma from an external source or contact of developing tissues within a restricted crypt space and DDMLI may result from inter-tooth contact (crowding?) within the dental arcade. The plane-type defects observed in the deciduous dentition of the bonobo may therefore be reflective of some form of contact and/or trauma within or to the dental arcade and the higher prevalence of the defect may be indicative of differential bone densities within/between primates resulting in the bonobo being more susceptible to the aforementioned forces.

Vertical Enamel Hypoplasia (VEH)

Of the 182 individual bonobo crania analyzed at the Royal Museum for Central Africa, approximately 1.6% of the collection expressed vertical enamel hypoplasia. VEH was only found unilaterally in the maxillary incisors. One infant expressed a vertical zone of hypoplasia while one adult's incisor and one infant's incisor expressed a vertical string of pits.

Darcy's Defect or DDMLI

Of the 182 individual bonobo crania analyzed at the Royal Museum for Central Africa, approximately 0.5% of the collection expressed Darcy's Defect. This defect was bilaterally expressed in one adult male in the collection. While Darcy's Defect has only recently been identified (Hannibal 2000b, 2003) and was found to the second most common type of enamel hypoplastic defect in a collection of common chimpanzees housed at the Smithsonian, the extremely low incidence in bonobos is significant when compared to these chimpanzees ($p<0.0001$). Differences in tooth eruption schedules between the two species of Pan are thought to be a factor in defect prevalence.

CHAPTER 5: TIMING AND PERIODICITY RESULTS

The timing and periodicity of linear enamel hypoplasia (LEH) are determined through location frequencies on tooth crown fractions, relative caliper measures of LEH location standardized against crown height, and perikymata counts. It is known from studies by Reid, Schwartz and Dean (2000) and Reid and Dean (2000) of perikymata density that crown formation is not strictly linear. Typically crown formation is rapid at the beginning and end of crown formation but quite regular for the majority of crown formation. Fortunately for the purposes of this study it was possible to show that the majority (85%) of rLEH occurred in the middle 60% of crown formation. Consequently, one of the first analyses performed in this section is of perikymata density per tenth of crown height (various teeth) followed by an analysis of the location on the imbricational enamel where LEH occur. The degree of error introduced by assuming uniformity of crown formation rate in the middle 60% of the imbricational enamel is determined by examining the departure in perikymata counts of each tenth of enamel surface (0.2 to 0.3,….., 0.7 to 0.8) from the average count. It is shown that it is reasonable to treat the rate at which that imbricational enamel exhibiting the vast majority of rLEH occurs is linear. This result supports the earlier analysis of relative imbricational enamel height to determine the span and timing of repetitive enamel defects.

Metrical analyses were used to determine the onset of LEH, the duration or span of the stress from first to last observable events on the tooth crown, and the intervals between onset of enamel defects. Relative values were obtained for these measurements by dividing them by the tooth crown height. This value, in turn, was multiplied by crown formation times for common chimpanzees to ascertain the time (in months or years) of the stressor(s) during crown formation; and by bonobo imbricational enamel formation estimates generated in this study from the same tooth or same tooth type (see Methods Section).

Lastly, perikymata counts were used to determine the timing of intervals between enamel defects and to elucidate the duration of an actual hypoplastic episode. An interval is defined as the beginning of one episode to the beginning of the second episode. The number of perikymata between each pair of LEH was counted and converted into days between episodes (based on the number of daily cross-striations observed to occur between striae of Retzius by a co-worker on an incisor and a canine tooth of *Pan paniscus* selected for this study – see Chapter 3 Methods Section).

CALIPER MEASUREMENTS

Materials and Methods

To determine the interval timing of LEH in bonobos, teeth from individual bonobos were chosen if they displayed two or more episodes of LEH on a single tooth. Teeth with only one defect were excluded. For this part of the analysis, 490 teeth from 68 individuals were examined [Table 5-1]. (A single individual could thus contribute several observations.)

Crown height, distance of the first episode from the CEJ, and distance distance of the last episode from the CEJ were measured using Mitutoyo digital calipers. The duration of stress (i.e., the spacing between the first and last episodes of LEH) was calculated by subtracting the last LEH measurement from the first. These measurements were converted to a relative span between first and last LEH by crown formation durations reported for the common chimpanzee [see Table 5-2] and from more precise estimates generated in this study of perikymata counts in imbricational enamel among *Pan paniscus* [see Table 5-3]. When these crown formation times are used, a *linear* rate of crown formation is assumed. Non-linear rates of crown formation will be examined later in this chapter.

Statistical analyses were performed in Statview 5.0.1 PowerPC Version on both Macintosh G4 and iMac computers. ANOVA tests were used to "test the null hypothesis

Table 5-1: Sample of Teeth Selected for LEH Spatial Measurement Study

Tooth	Side	Jaw	Female	Male	Unknown	Total
	Left	Upper	19	15	8	42
I1	Right	Upper	18	13	8	39
	Left	Lower	18	12	6	36
	Right	Lower	19	12	4	35
	Left	Upper	15	13	5	33
I2	Right	Upper	18	13	4	35
	Left	Lower	17	11	5	33
	Right	Lower	17	10	4	31
	Left	Upper	15	9	3	27
C	Right	Upper	13	9	3	25
	Left	Lower	17	17	5	39
	Right	Lower	18	14	3	35
	Left	Upper	10	5	4	19
P3	Right	Upper	11	4	3	18
	Left	Lower	12	6	1	19
	Right	Lower	6	4	2	12
	Left	Upper	0	2	0	2
P4	Right	Upper	0	2	1	3
	Left	Lower	2	1	1	4
	Right	Lower	2	1	0	3
Totals	-	-	247	173	70	490

that the mean of the dependent variable is the same regardless of the main effect" (SAS Institute 1999:79). The independent (nominal) variables were sex, tooth class, side, and jaw, whereas the dependent variable involved a particular spatial measurement such as the onset or duration of LEH.

Imbricational Enamel Formation Times

A modified version of Reid, Hillson, and Dean's (2000) methodology was employed in this study to determine the imbricational enamel formation times for fractions of 10 bonobo tooth crowns. (The specimens used in this study may be found in Table 3- 5). Imbricational enamel formation times were not determined from thin sections but from the SEM montages which showed perikymata ranging almost from the occlusal surface to the cervical margin.

Table 5-2: Common Chimpanzee Tooth Crown Formation Times in Years

Tooth	Jaw	Onset of Mineralization	Cuspal Enamel	Imbricational Enamel	Total Crown Formation[1]	Age at Crown Completion[2]
I1	Upper	0.24	0.48	4.81	5.29	5.53[3]
I2	Upper	1.06	0.46	4.32	4.78	5.84[3]
C	Upper	-	-	-	-	-
P3	Upper	1.24 ● 0.04	0.49	3.81	4.30	5.54[4]
P4	Upper	1.43 ± 0.34	0.50	3.11	3.61	5.04[4]
I1	Lower	0.30	0.80	3.58	4.38	4.68[3]
I2	Lower	0.39	0.39	4.59	4.98	5.37[3]
Cf	Lower	0.44	0.54	5.49	6.03	6.47[5]
Cf	Lower	-	0.47	5.38	5.85[4]	-
Cf	Lower	0.44	0.51 avg	5.44 avg	5.95	6.38 avg
Cm	Lower	0.44	0.54	6.34	6.88	7.32[3]
Cm	Lower	-	0.52	6.29	6.81[5]	-
Cm	Lower	0.44	0.53 avg	6.32 avg	6.85	7.29 avg
P3	Lower	1.20 ± 0.14	0.73 ± 0.10	4.29 ± 0.37	5.01 ± 0.45	6.21 ± 0.59
P4	Lower	1.63 ± 0.27	0.70 ± 0.10	3.20 ± 0.08	3.90 ± 0.14	5.53 ± 0.41

[1] total crown formation *time* = cuspal enamel + imbricational enamel
[2] *age* at crown formation = onset of minearalization + cuspal enamel + imbricational enamel
[3] Reid, Hillson, Dean (unpublished data)
[4] Reid *et al.* 1998: 440-441, Table 5 and Table 6
[5] Schwartz and Dean 2001

Table 5-3: Imbricational Enamel Formation Times for the Bonobo[1]

Cast	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	Total pkg count	Imbricational Formation Time (days)
Maxillary Central Incisor, I1 (n=3)												
49c	10	13	14	18	22	21	26	18	15	4	161	966
110a	9	13	13	19	25	23	28	32	31	21	214	1284
124a	13	13	13	19	18	19	22	12	13	16	158	948
Mean	11	13	13	19	22	21	25	21	20	14	178	1066
S.D.	2	0	1	1	4	2	3	10	10	9	32	189
Maxillary Lateral Incisor, I2 (n=1)												
120a	11	18	17	19	23	21	25	29	25	10	208	1248
Mandibular Lateral Incisor, I2 (n=1)												
126a	12	10	13	17	23	32	29	40	27	5	198	1188
Maxillary Canine, C (n=1)												
63c	11	42	34	31	38	28	32	30	26	2	273	1911

[1] perikymata counts for equal % zones occlusal (1) to Cervical (10) Margin.

From the SEM montages, the tooth crown surfaces were divided into tenths and the perikymata were counted for each zone [see Table 5-3]. To derive the time (in days) required to form imbricational enamel where LEH occurs, the summed perikymata counts were multiplied by 6 for incisors (applied to lowers and laterals) and 7 for the canine (also applied to the lower). From these cumulative counts, it was determined that the average imbricational enamel formation times were 1066 ± 189 days or 2.9 ± 0.5 years for the maxillary central incisor, 1248 days or 3.4 years for the maxillary lateral incisor, 1188 days or 3.3 years for the mandibular lateral incisor, and 1911 days or 5.2 years for the canine.

It is important to note, however, when implementing these imbricational enamel formation values that a large proportion of the sample is comprised of teeth from female and unsexed individuals. For the maxillary central incisor, 2 of the 3 teeth are from females with the remaining one from an unsexed individual. Of the 7 maxillary central incisors, 3 show perikymata in all the fractions ranging from the occlusal surface to the CEJ (see Table 5-3) and 4 display perikymata in 9 of the 10 zones (see Appendix D). The incomplete fractions show that the crown formation time for the central incisor is approximately 2.75 years and that the single value for the lateral incisor may be high as a result of individual variation.

Position of Enamel Hypoplasia on Tooth Crown Surface

Reid and Dean (2000) assert that tooth crown formation is not a linear process, and thus, by establishing the chronological crown formation times for respective fractions of a tooth crown, a more reliable age for enamel hypoplastic defects can be discerned. This is accomplished by determining in what fraction of the tooth the defect lies.

The position of LEH on the bonobo tooth crown was measured with calipers from the defect to the CEJ. This measurement was divided by the measured crown height to determine the placement or fraction of the defect relative to the crown height. Tooth crowns were divided into tenths and the percentages of enamel defects found in each fraction were then calculated.

Figure 5-1: Location of Linear Enamel Hypoplasia Position and Frequency (%) by Enamel Zones 1 to 10, Occlusal to Cervical Margins

zone	MAXILLARY RIGHT								MAXILLARY LEFT							
	M3	M2	M1	P4	P3	C	I2	I1	I1	I2	C	P3	P4	M1	M2	M3
1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
2	0	0	0	0	2	9	8	8	8	7	12	3	0	0	0	0
3	0	100	0	10	10	18	29	18	19	17	20	11	14	0	0	0
4	0	0	0	10	37	19	13	16	18	21	17	30	36	100	0	0
5	0	0	0	20	25	11	13	13	10	14	13	31	36	0	0	0
6	0	0	0	30	17	15	14	11	18	23	14	18	7	0	0	0
7	0	0	0	30	7	17	9	14	13	10	15	5	7	0	0	0
8	0	0	0	0	2	5	9	12	10	6	6	2	0	0	0	0
9	0	0	0	0	0	3	4	7	4	1	3	0	0	0	0	0
10	0	0	0	0	0	2	1	1	1	1	1	0	0	0	0	0
N	0	1	0	7	37	26	40	40	45	39	28	39	11	1	0	0

zone	MANDIBULAR RIGHT								MANDIBULAR LEFT							
	M3	M2	M1	P4	P3	C	I2	I1	I1	I2	C	P3	P4	M1	M2	M3
1	0	0	0	0	0	0	1	1	1	2	2	0	0	0	0	0
2	0	0	0	0	0	13	13	12	11	14	10	1	0	0	0	0
3	0	0	0	14	27	17	20	22	17	15	19	24	7	0	0	0
4	0	0	0	0	34	18	15	14	15	18	16	39	14	0	0	0
5	0	0	0	29	21	14	20	13	19	21	16	18	7	0	0	0
6	0	0	0	21	10	17	10	12	15	15	16	7	64	0	0	0
7	0	0	0	21	5	12	3	16	13	8	9	4	7	0	0	0
8	0	0	0	14	2	7	6	7	7	5	7	6	0	0	0	0
9	0	0	0	0	2	1	1	3	1	2	3	0	0	0	0	0
10	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0
N	0	0	0	11	44	35	37	41	41	40	39	46	9	0	0	0

The distribution of LEH on the tooth crown surface is shown in Figure 5-1. From this figure, it can be seen that the majority of the enamel defects show a large percentage (85%) between zones 3 and 8, i.e. the mid-60% of the tooth crown, for the maxillary incisors, canines, and premolars and between zones 2 and 7 for mandibular incisors, canines, and premolars. Thus, the average error in perikymata count per tenth of crown height between crown 3/10ths and crown 8/10ths (where the majority of these defects occur) is less than 0.5 perikymata. As a result of this average error, imbricational enamel formation rates where the great proportion of LEH occurs may be considered linear.

Skinner and Hopwood (2003) conducted a similar study of the spacing of repetitive linear enamel hypoplasia (rLEH) from the cusp/incisal edge to the cervix in chimpanzees and gorillas. The researchers found a greater percentage of hypoplastic episodes, 93.6%, in the middle 60% of anterior crown heights of incisors and canines. They calculated the average error in perikymata count between crown 2/10ths and crown 8/10ths to be less than 0.01 perikymata, therefore, Skinner and Hopwood argue that because the majority of hypoplastic defects fall within 60% of their crowns, imbricational enamel is deposited in this region at a "sufficiently regular rate so as not to significantly affect rLEH interval."

Results – Caliper Measurements

Timing of Apparent Onset of Stress from Location of First Episode of LEH

Overall, there is a slight difference in the commencement of stress in the incisors, canines, and premolars in terms of the first episode of LEH relative to the CEJ [Table 5-4 and Figure 5-2]. ANOVA shows this slight difference between tooth type (incisors, canines, premolars) and the relative onset of the first stress ($F=4.293$, $p=0.0142$). At this level, the difference is also slight between the onset of LEH and sex ($F=3.526$, $p=0.0611$).

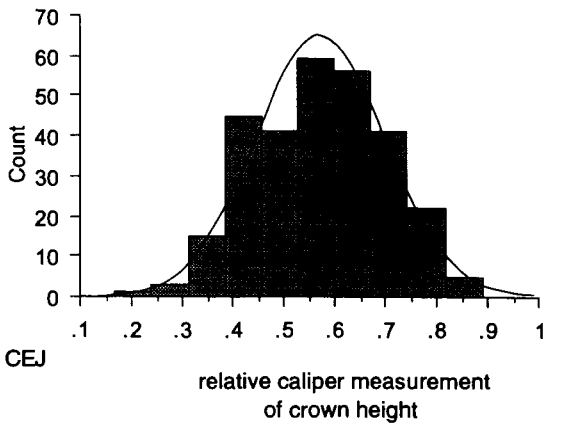
An examination of sex and tooth-type together does show a significant difference between canines ($F=10.676$, $DF=1$, $p=0.0015$) but not for central and lateral incisors ($F=0.164$, $DF=1$, $p=0.6858$) and premolars ($F=0.011$, $DF=1$, $p=0.9186$). That is, the

Table 5-4: Relative Distance from CEJ to First LEH and Onset of Stress (using a common chimpanzee model)

Tooth		Male	Female	Unknown	Mean/Total	Stress Onset In Years*
	Mean	.643	.602	.601	.616	
I1 max	S.D.	.122	.150	.069	.128	2.57
	S.E.	.023	.025	.017	.014	
	(n)	28	37	16	81	
	Mean	.559	.551	.502	.547	
I1 man	S.D.	.131	.123	.099	.123	2.72
	S.E.	.027	.020	.031	.015	
	(n)	24	36	10	70	
	Mean	.565	.604	.591	.587	
I2 max	S.D.	.086	.132	.148	.119	3.30
	S.E.	.017	.023	.047	.014	
	(n)	26	33	10	69	
	Mean	.537	.537	.543	.538	
I2 man	S.D.	.140	.122	.107	.124	2.90
	S.E.	.030	.021	.036	.015	
	(n)	21	35	9	65	
	Mean	.615	.567	.603	.587	
C max	S.D.	.152	.084	.120	.115	-
	S.E.	.036	.016	.054	.016	
	(n)	18	28	5	51	
	Mean	.612	.528	.617	.573	
C man	S.D.	.090	.108	.096	.107	3.42 (m)
	S.E.	.016	.018	.034	.012	3.52 (f)
	(n)	31	35	8	74	
	Mean	.528	.515	.497	.515	
P3 max	S.D.	.123	.089	.069	.093	3.58
	S.E.	.041	.019	.026	.015	
	(n)	9	22	7	38	
	Mean	.518	.558	.485	.538	
P3 man	S.D.	.101	.110	.136	.108	3.91
	S.E.	.032	.026	.079	.019	
	(n)	10	18	3	31	
	Mean	.535	-	.629	.554	
P4 max	S.D.	.144	-	-	.132	3.32
	S.E.	.072	-	-	.059	
	(n)	4	-	1	5	
	Mean	.601	.548	.503	.558	
P4 man	S.D.	.015	.117	-	.083	3.74
	S.E.	.011	.068	-	.034	
	(n)	2	3	1	6	

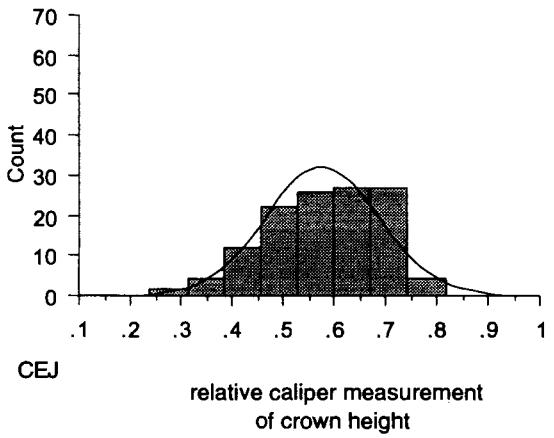
* e.g., For I1 max = [4.81 years chimpanzee imbricational enamel - (.616 x 4.81 years)] = 1.85 years + 0.48 years cuspal enamel +0.24 years onset of mineralization = 2.57 years of age.

Figure 5-2: Relative Onset of LEH in Tooth Types



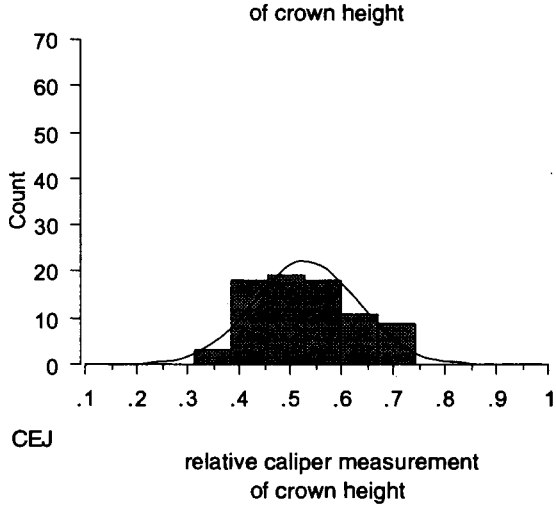
INCISORS

n = 288
mean = .573
std. dev. = .127
skewness = -105
kurtosis = -.469



CANINES

n = 124
mean = .578
std. dev. = .110
skewness = -.429
kurtosis = -.188



PREMOLARS

n = 78
mean = .532
std. dev. = .100
skewness = .332
kurtosis = -.799

onset of LEH in the canine appears to be delayed in females or males start to form tooth crowns later (see Table 5-4).

When calculating the relative onset of LEH using crown formation times for the common chimpanzee, the timing of the first LEH episode overall ranges from 2.6 to 3.9 years of age, depending on the tooth type [Table 5-4]. It is important to keep in mind that these timing estimates are based on onset of mineralization, cuspal enamel and imbricational enamel formation times for common chimpanzees. The bonobo has a smaller dentition and perhaps even a shorter crown formation period, i.e., cuspal enamel formation as well as the onset of mineralization times will also differ. A comparison of bonobo with common chimpanzee imbricational enamel formation times, shows that form imbricational enamel [see Table 5-5].

Table 5-5: Chimpanzee and Bonobo Imbricational Enamel Formation Times in Years

Tooth Type	Jaw	Bonobo	Chimpanzee
I1	Maxillary	2.92	4.81
I2	Maxillary	3.42	4.32
I2	Mandibular	3.25	4.59
C	Maxillary	5.24	-
C	Mandibular	-	6.32 avg

When the caliper measurements are standardized against imbricational enamel times for the bonobo, the relative onset of LEH ranges from at least 1 to 2 years depending on the tooth type [Table 5-6]. For the incisors, the onset of LEH closely ranges from at least 1.1 to 1.3 years while the onset of LEH in the canine is a minimum of 2.0 years. These values are somewhat misleading, i.e., the onset of LEH appears earlier, because the calculations are based on imbricational times only as the onset of mineralization and cuspal enamel formation times are not yet known for the bonobo. Assuming onset of mineralization and cuspal enamel formation times take approximately one year in the bonobo, as they do in the common chimpanzee, the onset of LEH in the bonobo is predicted to be around 2 years for the incisors and 3 years for the canine.

Table 5-6: Onset of Stress in Terms of Years Calculated from the Start of Imbricational Enamel Formation Times for the Bonobo

Tooth	Jaw	Years
I1	Maxillary	1.12*
I2	Maxillary	1.41
I2	Mandibular	1.50
C	Maxillary	2.16

* e.g., For I1 max = [2.92 years imbricational crown formation – (.616 x 2.92)] = 1.12 years

Comparison with Other Primates. In their examination of repetitive linear enamel hypoplasia (rLEH), Skinner and Hopwood (2003) studied the onset of LEH in common chimpanzees, gorillas, and organtuans. From their caliper measurements of the first hypoplastic episode standardized against crown height, they found few significant differences in the onset of LEH between the taxa – with the commencement of LEH beginning around 2.5 years of age in all taxa. A marked difference in onset was observed between the incisors and canines, however, this observation is attributed to differential crown formation times.

For the bonobo, the onset of LEH in incisors and canines was found to range from 2.6 to 3.5 years of age based on common chimpanzee crown formation times and 1 to 2 years based on bonobo imbricational data. It is suspected that the age of onset is lower for the bonobo as a marked difference has been observed in imbricational enamel formation times between the bonobo and common chimpanzee [see Table 5-5]. A difference in the onset of LEH was also found between the incisors and the canines. In the bonobo, the relative distance of the first episode of LEH to the cervix ranges from 0.538 to 0.616 for the incisor, 0.612 to 0.615 in male canines, and 0.528 to 0.567 in female canines. These values fall within in the ranges reported by Skinner and Hopwood (see Skinner and Hopwood 2003: Table 3).

Duration of Overall Stress on Tooth Crown

The relative duration of overall stress is determined from the span (i.e., time) between the first and final episode of LEH divided by the crown height for the same tooth [Table 5-7]. According to ANOVA, there are significant differences in the duration of

Table 5-7: Relative Distance between First and Last LEH and Duration of Stress (using a common chimpanzee model)

Tooth		Male	Female	Unknown	Mean/Total	Duration of Stress in Years
	Mean	.384	.351	.376	.367	
I1 max	S.D.	.115	.158	.091	.132	1.77*
	S.E.	.022	.026	.023	.015	
	(n)	28	37	16	81	
	Mean	.305	.305	.321	.307	
I1 man	S.D.	.169	.131	.151	.145	1.10
	S.E.	.035	.022	.048	.017	
	(n)	245	36	10	70	
	Mean	.276	.370	.389	.337	
I2 max	S.D.	.093	.146	.178	.140	1.46
	S.E.	.018	.025	.056	.017	
	(n)	26	33	10	69	
	Mean	.308	.315	.308	.312	
I2 man	S.D.	.149	.126	.100	.129	1.43
	S.E.	.033	.021	.033	.016	
	(n)	21	35	9	65	
	Mean	.380	.366	.433	.377	
C max	S.D.	.161	.120	.098	.133	-
	S.E.	.038	.023	.044	.019	
	(n)	18	28	5	51	
	Mean	.408	.345	.426	.380	
C man	S.D.	.119	.131	.062	.124	2.58 (m)
	S.E.	.021	.022	.022	.014	1.88 (f)
	(n)	31	35	8	74	
	Mean	.224	.192	.174	.196	
P3 max	S.D.	.090	.062	.059	.069	0.75
	S.E.	.030	.013	.022	.011	
	(n)	9	22	7	38	
	Mean	.231	.276	.226	.257	
P3 man	S.D.	.100	.094	.140	.099	1.10
	S.E.	.031	.022	.081	.018	
	(n)	10	18	3	31	
	Mean	.238	-	.173	.225	
P4 max	S.D.	.121	-	-	.019	0.70
	S.E.	.060	-	-	.049	
	(n)	4	-	1	5	
	Mean	.243	.236	.099	.216	
P4 man	S.D.	.014	.021	-	.059	0.69
	S.E.	.010	.012	-	.024	
	(n)	2	3	1	6	

* e.g., For I1 max = 4.81 years chimpanzee imbricational enamel x 0.367 = 1.77 years.

stress among tooth types, incisors, canines, and premolars, ($F=34.895$, $p<0.0001$) and specific teeth, I1, I2, C, P3, P4, ($F=18.508$, $p<0.0001$). These differences, however, may be attributed to differences in crown formation times. There is no significant difference in duration of stress between the sexes ($F=0.345$, $p=0.5575$) or between sex and tooth types for incisors ($F=0.552$, $p=0.4583$), canines ($F=3.121$, $p=0.0801$), and premolars ($F=0.001$, $p=0.9777$).

From the relative durations, it is estimated that the stress is recorded for 0.7 to 2.6 years, depending on the time of imbricational enamel formation for chimpanzees. Calculations for the duration of overall stress for those teeth, which have bonobo imbricational enamel formation data, yield a lower span of duration for all teeth [Table 5-8]. The span of LEH for the incisors is around 1.0 to 1.3 years while the defects for the canines span 2.0 years. The apparent stress duration in canines is approximately 2.0 years in males and 1.9 years in females.

Table 5-8: Duration of Stress for Bonobos in Terms of Years for Imbricational Perikymata Crown Formation

Tooth	Mean	Years
I1 max	0.367	1.1
I2 max	0.337	1.3
I2 man	0.312	1.0
C max	0.377	2.0

Comparison with Other Primates. Skinner and Hopwood (2003) researched the duration of stress in chimpanzees, gorillas, and orangutans by measuring the span between the first and last episodes of LEH and standardizing it against crown height. They found that differences in the sexes were not observed but orangutans demonstrated a longer span of episodes whereas chimpanzees and gorillas did not differ from each other in stress duration. The duration of stress in the African apes and the orangutans differed significantly for the tooth types, with defects on the canines spanning 1.6 to 2.9 years in African apes and 2.1 to 4.9 years for the orangutans.

The bonobo appears to follow a similar pattern with the chimpanzees, gorillas, and orangutans, i.e., no significant difference was found in the duration of stresses

between the sexes but one is found between the different tooth types. The duration of stress for the bonobo canine is approximately 1.9 years for the female and 2.6 years for the male based on calculations using chimpanzee imbricational data. The duration of stress based on bonobo imbricational data (based on a single male maxillary canine) is 1.9 years for the female and 2.0 years for the male. These values fall within the range provided by Skinner and Hopwood (2003) for the African apes.

Interval between Episodes of Stress

According to ANOVA, there are significant differences in the interval width between the successive episodes of rLEH stress ($F=33.006$, $df=9$, $p<.0001$) [see Table 5-9]. Differences were found between the sexes ($F=4.481$, $df=1$, $p=0.0345$) which may be suggestive of perikymata packing differences between the sexes or because tooth types differ rather than sex types. The relative spacing was also found to differ significantly between incisors, canines, and premolars ($F=99.954$, $df=2$, $p<.0001$) but not between the jaws ($F=0.004$, $p=0.9479$) or the sides ($F=0.989$, $p=0.3203$) . A closer examination of Table 5-9 shows that the intervals between earlier episodes of LEH are wider apart than those that occur later, specifically the relative spacing between interval 2-3 and interval 3-4 appears to drop for the incisors and canines. T-tests show that the decrease spacing between intervals 2-3 and 3-4 are not significant for the central incisor ($t=0.492$, $p=0.6237$), but they are significant for the lateral incisors ($t=1.996$, $p=0.0528$) and the canines ($t=2.031$, $p=0.0454$). For the premolars, the drop in relative spacing between intervals 1-2 and 2-3 was not found to be significant for the third premolar ($t=0.375$, $p=0.7130$) nor the fourth premolar ($t=2.693$, $p=0.2264$).

Calculations with imbricational data for the common chimpanzee yielded the interval between repeat episodes of LEH (in months) to be 8.2 ± 4.0 months for the maxillary central incisors, 7.8 ± 3.4 months for maxillary lateral incisors, 8.4 ± 3.6 for mandibular lateral incisors, and 7.4 ± 3.7 months the maxillary male canines and 6.4 ± 3.2 for the maxillary female canines (the canine values were calculated using mandibular canine estimates). For the incisors and canines, which have imbricational enamel crown formation times for the bonobo, the spatial measurements for the intervals between

Table 5-9: Relative Spacing between LEH Episodes (Jaws Combined)

Interval	Mean	SD	SE	Count
Central Incisor, I1				
Int 1-2	.173	.073	.006	151
Int 2-3	.145	.060	.006	101
Int 3-4	.120	.050	.007	59
Int 4-5	.111	.052	.010	25
Int 5-6	.099	.031	.012	7
Int 6-7	.071	-	-	1
Total	.150	.068	.004	344
Lateral Incisor, I2				
Int 1-2	.170	.070	.006	134
Int 2-3	.153	.061	.006	95
Int 3-4	.117	.049	.008	41
Int 4-5	.095	.029	.007	17
Int 5-6	.106	.019	.013	2
Total	.152	.066	.004	289
Canine, C				
Int 1-2	.129	.063	.006	125
Int 2-3	.116	.052	.005	115
Int 3-4	.097	.035	.004	85
Int 4-5	.081	.036	.005	47
Int 5-6	.076	.036	.006	31
Int 6-7	.064	.035	.008	20
Int 7-8	.064	.030	.007	17
Int 8-9	.059	.027	.009	10
Int 9-10	.069	.032	.014	5
Int 10-11	.059	.013	.008	3
Total	.103	.053	.002	458
Premolar, P3				
Int 1-2	.186	.070	.008	69
Int 2-3	.137	.057	.014	16
Int 3-4	.128	.097	.056	3
Total	.175	.071	.008	88
Premolar, P4				
Int 1-2	.198	.049	.015	11
Int 2-3	.119	.080	.057	2
Total	.186	.058	.016	13

episodes of LEH for these particular teeth yield timings closer to six months [see Table 5-10].

Comparison with Other Primates. Skinner and Hopwood (2003) found no significant differences in the relative spacing of LEH between the sexes but they did find

Table 5-10: Intervals between Episodes of Stress based on Bonobo Imbricational Data

Tooth	Mean	Interval (Months)
I1 max	0.142	4.98 ± 2.45
I2 max	0.151	6.19 ± 2.70
I2 man	0.153	5.97 ± 2.57
C max	0.098	6.16 ± 3.08

taxonomic differences between the Asian and African apes as well as differences in tooth type. Within the African apes, the relative spacing was found to differ significantly between the central incisors and the canines but not for the orangutan – an observation attributed differences in the rate of crown formation.

Attributing the repetitive nature of rLEH to a "regular semi-annual cycle of stress" in primates was first proposed by Skinner in 1986 and later with additional colleagues in 1995. These calculations were based on radiographic crown formation estimates. Using histological estimates, Skinner and Hopwood (2003) have calculated the mean interval between rLEH for the anterior teeth of chimpanzees, gorillas, and orangutans (with a few exceptions) to be close to 6 months. Median values were exactly 0.5 years. However, canines for three male orangutans proved to be an exception with the average/median interval between episodes of LEH to be closer to a year. It was also found that Bornean orangutans experienced semi-annual stress whereas Sumatran orangutans manifested stress yearly.

Based upon imbricational data for the bonobo, the interval between episodes of stress for the bonobo would appear to follow the semi-annual periodicity of the other African ape species studied by Skinner and Hopwood (2003). Differences between the intervals of stress were also observed between the tooth types and are attributed to differential crown formation times.

PERIKYMATA COUNTS FOR MACROSCOPIC OBSERVATIONS

Materials and Methods (for Macroscopic LEH Observations)

As noted earlier, in order to determine the timing of LEH in bonobos using perikymata counts, teeth were selected if they displayed prominent perikymata in combination with two or more LEH at the macroscopic level. From the collection of 189 crania at the Royal Museum for Central Africa, 24 crania had teeth that met the criteria. From these individuals, 51 teeth were impressed and casts made. From these teeth, 37 composite images were made and 22 were used for further analyses [see Table 3-4]. The number of individual bonobos used in this sample is 16.

The methods for creating the composite image from resin replicas of these teeth are described in Chapter 3. Counts were made directly from traced perikymata on the magnified composite image on the computer monitor. The counts for an enamel hypoplastic episode (i.e., its duration) were made from the "shoulders" of the defect. The interval between episodes was counted as the number of perikymata from the start of one LEH to the beginning adjacent defect. Perikymata counts on the 22 teeth were done twice and the counts averaged.

The time required to form one perikymata is 6 days for the incisor and 7 days for the canine. These values were obtained from cross striation counts of bonobo teeth collected in the year 2000 for Dr. Don Reid who thin sectioned and studied the teeth at the University of Newcastle's Department of Oral Biology (see Chapter 3).

Results

Interval between Stress Onsets – Perikymata from Macroscopic EH Observations

The number of perikymata between successive pairs of LEH was counted from onset to onset and averaged. The distribution of perikymata per rLEH is shown in Table 5-11 and the distribution within the different tooth classes was also determined with the results displayed in Table 5-12 and Figure 5-3.

ANOVA of the number of perikymata between successive episodes of rLEH does demonstrate a significance between the stress interval and the number of perikymata for

Table 5-11: Number of Perikymata per rLEH (Tooth Types Combined)

Interval	Mean	Median	Std. Dev.	Std. Error	Count
EH 1 – 2	34	36	18	4	20
EH 2 – 3	32	31	14	3	18
EH 3 – 4	28	26	10	3	14
EH 4 – 5	15	16	5	2	6
EH 5 – 6	17	16	3	1	5
EH 6 – 7	16	16	7	4	3
EH 7 – 8	19	19	-	-	1
EH 8 – 9	21	21	-	-	1
Averages	28	24	15	2	68

Table 5-12: Mean Number of Perikymata between rLEH per Tooth Type

Interval	Mean	Median	S.D.	S.E.	Count[1]	Months
Incisor						
Int 1-2	38	40	21	6	11	
Int 2-3	28	24	12	4	10	
Int 3-4	23	23	8	3	6	
Int 4-5	14	10	6	4	3	
Int 5-6	17	17	4	3	2	
Int 6-7	24	24	-	-	1	
Average	29	24	16	3	33	5.7[2]
Canine						
Int 1-2	29	32	12	4	7	
Int 2-3	38	38	16	6	7	
Int 3-4	32	26	12	4	7	
Int 4-5	17	18	3	2	3	
Int 5-6	17	16	3	2	3	
Int 6-7	13	13	4	3	2	
Int 7-8	19	19	-	-	1	
Int 8-9	21	21	-	-	1	
Average	28	23	14	2	31	6.4[3]
Premolar						
Int 1-2	29	29	20	14	2	
Int 2-3	24	24	-	-	1	
Int 3-4	26	26	-	-	1	
Average	27	25	12	6	4	-

1. N = Number of intervals observed from 23 teeth
2. [(29 perikymata x 6 days/perikymata) / 365 days] x12 months = 5.7 months
3. [(28 perikymata x 7 days/perikymata) / 365 days] x12 months = 6.4 months

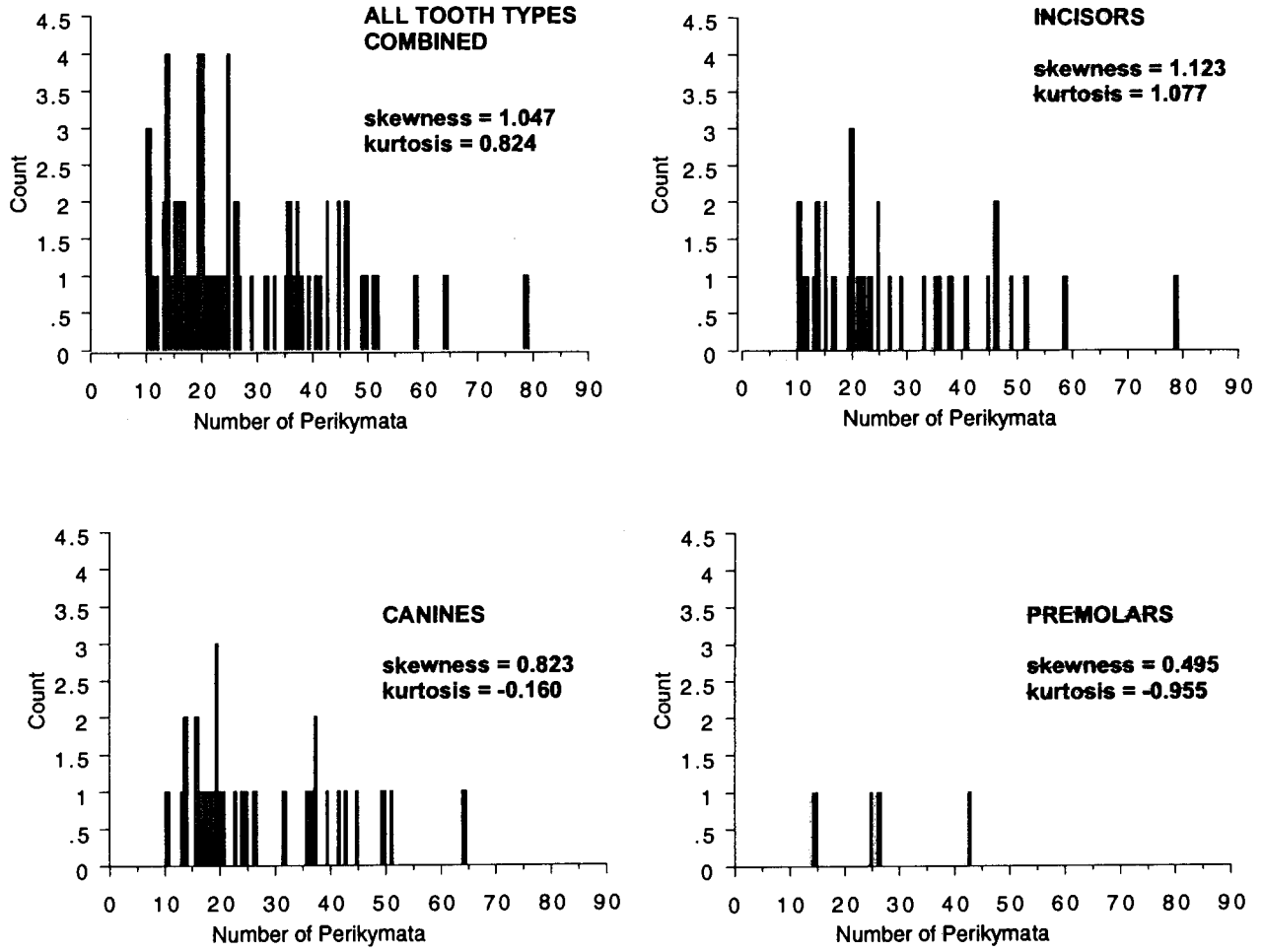
tooth types combined (F=2.374, df=7, p=0.0328). From Tables 5-11 and 5-12, a drop in the number of perikymata between intervals of rLEH can be observed. A statistical significant difference was observed in the number of perikymata compared between successive stress intervals such that the number of perikymata is greater in teeth with earlier episodes than later episodes. This decline in the number of perikymata is most apparent between intervals 3-4 and 4-5 (t=2.711, p=0.0143). Upon closer examination of the separate tooth classes, statistical differences are not observed for the incisors (t=1.575, p=0.1593) or the canines (t=2.217, p=0.0574) at these intervals. The mean number of perikymata from intervals 4-5 onwards is half the values expressed in the earlier episodes and may reflect added episodes of stress within an individual.

For all tooth types combined the average perikymata count between rLEH is 28 (SD=15, SE=2, n=68). These values are consistent with a stressor of approximately six-month periodicity. Because of skewing [see Figure 5-3], it is important to note that the median value is 24 perikymata, decreasing the periodicity to four months.

Comparison with Orangutans. In their study of rLEH among African and Asian apes, Skinner and Hopwood (2003) were able to study the distribution of perikymata on the anterior teeth of orangutans. They observed the mean number of perikymata between rLEH to differ between orangutans from Borneo and Sumatra for booth tooth types. It is thought that Bornean and Sumatran orangutans experience multimodal patterns of semi-annual and annual stress, respectively, based on differing imbrication cycles.

From perikymata counts between onsets of rLEH, bonobos also display a pattern of semi-annual stress of approximately six months. An examination of each interval within the incisors and canines reveals that the mean number of perikymata is halved after interval 4-5, implying added episodes of stress. If one considers median values, a multi-modal pattern of tri-annual stress within certain individuals could also reflect an additional episode of stress. These additional episodes observed in the bonobo contrast with Skinner and Hopwood's (2003) observation that some ape species demonstrate some resistance to stressors wherein the span between rLEH is longer, i.e., some individuals are skipping cycles of stress.

Figure 5-3: Distribution of Perikymata Counts between Macroscopic Episodes of LEH



Duration of Stress or Enamel Recovery Period for Macroscopically Observed LEH

From the composite SEM images, the duration of the actual hypoplastic event or period of enamel recovery can be determined by counting the number of perikymata within the hypoplastic event.

A statistically significant difference was not observed between the number of perikymata within a hypoplasia and type of tooth (F=1.140, p=0.3245) nor in the duration hypoplasias between the sexes (F=0.965, p=0.3296). Overall, a hypoplastic episode has a mean of 8.7 perikymata (median = 7.0, S.D.=5.2, S.E.= 0.5, n=90) or a duration of 6.8 or 8.0 weeks based on 6 or 7 cross-striation counts, respectively [Figure 5-4]. The results for the various tooth types are displayed in Table 5-13. The mean number of perikymata for the combined sexes including those of unknown sex for the incisors is 8.0 (median = 6.0, S.D.=5.6, S.E.= 0.8, n=46) and 9.3 (median =8.2, S.D.= 4.6, S.E.= 0.7, n=38) for the canines.

Figure 5-4: Duration of Hypoplastic Episode for Macroscopically Observed LEH

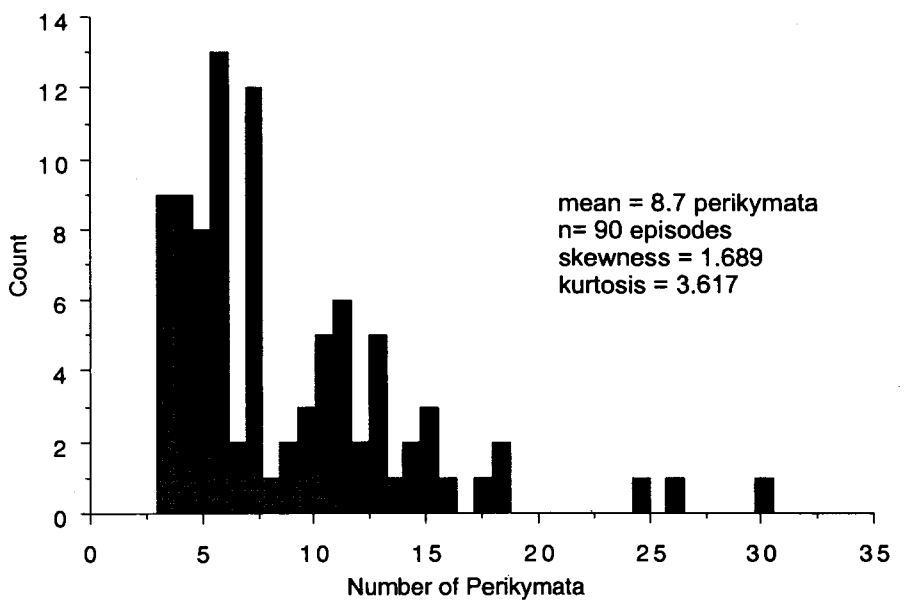


Table 5-13: Average Number of Perikymata within an LEH

Tooth		Male	Female	Unknown
Incisor	Mean	-	9.5	6.5
	S.D.	-	6.9	3.5
	S.E.	-	1.4	0.7
	(n)	-	23	24
	Median	-	7.0	5.8
Canine	Mean	11.2	8.7	-
	S.D.	3.7	4.8	-
	S.E.	1.2	0.9	-
	(n)	9	29	-
	Median	10.5	7.5	-
Premolar	Mean	-	10.8	-
	S.D.	-	5.1	-
	S.E.	-	2.1	-
	(n)	-	6	-
	Median	-	10.0	-

Comparison with Orangutans. Skinner and Hopwood (2003) were able to examine the distribution of perikymata within hypoplastic episodes on the anterior teeth of Bornean and Sumatran orangutans. They found that defects for both subspecies ranged from 6 to 7 weeks, a value shared by the bonobo.

PERIKYMATA COUNTS FOR MICROSCOPIC OBSERVATIONS

Materials and Methods (for Microscopic LEH Observations)

While it was the intent in the original perikymata study to only examine macroscopically observed hypoplastic episodes, additional enamel hypoplasias could be observed on the scanning electron micrographs. Thus, an examination of *all* hypoplasias on the SEM tooth crown surfaces was made to see if there was a difference in the timing of the defects when compared to the macroscopic observations. For this microscopic study, 35 SEM montages were utilized [see Table 5-14].

Table 5-14: Sample of Bonobo Teeth for Microscopic Perikymata Counts

Museum #	SFU Mould #	Sex	F.D.I. #
RG 9957	109a	unknown	1-1
RG 9997	110a	unknown	1-1
RG 11528	111a	female	1-1
RG 21742	112a	female	2-2
RG 21742	112a	female	2-1
RG 21742	112a	female	2-3
RG 23509	115a	male	1-3
RG 29029	118a	unknown	1-1
RG 29030	119a	female	2-1
RG 29031	120a	female	1-2
RG 29040	121a	female	2-4
RG 29040	122a	female	3-3
RG 29040	122a	female	3-4
RG 29055	123a	female	1-2
RG 29060	124a	female	2-1
84036 M11	125a	female	3-1
84036 M11	126a	female	3-2
84036 M11	127a	female	3-4
88041 M8	128a	unknown	2-1
88041 M8	129a	unknown	3-2
88041 M8	129a	unknown	3-1
RG 9338	49c	female	2-1
RG 9918	50c	unknown	3-3
RG 13201	51c	female	2-3
RG 14748	52c	unknown	3-3
RG 22908	53c	unknown	1-1
RG 26993	55c	female	1-3
RG 27698	58c	female	3-3
RG 28712	59c	male	3-3
RG 29040	60c	female	4-3
RG 29040	61c	female	1-1
RG 29040	61c	female	1-3
RG 29050	62c	male	3-3
RG 29053	63c	male	2-3
RG 29056	64c	male	1-1

Results

Interval between Stress Onsets – Perikymata from Microscopic EH Observations

As with the macroscopic study, the number of perikymata between successive pairs of all microscopically visible LEH was counted from onset to onset and averaged. The distribution of perikymata per rLEH is shown in Figure 5-5 and Table 5-15.

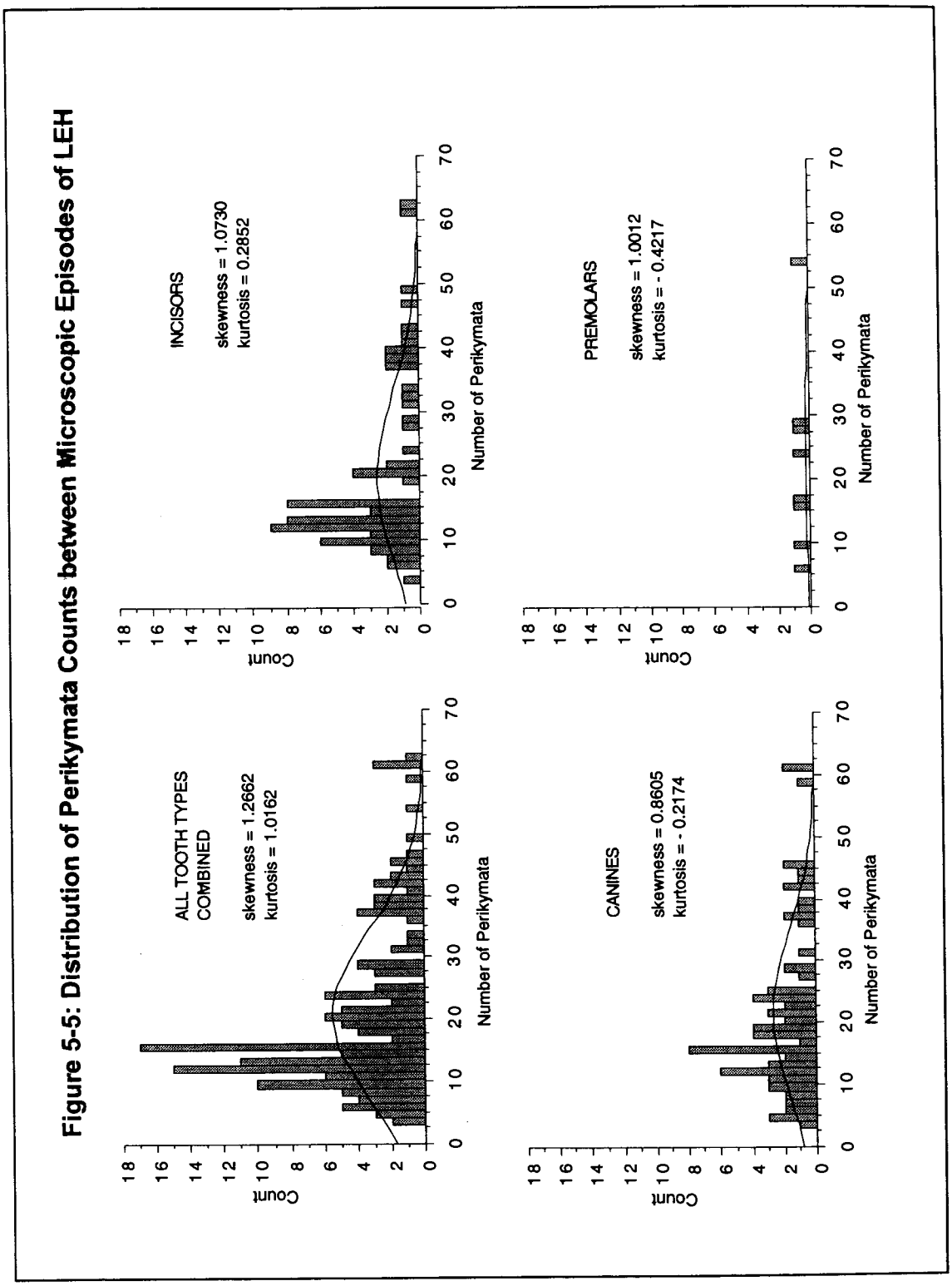


Figure 5-5: Distribution of Perikymata Counts between Microscopic Episodes of LEH
ALL TOOTH TYPES COMBINED
skewness = 1.2662
kurtosis = 1.0162
INCISORS
skewness = 1.0730
kurtosis = 0.2852
CANINES
skewness = 0.8605
kurtosis = - 0.2174
PREMOLARS
skewness = 1.0012
kurtosis = - 0.4217
Count
Number of Perikymata

Table 5-15: Number of Perikymata per rLEH (Microscopic)

Interval	Mean	Median	Std. Dev.	Std. Error	Count
EH 1 – 2	26	24	16	3	34
EH 2 – 3	20	16	12	2	32
EH 3 – 4	20	15	16	3	28
EH 4 – 5	21	17	12	2	23
EH 5 – 6	18	17	8	2	14
EH 6 – 7	23	20	14	5	10
EH 7 – 8	14	12	8	3	7
EH 8 – 9	10	10	2	1	3
EH 9 – 10	12	11	1	1	3
EH 10 – 11	10	10	-	-	1
Average	21	16	13	1	155*

* Includes events that were macroscopically observed.

ANOVA of the number of perikymata between successive episodes of rLEH shows no significant difference between the stress interval and the number of perikymata ($F=1.262$, $df=9$, $p=0.2628$) and no significant differences were found between the number of perikymata and tooth type (incisors, canines, premolars) ($F=0.143$, $df=2$, $p=0.8672$). A slight difference, however, was found between the number of perikymata between sucessive episodes of rLEH and the sexes ($F=4.980$, $df=1$, $p=0.0278$; $t=2.232$, $df=104$, $p=0.0278$). A closer examination of the tooth types shows the significance lying in the canine ($F=5.310$, $df=1$, $p=0.0247$) not the incisor ($F=0.327$, $df=1$, $p=0.5711$) with males expressing fewer perikymata between episodes, i.e., males are experiencing more frequent stress (see Table 5-16). This latter observation, however, is only based on 3 males.

The mean number of perikymata observed between *all* the LEH episodes visible on the SEM micrographs (i.e., a maximum of 11 episodes of LEH or 10 intervals) is 21

Table 5-16: Mean Number of Perikymata between rLEH by Sex

Tooth Type	Sex	Mean	S.D.	S.E.	N
Incisor	Female	23.8	14.8	2.6	33
	Male	19.5	7.1	3.6	4
	Total	23.4	14.1	2.3	37
Canine	Female	24.6	14.2	2.1	45
	Male	16.1	5.5	1.4	16
	Total	22.8	15.0	5.3	61

(S.D. = 13, S.E. = 1, n = 155) or 4.1 to 4.8 months if perikymata formation takes 6 or 7 days, respectively. The breakdown for each tooth-type is shown in Table 5-17. These values are thus suggestive of a 4-month cycle, which is less than the period for the macroscopic EH observations that averaged 6 months from the perikymata counts.

Further examination of the inter-LEH perikymata counts for incisors, canines, and premolars show a tighter clustering of increments around 10 perikymata or 2 months. Figure 5-4 shows the distribution of the perikymata counts between all episodes of the LEH observed on the electron micrographs.

A t-test comparing the difference in the mean counts of perikymata between rLEH intervals for macroscopically (mean=28) and microscopically (mean=21) observed LEH was found to be significant (t=3.705, p=0.0003).

Table 5-17: Mean Number of Perikymata Per Tooth Type

Tooth	Mean	S.D.	S.E.	N*	Months
Incisor	20	13	2	71	3.9
Canine	21	13	2	76	4.8
Premolar	23	15	5	8	-
Total	21	13	1	155	3.9 to 4.8

* N = Number of intervals observed from 35 teeth

Duration of Stress or Enamel Recovery Period for Microscopically Observed LEH

For this analysis, the duration of all the hypoplastic events (or period of enamel recovery) is determined by counting the number of perikymata within all the LEH observed on the SEM micrograph. Overall, a hypoplastic episode has a mean of 7.8 perikymata (S.D.=5.0, n=192) or a duration of 6.7 or 7.8 weeks based on 6 or 7 perikymata counts, respectively [Figure 5-6]. The results for the various tooth types are displayed in Table 5-18. Available median values for a variety of tooth types are 6 or 11. This would suggest then, the probable duration of LEH is about 5 weeks for incisors, approximately 6 to 10 weeks for female and male canines, respectively.

A t-test comparing the difference in the mean counts of perikymata within macroscopically (mean=9) and microscopically (mean=8) observed LEH found no statistical significance (t=1.355, p=0.1765). ANOVA also showed no statistical

Figure 5-6: Duration of Hypoplastic Episode for Microscopically Observed LEH

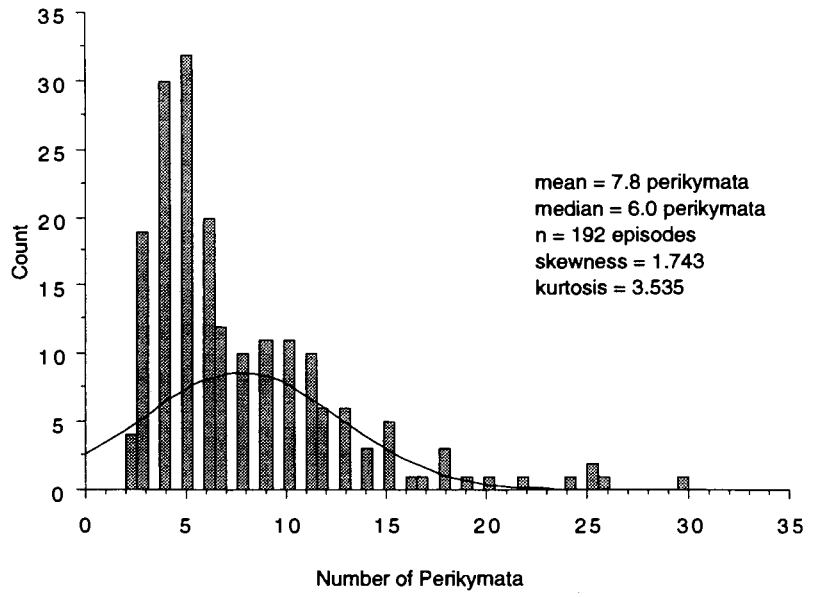


Table 5-18: Average Number of Perikymata within a Microscopically Observed LEH

Tooth		Male	Female	Unknown	Combined
Incisor	Mean	11.4	8.4	6.3	7.6
	S.D.	3.8	5.4	4.4	5.0
	S.E.	1.7	1.4	0.7	.5
	(n)	5	44	41	90
	Median	11.0	6.0	5.0	6.0
Canine	Mean	9.4	7.5	5.7	7.6
	S.D.	4.5	4.9	2.9	4.6
	S.E.	1.0	0.7	0.7	.5
	(n)	20	54	17	91
	Median	10.0	6.0	5.0	6.0
Premolar	Mean	-	10.3	-	-
	S.D.	-	7.3	-	-
	S.E.	-	2.2	-	-
	(n)	-	11	-	-
	Median	-	6.0	-	-

difference between the number of perikymata within hypoplastic grooves and sex ($F=3.308$, $p=0.0715$) or tooth type ($F=1.096$, $p=0.2974$) or sex and tooth type combined ($F=3.990$, $p=0.160$). Therefore, the stressor(s) must be uniform because teeth and sex are affected equally.

SUMMARY OF RESULTS

For the metrical analyses relative caliper measurements were compared to two crown formation times: crown formation times taken from the literature determined from striae in tooth crown sections for *Pan troglodytes* and imbricational enamel formation times determined from SEM montages for *Pan paniscus*. The imbricational enamel formation times for the bonobo were 1066 ± 189 days or 2.9 ± 0.5 years for the maxillary central incisor, 1248 days or 3.4 years for the maxillary lateral incisor, 1188 days or 3.3 years for the mandibular lateral incisor, and 1911 days or 5.2 years for the canine. The majority, 85%, of enamel hypoplastic defects were found to fall within the mid-60% of the tooth crown.

Bonobos were found to express their first episode of LEH between 2.6 to 3.9 years of age when calculating crown formation times for the common chimpanzee. Based on imbricational enamel formation times that are available for the bonobo, the onset of stress calculated from the start of imbricational enamel formation is approximately 1 to 2 years for the incisor and canine, respectively. Assuming that the onset of mineralization and cuspal enamel formation take approximately one year to form in the bonobo, as it does for *Pan troglodytes*, the age at onset of LEH in the bonobo is 2 to 3 years. Statistical tests show that there is a significant difference in the relative onset of the first stress and tooth type and slight difference was also observed for sex. A closer examination of sex and tooth type together reveals the statistical significance to be found in the canines and not in the incisors and premolars.

Depending on the method used (i.e., caliper measurements versus perikymata counting) the timing of intervals between LEH is not as clear. From the caliper measurements of defect interval spacing, the interval between rLEH is between 6.4 and

8.4 months when calculations are based upon *Pan troglodyte* schedules. Using bonobo imbricational data, however, brings the interval down to 5 to 6 months. Perikymata counts from SEMs reveal reconstructed stress intervals of 5.7 to 6.4 months for incisors and canines, respectively. These calculations are based on stress intervals between macroscopically visible enamel defects. Cross-striation counts were not available for premolars.

As for the duration of an enamel hypoplastic event, perikymata counts reveal a mean value of 6.8 to 8 weeks. Mean values for the probable duration of LEH in the incisors and canines are approximately 7 and 9 weeks, respectively, although the number of perikymata within a hypoplastic defect and the type of tooth was not found to be significant. It seems, then, that bonobos are affected by a cyclical stressor every 5 to 6 months and take between 7 and 8 weeks to recover from the stress.

Because additional enamel hypoplasias were thought to be observed on the scanning electron micrographs, an examination of *all* hypoplasias on the SEM tooth crown surfaces were made to see if there was a difference in the timing of the defects when compared to the macroscopic observations. From these micrographic observations, the stress intervals between repetitive episodes of LEH were found to range from 3.9 to 4.8 months for incisors and canines, respectively. The duration of these defects is about 6.5 weeks for incisors and 7.6 weeks for canines and a statistical significance was not found between the number of perikymata within a hypoplasia and the sex of an individual or the tooth type. The mean counts of perikymata between macroscopically and microscopically observed intervals of LEH were statistically significant, but the mean number of perikymata within LEH that were both macroscopically and microscopically observed were not found to be statistically significant.

CHAPTER 6: SUMMARY AND DISCUSSION

The objectives for the study of enamel hypoplasias (EH) in the bonobo, Pan paniscus, were two-fold: (1) to conduct systematic analyses of the distribution of this dental enamel defect, and (2) from a micro-incremental analysis of the tooth crown surfaces, correlate the timing of enamel hypoplastic defects to potential stressors.

RESULTS SUMMARY

Prevalence of Enamel Hypoplasia

The enamel hypoplasia inventory of the bonobo crania housed at the Royal Museum for Central Africa in Tervuren, Belgium yielded unexpected results. When compared to other studies of hypoplasia among large bodied apes, particularly the common chimpanzee (*Pan troglodytes*), the bonobo demonstrates an unusually high prevalence of enamel hypoplastic defects indicating these primates are more susceptible to physiological stressors. The bonobo is found to express its first episode of stress at 2.6 years and is affected by a cyclical stressor every 5 to 6 months and takes between 7 and 8 weeks to recover from the stress.

Linear enamel hypoplasia (LEH), pit hypoplasias, plane-type hypoplasia, vertical enamel hypoplasia (VEH) and other hypoplastic forms such as localized hypoplasia of the primary canine (LHPC) and Darcy's Defect have all been identified in the bonobo. The distribution of EH in the bonobo was consistent with other large apes, i.e., anterior teeth demonstrated a higher degree of EH, LHPC and pit defects were common in the deciduous dentition whereas LEH was more common in the permanent dentition. Because all the adult bonobos were affected with LEH, no significant difference in the expression of the defect between the sexes could be calculated. A statistical significance, however was not found in the severity of LEH expressed between the sexes in any of the age categories, nor was a statistical significance was found in the expression of LHPC, pits, or plane-type defects between the sexes of any age category.

When compared to other studies involving chimpanzees (Guatelli-Steinberg 1998b, Hannibal 2000b, Lukacs 1999a, Newell 1998, Skinner 1986b) there was a significant difference in the prevalence of many of these enamel defects. Bonobos demonstrated a higher prevalence of LEH, pitting, and LHPC but not for Darcy's Defect.

Particular teeth are known to express unique hypoplasias: the deciduous canine for LHPC and the permanent maxillary lateral incisor for Darcy's Defect. More often than not these defects are expressed bilaterally and, therefore, suggest a systemic stress. Of the 534 teeth from individuals expressing pits in their permanent dentition, 6% displayed pit-type defects. The lateral incisors possessed the greatest number of pits in the permanent dentition, particularly the left lateral maxillary incisor. These pit-type defects of the permanent maxillary lateral incisors may be indicative of a specific physiological stressor for this particular tooth. Hannibal (2000b) was first to observe a diagonal hypoplastic defect unique to this tooth type, i.e., Darcy's Defect, indicating this tooth was "clearly differentially susceptible." The few pits in the permanent dentition of the bonobo were by no means diagonal and were found on other tooth classes, but the prevalence of these pits on the maxillary lateral incisor in which there is a hypoplasia unique to this tooth is cause for speculation.

The fact that there are differences in the eruption of the second permanent incisor between the bonobos and common chimpanzees may account for prevalence of pits in this tooth for the bonobo. It could also explain why Darcy's Defect was only observed in one individual and why the bonobo has the lowest prevalence of this defect in the large apes. Kinzey (1984:81) observed a "marked difference between the two species of chimpanzees in the timing of eruption of the second permanent incisor" with the most common eruption sequence for the bonobo being: M1, I1, M2, I2, P3, P4, C, and M3. The usual order of eruption of permanent teeth in the apes is: M1, I1, I2, M2, P3, P4, C, and M3 (Kinzey 1984:81). Hublin, Braga, and Triel (2001) examined the permanent tooth crowns and root calcification in the bonobo skeletal collection at the Royal Museum for Central Africa. They found that overlapping crown formation between permanent molars (M1/M2) is not demonstrated by all bonobos (as it frequently occurs in humans) and canine-premolar eruption does not appear to be delayed like the common chimpanzee.

Timing of Enamel Hypoplasia

From SEM montages of bonobo teeth, imbricational enamel formation times were created for the maxillary central incisor (I1), maxillary lateral incisor (I2), mandibular lateral incisor (I2) and mandibular canine (C) by adding together perikymata counts from fractions of the tooth crown. From these counts it was determined that the imbricational enamel formation periods for the maxillary I1 is 2.92 ± 0.5 years, 3.4 years for maxillary I2, 3.2 years for mandibular I2, and 5.2 years for the canine.

At the macroscopic level, the age at which bonobos expressed LEH was determined through caliper measurements of relative crown heights, which were then multiplied by tooth crown calcification schedules reported for the common chimpanzee and by imbricational enamel formation times for the bonobo.

From these caliper measurements, the onset of LEH was found to range from 2.6 to 3.9 years of age based on *P.troglodytes* schedules and 2 to 3 years of age based on imbricational bonobo data. There was a slight difference in the onset of LEH between tooth types that may be attributed to differential crown formation times. A significant difference was found between the onset of LEH and the sexes, specifically for the canine, but again this difference may be attributed to differential periods of crown formation with males possessing earlier formation schedules.

Calculating the onset of LEH in the bonobo is difficult because analyses in this thesis have demonstrated that histological crown formation times are incomplete for the bonobo and imbricational enamel formation times for the common chimpanzee and the bonobo are not similar (see Table 5-5). It has been shown that the bonobo has a smaller dentition (Almquist 1974, Johanson 1974a,b, Kinzey 1984, White 1996) thus it is realistic to expect shorter crown formation periods. Skinner and Hopwood (2003) calculate the onset of LEH in common chimpanzee, gorillas, and orangutans to be 2.5 years in all taxa. If one assumes that the onset of mineralization and cuspal enamel formation times are roughly a year in the bonobo, then the onset of stress for this primate is similar to other primates.

The repetitive nature of LEH suggests this defect is episodic in nature. From the perikymata counts between intervals of macroscopically observed LEH, the mean

number of perikymata is 28 or 5.7 to 6.4 months depending on the number of cross-striations, suggestive of a bi-annual cycle. A cursory examination of the average number of perikymata for the various intervals between macroscopically visible hypoplastic episodes does show a gradation in the number of perikymata as defects approach the CEJ. A marked decline in the mean number of perikymata was found between intervals 3-4 and 4-5, with the mean number of perikymata from interval 4-5 onwards to be half of the values expressed in earlier episodes. This phenomenon is thought to reflect added episodes of stress within an individual.

Skinner and Hopwood (2003) were able to examine the distribution of perikymata between rLEH of orangutans from Borneo and Sumatra and found that the orangutans experienced multimodal patterns of semi-annual and annual stress, respectively. The researchers were also able to take caliper measurements of the rLEH intervals and calculated the mean interval to be close to 6-months in chimpanzees, gorillas, and orangutans.

Comparing caliper measures of rLEH on bonobo teeth and calculating the measurements relative to imbricational enamel formation in the bonobo also yielded a periodicity of 6 months (see Table 5-10). (Calculations against crown formation times for the common chimpanzee yielded higher intervals of 7.5 to 8 months for incisors and 6.5 for canines). Thus, the bonobo demonstrates a 6-month periodicity that complements similar observations for apes across the globe. An examination of the relationship of even to odd episodes of LEH in the bonobo is not shared with those observed by Skinner (1986) with Hopwood (2003). Bonobos do not demonstrate a greater preference for even over odd episodes like the other ape species.

Perikymata can also be identified and counted within the enamel hypoplastic grooves. Overall, the average number of perikymata in a hypoplastic groove was 8.7 or 6.8 to 8.0 weeks depending on the number of cross-striations. A similar duration, 6 to 7 weeks, also observed in orangutans (Skinner and Hopwood 2003). Whether these grooves are indicative of the actual duration of the stress or the time required for the enamel to recover from the initial systemic insult is the subject of debate (Hillson and Bond 1997, Dr. Don Reid, personal communication March 24, 2001).

From the scanning electron micrographs taken of bonobo teeth, additional enamel hypoplasias were observed on the micrographic images. The mean number of perikymata counts between intervals of *all* defects visible on the SEMs, is 21 or 4.1 to 4.8 months depending on the number of cross-striations, suggestive of a tri-annual cycle. Overall, the average number of perikymata in all microscopically observed hypoplastic grooves was 7.8 or 6.7 to 7.8 weeks depending on the number of cross-striations. The mean counts of perikymata between macroscopically and microscopically observed rLEH were statistically significant but the means within macroscopic and microscopic episodes were not found to be statistically significant.

Summary of Findings

1. Bonobos demonstrate a high prevalence of enamel hypoplastic defects compared to other apes suggesting these primates are more susceptible to physiological stressors.
2. The distribution of hypoplasias in the bonobo is consistent with other apes except for Darcy's Defect. The lack of this defect in the bonobo is attributed to differences in eruption schedules between common chimpanzees and bonobos.
3. Bonobos have shorter imbricational enamel formation times when compared to the common chimpanzee. Imbricational enamel formation times for the bonobo were found to be 2.9 years for maxillary the I1, 3.4 years for the maxillary I2, 3.2 years for the mandibular I2, and 5.2 years for the mandibular canine.
4. The onset of LEH ranged from 2.6 to 3.9 years based on *P.troglodytes* schedules and 2 to 3 years based on *P.paniscus* schedules.
5. LEH is episodic in nature with bonobos demonstrating a 6-month periodicity for macroscopically observed defects based on perikymata counts and caliper measurements compared against bonobo imbricational enamel formation times.
6. The mean number of perikymata between successive episodes of stress is halved as defects approach the CEJ reflecting added episodes of stress.
7. The duration of stress ranges from 6.8 to 8 weeks, depending on the number of cross-striations.

8. In addition to macroscopically observed defects, other hypoplastic defects can be observed on SEM montages. A tri-annual cycle of stress is observed when *all* hypoplastic defects visible on SEM montages are considered, with the stressor lasting from 6 to 7 weeks.

DISCUSSION

It was expected that the prevalence of EH within the bonobo, *Pan paniscus*, would parallel the prevalence of EH within the common chimpanzee, *Pan troglodytes*. This was clearly not the case. Since the bonobo appears to be perpetually stressed, the issue then becomes whether the high prevalence of EH in *Pan paniscus* is indicative poor health status or a lower threshold, in this primate, to biological and environmental stressors overall. Such low thresholds have already been suggested by Hannibal (2000b) for the Great Ape species as a whole and models of susceptibility thresholds have been put forward by Newell (1998) for primates at the levels of the individual, tooth, and taxa.

The bonobos' social interaction is one aspect of their behaviour that would indicate that they do experience a higher level of stress when compared to other primates. The "sexual" receptivity of bonobos is not necessarily affiliated with periods of ovulation. Kano (1992:162) recognizes the "excessive copulatory behaviour" as social behaviour, which enables male-female coexistence. The "promiscuous" behaviour has been suggested to act as a tool to reduce social tension (Takahata *et al.* 1996:153). Considering that they "reduce tension" rather frequently, bonobos should be relatively stress-free and hence display a low prevalence of EH. Conversely, if bonobos feel the need to constantly "reduce tension" then perhaps they are experiencing a higher level of stress and therefore manifest a greater prevalence of EH.

The repetitive nature of LEH has been attributed to semi-annual and annual rains, which are believed to have a role in the cyclical stress that is manifested in the dentition of non-human primates (Guatelli-Steinberg and Skinner 2000, Skinner 1986b, Skinner *et al.* 1995, Skinner 2000, and Skinner and Hopwood 2003). Skinner and Hopwood (2003) compared rLEH between African and Asian apes and found that "the semiannual stressor transcends geographic and temporal boundaries, and is attributed to regular moisture cycles associated with the intertropical convergence zone modified by the monsoon."

The researchers hypothesize that "the combination of seasonal variation in fruiting cycles with specific stressors (malaria and/or intestinal parasites, especially hookworm)" results in the widespread phenomenon of rLEH. While bonobos shares a similar periodicity with other ape species across the globe, here it is hypothesized that periodic stress manifested in the bonobos' dentition is a result of rainy seasons which in turn synchronize disease cycles (particularly parasitic infection, namely *Oesophagostomum*), rather than nutritional/seasonal fruiting cycles. White's (1998) study of the bonobos at Lomako found no significant correlation between monthly rainfall and total fruit abundance nor a particular reliance on specific food types during specific months. She, therefore, hypothesizes bonobos "do not undergo a seasonal dietary crunch period when large parities and female sociality are not feasible" (White 1998:1024). The roles of moisture cycles, parasitic infection, and nutritional stress that may or may not play in rLEH are further discussed below.

Moisture Cycles

At the beginning of this study, it was hypothesized that the bonobos, whose habitat is close to the equator, would have a lower frequency of EH because the marked difference in rain cycles decreases with latitudes approaching the equator. With the help of scanning electron microscopy and microincremental structures of the dentition, a closer examination of LEH revealed that the defect in bonobos was episodic in nature with the hypoplasias being expressed at 6-month intervals on average. This biannual stress may be reflective of semi-annual and annual rains, which are believed to have a role in the cyclical stress that is manifested in the dentition of non-human primates (Guatelli-Steinberg and Skinner 2000, Skinner 1986b, Skinner *et al.* 1995, Skinner 2000, and Skinner and Hopwood 2003).

The Democratic Republic of the Congo's (DRC) position along the equator (Meditz and Merrill 1994:69) is affected by the seasonal pattern of rainfall. In the equatorial rain forest "there is no clear distinction... between a rainy and a dry season; rain can fall in any month, several times a day, nearly 100 inches of it a year" (Forbath 1977:8-9). The vast majority of bonobo field investigations are made at Wamba and Lomako which are situated at latitudes of 0°10'N and 0°51'N, respectively (de Waal and

Lanting 1997:59). Kano (1992:49) described the climate and topography in the bonobo's area of distribution as "invariable." Yet, he continues to describe the rainfall in their habitat, which is anything but uniform,

> ...in the northern part of [the bonobo's] distribution, the annual rainfall is between 1,600 and 2,000 mm and, in general, is evenly distributed among months. From the equator southward, rainfall clearly follows the pattern of the southern hemisphere, with the minimum amount of rain falling between the months of June and July. There are more than six months when the amount of rainfall per month exceeds 100 mm and only two to three months of less than 25 mm of rainfall, satisfying the conditions for the formation of a tropical rainforest (Kano 1992:53).

Kano (1992:50-51) also provides graphs of meteorological data, collected by Vuanza and Grabbe (1975) in the bonobo's region of distribution, showing a bi-modal distribution of rainfall [see Figure 6-1].

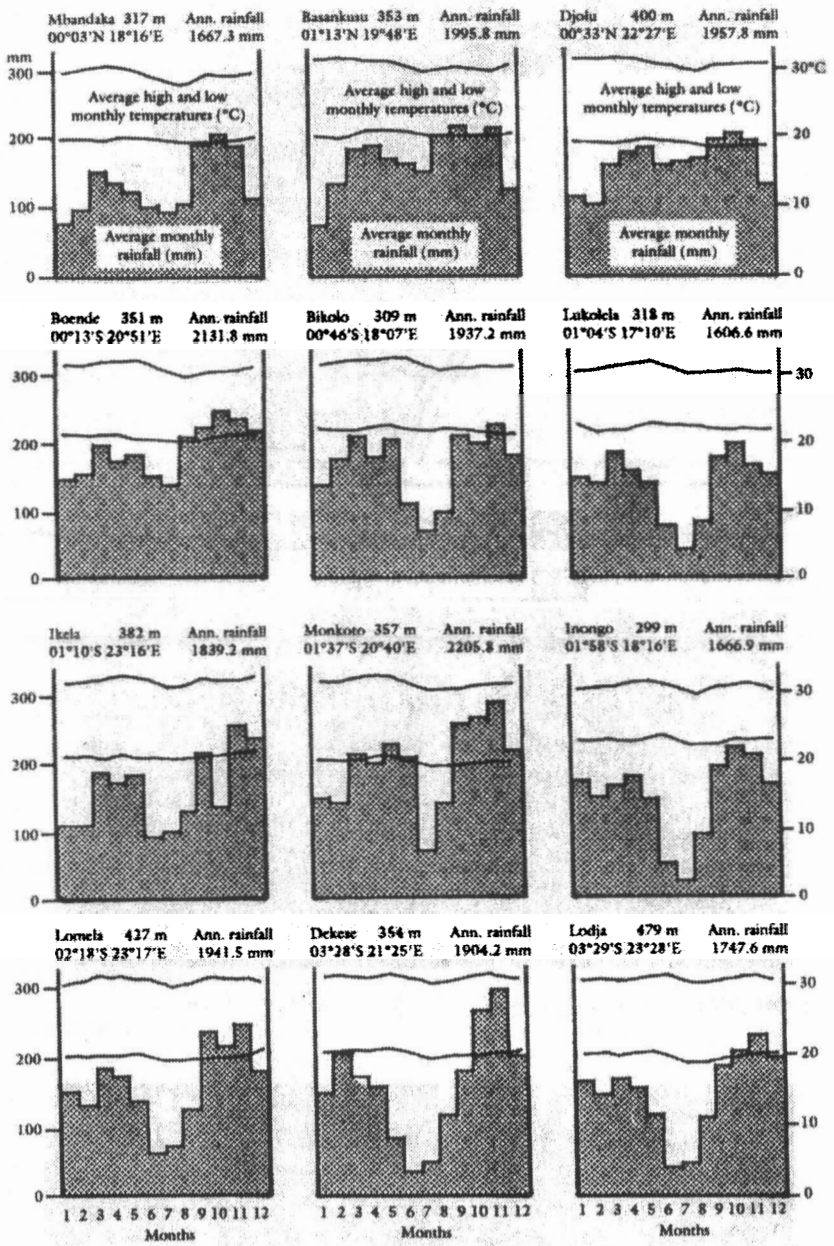
White (1998:1023) identifies two wet and two dry seasons at Lomako per year but notes "considerable variation" in annual precipitation over the years. White does acknowledge that the area is "less seasonal" with respect to rainfall at other chimpanzee regions.

Macho and colleagues (1996) attribute moisture cycles, which in turn influence seasonal availability of food, to an annual occurrence of accentuated striae of Retzius manifested in the molars (n=11) of *Theropithecus*. While none of these specimens displayed enamel hypoplasias, an annual periodicity of accentuated striae was observed in the teeth. Two periodicities were observed: one with an annual cycle and the other with an annual cycle that was further divided into two longer and shorter periods of episodic stress. The researchers attribute the later cycle to the "onset of each rainy season and the height of the dry season when the available food sources were at a minimum" (Macho *et al.* 1996:68).

White's (1998) double rainy and dry seasons recorded at Lomako and Macho's (*et al.* 1996) hypothesis of tri-seasonality manifested as accented striae in *Theropithecus* molars does lend credence to the 4 to 5 month periodicity observed on SEM montages of bonobo teeth. Because this shorter periodicity was only identified at the microscopic level, it is possible that bonobos are subtlely picking up changes in moisture cycles.

Figure 6-1: Altitude ASL, Rainfall, and Temperature with Bonobo Regions of Distribution

(source: Kano 1992:50-51 citing Vuanza and Grabbe 1975)



Parasitic Infection

Parasitism becomes extremely significant when the host becomes immunosuppressed or is susceptible to other diseases as a result of these infections. Hypoplastic enamel defects have been experimentally produced in sheep incisors through induced parasitism (Suckling and Thurley 1984, Suckling, Elliott, and Thurley 1986, 1983). Kuntz (1982:185) identifies the cyclical nature of the intermediate hosts (as well as the stress of transferring wild primates into a captive environment) as potential inducers of parasitic infections.

Skinner (1986b) first proposed the distribution of LEH in chimpanzees was episodic in nature, with timing between these episodes calculated at 6 months on average. This time interval coincides with the major and minor rainy season in the subtropics. These rainy periods were later paralleled with the seasonal prevalence of malaria throughout Africa (Skinner *et al.* 1995). Later Skinner with Hopwood (2000) elaborated upon the etiologies of repetitive and prevalent LEH and suggested common diseases, such as pneumonia, hookworm, and malaria, as possible agents. Guatelli-Steinberg with Skinner (2000:128) also refer to past studies (Skinner *et al.* 1995) linking the periodicity of malaria with hypoplasias but also hypothesize that intestinal parasites, particularly hookworm with its marked seasonal variation in egg counts, could also be responsible for repetitive episodes of LEH. Hannibal (2000b:3), Guatelli-Steinberg (1998:195), and Newell (1998) also refer to parasite infections in their theses on EH in non-human primates. Skinner and Hopwood (2003) provide the latest hypothesis regarding rLEH and parasitic infection. These researchers feel that it is a combination of malnutrition (i.e., seasonal variation in fruiting cycles) with specific disease stressors (malaria and hookworm) that contribute to rLEH.

In chimpanzees, numerous protozoa and helminthes have been observed. The chimpanzee is particularly susceptible to *Strongyloides* nematode infections in the wild and captivity (Kuntz 1982:187, 191). The parasite literature for the bonobo is ephemeral, with brief reports (Fain 1957, 1959, 1962, 1963, Mortelmans *et al.* 1971, Stam 1960) and reference in parasite reviews (Benoit 1961, Myers and Kuntz 1972, Vuylsteke 1964, Webber 1955, Yamashita 1963, Zumpt 1961). Fortunately, there are a few surveys of parasites in wild bonobos from the field sites of Wamba (Hasegawa and Kano 1983,

Kano 1992) and Lomako (Dupain *et al.* 2000, Dupain *et al.* 2002). Researchers at this latter site have recently reported on new evidence for medicinal plant use for *Oesophagostomum* infections (Dupain *et al.* 2002). A taxonomic breakdown of the parasites found in the bonobo may be found in Appendix E.

Kano collected several hundred fecal samples while he was researching the bonobos' exploitation of food resources at Wamba (Kano 1992:113). These samples were analyzed by Hasegawa at the Ryukyus University School of Medicine's Laboratory of Parasitology in Japan. During the analysis, it was discovered that bonobos were infected with an enormous number of protozoans, their numbers and size so great that Hawegawa had first thought they were fragments of food (Kano 1992:113). *Troglodytella* species were the most prevalent parasite in the fecal study, with almost all of the bonobos infected (Hasegawa *et al.* 1983:420). *Strongyloides* was the most prevalent nematode found in the survey, with 53% of the samples affected. Hasegawa and his colleagues (1983: 422) speculated that the *Strongyloides* species could be *S. fülleborni* due to the similarity in egg size.

Almost 20 years later, a team of European researchers (Dupain *et al.* 2000) conducted analyses of parasites in bonobos and humans at Lomako. The overall parasite loads were found to be lower a Lomako than at Wamba although it was not reported if this finding was statistically significant. The types of parasites and their prevalence were comparable to Hasegawa's (1983) study at Wamba. Once again, *Troglodytella* species were the dominant parasite, with 75% of the samples affected, and 39% of the samples had *Strongyloides*. However, the frequency of *Oesophagostomum* was greater at Lomako, 49%, than at Wamba, 18%, and the trematode species were found to be different at both sites.

Troglodytella is a common parasite of anthropoid apes (Hasegawa *et al.* 1983:421, Mortelmans *et al.* 1970:190) and is the largest protozoan parasite known in apes (Mortelmans *et al.* 1970:190). Mortelmans and his colleagues (1971:90) observed chimpanzees which were heavily infected with *Troglodytella abrassarti* and suggested that repeated intestinal disorders, such as liquid or hemorrhagic diarrhea, induced by these parasites could "open the door" for other more severe bacterial, parasitic, or viral

infections. Kuntz and Myers (1969:187) also state "the strongyles, *Oesophagostomum*, and hookworms, are undoubtedly the most important helminths as far as the welfare of the host is concerned."

The majority of the parasite studies for the bonobo involved fecal analyses or autopsies. Skinner (1995:209) does cite Bray (1963) who states that the bonobo is susceptible to malaria like the other great apes. In gorillas, malaria has been known to induce pneumonia (Skinner 1995:209), an inflammatory condition cited as a leading cause of death in chimpanzees (Schmidt 1978:12). Bonobos are also known to be susceptible to respiratory disease (de Waal and Lanting 1997:12).

A review of the parasite literature for the bonobo (see Appendix F) does reveal that bonobos have *Necator americanus*, *Oesophagostomum, Strongyloides*, and perhaps even *Plasmodium* – all parasites thought to induce repetitive and prevalent hypoplasias. The extremely high prevalence of *Troglodytella* – if severe enough – could also provide an opportunity for other, more severe infections, to take hold.

Self-medication by leaf-swallowing *Manniophyton* species was recently observed in bonobos during the October rainy season (Dupain *et al.* 2002). The onset of this rainy season is also the time in which the prevalence of *Oesophagostomum* increases in bonobos (Dupain *et al.* 2002:1053). Researchers were able to correlate the time and rate of infection and observed a significant increase in the prevalence of *Oesophagostomum* from the months of October and November but found no correlation with time and rate of infection with *Troglodytella* and *Strongyloides* – these species demonstrated an even distribution of the rainy season (Dupain *et al.* 2002:1059).

The leaf-swallowing mode of treatment for parasitic infection was initially observed in *Pan troglodytes* (Dupain *et al.* 2002:1054) and Huffman and colleagues (1997) have examined the seasonal trends of nematode infection that precipitate medicinal plant use. These researchers found that the re-infection of chimpanzees from Mahale with *Oesophagostomum* was synchronized with annual rainfalls, and these infections increased dramatically within the first two months of the onset heavy rains and the timing complements the parasites reproductive cycle.

The increased prevalence of *Oesophagostomum* observed in the bonobo from the months of October and November and the rapid re-infection of common chimpanzees with *Oesophagostomum* within two-months of the heavy rain season parallels the duration or recovery period of hypoplastic episodes found on bonobo teeth. The number of perikymata found within hypoplastic grooves on bonobos averaged 7 to 8 weeks, or approximately 2 months – a period of time in which bonobos may contract and begin to recover from the stress of a parasitic infection.

Nutritional Stress

The debate concerning whether EH is a poor or respectable indicator of dietary stress is a passionate one (see Goodman 1991, Neiberger 1990, 1991, Ogilvie and Trinkaus 1990). Regardless, in many non-human primate EH studies, availability and exploitation of environmental resources is considered a potential factor (see Appendix B Table A-2). Exploitation and availability of food resources is an important aspect of primate behaviour and nutrition, since feeding is considered one of the primates' major activities (Thorington 1970:15).

Rainfall is the greatest environmental variable in tropical rainforests and this variable, in turn, affects food supply and vectors of disease (Skinner *et al.* 1995:205). However, the constant availability of food is a key factor in the socio-ecology of bonobo behaviour (White 1996, 1998).

According to White (1996:11), "the crucial ecological basis for [the] differences from the male-bonded system of chimpanzees is that [bonobos] do not go through an extended season of low food availability during which party sizes fall to low levels." White (1998:1023) later observed that while monthly variations in fruit availability did exist, there was not a consistent seasonal variation. Thus, despite "some variation in fruit abundance, there was no period when fruit shortage prevented female bonobos from being social" (White 1998:1024). This would imply, then, that bonobos are not as affected by nutritional stress as chimpanzees and should, therefore, demonstrate a lower prevalence of EH within its permanent and deciduous dentitions. The results from this

study were contrary to this expectation, however, with the bonobo displaying the extremely high prevalence of EH when compared to the common chimpanzee.

The bonobo has been observed to eat many species of plants and "various parts of the plant as it changes form in response to the season" (Kano 1992:93). At Wamba, 147 food items have been inventoried and from these, fleshly fruits and seeds comprise the core of the bonobo's diet (Kano 1992:93). Non-plant foods listed in this inventory include: flying squirrels, insect larvae, earthworms, honey, eggs, and soil. In his preliminary field investigations, Kano (1992:96-108) made a number of important observations regarding the dietary habits of the bonobo:

- While its diet is diverse, at feeding times only a few foods are consumed at one time.
- Staple food items fluctuate according to the fruiting season and some fruits lack an obvious fruiting cycle resulting "bumper crops" in irregular cycles.
- A unique feature of the principal foods the bonobo consumes is that they are produced in large quantities in one food patch.
- Bonobos demonstrate a "food culture" – i.e., they display "regional differences in food habits." For example, bonobos at Lomako consume more animal species than those at Wamba.
- The major consumable foods are distributed over a wide range as the rain forest is relatively uniform in composition.
- Bonobos are conservative when it comes to exploiting new food resources.

Bonobos were found to exploit three types of vegetation coverage (western swamp forest, eastern dry forest, and mixed forest) on a daily, seasonal, and yearly basis (Hashimoto *et al.* 1998). Hashimoto and his colleagues (1998:1058) found that while parties of bonobos changed their home ranges over the years, the proportions of exploited vegetation zones were relatively static. The dry forest constituted the majority of the home range yet the other two zones were regularly exploited. The researchers attributed the "frequent" exploitation of the dry forest during the rainy season to the seasonal availability of their "favourite fruits" during this time.

As a result of its apparently diverse diet and exploitation of vegetation zones, the bonobo does not appear to be vulnerable to nutritional stress on a large scale. The high

prevalence of hypoplasias and periodic nature of LEH found in this primate is thus attributed to social behaviour and disease influenced by seasonal rains.

CONCLUSIONS

This study of enamel hypoplasias among the bonobo, *Pan paniscus,* has revealed that this primate expresses an extremely high prevalence of this enamel defect in both its permanent and deciduous dentitions. From caliper measurements and SEM images, the onset of LEH was found to be around 2 to 2.6 years and continuing through the tooth crown formation period. Repetitive episodes of LEH occur every 6-months on average and from perikymata counts within the hypoplastic grooves, the stress or at the very least the enamel recovery period, lasts 6 to 8 weeks. Rainfall and seasonal cycles of parasite infection are thought to be the likely factors rather than nutritional stress.

Suggestions for Further Research

(1) The Royal Museum for Central Africa possesses a number of *Pan troglodytes* specimens that were captured during the bonobos procurement expedition (see Chapter 3 Notes). An examination of these chimpanzees from adjacent areas to the north and east of the bonobos' habitat of the Democratic Republic of the Congo (formerly Zaire) would provide a unique insight into the prevalence and timing of enamel hypoplasias for apes from this region. An examination of these animals in this collection would also provide a rare opportunity to examine apes that were collected in the same small time frame.

(2) Preliminary analyses in this study revealed the bonobo manifesting a large number of hypoplastic defects (pits, LHPC, plane-type defects) in its deciduous dentitions. A re-examination of the deciduous dentition with a focus upon the timing of these defects is suggested.

(3) There is potential for a non-destructive SEM examination of imbricational enamel formation for number of tooth classes in a single individual, 84036M11, that has perikymata prominently displayed on a number of tooth crown surfaces. Because the tooth crowns were removed from the jaws and imbedded in putty, the impressive preservation of the perikymata is attributed to the crypts that covered and protected

the teeth. A microscopic examination of these teeth would also allow for the comparison of rLEH between the different tooth types within an individual.

(4) Lastly, radiographic examination of a cross-section of the bonobo collection housed at the Royal Museum is required for comparative purposes with other chimpanzee dental studies.

REFERENCES CITED

African Museum
1998 *Welcome to the Royal Museum for Central Africa*. Retrieved October 1, 1999, from the World Wide Web:www.africanmuseum.be/Uk/index.html

Aimi, Misuru and Yasuo Nogami
1991 Dental Incremental Markings in Enamel of Japanese Macaques. In *Primatology Today: Proceedings of the XIIIth Congress of the International Primatological Society, Nagoya and Kyoto, 18-24 July 1990*, edited by Akiyoshi Ehara, Tasuku Kimura, Osamu Takenaka, and Mitsuo Iwamoto, pp.455-458. Elsevier, Amsterdam.

Almquist, Alan J.
1974 Sexual Differences in the Anterior Dentition in African Primates. *American Journal of Physical Anthropology* 40:359-368.

Alt, Kurt W., Friedrich W. Rösing, and Maria Teschler-Nicola (editors)
1998 *Dental Anthropology: Fundamentals, Limits, and Prospects*. Springer-Verlag/Wein, New York.

Anemone, Robert L., Elizabeth S. Watts, and Daris R. Swindler
1991 Dental Development of Known-Age Chimpanzees, *Pan troglodytes* (Primates, Pongidae). *American Journal of Physical Anthropology* 86(2):229-241.

Benoit, P.L.G.
1961 Anaploures du Centre African. *Revue Zoologique du Botanique Africaines*. 63 (2-4):231-241.

Bermúdez de Castro, José and Pilar J. Pérez
1995 Enamel Hypoplasia in the Middle Pleistocene Hominids from Atapuerca (Spain). *American Journal of Physical Anthropology* 96(3):301-314.

Berti, P.R. and M.C. Mahaney
1992 Quantification of the Confidence Intervals for Linear Enamel Hypoplasia Chronologies. In *Recent Contributions to Study of Enamel Developmental Defects*, edited by L. Capasso and A.H. Goodman, pp.19-30. Journal of Paleopathology Monographic Publications 2, Teramo (Italy).

Berten, J.
1895 Hypolasie des Schmelzes (Congenitale Schmelzdefecte; Erosionen). *Deutsche Monatsschrift für Zahnheilkunde* 13:425-439; 483-498; 533-548; 587-606.

Beynon, A. David
1987 Replication Techniques for Studying Microstructure in Fossil Enamel. *Scanning Microscopy* 1:663-669.

Beynon, A. David and M. Christopher Dean
1989 Histological Estimates of Crown Formation Times in Great Apes [abstract]. *American Journal of Physical Anthropology* 78(2):192.
1988 Distinct Dental Development Patterns in Early Fossil Hominids. *Nature* 335(6190):509-514.

Beynon, A. David and Donald J. Reid
1987 Relationships Between Perikymata Counts and Crown Formation Times in the Human Permanent Dentition [abstract]. *Journal of Dental Research* 66:889.

Beynon, A.D. and B.A. Wood
1987 Patterns and Rates of Enamel Growth in the Molar Teeth of Early Hominids. *Nature* 326(6112):493-496.

Beynon, A. David, M. Christopher Dean, and Donald J. Reid
1991 Histological Study on the Developing Dentition in Gorilla and Orangutan. *American Journal of Physical Anthropology* 86(2):189-204.

Bolser, L. and S.E. Savage-Rumbaugh
1989 Periparturitional Behaviour of a Bonobo (*Pan paniscus*). *American Journal of Primatology* 17(1):93-103.

Boyde, Alan
1990 Developmental Interpretations of Dental Microstructure. In *Primate Life History and Evolution*, edited by C.J. DeRousseau, pp.229-267. Wiley-Liss, New York.
1971 Scanning Electron Microscopy Studies of the Completed Enamel Surface. In *Tooth Enamel II*, edited by R.W. Fearnhead and M.V. Stack, pp.39-42. John Wright, Bristol.
1970 The Surface of Enamel in Human Hypoplastic Teeth. *Archives of Oral Biology* 15:897-898.

Boyde, A. and L. Martin
1984a The Microstructure of Primate Dental Enamel. In *Food Acquisition and Processing in Primates*, edited by D.J. Chivers, B.A. Wood, and A. Bilsborough, pp.341-367. Plenum Press, New York.

Boyde, A. and L. Martin
1984b A Non-Destructive Survey of Prism Packing Patterns in Primate Enamels. In *Tooth Enamel IV*, edited by R.W. Fearnhead and S. Suga, pp.417-421. Elsevier Science Publishers, Amsterdam.
1982 Enamel Microstructure Determination in Hominoid and Cercopithecoid Primates. *Anatomical Embryology* 165:193-212.

Boyde, A., M. Fortelius, K.S. Lester, and L.B. Martin
1988 Basis of the Structure and Development of Mammalian Enamel as Seen by Scanning Electron Microscopy. *Scanning Microscopy* 2(3):1479-1490.

Bray, R.S.
1963 The Malaria Parasites of Anthropoid Apes. *The Journal of Parasitology* 49(6):888-891.

Bromage, Timothy G.
1991 Enamel Incremental Periodicity in the Pig-Tailed Macaque: A Polychrome Fluorescent Labeling Study of Dental Hard Tissues. *American Journal of Physical Anthropology* 86(2):205-214.
1989 Experimental Confirmation of Incremental Periodicity in the Pigtailed Macaque [abstract]. *American Journal of Physical Anthropology* 78(2):197.

Bromage, Timothy G. and M. Christopher Dean
1985 Re-Evaluation of the Age at Death of Immature Fossil Hominids. *Nature* 317(6037):525-527.

Buikstra, Jane E. and Douglas H. Ubelaker (editors)
1994 *Standards for Data Collection From Human Skeletal Remains*. Arkansas Archaeological Survey, Fayetteville.

Capasso, L. and A.H. Goodman
1992 Introduction. In *Recent Contributions to Study of Enamel Developmental Defects*, edited by L. Capasso and A.H. Goodman, pp.11-15. Journal of Paleopathology Monographic Publications 2, Teramo (Italy).

Clarkson, J.
1989 Symposium II: Review of Terminology, Classifications and Indices of Developmental Defects of Enamel. *Advances in Dental Research* 3(2):95-96.

Cohen, B. and W. H. Bowen
1999 Diseases of the Oral Cavity. In *Pathology of Simian Primates, Part I*, edited by R.N.T-W-Fiennes, pp.639-670. Karger, Basel.

Colyer, James Frank
1936 *Variations and Diseases of the Teeth of Animals*. J. Bale, Sons and Danielsson, London.

Coolige, H.
1984 Historical Remarks Bearing on the Discovery of *Pan paniscus*. In *The Pygmy Chimpanzee: Evolutionary Biology and Behavior*, edited by Randall L. Susman, pp.ix-xiii. Plenum Press, New York.
1933 *Pan paniscus*: Pygmy Chimpanzee from South of the Congo River. *American Journal of Physical Anthropology* 18(1):1-57.

Cramer, Douglas, Lloyd
1977 *Craniofacial morphology of Pan paniscus: A Morphometric and Evolutionary Appraisal*. Contributions to Primatology, Volume 10. S. Karger, New York.

Dean, M.C.
1998 A Comparative Study of Cross Striation Spacings in Cuspal Enamel and of Four Methods of Estimating the Time Taken to Grow Molar Cuspal Enamel in *Pan, Pongo,* and *Homo. Journal of Human Evolution* 35(4-5):449-462.
1989 The Developing Dentition and Tooth Structure in Hominoids. *Folia Primatologica* 53(1-4):160-176.
1987 Growth Layers and Incremental Markings in Hard Tissues: A Review of the Literature and Some Preliminary Observations About Enamel Structure in *Paranthropus boisei. Journal of Human Evolution* 16(2):157-172.

Dean, M.C. and A.D. Beynon
1989 Estimates of Age at Death Using Histological Techniques in a Modern Population of Known Age and Death and Some Comparisons with Great Ape and Fossil Hominid Developing Dentitions [abstract]. *American Journal of Physical Anthropology* 78(2):211.

Dean, M.C. and D.J. Reid
2001 Perikymata Spacing and Distribution on Hominid Anterior Teeth. *American Journal of Physical Anthropology* 116(3):209-215.

Dean, M.C. and B.A. Wood
1981 Developing Pongid Dentition and Its Use for Ageing Individual Crania in Comparative Cross-Sectional Growth Studies. *Folia Primatologica* 36:111-127.

Dean, M.C., C.B. Stringer, and T.G. Bromage
1986 A New Age at Death for the Neanderthal Child from Devil's Tower, Gibraltar and the Implications for Studies of General Growth and Development in Neanderthals. *American Journal of Physical Anthropology* 70(3):301-309.

Dettwyler, K.A. and C. Fishman
1992 Infant Feeding Practices and Growth. *Annual Review of Anthropology* 21:171-204.

de Waal, Frans and Frans Lanting
1997 *Bonobo: The Forgotten Ape*. University of California Press, Berkeley.

Dirks, Wendy
1998 Histological Reconstruction of Dental Development and Age at Death in a Juvenile Gibbon (*Hylobates lar*). *Journal of Human Evolution* 35(4-5):411-425.

Dobney, Keith and Anton Ervynck
1998 A Protocol for Recording Linear Enamel Hypoplasia on Archaeological Pig Teeth. *International Journal of Osteoarchaeology* 8(4):263-273.

Donat, L.D. and J.C. Rose
1991 Enamel Hypoplasia: A Morphological Classification [abstract]. *American Journal of Physical Anthropology* 84(S12):69.

Dupain, J., P. Garcia, C. Nell, L van Elsacker, and F. Ponce
2000 A Survey of Intestinal Parasites of a Sympatric Population of Bonobos (*Pan paniscus*) and Humans (*Homo sapiens*) in the Democratic Republic of Congo [abstract]. *Folia Primatologica* 71:222.

Dupain, Jef, Linda van Elsacker, Carlos Nell, Poala Garcia, Francisco Ponce, and Michael A. Huffman
2002 New Evidence for Leaf Swallowing and *Oesohagostomum* Infection in Bonobos (*Pan paniscus*). *International Journal of Primatology* 23(5):1053-1062.

Eckhardt, R.B.
2000 Primate Enamel Hypoplasias as Indicators of Metabolic Stress, Adaptation, and Evolution [abstract]. *American Journal of Physical Anthropology* 111 (S30):144.
1992 Tooth Crown Development: Nonhuman Primate Perspectives on the Interpretation of Linear Enamel Hypoplasia Frequencies in Present and Past Hominid Populations. In *Recent Contributions to Study of Enamel Developmental Defects*, edited by L. Capasso and A.H. Goodman, pp.293-306. Journal of Paleopathology Monographic Publications 2, Teramo (Italy).

Eckhardt, R.B. and R. Protsch von Zieten
1993 Enamel Hypoplasias as Indicators of Developmental Stress in Pongids and Hominids. *Human Evolution* 8(2):93-99.

Eckhardt, R.B., A. Protsch and R. Protsch von Zieten
1992 Vertical Enamel Hypoplasias in a Free-Living Population of Liberian Chimpanzees: Variations in Frequency and Incidence. In *Recent Contributions to Study of Enamel Developmental Defects*, edited by L. Capasso and A.H. Goodman, pp.107-114. Journal of Paleopathology Monographic Publications 2, Teramo (Italy).

El-Najjar, M., M.DeSanti, and L. Ozebek
1978 Prevalence and Possible Etiology of Dental Enamel Hypoplasia. *American Journal of Physical Anthropology* 48(2):185-19.

Fain, A.
1957 L'Acariase Pulmonaire Chez le Chimpanzé et le Gorille par des Acariens du Genre *Pneumonyssus* Banks. *Revue Zoologique et Botanique du Africaines* 56(3-4):234-242.
1959 Les Acariens du Genre *Pneumonyssus* Banks Parasites Endopulmonaires des Singes au Congo Belge (Halarachnidae: Mesostigmata). *Annals of Parasitology* 34(1-2):126-148.
1962 *Pangorillalges pani* g.n., sp.n., Acarien Psorique du Chimpanzé (Psoralgidae-Sarcoptiformes). *Revue Zoologique et Botanique du Africaines* 66(3-4):283-290.
1963 Les Acariens Porducteurs de Gale Chez les Lemuriens et les Singes avec une Etude des Psoroptidae (Sarcoptiformes). *Bull. Inst. Roy. Sc. Nat. Belgique* 39(32):125.

FitzGerald, Charles M. and Jerome C. Rose
2000 Reading Between the Lines: Dental Development and Subadult Age Assessment Using the Microstructural Growth Markers of Teeth. *In Biological Anthropology of the Human Skeleton*, edited by M. Anne Katzenberg and Shelley R. Saunders, pp.163-186. Wiley-Liss, New York.

Forbath, Peter
1977 The River Congo: The Discovery, Exploration, and Exploitation of the World's Most *Dramatic River*. Harper ad Row Publishers, New York.

Fukuhara, T.
1959 Comparative Anatomical Studies of the Growth Lines in the Enamel of Mammalian Teeth. *Acta Anat. Nipp.* 34:322-332.

Furuichi, Takeshi
1992 The Prolonged Estrus of Females and Factors Influencing Mating in Wild Group of Bonobos (*Pan paniscus*) in Wamba, Zaire. In *Topics in Primatology: Behavior, Ecology, and Conservation*, Volume 2, edited by N. Itoigawa, Y. Sugiyama, G.P. Sakett, and R.K.R. Thompson, pp.179-190. University of Tokyo Press, Tokyo.

Garn, Stanley M.
1976 The Insides of the Bones and Mass of Humanity. *Annals of the XLI International Congress of Americanists* 3:453-457.

Gerloff, U., B. Hartung, B. Fruth, G. Hohmann, and D. Tautz
1999 Intracommunity Relationships, Dispersal Pattern and Paternity Success in a Wild Living Community of Bonobos (*Pan paniscus*) Determined from DNA Analysis of Faecal Samples. *Proceedings of the Royal Society of London Series B – Biological Sciences* 266(1424):1189-1195.

Goldsmith, E.I. and J. Moor-Jankowski
1978 Some Aspects of the Proposed Captive Breeding of Dwarf Chimpanzees *(Pan paniscus)*. *Journal of Medical Primatology* 7:65-69.

Goodman, A.H.
1991 Paleoepidemiological Inference and Neanderthal Dental Enamel Hypoplasia: A Reply to Neiburger. *American Journal of Physical Anthropology* 85(4):461-462.

Goodman, Alan H. and Rhan-Ju Song.
1999 Sources of Variation in Estimated Ages at Formation of Linear Enamel Hypoplasias. In *Human Growth in the Past: Studies from Bones and Teeth*, edited by Robert D. Hoppa and Charles M. FitzGerald, pp.210-240. Cambridge University Press, Cambridge.

Goodman, A.H. and J.C. Rose
1991 Dental Enamel Hypoplasias as Indicators of Nutritional Status. In *Advances in Dental Anthropology*, edited by M. Kelley and C. Larsen, pp.279-293. Wiley-Liss, New York.
1990 Assessment of Systematic Physiological Perturbations from Dental Enamel Hypoplasias and Associated Histological Structures. *Yearbook of Physical Anthropology* 33:59-110.

Guatelli-Steinberg, Debbie
2000 Linear Enamel Hypoplasia in Gibbons (*Hylobates lar carpenteri*). *American Journal of Physical Anthropology* 112(3):395-410.
1998a Linear Enamel Hypoplasia and Life History in Cayo Santiago Rhesus Monkeys [abstract]. *American Journal of Physical Anthropology* 115(S26):89.
1998b *Prevalence and Etiology of Linear Enamel Hypoplasia in Non-Human Primates.* Unpublished Ph.D. dissertation, Department of Anthropology, University of Oregon.

Guatelli-Steinberg, D. and W. Dirks
2001 The Timing of Hypoplastic Events in Gibbons [abstract]. *American Journal of Physical Anthropology* 113(S32):73-74.

Guatelli-Steinberg, Debbie and John R. Lukacs
1999 Interpreting Sex Differences in Enamel Hypoplasia in Human and Non-Human Primates: Developmental, Environmental Considerations. *Yearbook of Physical Anthropology* 42:73-126.
1998 Preferential Expression of Linear Enamel Hypoplasia on the Sectorial Premolars of Rhesus Monkeys (*Macaca mulatta*). *American Journal of Physical Anthropology* 107(2):179-186.

Guatelli-Steinberg, D. and E.A. Newell
2000 Interpreting Sex Differences in Linear Enamel Hypoplasia in Non-Human Primates [abstract]. *American Journal of Physical Anthropology* 111(S30):168.

Guatelli-Steinberg, Debbie and Mark Skinner
2000 Prevalence and Etiology of Linear Enamel Hypoplasia in Monkeys and Apes from Asia and Africa. *Folia Primatologica* 71(3):115-132.

Hannibal, D.L.
2003 An Unusual Hypoplastic Defect of the Maxillary Lateral Incisors in Great Apes [abstract]. *American Journal of Physical Anthropology* 120(S36):108.
2000a Linear Enamel Hypoplasia in the Great Apes: Analysis by Genus and Locality [abstract]. *American Journal of Physical Anthropology* 111(S30):172.
2000b *Enamel Hypoplasia in the Great Apes*. Unpublished Masters Thesis, Department of Anthropology, University of Oregon.

Hasegawa, Hideo and Takayoshi Kano
1983 A Parasitological Survey on the Feces of the Pygmy Chimpanzees, *Pan paniscus*, at Wamba, Zaire. *Primates* 24(3):419-423.

Hashimoto, C. Y. Tashiro, D. Kimura, T. Enomoto, E.J. Ingmanson, G. Idani, and T. Furuichi
1998 Habitat use and Ranging of Wild Bonobos (*Pan paniscus*) at Wamba. *International Journal of Primatology* 19(6):1045-1060.

Hillson, S.W.
1996 *Dental Anthropology*. Cambridge University Press, Cambridge.
1994 The Relationship of Enamel Hypoplasias to the Pattern of Tooth Crown Growth [abstract]. *American Journal of Physical Anthropology* 94(S18):107-108.
1992a Dental Enamel Growth, Perikymata, and Hypoplasia in Ancient Tooth Crowns. *Journal of Royal Society of Medicine* 85(8):460-466.

Hillson, S.W.
1992b Impression and Replica Methods for Studying Hypoplasia and Perikymata on Human Tooth Crown Surfaces from Archaeological Sites. *International Journal of Osteoarchaeology* 2(1):65-78.
1986 *Teeth*. Cambridge University Press, Cambridge.

Hillson, S.W. and S. Bond
1997 Relationship of Enamel Hypoplasia to the Pattern of Tooth Growth: A Discussion. *American Journal of Physical Anthropology* 104(1): 89-104.
Hillson, S.W. and B.K. Jones
1989 Instruments for Measuring Surface Profiles: An Application in the Study of Ancient Tooth Crown Surfaces. *Journal of Archaeological Science* 16(1):95-105.

Hindle, Aimee
1998 Problems in the Methodology of Dental Enamel Hypoplasia Analyses. *Nexus* 13(1):1-8. Retrieved September 28, 2000, from the Word Wide Web: http://socserv.socsci.mcmaster.ca/~nexus/hindle.htm

Hochschild, Adam
1998 *King Leopold's Ghost: A Story of Greed, Terror, and Heroism in Colonial Africa.* Houghton Mifflin, Boston.

Hodges, D.C. and R.G. Wilkinson
1990 Effect of Tooth Size on the Ageing and Chronological Distribution of Enamel Hypoplastic Defects. *American Journal of Human Biology* 2:553-560.
1988 Determination of Chronological Distributions from Enamel Hypoplasias [abstract]. *American Journal of Physical Anthropology* 75(2):223.

Hooper, Edward
1999 *The River: A Journey Back to the Source of HIV and AIDS*. Allen Lane, London.

Horn, A.
1979 The Taxonomic Status of the Bonobo Chimpanzee. *American Journal of Physical Anthropology* 51(2):273-282.

Hublin, J.J., J. Braga, and J. Treil
2001 Dental Maturation Assessment Using Computed Tomography in *Pan paniscus*. Implications for Early Hominid Studies [Abstract]. *American Journal of Physical Anthropology* S32:82.

Huda, Tushar F.J. and J.E. Bowman
1994 Variation in Cross-Striation Number between Striae in an Archaeological Population. *International Journal of Osteoarchaeology* 4:49-52.

Huffman, M.A., S. Gotoh, D. Izutsu, K. Koshimizu, and M.S. Kalunde
1997 Seasonal Trends in Intestinal Nematode Infection and Medicinal Plant Use Among Chimpanzees in the Mahale Mountains, Tanzania. *Primates* 38(2):111-125.

Idani, G., S. Kuroda, T. Kano, and R. Asato
1994 Flora and Vegetation of Wamba Forest, Central Zaire with Reference to Bonobo (*Pan paniscus*) foods. *Tropics* 3(3/4):309-332. Retrieved September 27, 1999, from the World Wide Web: www.primate.wisc.edu/pin/tro6

Johanson, D.C.
1974a Some Metric Aspects of the Permanent and Deciduous Dentition of the Pygmy Chimpanzee (*Pan paniscus*). *American Journal of Physical Anthropology* 41(1):39-48.
1974b *An Odontological Study of the Chimpanzee with Some Implications for Hominoid Evolution.* Unpublished Ph.D. dissertation, Department of Anthropology, University of Chicago, Chicago.

Jurmain, Robert
1997 Skeletal Evidence of Trauma in African Apes, with Special Reference to the Gombe Chimpanzees. *Primates* 38(1):1-14.

Kano, Takayoshi
1992 *The Last Ape: Pygmy Chimpanzee Behaviour and Ecology*. Translated by Evelyn Ono Vineberg. Stanford University Press, Stanford, California.
1984 Observations of Physical Abnormalities Among the Wild Bonobos (*Pan paniscus*) of Wamba, Zaire. *American Journal of Physical Anthropology* 63(1):1-11.

Katzenberg, M.A., D.A. Herring, and S.R. Saunders
1996 Weaning and Infant Mortality: Evaluating the Skeletal Evidence. *Yearbook of Physical Anthropology* 39: 177-199.

Kinzey, W.G.
1984 The Dentition of the Pygmy Chimpanzee, *Pan paniscus*. In *The Pygmy Chimpanzee: Evolutionary Biology and Behavior*, edited by Randall L. Susman, pp.65-88. Plenum Press, New York.

Kreshover, S.J.
1992 The Histopathology of the Incisor Teeth of Mice with Experimentally Produced Tuberculosis. *Journal of Dental Research* 21:27-34.

Kuntz, R.E.
1982 Significant Infections in Primate Parasitology. *Journal of Human Evolution* 11(3): 185-194.

Kuntz, R.E. and Betty June Myers
1969 Parasitic Protozoa, Commensals and Helminths of Chimpanzees Imported from the Republic of the Congo. In *Proceedings of the Second International Congress of Primatology*, Atlanta, Georgia 1968, volume 3: Neurology, Physiology, and Infectious Diseases, edited by C.R. Carpenter, pp.184-190. S. Karger, Basel and New York.

Kuykendall, Kevin L.
1996 Dental Development in Chimpanzees (*Pan troglodytes*): The Timing of Tooth Calcification Stages. *American Journal of Physical Anthropology* 99(1):135-157.

Kuykendall, K.L. and J. Bozic
2001 An Assessment of Deciduous Tooth Mineralization in Chimpanzees (*Pan troglodytes*) [abstract]. *American Journal of Physical Anthropology* 114(S32):94.

Larsen, Clark Spencer
1964 *Bioarchaeology: Interpreting Behaviour from the Skeleton*. Cambridge University Press, Cambridge.

Lavelle, C.L.B., R.P. Shellis, and D.F.G. Poole (editors)
1977 The Calcified Dental Tissues of Primates. In *Evolutionary Changes to the Primate Skull and Dentition*, pp.197-279. Charles C. Thomas, Springfield.

Lovell, Nancy C.
1999 Patterns of Dental Enamel Defects at Ancient Mendes, Egypt. *American Journal of Physical Anthropology* 110(1):69-80.
1991 An Evolutionary Framework for Assessing Illness and Injury in Non-Human Primates. *Yearbook of Physical Anthropology* 34:117-155.
1990 *Patterns of Injury and Illness in Great Apes: A Skeletal Analysis*. Smithsonian Institution Press, Washington, D.C..

Lovell, Nancy, Robert Jurmain, and Lynn Kilgore
2000 Skeletal Evidence of Probable Treponemal Infection in Free Ranging African Apes. *Primates* 41(3):275-290.

Lukacs, J.R.
2001a Enamel Hypoplasia in the Deciduous Teeth of Great Apes: Variation in Prevalence and Timing of Defects. *American Journal of Physical Anthropology* 116(3):199-208.
2001b Enamel Hypoplasia in the Deciduous Teeth of Early Miocene Catarrhines: Evidence of Perinatal Physiological Stress. *Journal of Human Evolution* 40(4):319-329.

Lukacs, J.R.
2000 Enamel Hypoplasia in Deciduous Teeth of Great Apes: Variation in Prevalence and Timing of Defects [abstract]. *American Journal of Physical Anthropology* 111(S30):215-216.
1999a Enamel Hypoplasia in Great Ape Deciduous Canines: Do Differences in Defect Prevalence Imply Differential Levels of Physiological Stress? *American Journal of Physical Anthropology* 110(3):351-363.
1999b Interproximal Contact Hypoplasia (IPCH) in Primary Teeth: A New Enamel Defect with Anthropological and Clinical Relevance. *American Journal of Human Biology* 11(6):718-734.

Macho, G.A., D.J. Reid, M.G. Leakey, N. Jablonski, and D. Beynon
1996 Climatic Effects on Dental Development of *Theropithecus oswaldi* from Koobi Fora and Olorgesailie. *Journal of Human Evolution* 30(1):57-70.

Malenky, R.K. and E.W. Stiles
1991 Distribution of Terrestrial Herbaceous Vegetation and Its Consumption by *Pan paniscus* in the Lomako Forest, Zaire. *American Journal of Primatology* 23(3):153-169.

Mann, A.E., J.M. Monge, and M. Lampl
1991 Investigation Into the Relationship Between Perikymata Counts and Crown Formation Times. *American Journal of Physical Anthropology* 86(2): 175-188.

Mann, A., M. Lampl, and J. Monge
1990 Patterns of Ontogeny in Human Evolution: Evidence from Dental Development. *Yearbook of Physical Anthropology* 33:111-150.

Marks, M.K.
1992 Developmental Dental Enamel Defects: A SEM Analysis. In *Recent Contributions to Study of Enamel Developmental Defects*, edited by L. Capasso and A.H. Goodman, pp.79-90. Journal of Paleopathology Monographic Publications 2, Teramo (Italy).
1988 Enamel Hypoplasia and Hypocalcification: Correlation of Defects Identified by Light or Scanning Electron Microscopy [abstract]. *American Journal of Physical Anthropology* 75(2):246.

Martin, L.B., A. Boyde, and F.E. Grine.
1988 Enamel Structure in Primates: A Review of Scanning Electron Microscope Studies. *Scanning Microscopy* 2(3):1503-1526.

Massler, M, I. Schour, and H.G. Poncher
1941 Developmental Pattern of the Child as Reflected in the Calcification Pattern of the Teeth. *American Journal of Diseases of Children* 62:33-67.

Mellanby, M.
1929 *Diet and Teeth: An Experimental Study. Part I: Dental Structure in Dogs*. Medical Research Council, Special Report Series, No.140. His Majesty's Stationery Office, London.

Meditz, Sandra and Tim Merrill
1994 *Zaire: A Country Study*, 4th edition. Federal Research Division, Library of Congress (Country Studies/Area Handbook Program), Department of the Army, Washington, DC.

Miles, A.E.W. and Caroline Grigson
1990 *Colyer's Variations and Diseases of the Teeth of Animals*. Cambridge University Press, Cambridge.

Mimura, Tasuka
1939 The Circulation of Growth Lines in Enamel. *Kobyo-Shi* 13:454-455.

Moggi-Cecchi, J.
2000 Enamel Hypoplasia in South African Early Hominids: A Reappraisal [abstract]. *American Journal of Physical Anthropology* 111(S30):230-231.
1989 *Analysis of the Enamel Macrodefects in the Dentition of Man and Non-Human Primates, and its Relevance for Paleoanthropological Studies* (in Italian). Testi di dottorato di ricerca, University of Florence.

Moggi-Cecchi, Jacopo and Sergio Crovella
1992 Dental Developmental Defects in the Dentitions of *Dryopithecus* from Spain. *Anthropologica Contemporanea* 15:47-50.
1991 Occurrence of Enamel Hypoplasia in the Dentitions of Simian Primates. *Folia Primatologica* 57(2):106-110.

Moggi-Cecchi, J., P.V. Tobias, and A.D. Beynon
1998 The Mixed Dentition and Associated Skull Fragments of a Juvenile Fossil Hominid From Sterkfontein, South Africa. *American Journal of Physical Anthropology* 106(4):425-465.

Molnar, S. and S.C. Ward
1975 Mineral Metabolism and Microstructural Defects in Primate Teeth. *American Journal of Physical Anthropology* 43(1):3-18.

Moreland, A.F.
1970 Nutritional Status and Problems of Feeding Newly Imported Primates. In *Feeding and Nutrition of Nonhuman Primates*, edited by R.S. Harris, pp.29-43. Academic Press, New York.

Mortelmans, J., J. Vercruysse and P. Kageruka
1971 Three Pathologic Intestinal Protozoa of Anthropoid Apes: *Entamoeba histolytica, Balantidium coli* and *Troglodytella abassarti*. In *Proceedings of the 3rd International Congress of Primatology (1970 Zurich)*, Volume 2: Neurobiology, Immunology, Cytology, edited by J. Biegert and W. Leutenegger, pp.187-191. S. Karger, Basel.

Myers, Betty June and Robert E. Kuntz
1972 A Checklist of Parasites and Commensals Reported for the Chimpanzee (Pan). *Primates* 13(4):433-471.

Neiburger, E.J.
1991 Reply to Dr. Goodman. *American Journal of Physical Anthropology* 85(4):462-464.
1990 Enamel Hypoplasias: Poor Indicators of Dietary Stress. *American Journal of Physical Anthropology* 82(2):231-232.
1988 Enamel Hypoplasia – A Poor Indicator of Nutritional Stress [abstract]. *American Journal of Physical Anthropology* 75(2):253.

Newberne, P.M.
1970 Syndromes of Nutritional Deficiency Disease in Non-Human Primates. In *Feeding and Nutrition of Nonhuman Primates*, edited by R.S. Harris, pp. 205-232. Academic Press, New York.

Newell, Elizabeth Anne
2003 Intertooth Distribution of Liner Enamel Hypoplasia in Non-Human Primates [abstract]. *American Journal of Physical Anthropology* 120(S36):157.
2000 The Relationship of Maturation Length to Interspecific Variation in Enamel Hypoplasia [abstract]. *American Journal of Physical Anthropology* 111(S30):236-237.
1998 *Dental Enamel Hypoplasia in Non-Human Primates: A Systematic Assessment of Its Occurrence and Distribution.* Unpublished Ph.D. dissertation, Department of Anthropology, Temple University, Philadelphia.

Nikiforuk, G. and D. Fraser
1981 The Etiology of Enamel Hypoplasia: A Unifying Concept. *Journal of Pediatrics* 98:888-893.

Nissen, H.W. and A.H. Riesen
1964 The Eruption of the Permanent Dentition in the Chimpanzee. *American Journal of Physical Anthropology* 22:285-294.

Ogilvie, M. and E. Trinkaus
1990 Reply to Neiburger. *American Journal of Physical Anthropology* 82(2):232-233.

Okada, M.
1943 Hard Tissues of Animal Body – Highly Interesting Details of Nippon Studies in Periodic Patterns of Hard Tissue are Described. *Shanghai Evening Post Medical Edition* September:15-31.

Pedersen, P.O. and D.B. Scott
1951 Replica Studies of the Surface of Teeth from Alaskan Eskimos, West Greenland Natives, and American Whites. *Acta odontologica Scandinavica* 9:262-292.

Peel, E. and M. Chardome
1947 Note Complémentaire sur des Filaridés de Chimpanés *Pan paniscus* et *Pan satyrus* au Congo Belge. *Ann. Soc. Belge Méd. Trop.* 27(2):241-250.
1946 Sur des Filaridés de Chimpanzés (*Pan paniscus* et *Pan satyrus*) au Congo Belge. *Ann. Soc. Belge Méd. Trop.* 26(3):117-156.

Puech, Pierre-Francois and Henri Albertini
1981 Enamel Pits of the Lazaret Man. *Journal of Human Evolution* 10(6):449-452.

Protsch von Zieten, R.R. and R.B. Eckhardt
1991 Transverse Enamel Hypoplasia as an Indicator of Developmental Stress in Pongids and Hominids [abstract]. *American Journal of Physical Anthropology* 84(S12):146.

Purdell,-Lewis, D.J., G.W. Suckling, M. Triller, and W.L. Jongebloed
1987 Artificially Induced Developmental Defects in Sheep Enamel Examined by Scanning Electron Microscopy. *Journal de Biologie Buccal* 15(2):119-124.

Reid, D.J. and M.C. Dean
2000 Brief Communication: The Timing of Linear Hypoplasias on Human Anterior Teeth. *American Journal of Physical Anthropology* 113(1):135-139.

Reid, D.J., S.W. Hillson, M.C. Dean
2000 Defining Chronological Growth Standards for Known Fractions of Tooth Crown Height in Primate Anterior Teeth [Abstract]. *American Journal of Physical Anthropology* 111(S30):260.

Reid, D.J., S.W. Hillson, M.C. Dean
n.d. Defining Chronological Growth Standards for Known Fractions of Tooth Crown Height. Unpublished paper.

Reid, Donald J., Gary T. Schwartz, Christopher Dean and Malkanthi S.S. Chandrasekera
1998 A Histological Reconstruction of Dental Development in the Common Chimpanzee, *Pan troglodytes*. *Journal of Human Evolution* 35(4-5):427-448.

Risnes, Steinar
1985 Circumferential Continuity of Perikymata in Human Dental Enamel Investigated by Scanning Electron Microscopy. *Scandinavian Journal of Dental Research* 93: 185-191.

Rose, Jerome C.
1979 Morphological Variations of Enamel Prisms within Abnormal Striae of Retzius. *Journal of Human Biology* 51:139-151.

Rose, J.C., K. Condon, A.H. Goodman
1985 Diet and Dentition: Developmental Disturbances. In *The Analysis of Prehistoric Diets*, edited by R.I. Gilbert and J.H. Mielke, pp.281-306. Academic Press, New York.

Sabater Pi, Jordi and Joaquim J. Veà
1994 Comparative Inventory of Foods Consumed by the Wild Pygmy Chimpanzee (*Pan paniscus,* Mammalia) in the Lilungu-Lokofe Region of the Republic of Zaire. *Journal of African Zoology* 108(4):381-396.

Sarnat, B.G. and I. Schour
1941 Enamel Hypoplasia (Chronologic Enamel Aplasia) in Relation to Systemic Disease: A Chronologic, Morphologic, and Etiologic Classification. *Journal of the American Dental Association* 28:1989-2000.

Schmidt, R.E.
1978 Systemic Pathology of Chimpanzees. *Journal of Medical Primatology* 7:274-318.

Schouteden, H.
1931 Quelques Notes sur le Chimpanze de la Rive Gauche du Congo, *Pan satyrus paniscus*. *Revue Zoologique du Botanique Africannes* XX(3):310-314.
1929 A Propos du Chimpanze de la rive gauche du Congo. *Cercle Zoologique Conglais* 6(2):29.
1928 Le Chimpanze du Congo Central. *Cercle Zoologique Conglais* 6:108.

Schultz, Michael, Petra Carli-Thiele, Tyede H. Schmidt-Schultz, Uwe Kierdorf, Horst Kierdorf, Wolf-Rüdiger Teegen and Kerstin Kreutz
1998 Enamel Hypoplasias in Archaeological Skeletal Remains. In *Dental Anthropology: Fundamentals, Limits, and Prospects*, edited by Kurt W. Alt, Friedrich W. Rösing, and Maria Teschler-Nicola, pp.293-311. Springer-Verlag/Wien, New York.

Schuman, E.L. and R.F. Sognnaes
1956 Developmental Microscopic Defects in the Teeth of Subhuman Primates. *American Journal of Physical Anthropology* 14(2):193-209.

Schwartz, Gary T. and M.C. Dean
2000 The Ontogeny of Canine Dimorphism in Extant Hominoids [abstract]. American *Journal of Physical Anthropology* 111(S30):275.

Schwartz, G.T., M.C. Dean, D.J. Reid, and A.L. Zihlman
2001a Histological Analysis of Dental Development in *Gorilla* [Abstract]. *American Journal of Physical Anthropology* 113(S32):134.

Schwartz, G.T., M.C. Dean, D.J. Reid, and A.L. Zihlman
2001b Histological Analysis of Dental Development in *Gorilla*. Paper presented at the 70th Annual Meeting of the American Association of Physical Anthropologists, Kansas City Missouri, March 28-31, 2001.

Schwarz, E
1929 Das Vorkommen des Schimpansen auf den linken Kongo-Ufer. *Revue de Zoologique et de Botanique Africaines* 16:425-426.
1928 Le Chimpanze de la Rive Gauche du Congo. *Cercle Zoologique Conglais* 5:70-71.

Skinner, Mark F.
2000 Periodicity of Repetitive Linear Enamel Hypoplasia in Asian and African Apes [abstract]. *American Journal of Physical Anthropology* 111(S30):283.
1996 Developmental Stress in Immature Hominines from Late Pleistocene Eurasia: Evidence from Enamel Hypoplasia. *Journal of Archaeological Science* 23(6):833-852.
1986a An Enigmatic Hypoplastic Defect of the Deciduous Canine. *American Journal of Physical Anthropology* 69(1):59-69.
1986b Enamel Hypoplasia in Sympatric Chimpanzee and Gorilla. *Human Evolution* 1(4):289-312.

Skinner, Mark F. and Gail Anderson
1991 Individualization and Enamel Histology: Case Report in Forensic Anthropology. *Journal of Forensic Science* 36(3):939-948.

Skinner, Mark F. and Alan H. Goodman
1992 Anthropological Uses of Developmental Defects of Enamel. In *Skeletal Biology of Past Peoples: Research Methods*, edited by S.R. Saunders and M.A. Katzenberg, pp.153-174. Wiley-Liss, New York.

Skinner, Mark F. and Dave Hopwood
2003 An Hypothesis for the Causes and Periodicity of Repetitive Transverse Enamel Hypoplasia (rTEH) in Large, Wild African (*Pan troglodytes* and *Gorilla gorilla*) and Asian (*Pongo pygmaeus*) Apes. *American Journal of Physical Anthropology*. (Early View – Article Online in Advance of Print). Retrieved December 9, 2003, from the World Wide Web: http://www.3.interscience.wiley.com/cgi-bin/fulltext/104539368/HTMLSTART

Skinner, Mark F. and Elizabeth A. Newell
2003 Localised Hypoplasia of the Primary Canines in Bonobos, Orangutans, and Gibbons. *American Journal of Physical Anthropology* 120(1):61-72.

Skinner, M.F., T.L. Durpas, and S. Moya-Sola
1995 Periodicity of Linear Enamel Hypoplasia Among Miocene *Dryopithecus* from Spain. *Journal of Paleopathology* 7(3):195-222.

Skinner, M.F., W. Hadaway, and J. Dickie
1994 Effects of Ethnicity and Birth Month on Localized Enamel Hypoplasia of the Primary Canine. *Journal of Dentistry for Children* (Mar/Apr) 61:109-113.

Stam, A.B.
1960 Un Cas Mortel d'Ascaridiose (*Ascaris lumbricoides,* L.) Chez le Chimpanzé Nain (*Pan paniscus* Schwarz). *Annals of Parasitology* 35:675.

Stottlemire, M.M.
1998 Presence of Dental Enamel Hypoplasia in Wild-Shot Chimpanzees (genus *Pan*) and Gorillas (genus *Gorilla*) [abstract]. *American Journal of Physical Anthropology* 105(S26):212.

Suckling, Grace
1989 Developmental Defects of Enamel: Historical and Present-Day Perspectives of Their Pathogenesis. *Advances in Dental Research* 3(2):87-94.

Suckling, Grace and D.C. Thurley
1984 Developmental Defects of Enamel: Factors Influencing Their Macroscopic Appearance. In *Tooth Enamel IV*, edited by R.W.Fearnhead and S.Suga, pp.357-362. Elsevier Science Publishers, Amsterdam.

Suckling, G., D.C. Elliott, and D.C. Thurley
1986 The Macroscopic Appearance and Associated Histological Changes in the Enamel Organ of Hypoplastic Lesions of Sheep Incisor Teeth Resulting from Induced Parasitism. *Archives of Oral Biology* 31(7):427-439.
1983 The Production of Developmental Defects of Enamel in the Incisor Teeth of Penned Sheep Resulting from Induced Parasitism. *Archives of Oral Biology* 28(5):393-399.

Swärdstedt, T.
1966 *Odontological Aspects of a Medieval Population in the Province of Jämtland/Mid Sweden*, University of Lund, Sweden. Akademisk Avhandling som med vederbörligt tillstand av Odontologiska Fakuleten vid Lunds Universitet försvaras I Tandläkarhöskolans Aula, Malmö, Fredagen den 9 December 1966 K19 CT.

Susman, R.L.
1984 Preface. In *The Pygmy Chimpanzee: Evolutionary Biology and Behavior*, edited by Randall L. Susman, pp.xv-xx. Plenum Press, New York.

Takahata, Y., H. Ihobe, and G. Idani
1996 Comparing Copulations of Chimpanzees and Bonobos: Do Females Exhibit Proceptivity or Receptivity. In *Great Ape Societies*, edited by W.C. McGrew, L.F. Marchant, and T. Nishida, pp.146-155. Cambridge University Press, Cambridge.

Teaford, M.F. and O.J. Oyen
1989 In Vivo and In Vitro Turnover in Dental Microwear. *American Journal of Physical Anthropology* 80(4):447-460.

Teare, J.A. , B. Bell, R. Kuhlmann, and G.Geanon
1996 Ultrasonographic Measurement of Fetal Growth in a Bonobo (*Pan paniscus*). *Journal of Zoo and Wildlife Medicine* 27(4):477-481.

Thorington Jr., R.W.
1970 Feeding Behaviour of Nonhuman Primates in the Wild. In *Feeding and Nutrition of Nonhuman Primates*, edited by R.S. Harris, pp. 15-27. Academic Press, New York.

Uchida, Akiko
1996 *Craniodental Variation Among the Great Apes*. Peabody Museum Bulletin No.4, Harvard University, Cambridge.

van den Audenaerde, D.F.E.T.
1984 The Tervuren Museum and the Pygmy Chimpanzee. In *The Pygmy Chimpanzee: Evolutionary Biology and Behavior*, edited by Randall L. Susman, pp.3-12. Plenum Press, New York.

Vervaecke, H., L. van Elsacker, U. Mohle, M. Heistermann, and R.F. Verheyen
1999 Inter-menstrual Intervals in Captive Bonobos (*Pan paniscus*). *Primates* 40(2)283-289.

Vitzthum, V.J. and R. Wikander
1988 Incidence and Correlates of Enamel Hypoplasia in Nonhuman Primates [abstract]. *American Journal of Physical Anthropology* 75(2):284.

Vuanza, P.N. and M.Grabbe
1975 *Les Régimes Moyens et Extrêmes des Climats Principaux du Zaire*. Centre Meteorologique de Kinshasa.

Vuylsteke, C.
1964 Mission de Zoologie Medicale au Maniema (Congo, Leopoldville). 3. Vermes. Nematoda. *Ann. K. Mus. Midden-Africa, Tervuren, s.8, Zool. Wetensch.* 132:41-66.

Waldron, Tony
1994 *Counting the Dead: The Epidemiology of Skeletal Populations*. John Wiley, New York.

Walker, A. and M. Teaford
1989 Inferences from Quantitative Analysis of Dental Microwear. *Folia Primatologica* 53(1-4):177-189.

Webber, W.A.F.
1955 The Filarial Parasites of Primates: A Review. I. *Dirofilaria* and *Dipetalonema*. *Ann. Trop. Med. & Parasitol.* 49(2):123-141.

White, Frances
1998 Seasonality and Socioecology: The Importance of Variation in Fruit Abundance to Bonobo Sociality. *International Journal of Primatology* 19(6):1013-1027.
1996 *Pan paniscus* 1973 to 1996: Twenty-three Years of Field Research. *Evolutionary Anthropology* 5(1):11-17.

World Wildlife Fund
n.d. *Bonobo*. Retrieved September 27, 1999, from the World Wide Web: www.panda.org/resources/publication/species/threatened/bonobo/page7.htm

Yamashita, Jiro
1963 Ecological Relationships between Parasites and Primates. *Primates* 4(1):1-96.

Yanagisawa, T., H. Tohda, Y. Miake, and S. Takuma
1984 Quantitative Microradiography and Electron Microscopy of Hypoplastic Human Enamel. In *Tooth Enamel IV*, edited by R.W.Fearnhead and S.Suga, pp.373-377. Elsevier Science Publishers, Amsterdam.

Yuan, M., R. Holloway, L. Moss-Salentijn, M.Yoder, and D. Broadfield
1998 Perikymata Counts in Two Modern Human Sample Populations [abstract]. *American Journal of Physical Anthropology Supplement* 105(S26):237-238.

Zsigmondy, Otto
1893 On Congenital Defects of the Enamel. *Dental Cosmos* 35:709-717.

Zumpt, F.
1961 The Arthropod Parasites of Vertebrates in Africa South of the Sahara (Ethiopian Region). Vol. 1 (Chelicerata). *Publications South African Institute Medical Research* 9:1-457.

APPENDIX A:
DIFFERENT DESCRIPTIVE CATEGORIES OF ENAMEL HYPOPLASIAS

Table A-1: DIFFERENT DESCRIPTIVE CLASSIFICATIONS OF ENAMEL HYPOPLASIAS

Hillson's Types[1] (after Berten 1895)	EH Category	DDE Index[1]	Arrangement	Number	Description	Alternative Descriptions
PIT-TYPE	PIT	FDI type 3 Type D	Linear, Non-Linear	Single Multiple	Mild Severe	G (Gross) Hypoplasia[2]
FURROW-TYPE	LEH	FDI type 4 Type E	Grooves – Horizontal	Single Multiple	Mild Moderate Severe	TEH (Transverse EH)[3] M (Melanby) Hypoplasia[2] Lines[4]
	VEH	FDI type 5 Type F	Grooves – Vertical			
PLANE-TYPE	-	FDI type 6 Type G	Missing Enamel			Grooves?[4]
	LHPC	n/a	Localized	Single		
	DDMLI[5]	n/a	Diagonal	Single	Mild Moderate Severe	Darcy's Defect
	IPCH[6]	n/a	Mesial, Distal	Single Multiple	Pin-point Plane-form Extensive plane-form	Interproximal Contact Enamel Hypoplasia[6,7]

Sources: (1) Hillson 1996, (2) Hillson 1986, (3) Eckhardt and Protsch von Zieten 1991, (4) El-Najjar *et al.* 1978, (5) Hannibal 2001b, 2003, (6) Lukacs 1999b, (7) Skinner 1996

APPENDIX B:
STUDIES OF EH IN CHIMPANZEES

Table A-2 STUDIES OF EH IN CHIMPANZEES

DATE	RESEARCHER	CHIMP COLLECTION	EH	TEEH	METHOD	ETIOLOGY/ FACTORS
2003	Skinner & Hopwood	Powell Cotton Museum n=44	rTEH	Incisors Canines	Gross examination Caliper measurements SEM	Seasonal variation in fruiting cycles with specific stressors (infections?)
2003	Hannibal	Smithsonian Natural History Museum n=25	DDMLI	Central incisor (35.71%)	Gross examination	Physiological systemic stress, genetic
2003	Skinner & Newell	Royal Museum for Central Africa (bonobos n=39)	LHPC	Canine (61.5%)	Gross examination	Problem of bone mass
2001	Lukacs	Powell Cotton Museum n=42	LHPC	Canine (48%)	Gross examination Caliper measurements	Environmental factors, interspecific differences
2000b	Hannibal	Smithsonian Natural History Museum n=25	LEH (68%) DDMLI (37.2%)	N/A	Gross examination (Goodman & Rose)	Biological, environmental
2000	Skinner & Guatelli-Steinberg	Powell Cotton Museum n=44	rTEH	Incisors Canines	Caliper measurements	Semi-annual stress
1999	Lukacs	Hamann-Todd Collection (Cleveland Museum) deciduous = 40 Smithsonian Natural History Museum deciduous =11	LHPC	Canines (22%)	Gross examination (Goodman & Rose)	Genetic, developmental, morphological
1998	Stottlemire	Hamann-Todd Collection (Cleveland Museum) n=98	LEH	80.6%		

Table A-2 STUDIES OF EH IN CHIMPANZEES cont'd...

DATE	RESEARCHER	CHIMP COLLECTION	EH	TEEH	METHOD	ETIOLOGY/ FACTORS
1998	Newell	Unspecified permanent n = 7 deciduous n=39	LEH pitting	~50%^{P}, 5.1%^{d} 11.4%^{P}, 15.4%^{d}	Gross examination (Goodman & Rose)	Unquantified (genetic, behavioural, anatomical, nutritional, illness
1998	Guatelli-Steinberg	Museum of Comparative Zoology (Harvard) n=17 Museum of vertebrate Zoology (Berkeley) n=11	LEH rr:LEH	I,C,PM, M 22/28 = 82% 4/28 = 14%	Gross examination (Goodman & Rose)	Sexual dimorphism
1993	Eckhardt & Protsch von Zieten	Liberian Chimpanzees (Frankfurt Anthropological Institute), deciduous = 70	Pits FDI type 3	Max & man dentitions	Gross examination (Goodman & Rose)	
1992	Eckhardt	Liberian Chimpanzees (Frankfurt Anthropological Institute), n = 280	LEH (present, Absent)	I (n=70/280) C (n=59/280)	Gross examination	Genetic, environmental
1991	Protsch von Zieten & Eckhardt	Liberian Chimpanzees (F.A.I.)	VEH	Max C (n=2)	Gross examination	Genetic
1991	Moggi-Cecchi & Crovella	Pan n=24	Grooves Lines Pits	Max & man dentitions	Gross examination (El-Najjar *et al.*1978)	Nutritional, metabolic disruptions, infections, localized trauma, other
1986	Skinner	Powell-Cotton (Kent, UK) n=110	Grooves (deep, mild)	C, M3	Gross examination	Cyclical seasonal stress
1975	Molnar & Ward	Captive Wild Wild	Scattered Numerous Numerous	M^1, M_1 M^1, M_1 M_1	Thin section (Bohatirchuk 1957)	Diet, unknown factors (infectious disease, gestation periods)

Table A-2 STUDIES OF EH IN CHIMPANZEES cont'd...

DATE	RESEARCHER	CHIMP COLLECTION	EH	TEEH	METHOD	ETIOLOGY/ FACTORS
1960	Jones & Cave (cited by Eckhardt 1992)	Sierra Leone Chimpanzees n=6/13	Faint transverse grooving, very slight degree of EH, severe/deeply grooved			
1956	Schulman & Sognanaes	Liberian Chimpanzee (Harvard Peabody) n=78	LEH slight moderate	I, C P, M1, M3	Gross examination (Mellanby'29,'30,'34) Microscopic (Scott & Wyckoff 1956) Histological ground section (Sognnaes 1947)	
1936	Widdowson (cited by Eckhardt 1992)	Unspecified	LEH	incisor		
1936	Coyler (cited by Schuman & Sognnaes 1956)	Museum of Royal College of Surgeons n=1 Unspecified n=2	Transversely grooved grossly visible pits pitted or grooved enamel			Food shortages

APPENDIX C:
DATA COLLECTION FORM

Pan paniscus Skeletal Collection ROYAL MUSEUM for CENTRAL AFRICA, Tervuren, Belgium

DATA COLLECTION FORM

Date of Examination_____ Specimen #_____

Developmental Age_____ Sex_____

NOTES_____

Tooth Class	Type of EH	Episode #	CEJ measurement	Severity	Comments

Specimen # _____ **Page** _____ **of** _____

Pan paniscus Skeletal Collection ROYAL MUSEUM for CENTRAL AFRICA, Tervuren, Belgium

Date of Examination_____Specimen #_____

Tooth Class	Type of EH	Episode #	CEJ measurement	Severity	Comments

Specimen # _____ Page _____ of _____

Pan paniscus Skeletal Collection ROYAL MUSEUM for CENTRAL AFRICA, Tervuren, Belgium

Date of Examination_____ Specimen # _____

MAX. RIGHT	Include/ Exclude	COMMENTS	MAX. RIGHT	Include/ Exclude	COMMENTS
I1			i1		
I2			i2		
C			c		
P3			dm1		
P4			dm2		
M1					
M2					
M3					
MAX. LEFT			MAX. LEFT		
I1			i1		
I2			i2		
C			c		
P3			dm1		
P4			dm2		
M1					
M2					
M3					
MAN. RIGHT			MAN. RIGHT		
I1			i1		
I2			i2		
C			c		
P3			dm1		
P4			dm2		
M1					
M2					
M3					
MAN. LEFT			MAN. LEFT		
I1			i1		
I2			i2		
C			c		
P3			dm1		
P4			dm2		
M1					
M2					
M3					

APPENDIX D:
MAXILLARY CENTRAL INCISOR CROWN FORMATION CHRONOLOGIES

APPENDIX D: *P. paniscus* Maxillary Central Incisor Crown Formation Chronologies

A-3: Distribution of Perikymata in Equal % Zones Occlusal (1) toCervical (10) Margin

Cast	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	Total
53c	3	13	14	16	19	20	16	24	14	-	139
61c	9	18	15	22	28	23	22	27	17	-	181
118a	-	14	16	27	27	19	38	29	22	3	195
119a	-	13	16	18	19	21	18	23	21	6	155
Mean	6	15	15	21	23	21	24	26	19	5	168
S.D.	4	2	1	5	5	2	10	3	4	2	25

A-4: Distribution of Perikymata (in Days) in Equal % Zones Occlusal (1) to Cervical (10) Margin

Cast	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	Total
53c	18	78	84	96	114	120	96	144	84	-	139
61c	54	108	90	132	168	138	132	162	102	-	181
118a	-	84	96	162	162	114	228	174	132	18	195
119a	-	78	96	108	114	126	108	138	126	36	155
Mean	36	87	92	125	140	125	141	155	111	27	168
S.D.	25	14	6	29	30	10	60	17	22	13	25

A-5: Cumulative Perikymata Counts in Equal % Zones Occlusal (1) to Cervical (10) Margin

Cast	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	Total
53c	3	16	30	46	65	85	101	125	139	-	139
61c	9	27	42	64	92	115	137	164	181	-	181
118a	-	14	30	57	84	103	141	170	192	192	195
119a	-	13	29	47	66	87	105	128	149	155	155
Mean	6	18	33	54	77	98	121	147	165	174	168
S.D.	4	6	6	9	13	14	21	24	25	26	25

A-6: Cumulative Perikymata Counts (in Days) Equal % Zones Occlusal (1) to Cervical (10) Margin

Cast	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	Total
53c	18	96	180	276	390	510	606	750	834	-	834
61c	54	162	252	384	552	690	822	984	1086	-	1086
118a	-	84	180	342	504	618	846	1020	1152	1170	1170
119a	-	78	174	282	396	522	630	768	894	930	930
124a	78	156	234	348	456	570	702	774	852	948	948
Mean	36	105	197	321	461	585	726	881	992	1050	1005
S.D.	25	39	37	51	80	85	125	141	152	170	151

APPENDIX E:
PARASITES FOUND IN *Pan paniscus*

Table A- 7: Summary of Parasites Identified in Bonbos, *Pan paniscus*

PARASITE	SAMPLE SOURCE	RESEARCHER
Phylum: APICOMPLEXA		
Order: Haemosporida	**(malarias)**	
Plasmodium reichenowi	North of Congo Republic	Bray 1963
Plasmodium vivax schwetzi	North of Congo Republic	Bray 1963
Plasmodium malariae	North of Congo Republic	Bray 1963
Phylum: ARTHROPODA		
Class: Insecta		
Order: Anoplura	**(sucking lice)**	
Family: Pediculidae		
Pediculus shaeffi (chimpanzee louse)		Benoit 1961†
Class: Arachnida		
Suborder: Mesostigmata		
Family: Pneumonyssus	**(nasal or lung mites)**	
Pneumonyssus duttoni		Fain 1957
Pneumonyssus longus		Fain 1959† Zumpt 1961†
Pneumonyssus oudemansi		Fain 1957†
Suborder: Astigmata		
Family: Sarcoptidae	**(mange/itch mite)**	
Sarcoptes scabei		Fain 1963†
Family: Psoralgidae	**(primate mite?)**	
Pangorillalges pani		Fain 1962, 1963†

Phylum: CILIOPHORA?	**(CILIATES)**	
Troglodytella sp. - 75.3%	Fecal, wild – Lamoko	Dupain *et al.* 2000
- 74%	Fecal, wild – Lamoko	Dupain *et al.* 2002
- 99.0%	Fecal, wild - Wamba	Hasegawa & Kano 1983
*Troglodytella abrassarti**	Fecal – newly captured	Mortelmans *et al.* 1971
Phylum: NEMATODA	**(ROUNDWORMS)**	
Class: Chromadorea		
Order: Ascaridida	**(large roundworm)**	
Family: Ascarididae		
Ascaris lumbricoides	Necropsy	Stam 1960†
Ascarids - 3.4%	Fecal wild – Lamoko	Dupain *et al.* 2000
Order: Oxyurida	**(pinworm)**	
Family: Oxyuridiae		
Enterobius ? - 6.2%	Fecal, wild – Wamba	Hasegawa & Kano1983
Order: Rhabditida		
Family: Ancylostomatidae	**(hookworm)**	
Necator americanus	Fecal	Stam 1960†
Hookworm - 13.5%	Fecal Wild – Lamoko	Dupain *et al.* 2000
- 21.0%	Fecal, Wild – Wamba	Hasegawa & Kano1983
Family: Chabertiidae	**(nodular worm)**	
Oesophagostomum - 49.4%	Fecal, wild – Lamoko	Dupain *et al.* 2000
- 50 %	Fecal, wild – Lamoko	Dupain *et al.* 2002
- 17.9%	Fecal, wild – Wamba	Hasegawa & Kano1983
O. stephanostomum		Vuylsteke 1964‡
Family: Strongyloididae	**(threadworm)**	
Strongyloides sp. - 39.2%	Fecal, wild – Lamoko	Dupain *et al.* 2000
- 36 %	Fecal, wild – Lamoko	Dupain *et al.* 2002
- 52.9%	Fecal, wild – Wamba	Hasegawa & Kano1983

Order: Spirurida		
Family: Onchocercidae		
Dipetalonema rodhaini		Webber 1955†
Dipetalonema streptocerca		Peel & Chardome 1946a,b†
		Peel & Chardome 1947†
Dipetalonema vanhoofi		Peel & Chardome 1946a,b†
		Peel & Chardome 1947†
? Microfilaria streptocara		Peel & Chardome 1946a,b†
		Peel & Chardome 1947†
Order: Trichocephalida	**(whipworm)**	
Family: Trichuridae		
Trichuris sp. - 2.2%	Fecal, wild – Lamoko	Dupain *et al.* 2000
- 3.3%	Fecal, wild – Wamba	Hasegawa & Kano1983
Capillaria sp. - 21.0%	Fecal, wild – Wamba	Hasegawa & Kano1983
- 0.0%	Fecal, wild – Lomako	Dupain *et al.* 2000
Phylum: PLATYHELMINTHES	**(FLATWORMS)**	
Class: Trematoda		
Trematoda sp.1 – 18.0%	Fecal, wild – Lamoko	Dupain *et al.* 2000
Trematoda sp.2 – 1.1%	Fecal, wild – Lamoko	Dupain *et al.* 2000
Subclass: Digenea		
Family: Dicrocoeliidae		
Dicrocoeliid – 45.1%	Fecal, wild - Wamba	Hasegawa &Kano1983
Phylum: PROTOZOA Subphylum: SARCOMASTIGOPHORA		
Order: Amoebida		
Family: Endamoebidae		
Entamoeba histolytica (including clinical symptoms)	Fecal – newly captured	Mortelmans *et al.* 1971

* largest protozoan parasite known in apes (Mortelmans *et al.* 1971)
† source: from Myers & Kuntz 1972
‡ source: from Hasegawa & Kano 1983