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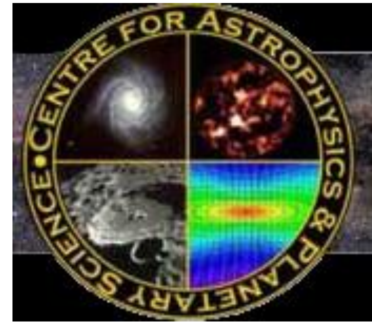

Cuspal Enamel Growth and Crown Enamel Thickness in Modern-Day African Pastoralists and Farmers

MSc (Research) Anthropology

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// Abstract

European and African populations have exhibited limited variation in dental development. However, it remains unclear whether tooth growth, specifically enamel formation, varies between modern human populations with differing diets. Enamel growth and thickness were compared between two modern human ethnic populations, the Fulbe and Nso, from Cameroon. There is a cultural focus on milk-drinking and consuming milk-based products in the Fulbe which is not present in the Nso, who have a broad-based modern diet including agricultural produce. This study aimed to determine whether enamel formation differed between these populations and, if so, whether these variations correlated with their diets.

Standard histological methods were used to analyze cuspal enamel growth rates, average enamel thickness and tooth crown size in 35 permanent molars (Fulbe n= 9; Nso n= 26). Prism widths were measured using scanning electron microscopy. Cuspal enamel growth rates and average enamel thickness were also measured in a comparative European (British) sample.

The speed at which tooth crowns extended in height was significantly (p= 0.005) faster in the Fulbe (n= 7, mean= 23.61µm/day) than the Nso (n= 7, mean= 14.41µm/day). Prism widths were also larger (Fulbe mean= 5.68µm; Nso mean= 5.24µm). The comparative European sample had accelerated extension rates (n= 20, mean= 28.39µm/day) which were more similar to the results for the Fulbe than the Nso. Tooth crown size was significantly (p< 0.005) larger in the Nso molars (n= 16, mean= 54.96mm^2) than the Fulbe molars (n= 5, mean= 48.07mm^2). Crown enamel thickness was greater in the combined

African (Fulbe and Nso) molars (mean= 1.49mm) than the European molars (mean= 1.14mm) from this study, as well as other comparative datasets from previous publications. No consistent differences were found between pre-weaning (first molars) and post-weaning (second and third molars) cuspal enamel growth rates in either the pastoralists or farmers.

These results imply there is more variation in modern human tooth growth than previously reported. Further research into cuspal enamel growth rates across geographic and regional populations is necessary to establish the full extent of human variation. The lack of difference between the first and distal molars suggests post-weaning diets, including those which emphasize the continued consumption of milk and milk-based products, have a limited effect on enamel formation. This means dietary shifts, such as weaning, may not be easily detected from dental samples in the fossil hominin record using histological techniques.

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Contents

ABSTRACT II

ACKNOWLEDGEMENTS IV

CONTENTS V

LIST OF TABLES VIII

LIST OF FIGURES IX

GLOSSARY....................XI

DIRECTIONAL TERMINOLOGY....................XI

ANATOMICAL TERMINOLOGY XII

CHAPTER 1: INTRODUCTION 1

1.1. Background 1

1.2. RESEARCH QUESTIONS 3

1.3. SUMMARY OF CHAPTERS 5

CHAPTER 2: LITERATURE REVIEW 7

2.1. Outline 7

2.2. ENAMEL 7

2.2.1. Enamel Anatomy....................7

2.2.2. Dental Development and Enamel Formation 9

2.2.3. Incremental Markings in Enamel 16

2.3. MODERN HUMAN VARIATION IN ENAMEL THICKNESS 19

2.4. MODERN HUMAN VARIATION IN ENAMEL FORMATION 23

2.5. FACTORS INFLUENCING ENAMEL THICKNESS AND FORMATION 27

2.5.1. Proteins and calories 30

2.5.2. Calcium, phosphorous and vitamin pathways....................32

2.6. APPLYING ENAMEL INCREMENTAL MARKINGS TO STUDIES OF HUMAN LIFE HISTORY.36

2.6.1. Life history theory....................36

2.6.2. Evolution of Human Life History 37

CHAPTER 3: MATERIALS 40

3.1. Outline 40

3.2. FULBE AND NSO 40

3.2.1. Background 40

3.2.2. Samples....................42

3.3. EUROPEAN 44

3.3.1. Background 44

3.3.2. Samples....................44

CHAPTER 4: METHODS 46

4.1. Outline 46
4.2. HISTOLOGY..46
4.2.1. Sample selection..46
4.2.2. Embedding..47
4.2.3. Sectioning..49
4.2.4. Mounting..50
4.2.5. Grinding and Polishing..51
4.2.6. Cover Slips..53
4.3. MICROSCOPY..54
4.3.1. Transmitted and Polarized Light Microscopy..54
4.3.2. Scanning Electron Microscopy..56
4.4. MEASUREMENTS..58
4.4.1. Average Enamel Thickness..58
4.4.2. Tooth Crown Size..59
4.4.3. Daily Secretion Rate..59
4.4.4. Enamel Extension Rate..61
4.4.5. Prism Width..64
4.4.6. Intraobserver error..65
4.5. STATISTICS..66
4.5.1. Descriptive Statistics..66
4.5.2. Inferential Statistics..67
4.5.2.1. Correlations..67
4.5.2.2. Analysis of Variance post hoc Pairwise Comparisons..68
4.5.2.3. Regressions..69
CHAPTER 5: RESULTS..71
5.1. Outline..71
5.2. FULBE AND NSO POPULATIONS..71
5.2.1. Comparisons across the Molar Row..76
5.2.2 Comparisons between the Cusps..81
5.2.3. Comparisons between the Sexes..85
5.3. AFRICAN AND COMPARATIVE POPULATIONS (FIRST MOLAR AND EUROPEAN)..86
5.3.1. AET..86
5.3.2. Tooth Crown Size..88
5.3.3. DSR..89
5.3.4. EER..92
5.3.5. Correlations and Regressions of combined (Fulbe and Nso) African and European samples..94
CHAPTER 6: DISCUSSION..97
6.1. Outline..97
6.2. DOES ENAMEL FORMATION DIFFER BETWEEN THE FULBE (PASTORALISTS) AND NSO (AGRICULTURALISTS)?..97
6.2.1. EER, Prism width and the Stretching Effect model..97

6.2.2. Tooth size 102
6.2.3. Enamel secretion and development 105
6.2.4. First and Distal Molars 108
6.3. Does variation in enamel growth between the two African populations correspond with diet after weaning? 110
6.3.1. The impact of pastoralist and agriculturalist diets on amelogenesis 110
6.3.2. The impact of the weaning process on enamel formation 116
6.4. Does enamel growth and thickness in the African first molars (both Fulbe and Nso combined) differ compared to the European first molars? 120
6.4.1. African and European first molars 120
6.4.2. New patterns of modern human variation 126
6.5. Limitations 131
6.6 Recommendations for Future Research 134
CHAPTER 7: CONCLUSION 137
REFERENCES 139
APPENDIX 151

List of Tables

Table 1- Frequency of each molar type from the Fulbe, Nso, combined African and European populations examined in this study……………………………………………….45

Table 2- Mean values for the five main variables for the Fulbe and Nso populations…………………………………………………………………………………….............71

Table 4- Mean DSRs (in µm/day) for three regions of cuspal enamel for the Fulbe and Nso populations…………………………………………………………………………………………………73

Table 6- Mean EERs (in µm/day) for three regions of the enamel cap for the Fulbe and Nso populations………………………………………………………………………………………74

Table 7- Mean values for the four main variables for the Fulbe and Nso populations separated by tooth type……………………………………………………………………………….76

Table 8- Mean DSRs (in µm/day) for three regions of cuspal enamel for the Fulbe and Nso populations separated by tooth type……………………………………………………………78

Table 9- Mean EERs (in µm/day) for three regions of enamel cap for Fulbe and Nso populations separated by tooth type…………………………………………………………….79

Table 10- Mean values for the four main variables for the Fulbe and Nso populations divided by molar type………………………………………………………………………………….80

Table 11- Mean AET (in mm) for the Fulbe and Nso populations

separated by cusp..……………………………………………………………………………………81

Table 12- Mean DSRs (in µm/day) for the Fulbe and Nso populations

separated by cusp ……………………………………………………………………………………..82

Table 13- Mean EERs (in µm/day) for the Fulbe and Nso populations

separated by cusp ……………………………………………………………………………………..84

Table 15- Mean AET (in mm) for the African and European molars examined in this study alongside the values calculated for previously published modern human samples……………………………………………………………………………………………………….86

Table 16- Mean tooth crown size (in mm^2) for all molars from the combined African population examined in this study and comparative samples from previous publications…………………………………………………………………………………………………….88

Table 17- Mean DSR (in µm/day) for the first molars of the combined African and European samples from this study and a comparative sample from Mahoney (2008)…………………………………………………………………………………….90

Table 18- Mean DSRs (in µm/day) for all molars from the combined African population from this study and comparative human samples from previous publications…..91

Table 19- Mean values of EER (in µm/day) for the first molars of the African (Nso) and European populations from this study……………………………………………………………92

List of Figures

Fig. 1- Diagram of amelogenesis adapted from Bronckers *et al* (2009)…………………11

Fig. 2- Photo of the cuspal enamel of a Fulbe M_3 …………………………………………….16

Fig. 3- Map of Cameroon……………………………………………………………………………41

Fig. 4- Diagram of a molar section……………………………………………………………..59

Fig. 5- a) Diagram of a molar section b) Photo of a Nso M^2 taken at 4x c) Photo of the Nso M^2 taken at 40x…………………………………………………………………60

Fig. 6- a) Photo of the cuspal enamel of a Fulbe M_3 b) Photo of the Fulbe M_3 taken at 10x……………………………………………………………………62

Fig. 7- a) Diagram of a molar section b) Photo of the cuspal enamel of a Fulbe M_2 taken at 698x c) Photo of Fulbe M_2 taken at 1000x, rotated 90° clockwise……………………….64

Fig. 8- Graph of average EER (in µm/day) for the Fulbe and Nso……………………………..72

Fig. 9- Graph of average DSR (in µm/day) for Fulbe and Nso split by tooth type……..77

Fig. 10- Graph of average EER (in µm/day) for Fulbe and Nso split by tooth type……79

Fig. 11- Graph of average EER (in µm/day) for all of the Fulbe, Nso and European molars...93

Fig. 12- Plot of log-transformed buccal cusp AET against log-transformed tooth crown size for all molars from the combined African (Fulbe and Nso) sample………………………..96

Fig. 13- Diagram of the stretching effect model adapted from Shellis (1984)……101

Glossary

Definitions for the dental terminology used in this study are provided below. All definitions are taken from White *et al* (2012, pp.13–15; 104-107).

Directional terminology

- *Anterior*- toward the front of the human body
- *Posterior*- opposite of anterior; for humans, toward the back of the individual
- *Medial*- toward the midline of the human body
- *Lateral*- opposite of medial; away from the midline of the human body
- *Mesial*- toward the midline point of the dental arch where the central incisors contact each other. The anterior portion of molars and premolars are called the mesial parts of these teeth
- *Distal*- opposite of mesial; away from the midline point of the dental arch
- *Lingual*- toward the tongue
- *Labial*- opposite of lingual; toward the lips; usually reserved for incisors and canines
- *Buccal*- opposite of lingual; toward the cheeks; usually reserved for premolars and molars
- *Occlusal*- facing the opposing dental arch, usually the chewing surface of each tooth
- *Apical*- at or towards the tip of the root of the tooth
- *Cervical*- at, around, or pertaining to either the neck proper (the portion of the body between the head and shoulders) or to any of a number of anatomical constrictions referred to as 'cervix'.

Anatomical terminology

- *Crown*- the part of the tooth covered by enamel
- *Root*- the part of the tooth that anchors the tooth in the alveolus of the mandible or maxilla
- *Enamel*- the specialized hard tissue that covers the tooth crown. It is about 97% mineralized, essentially fossilized once it is formed
- *Cementum*- a bone-like tissue that covers the external surface of tooth roots
- *Dentin*- the tissue that forms the core of the tooth. This tissue has no vascular supply but is supported by the vascular system in the pulp and is lined on the inner surface (the walls of the pulp cavity) by odontoblasts, dentin-producing cells. It underlines the enamel of the crown and encapsulates the pulp cavity, the central soft tissue space within a tooth.
- *Pulp*- the soft tissue within the pulp chamber. This includes nerves and blood vessels.
- *Cervix*- the constricted part of the tooth at the junction of the crown and root
- *Enamel-dentin junction (EDJ)*- the boundary between the enamel cap and the underlying dentin.
- *Cusp*- an occlusal projection of the crown. The tip of the cusp is the apex. The protoconid is the mesiobuccal cusp and metaconid is the mesiolingual cusp on the lower molars. The protocone is the mesiolingual cusp and paracone is the mesiobuccal cusp on the upper molars.

Chapter 1: Introduction

1.1. Background

Enamel growth has been shown to be consistent across modern human geographic populations. Reid and colleagues have repeatedly stated there are only minor differences between northern European and southern African dental samples, especially molars (Reid and Dean 2006; Guatelli-Steinberg, Reid and Bishop 2007; Feeney *et al.* 2010; Reid, Guatelli-Steinberg and Walton 2008; Guatelli-Steinberg *et al.* 2012). As a result, they have suggested the chronology of enamel formation is constrained within humans, regardless of genetic background (Reid and Dean 2006; Reid, Guatelli-Steinberg and Walton 2008). Also, Feeney e*t al* (2010) argued data from multiple populations could be combined to create a sample that represents the full range of variation in modern human enamel growth, and then compared to other hominin species. Guatelli-Steinberg and colleagues have done so by incorporating the results of the southern African and northern European populations, comparing them to Neanderthal molar samples, and then using them to extrapolate the somatic growth and life history patterns of our most recent fossil ancestor (Guatelli-Steinberg *et al.* 2005; Guatelli-Steinberg, Reid and Bishop 2007; Guatelli-Steinberg and Reid 2008).

However, it is unclear whether enamel formation differs within populations. All of the studies cited above used relatively isolated samples to represent entire geographic populations, without testing local levels of variation. Also, the extent to which enamel growth varies in populations with different diets is unknown. Modern human populations adopt diverse diets after weaning, which marks the dietary transition from breastfeeding to mixed-feeding and finally the consumption of solid foods after lactation ends. The influence of varied post-weaning diets on enamel growth is unclear. These issues directly

impact evolutionary studies of life history. Dental development, specifically the eruption of the first permanent molar, has been found to correlate with traits like age-at-weaning across the primate order (Smith 1989; Smith 1992). Consequently, it has been used to infer aspects of hominin life history (Kelley and Smith 2003). Yet, this correlation is not present in modern humans, who finish the weaning process at a uniquely early age (Humphrey 2010). Currently, there is no method for identifying weaning from the fossil record and so it is unclear when this unique feature of human life history evolved.

This study explored the extent to which enamel formation varied on a local level and in populations with different post-weaning diets. Reid and Dean (2006) suggested that if such variation exists it is most likely to emerge in the cuspal enamel of molars from geographically diverse populations. With this in mind, cuspal enamel growth and morphology were compared between molar samples from two ethnic groups from Cameroon, the Fulbe and the Nso. The Fulbe are pastoralists who continue consuming milk and milk-based products after weaning whilst the Nso are agriculturalists who incorporate farmed foods. This comparison could show whether the limited variation in enamel growth between geographically diverse populations (e.g. Europeans and Africans) is also present between neighbouring populations. It could also highlight the influence of diet on modern human enamel formation. Finally, comparing the Fulbe and Nso molar samples could determine whether weaning can be detected from enamel growth, which would impact studies of hominin life history.

1.2. Research Questions

This study aimed to answer three main questions:

1. *Does enamel formation differ between the Fulbe (pastoralists) and Nso (agriculturalists)?*

The Fulbe and Nso are neighbouring populations living in western Cameroon. Their dietary practices differ in that the Fulbe exhibit a cultural focus on milk-drinking which is not present in the Nso. Histology and scanning electron microscopy can be used to analyse incremental growth lines within enamel, which represent a timeline of enamel formation (Smith 1991; Antoine, Hillson and Dean 2009). To answer the first question, this study analysed cuspal enamel morphology and growth rates in a sample of permanent molars from the Fulbe and Nso. This would determine whether enamel formation varies between these two populations.

2. *Does variation in enamel growth between the two African populations correspond with diet after weaning?*

To answer the second question, cuspal enamel growth rates were compared between the first molars and combined distal (second and third) molars of the Fulbe and Nso populations. In humans, the first molars start growing before weaning whilst the second and third molars begin developing after it has ended (Smith 1991; Hill and Kaplan 1999; Humphrey 2010; Smith *et al.* 2013). Since the Fulbe and Nso share similar environmental conditions, any significant differences in the cuspal enamel growth rates of the combined distal molars between these populations might be attributed to their post-weaning diets. As such, this study aimed to show whether diet has a direct influence on modern human enamel formation. Any changes in cuspal enamel growth rates between the first and combined distal molars of the Fulbe and Nso could be indicative of weaning, a major

dietary shift. Thus, this study also tested whether histological analyses of enamel incremental markings can be used to identify weaning in the fossil record.

3. *Does enamel growth and thickness in the African first molars (both Fulbe and Nso combined) differ compared to the European first molars?*

To answer the final question, enamel thickness and growth rates were compared between the combined African (Fulbe and Nso) first molars and a comparative European sample of first molars taken from British Bronze Age and medieval individuals. This would place the results from the Fulbe and Nso populations into the wider anthropological context. It would show whether the limited variation in enamel growth between African and European populations found by Reid and colleagues (Reid and Dean 2006; Guatelli-Steinberg, Reid and Bishop 2007; Feeney *et al.* 2010; Reid, Guatelli-Steinberg and Walton 2008; Guatelli-Steinberg *et al.* 2012) was also present in these samples.

1.3. Summary of Chapters

Chapter 2: Literature Review

The literature review presents a detailed evaluation of previous anthropological research on enamel growth. It is split into five sections. The first outlines the key features of enamel anatomy and the process of enamel formation. The second and third sections review how enamel thickness and formation vary between modern humans, fossil hominins and extant primates. The fourth section describes the influence of genetic and environmental factors, specifically diet, on enamel formation. The application of dental enamel histology to life history studies, especially investigations of age-at-weaning, is reviewed in the final section.

Chapter 3: Materials

This chapter presents the human molar samples (Fulbe, Nso and European) selected for this study.

Chapter 4: Methods

Methods is split into four sections, one for each technique used during data collection: histology, microscopy (including scanning electron microscopy), data recording and statistical analysis.

Chapter 5: Results

This chapter presents all of the significant results for the descriptive and inferential statistical analyses. It is split into two sections. The first outlines the results for the comparison of cuspal enamel morphology and growth rates between the Fulbe and Nso molar samples. It also includes a comparison of results for the first and combined distal

(second and third) molars from each group. The second section compares the combined African (Fulbe and Nso) population with the European sample examined in this study as well as comparative human datasets from previous publications.

Chapter 6: Discussion

The discussion addresses the three main research questions. First, the results for the Fulbe and Nso molar samples, including the first and combined distal molars, are reviewed. The implications of these results with regards to dietary influences on enamel formation and studies of hominin life history are then considered. Following this, the results of the combined African and comparative European populations are discussed, and the current understanding of how enamel growth varies in modern human populations is explored. Then, the study limitations are briefly outlined. Lastly, recommendations for future research are discussed at the end of this chapter.

Chapter 7: Conclusion

An overview of the key findings of this study is provided in the conclusion.

Chapter 2: Literature Review

2.1. Outline

This chapter presents a comprehensive review of the anthropological literature pertaining to dental enamel. The first section includes a detailed explanation of enamel anatomy and formation. This is followed by contextual information from previous studies on how enamel thickness and formation varies between hominoid species and modern human populations. The genetic and environmental factors like diet that influence enamel development are also reviewed. Finally, a brief outline is provided on the use of dental enamel in hominin life history studies.

2.2. Enamel

2.2.1. Enamel Anatomy

Dental enamel is an avascular (lacks blood vessels) and acellular (lacks cells) layer of tissue that covers the tooth crown, and gives it a polished white appearance (Ross and Pawlina 2006; White 2012). Roughly 4% of enamel is made of organic materials and water (Permar 1963). However, the most important anatomical feature of enamel is that it is highly mineralized. This is due to the almost exclusive presence of inorganic material (roughly 96-98% by weight or 88-91% by volume), namely calcium phosphate in the form of crystalline hydroxyapatite (Boyde 1989). These crystals are tightly packed into the loose organic matrix, which includes proteins that provide a framework for the structure and help to prevent brittle fractures (Permar 1963; Aiello and Dean 1990). Its high level of mineralization makes enamel the hardest substance in the human body and allows it to protect the teeth throughout life. This is important because the cells responsible for enamel formation, ameloblasts, undergo morphological changes and transform into

maturational ameloblasts once enamel secretion ends (Dean 2004). Although the cells continue to raise the mineral content to its peak, the loss of the cells' enamel secretion and formation functions means that the enamel surface cannot heal itself when damaged (Ross and Pawlina 2006; Nanci 2008). The significant level of inorganic material in enamel also explains why teeth are more common in the archaeological record than other biological tissues. Whilst soft tissues and some parts of the human skeleton degrade easily, enamel can be preserved in most burial contexts and does not change significantly during fossilization (Aiello and Dean 1990). However, it may decompose in very acidic conditions (Hillson 1996).

On a microscopic level, the hydroxyapatite crystals found in enamel are tightly packed into bundles known as rods or prisms, which can be seen in scanning electron microscopy (SEM) images (Hillson 1996; Ross and Pawlina 2006; Antoine, Hillson and Dean 2009). In humans, each prism is approximately 4-7µm in diameter (Boyde 1989; Höhling 1989; Berkovitz, Holland and Moxham 2002; Hillson 2014). These structures are encased in a sheath and held together by interrod or interprism substance, which is formed from the intercellular material between adjacent ameloblasts as they migrate through the enamel during its formation (Permar 1963; Risnes 1998). As Boyde (1964) explained, ameloblasts and Tomes' process pits, which form around the secretory appendage on the ameloblasts, are packed hexagonally. However, this packing can exhibit minor variations, and the subsequent prism arrangement within these hexagons can occur in three main patterns. The first, Pattern 1, is where prisms have clear, complete boundaries which form a circular transverse section. Patterns 2 and 3 have incomplete prism boundaries with a horseshoe-shaped appearance (Risnes 1998). In these instances, the hexagonal cross-sections containing the prism boundaries can either be joined floor-to-side (Pattern

2) or corner-to-corner (Pattern 3) (Boyde 1989). The first and third patterns are most common in humans, especially Pattern 3, in which there are no abrupt changes in crystallite orientation at the centre of the prism.

During enamel formation, prisms are orientated to be roughly perpendicular to the enamel-dentin junction (EDJ) by ameloblasts as they migrate towards the future outer surface of the tooth crown (Permar 1963; Aiello and Dean 1990; Mahoney 2008). However, as the ameloblasts migrate, the enamel prisms deviate from this straight path by moving in a sinusoidal or helicoidal manner during a process commonly referred to as enamel prism decussation (Risnes 1998; Dean 2004). Prism decussation is most evident under the tips of the cusps of teeth in an area referred to as gnarled enamel, which is where prisms appear to be spiralling (Antoine, Hillson and Dean 2009). The purpose of enamel decussation seems to vary between species. Aiello and Dean (1990) suggested decussation in rodents is an adaptation suited to shearing off excess enamel and leaving a sharp edge on the incisors, which grow continuously. On the other hand, Macho *et al* (2003) argued enamel decussation is a functional adaptation that prevents the cracking of enamel in species with thick enamel and large bite forces, such as large-bodied apes. Thus, the complex arrangement of prisms not only adds to the unique structure of enamel, but it may also enhance its ability to protect the teeth from mechanical stresses during mastication (chewing).

2.2.2. Dental Development and Enamel Formation

The majority of dental development takes place in utero. As early as five weeks after fertilization, mesenchyme cells outline the shape of the future dental arch. As Aiello and Dean (1990) explained, mesenchyme is an embryonic connective tissue that also defines

the final tooth shape. Oral epithelium cells, which line the mouth cavity, then accumulate into the arch and form a horseshoe-shaped layer of tissue. This is separated into the vestibular lamina and the dental lamina (Hillson 1996; Ross and Pawlina 2006).

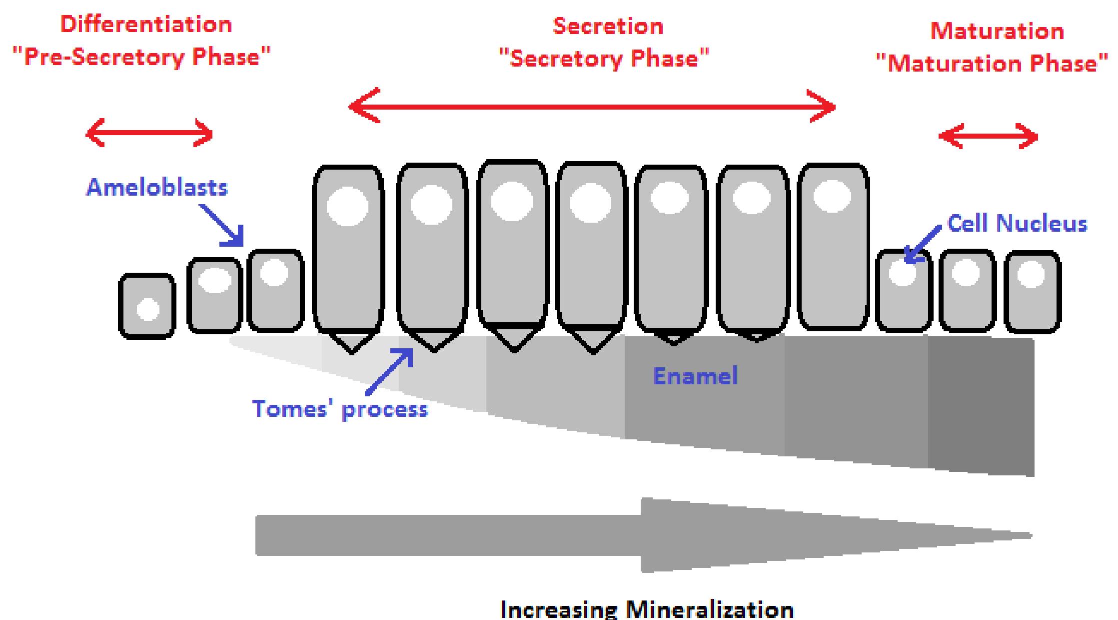
Roughly eight weeks after fertilization, during the first stage of dental development, the bud stage, ten swellings appear along the edge of the dental lamina as oral epithelium cells grow into the underlying mesenchyme (Permar 1963; Hillson 2014). These swellings are called tooth buds. Each one forms a domed structure called an enamel organ, which subsequently develop into the enamel caps for each tooth (Ten Cate 1959; Ross and Pawlina 2006; White 2012). The tooth buds for the deciduous teeth are formed at 8 weeks after fertilization and the buds for the permanent teeth are formed at 14 weeks (Hillson 2014). The enamel organs then appear at 10 and 16 weeks after fertilization, respectively (Hillson 1996).

The enamel organs develop further during the cap stage. A hollow forms on the opposite side to where the enamel organ emerged from the dental lamina (Ross and Pawlina 2006). This is filled with connective tissue that forms dental papilla (Permar 1963), which produces dentin-forming odontoblasts. The enamel organ also differentiates a layer of cuboidal cells called the internal enamel epithelium, which is involved in enamel formation. These structures are separated by the basement membrane that marks the line of the future EDJ (Permar 1963).

Enamel formation, or amelogenesis, takes place late during the bell stage of dental development, so called because of the shape the enamel organ adopts as cell differentiation takes place. It occurs after the beginning of dentinogenesis, the process of

dentin formation. Although amelogenesis and dentinogenesis appear to be separate processes, they are actually co-dependent. The differentiation of ameloblasts along the inner enamel epithelium induces the differentiation of odontoblasts from the dental papilla (Aiello and Dean 1990). Once odontoblasts begin to secrete dentin matrix, signals travel from the dental papilla to the ameloblasts at the inner enamel epithelium to induce the secretion of enamel (Hillson 2014). This reciprocal signalling controls the sequence of development for both enamel and dentin. As Dean (2004) explained, ameloblasts do not start to secrete enamel until at least 30 hours after dentin matrix secretion. Thus, the two hard tissues of the tooth, dentin and enamel, develop simultaneously as the two cell types travel in opposite directions from the future EDJ (Aiello and Dean 1990).

Fig. 1- Diagram of amelogenesis adapted from Bronckers *et al* (2009)



As shown in Fig. 1, like the continuous process of dental development, amelogenesis can be separated into artificial stages for the purposes of explanation and analysis (Reid and Dean 2006). First, the hollow within the enamel organ deepens and adopts several folds

that outline the future tooth crown. These folds occur because cells in the inner enamel epithelium divide and expand at different rates, which causes the structure to "buckle and bulge at sites of the future cusps" (Aiello and Dean 1990, p.108). During the pre-secretory phase of amelogenesis, cells from the inner dental epithelium differentiate into pre-ameloblasts, beginning at the tip of the developing tooth cusp (Berkovitz, Holland and Moxham 2002; Ross and Pawlina 2006; Mahoney 2008). As shown in Fig. 1, this process can be recognised as the cells appear to elongate into a column-like shapes (Permar 1963). These cells deposit a thin layer of aprismatic enamel matrix on the surface of the pre-dentin, which has already been deposited into the developing folds. This layer includes enamel matrix proteins, the majority of which (roughly 90%) are amelogenins that are unique to enamel matrix (Hillson 1996; Diekwisch *et al.* 2009; Kierdorf *et al.* 2014). This initiates a "wave of differentiation" (Simmer *et al.* 2010, p.1027), as adjacent cells differentiate into pre-ameloblasts along the EDJ and increase the height of the tooth crown (Mahoney 2008).

After the initial layer is deposited, the pre-ameloblasts differentiate further into secretory ameloblasts. As shown in Fig. 1, the key difference between these two cell types is that the latter has an additional pyramid-shaped appendage at its apical pole or secretory end called a Tomes' process (Berkovitz, Holland and Moxham 2002; Ross and Pawlina 2006). During the secretory phase, ameloblasts produce a thin, partially mineralized prismatic enamel matrix, made of hydroxyapaptite crystals, through the Tomes' process. Under microscopic observation, it is possible to see Tomes' process pits, where the Tomes' process fits into the enamel surface as it deposits enamel matrix (Aiello and Dean 1990).

As the enamel matrix is deposited, the Tomes' process uses the enamel proteins, particularly amelogenin, to orientate the hydroxyapatite crystals into prisms (Simmer *et al.* 2010; Mahoney 2011) and organise the spacing between them (Ross and Pawlina 2006). The developing layer of enamel increases in thickness as the cells make successive depositions whilst migrating away from the EDJ towards the future outer surface of the tooth crown (Simmer and Hu 2001; Mahoney 2008). A record of the movement of secretory-stage ameloblasts is preserved in mature enamel as this migration determines the path of prisms in the final structure (Risnes 1998; Ross and Pawlina 2006).

Simmer *et al* (2010) suggested the orientation of enamel prisms perpendicular to the ameloblasts as they move towards the tip of the cusp allows for complete coverage of the expanding enamel surface. Yet, Beynon *et al* (1991) stated that when the tips of the cusps finally achieve their full thickness, the height of the tooth crown is only up to two-thirds of its final value. This is because ameloblasts at the tip cease enamel deposition whilst those at the cervix only just begin enamel secretion. These ameloblasts secrete enamel matrix down the sides of the developing tooth in an imbricational manner, as successive layers overlap, rather than fully cover each other, at the surface of the tooth (Aiello and Dean 1990). As a result, the rate of enamel secretion, which refers to the increase in enamel thickness, is separated from the speed of enamel extension, or the increase in the height of the enamel layer (Mahoney 2015). To end the secretory phase, ameloblasts withdraw their Tomes' process and deposit a layer of aprismatic enamel (Berkovitz, Holland and Moxham 2002).

At the end of the secretory stage, newly formed enamel is only partially mineralized. It is comprised of "65% water, 20% organic material and 15% inorganic hydroxyapatite

crystals by weight" (Berkovitz, Holland and Moxham 2002, p.311). To fully mineralise the enamel, the secretory ameloblasts go through substantial morphological changes to become maturational ameloblasts. In addition to losing their Tomes' process, they switch polarity and adopt a ruffle-ended morphology, which has deep pleating at the distal end of their plasma membrane (Dean 2004; Berkovitz, Holland and Moxham 2002). Josephsen *et al* (2010) asserted that mature ameloblasts continuously alternate between this ruffle-ended shape and a smooth-ended structure, which does not have pleating.

After the cells differentiate into maturational ameloblasts, they form a mineralization front to break down and remove organic substances and water. Degraded proteins, mostly amelogenin, are also removed and replaced with enamelin, which is a more stable protein with a higher molecular weight (Boyde 1989; Ross and Pawlina 2006). At the same time, the ameloblasts incorporate calcium and phosphate ions onto the sides of the hydroxyapatite crystallites, which increases their width (Ross and Pawlina 2006; Mahoney 2008). Mature ameloblasts control this exchange through the continuous cycling between ruffle-ended and smooth-ended morphologies. Ions are brought into the enamel during the ruffle-ended stage whilst organic materials and water are released during the smooth-ended stage (Berkovitz, Holland and Moxham 2002). During this process, enamel hardens as the diameter of the hydroxyapatite crystals increases to between 50-100nm (Hillson 1996).

This explanation has separated the secretion and maturation stages of amelogenesis, but it is important to remember these are artificial divisions. Beynon and Dean (1988) argued these processes occur simultaneously within the tooth crown, as secondary mineralization begins at the tips of the cusps relatively early in amelogenesis, and then

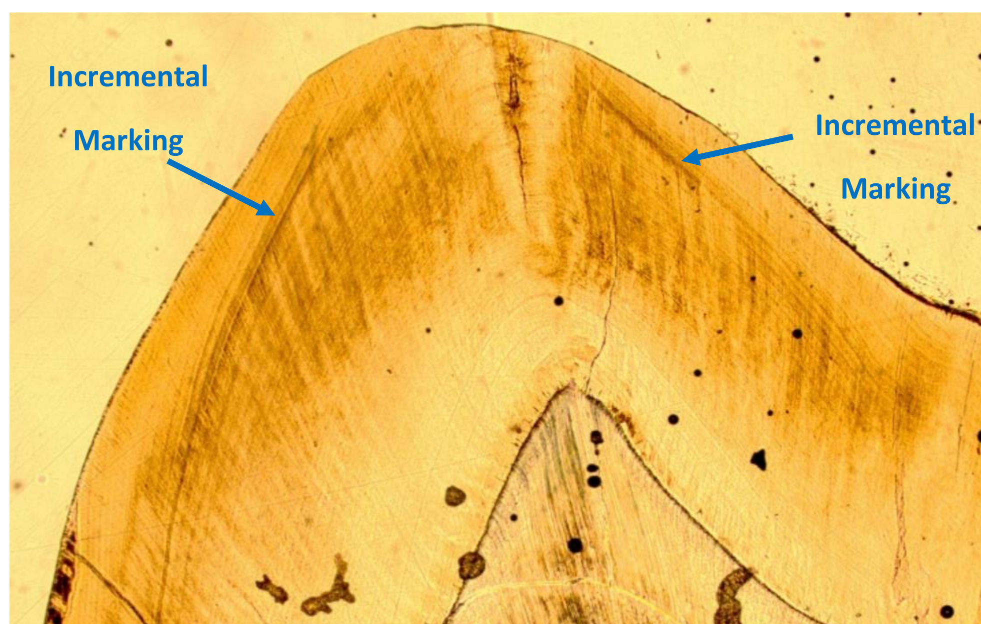
spreads down the sides of the crown along the EDJ. Thus, the tooth crown grows in height through the cervical extension of ameloblast differentiation along the EDJ at the same time as it grows in width through the migration of secretory and mineralizing ameloblasts from the EDJ to the outer surface of the crown (Smith, Martin and Leakey 2003; Simmer *et al.* 2010). The final thickness of the enamel is dependent upon the "number of active ameloblasts, the amount of enamel they secrete, and the length of time they remain active" (Mahoney, Miszkiewicz, *et al.* 2016, p.921). It has been suggested that the first set of ameloblasts induced to differentiate into pre-ameloblasts during the pre-secretory phase are also responsible for terminating amelogenesis (Simmer *et al.* 2010). At the end of amelogenesis, a proportion of the ameloblasts undergo apoptosis, whilst the majority differentiate into flattened epithelial cells and blend with cells in the remaining enamel organ to form the reduced enamel epithelium (Permar 1963).

It is important to note that the timing of enamel formation is not always consistent. Simmer *et al* (2010) suggested appositional growth rates, which are the daily increase in the thickness of the enamel layer perpendicular to the EDJ, vary throughout amelogenesis. For example, it has been observed that enamel growth resembles long bone growth, as both processes are faster earlier in the second trimester and slow down closer to birth (Mahoney 2015). This could be linked to the proliferation of ameloblasts at these times, as the number of ameloblasts actively forming enamel is directly linked to rates of tooth formation. As Aiello and Dean (1990) highlighted, the fewer the number of active ameloblasts present, the longer it takes to complete enamel formation, and vice versa. In this way, enamel formation can fluctuate within individual teeth.

2.2.3. Incremental Markings in Enamel

Ameloblasts deposit enamel matrix in a series of layers. When viewed microscopically, these layers can be seen as lines within the fully formed enamel, as shown in Fig. 2, which are referred to as incremental growth lines or markings (Bromage and Dean 1985; Beynon and Dean 1988; Boyde 1989; Dean 2009; Simmer *et al.* 2010). Most incremental markings are caused by rhythmic changes in enamel formation (Dean and Scandrett 1996; Smith, Martin and Leakey 2003; Antoine, Hillson and Dean 2009). These fluctuations are controlled by an individual's biological clock, and can range from sub-daily to annual rhythms (Smith *et al.* 2013). Thus, incremental markings in enamel are said to resemble growth rings found in the cross-sections of tree stumps (Beynon, Dean and Reid 1991; Dean 2010). Smith (1991) explained that enamel formation follows particular rhythms so faithfully that it is possible to decipher the timeline of amelogenesis. The rhythmic incremental markings relevant to this study are cross striations and Retzius lines. Accentuated markings, which occur when psychological or physical stress disrupt enamel formation, were also used. All three are discussed in detail below.

Fig. 2- Photo of the cuspal enamel of a Fulbe M_3. The sample has been sectioned and mounted onto a slide using histology (see Methods).



Cross striations are formed by the interchange between varicosities (formed at the peak of enamel secretion) and constrictions (formed when enamel secretion is at its slowest) along the length of enamel prisms during amelogenesis (Beynon and Dean 1988; Aiello and Dean 1990; Dean and Scandrett 1996). This oscillation occurs over a 24-hour cycle that corresponds with a circadian rhythm (Zhang *et al.* 2009), which also dictates the mineralization of teeth, shells and coral in a variety of organisms around the world (Aiello and Dean 1990; Dean 2006). Thus, each cross striation represents one day of enamel matrix secretion. The cause of these daily fluctuations in enamel secretion is unclear, but they have been linked to the cyclical changes in the carbonate, phosphate and hydrogen ion concentration of enamel matrix (Boyde 1989; Mahoney 2008; Simmer *et al.* 2010). When viewed microscopically, cross striations are perpendicular to the long axis of the tooth crown and appear as light and dark bands along the enamel prisms (FitzGerald 1998; Kierdorf *et al.* 2014). They are approximately 2-5.5µm apart in humans (Smith, Martin and Leakey 2003; Antoine, Hillson and Dean 2009; Hillson 2014). The spacing between successive cross striations gradually increases from the inner enamel near the EDJ to the outer enamel surface, which reflects the progressive increase in secretory activity by migrating ameloblasts (Reid, Beynon and Ramirez Rozzi 1998).

The second category of incremental markings used in this study is Retzius lines or brown striae of Retzius, which are named after the scientist who first observed them, Anders Retzius (Antoine, Hillson and Dean 2009). Retzius lines are less frequent than cross striations because they do not represent a daily, circadian cycle of variation in enamel formation, but instead are dictated by a long-period circaseptan cycle (Beynon and Dean 1988; Antoine, Hillson and Dean 2009). Thus, the number of days of amelogenesis between each Retzius line can range from six to twelve days in human populations, with

an average of seven to nine days, although this periodicity remains constant within each individual (Bromage and Dean 1985; Bromage 1991; Dean and Scandrett 1996; FitzGerald 1998; Berkovitz, Holland and Moxham 2002; Dean 2010; Mahoney 2012). Each Retzius line denotes the specific location of the migrating enamel formation front at a given time during amelogenesis (Beynon and Dean 1988; Risnes 1998; Smith, Martin and Leakey 2003; Antoine, Hillson and Dean 2009; Simmer *et al.* 2010; Kierdorf *et al.* 2014). The lines formed at the start of amelogenesis remain in the cuspal or appositional enamel, which covers the dentin horns (Beynon and Dean 1988). However, Retzius lines produced later in amelogenesis may terminate at the enamel surface to form structures called perikymata. Perikymata are broad, shallow, horizontal furrows that appear in the lateral or imbricational enamel (at the sides of the cap) and stretch around the circumference of the tooth crown (Smith, Martin and Leakey 2003; Mahoney 2012). Bromage and Dean (1985) stated that the first 20 to 30 Retzius lines do not reach the outer enamel surface, and so roughly six months of amelogenesis takes place before perikymata can be observed on the tooth crown.

Finally, accentuated markings, also known as irregular striae of Retzius or Wilson Bands (Smith, Martin and Leakey 2003; Simmer *et al.* 2010; Mahoney 2012) were also analysed in this investigation. Unlike the other markings, these lines do not represent regular increments or rhythmic variations in enamel formation. Instead, accentuated markings are caused by the temporary disruption or termination of enamel formation by ameloblasts as a result of pathology or stress (Dean and Scandrett 1996; Mahoney 2008; Mahoney 2015). Since enamel formation in humans takes place over several years, it is possible for a variety of stresses, physical or psychological, to disrupt it, including periods of fever, injury and nutritional deficiencies. Antoine *et al* (2009) explained that

accentuated markings either appear between or enhance regular Retzius lines within the enamel, and are associated with enamel hypoplasia on the outer surface. Enamel hypoplasia appears as furrows or pits on the tooth crown, which are formed when ameloblasts are disrupted and pulled away from the enamel formation front (Hillson 1996).

The first and most noteworthy accentuated marking is the neonatal line. Prenatal enamel does not contain accentuated markings and so the first irregular striae of Retzius is believed to be indicative of the stressful disturbance that occurs as a result of birth (Hillson 1996; Mahoney 2008; Mahoney 2011). This disturbance could be caused by the trauma of the birthing process, the nutritional transition between pre- and postnatal life (Berkovitz, Holland and Moxham 2002; Ross and Pawlina 2006) or it could be caused by a temporary onset of hypocalcaemia, which is when calcium levels in the blood plasma decrease in the first 48 hours after birth (Antoine, Hillson and Dean 2009). The neonatal line can be observed in deciduous teeth and occasionally permanent first molars using light microscopy and SEM (Hillson 1996). However, it appears to vary between individuals due to the differences between their births, which can make it difficult to identify.

2.3. Modern human variation in enamel thickness

Enamel thickness varies on the individual, population and species levels. The modern human dentition includes two sets of teeth, the deciduous and permanent dentition, both of which develop sequentially so as to establish a functional occlusion for mastication (Smith 1991). Enamel thickness has been shown to be significantly thinner in deciduous teeth compared to permanent teeth (Boyde 1989; Grine 2005; Mahoney 2010). For example, Smith *et al* (2012) cited modern human average enamel thickness

values, which represent the mean straight-line distance between the EDJ and the outer enamel surface, of approximately 1.08mm for the mandibular first permanent molars and 1.22mm for the maxillary first molars. On the other hand, Mahoney (2010) found enamel thickness ranged between 0.35-0.55mm in deciduous first molars. The variation in crown enamel thickness between deciduous and permanent teeth is most likely linked to their differing enamel formation times (Smith, Martin and Leakey 2003; Mahoney 2011).

This study focuses on the permanent molars, which develop in succession in all great apes. In humans, the first molar begins forming at birth and emerges at approximately 6 years of age, the second molar starts to form at 3 years and erupts around 12 years of age, and the third molar starts forming at 8 years and erupts around 18 years of age (Dean 2006; Dean 2010). As such the first molar begins developing before weaning, while the growth of the second and third molars occurs after weaning (Smith 1991; Hill and Kaplan 1999; Humphrey 2010; Smith *et al.* 2013).

Enamel thickness appears to increase in each successive tooth along the permanent molar row (Macho and Berner 1993; Schwartz 2000a; Mahoney 2013). Smith *et al* (2003) attributed this variation to the relative increase in the area of the enamel cap, as well as a decrease in the area of the dentin core, in posterior molars. Macho and Berner (1993) suggested the first molars may be expected to have the thickest enamel because they are in the functional occlusion, and so must resist environmental stresses, longer than the second and third molars. However, the authors explained that an increase in enamel thickness allows posterior molars to be functionally adapted to "sustain enhanced and prolonged masticatory loads" (Macho and Berner 1993, p.198). Other studies of enamel thickness and tooth function have supported the separation of the first and posterior

molars (Macho 1994; Macho and Berner 1994; Schwartz 2000a), and demonstrated the importance of analysing molar types separately before comparing overall enamel thickness between different populations or species.

Molar enamel thickness varies between extant great apes and fossil hominin species. Martin (1985) applied enamel thickness categories to the great apes based upon their relative enamel thickness (RET), which is a dimensionless index of enamel volume scaled to body size. The “thin” enamel (mean RET= 8.90-11.30) of chimpanzees, gorillas and gibbons was separated from the “intermediate/thick” enamel (mean RET= 14.65-17.49) found in organutans and the “thick” enamel (mean RET= 17.50-26.20) of humans (Martin 1985, p.261). The three-dimensional study of enamel thickness by Olejniczak e*t al* (2008) supported these categories, although chimpanzees were found to overlap with both gorillas and orangutans.

In another study, Olejniczak *et al* (2008) suggested Neanderthals had relatively thick enamel, like modern humans, and so the two species should be placed in the same category. This contradicts the assertion from Grine (2004) that European molars do not share relatively thin enamel with Neanderthals, which the author states is a unique feature separating Neanderthals from humans and other fossil hominins. Yet, Olejniczak *et al* (2008) explained that whilst Neanderthal molars have a larger dentin volume and EDJ surface area than modern human molars, which causes enamel to be thinner in the former than the latter, the volume of molar enamel in the two species does not differ. Thus, there is significant variation in crown enamel thickness across extant great apes, but less so between modern humans and recent fossil hominins.

Previous studies have shown molar enamel thickness may vary within modern human populations. Harris *et al* (2001) examined enamel thickness in black and white American children between the ages of 3 and 6 years old. They found deciduous molar enamel was thicker in black children than white children. The authors suggested this variation may be caused by an increased level of secretory activity in black individuals, as the period of crown formation is shorter but enamel is "disproportionately thick" compared to white individuals (Harris, Hicks and Barcroft 2001, p.223). The authors also discovered male children had larger molar crowns, but not thicker enamel caps, than female children. Although this study only examined deciduous teeth, it is supported by the analysis of permanent mandibular molars and canines by Schwartz and Dean (2005). In this study, Schwartz and Dean (2005) found molar crowns were larger in males than females, but this variation was attributed to the increased amount of dentin in male molars, as enamel thickness did not vary between the sexes.

Several anthropologists have also explored enamel thickness between modern human populations. In addition to comparing modern human molars with Neanderthal samples, Grine (2004) also contrasted enamel thickness in the permanent second molars of the European sample with a sub-Saharan African population. After combining maxillary and mandibular molars, Grine (2004) found RET did not differ between these two geographic populations. However, Smith *et al* (2006), who analysed permanent molars from North America, southern Africa, northern Europe (England) and a medieval population from Denmark, found enamel thickness was larger in the three modern human samples compared with the medieval Danish population. The authors stated this variation was linked with morphological differences, such as EDJ length. As a result, the authors contradicted Grine (2004) by suggesting enamel thickness may differ "among

regionally (and, to a degree, temporally) diverse modern human populations" (Smith *et al.* 2006, p.985).

Molar crown sizes have also been shown to vary between modern human populations. The study by Smith *et al* (2006) showed bi-cervical diameter was shorter in the Danish molars, and so tooth crown size was smaller, compared to the North American, southern African and northern European populations. The southern African molars exhibited the largest bi-cervical diameter and the third molars had significantly greater diameters than both the North American and northern European third molars. In a global comparison of dental samples, Hanihara and Ishida (2005) determined Australian teeth were the largest, followed by Melanesian, native American and sub-Saharan African samples. Teeth from western Eurasia and the Philippines were found to be the smallest. On the other hand, Górka *et al* (2015) discovered there was no significant difference in first molar crown size between global populations with diverse subsistence strategies, namely hunter-gatherers and agriculturalists, including populations from South Africa and the Congo. Thus, the authors suggested their results showed enamel morphology is not influenced by subsistence strategies or the associated food processing techniques.

2.4. Modern human variation in enamel formation

Like enamel thickness, enamel formation has been found to vary within individuals, as well as between hominin species and modern human populations. Within each permanent molar, ameloblast secretory activity increases across the enamel cap. This means the amount of enamel matrix secreted by ameloblasts is greatest towards the end of the secretory phase, at the outer enamel surface (Reid *et al.* 1998; Smith 2008; Kierdorf *et al.* 2014). This variation is evident in the comparative spaces between successive cross

striations, which correspond to the daily rate of enamel secretion (see Methods for Daily Secretion Rates). Smith (2008) recorded a lower limit of between 2-3µm/day and upper limit of 6-7µm/day for hominoid secretion rates. Whilst these ranges are consistent with those recorded for *Pan* (Dean and Shellis 1998; Lacruz and Bromage 2006; Dean 2010), early *Homo* (Beynon and Wood 1987) and the Neanderthals (Smith *et al.* 2007; Smith 2008), they are slightly lower than the maximum rate of 7.4µm/day in *Pongo* (Dean and Shellis 1998) and 7.3µm/day in *Australopithecus* (Beynon and Wood 1987).

Generally, modern human secretion rates are between 2-5.5µm/day (Risnes 1986; Beynon and Wood 1987; Boyde 1989; Risnes 1998; Reid, Beynon and Ramirez Rozzi 1998; Lacruz and Bromage 2006; Mahoney 2008; Birch and Dean 2009). This is because humans display less variation across the enamel cap than other hominoids. For example, Smith *et al* (2009) found the amount of enamel matrix secreted ranged from 2.99µm/day near the EDJ to 4.11µm/day at the outer enamel surface in seven mandibular third molars acquired from German dental practices. Similarly, Reid *et al* (1998) discovered outer secretion rates in the cuspal enamel of four French medieval (1150-1550AD) individuals were between 4µm/day in anterior teeth and 5.8µm/day in third molars, but secretion rates near the EDJ were consistent at around 3µm/day for the entire dentition.

The speed at which enamel-forming cells differentiate along the EDJ, which corresponds to the rate that teeth grow in height or extend, also varies within each molar (see Methods for Enamel Extension Rates). As Shellis (1984) explained, the rate of extension is highest near the cusp, where amelogenesis begins, and falls to a minimum value further down the EDJ towards the cervix. This variation reflects the "progressive slowing of enamel production towards the end of crown formation" (Reid *et al.* 1998, p.472). Dean

(2009) found cuspal extension rates ranged between 20-30µm/day in human canines and first molars. Yet, during their examination of British and southern African anterior teeth and molars, Guatelli-Steinberg *et al* (2012) discovered extension rates in the lateral enamel only reached a maximum of 16µm/day. These values are considerably lower than those provided for deciduous teeth. Mahoney (2015) recorded average extension rates of 52.03µm/day for the deciduous central incisors and between 35.75-40.05µm/day for the deciduous canines and molars of medieval British and modern Swedish populations. Since faster extension rates have been associated with shorter enamel formation times (Ramirez Rozzi and Bermudez de Castro 2004), it is unsurprising the deciduous dentition displays more rapid ameloblast differentiation at the start of amelogenesis, as these teeth develop over a shorter period of time than the permanent dentition.

Several studies have shown that enamel growth rates vary more between the anterior teeth than the molars of modern human populations. Reid and Dean (2006) and Guatelli-Steinberg *et al* (2007) examined growth rates in northern European and southern African dental samples [the same as those examined by Smith *et al* (2006)]. Both studies uncovered only minor variations between the molars from these two populations. As a result, Reid and colleagues have suggested that molar enamel formation does not vary across modern human populations. However, enamel growth rates in the anterior teeth, specifically the periodicity of perikymata and inferred formation times, were found to be significantly faster in the southern African samples. Both studies, as well as a later investigation by Reid, Guatelli-Steinberg and Walton (2008), also found growth rates were more likely to vary in the lateral enamel than the cuspal enamel. Most recently, Modesto-Mata *et al* (2015) observed significant differences in the lateral enamel formation of anterior teeth from northern European and southern African populations.

During their study of incisors from Spanish cave sites dating to the Copper and Bronze Ages, the authors inferred EERs by measuring the frequency and periodicity of perikymata on the outer surface of the lateral enamel. They discovered that extension rates varied significantly between, but not within, the Spanish and southern African samples. Despite these differences, Modesto-Mata *et al* (2015) suggested extension rates exhibited a shared pattern across modern humans which was distinct from fossil hominins.

Patterns of variation in enamel formation have been linked to evolutionary models within biological anthropology. As Stringer and Andrews (1988) explained, the model for the recent African origin of the human species, which is widely accepted in paleoanthropology, predicts genetic and morphological variation should be greatest within African populations. This prediction is supported by genetic evidence. Hunley *et al* (2016) found the diversity of genes steadily decreased as over-land distance from east Africa increased. The highest level of gene diversity was present in the South African Bantu population and the lowest in the south American Suruí samples. Similarly, Hanihara and Ishida (2005) found intraregional variation in dental morphology was largest in the sub-Saharan African population.

However, despite the evidence for an imbalanced proportion of variation across modern human populations, Reid and Dean (2006) argued a similar pattern of variation is not present in molar enamel formation. The authors stipulated the average duration of amelogenesis for molar samples with comparatively similar genetic origins (North Americans and northern Europeans) showed the same level of variation as molar samples from populations with more diverse genetic backgrounds (southern Africans and northern Europeans). Furthermore, the results of previous studies, as outlined above,

appear to support Reid and Dean's suggestion that enamel formation varies consistently across the human species, and so it would be "reasonable to expect similar variation(s) among other past and present populations of modern humans" (2006, p.344).

2.5. Factors influencing enamel thickness and formation

Tooth growth is largely determined by genetics (Lewis and Garn 1960). Genetic conditions such as amelogenesis imperfecta, which causes hypomaturation and hypocalcification of dental enamel, show the direct influence that specific genes have on ameloblast growth and function, and consequently on enamel thickness and formation. For example, Horvarth *et al* (2014) outlined the role of the MMP20 gene. This is responsible for producing the enzyme enamelysin, which remodels the enamel matrix proteins that act upon hydroxyapatite crystals. As such, this specific gene has been shown to have a key role in enamel formation.

However, even though enamel formation is relatively resilient to environmental stresses, all growth is liable to environmental restrictions (Tonge and McCance 1973). It is important to remember teeth are the only part of the human skeleton that directly interacts with the environment through the process of mastication (White 2012). Several authors have argued enamel thickness varies between molar cusps in response to functional differences during mastication (Macho and Berner 1993; Spears and Macho 1998; Schwartz 2000a; Schwartz 2000b; Mahoney 2008). Buccal cusps appear to have a thicker layer of enamel than lingual cusps on the mandibular molars, and vice versa for the maxillary molars, which is believed to provide an increased resistance to wear and mechanical loading during phase II (crushing and grinding) of mastication (Kay and Hiiemae 1974). Grine (2005) also suggested enamel is thicker over the lateral aspect of

these cusps because it protects against the pressures generated at the tips of the occlusal surfaces and so prevents fractures. These variations show how human enamel thickness has evolved to cope with environmental stresses, although the biomechanical behaviour of mandibular and maxillary molars is unclear (Spears and Macho 1998).

This study focused on the impact of environmental factors, specifically diet, on amelogenesis across modern human populations. Several studies have shown how enamel formation and thickness vary in response to broad dietary patterns. For example, folivorous primates (herbivores primarily eating leaves) have been shown to have thin-enamelled molars but thick-enamelled incisors (Macho and Berner 1993). Macho and Shimizu (2009) suggested this is because tough leaves are broken down through vertical mastication whereas more plastic foods require grinding and crushing. Similarly, Dirks (2003) determined molar development was faster in folivores than frugivores (herbivores primarily eating fruit) because the ecological risks of prolonged development were lower for the frugivores. The thick enamel of humans enables our species to take advantage of an omnivorous diet, including a variety of soft, hard and fibrous food sources from plants and other animals. Yet, there is some debate over the impact these broad dietary preferences have on human enamel formation and thickness. Macho and Berner stated even though enamel thickness is broadly indicative of dietary adaptations, it may provide more insight into the "biomechanical constraints on the masticatory apparatus" (1993, p.198).

One particular study suggests seasonal climatic variations, as well as dietary changes, may influence modern human enamel thickness and formation. Żądzińska *et al* (2013) discovered the season of birth of modern human children from Poland was significantly

correlated with the prenatal enamel thickness of their deciduous incisors. The thickest prenatal enamel was present in children born in autumn (September to November). The authors suggested seasonal variations in insolation (amount of solar radiation reaching the Earth's surface) may be responsible for this variation. They described how during periods of limited insolation, the biological production of vitamin D is restricted, so enamel growth may be slower and enamel thickness may be reduced (Żądzińska *et al.* 2013). This is important as vitamin D is necessary for calcium metabolism (see below). As such, insolation could explain why prenatal enamel was thickest in the children born in autumn, whose prenatal enamel formation encompassed the summer months. Yet, Żądzińska *et al* (2013) also asserted pregnant mothers are more likely to suffer from a nutritional vitamin D deficiency in the winter months, and so the thinner prenatal enamel of children born in spring and summer months could be linked to a lack of vitamin D in their mother's diet. Although this study presented an interesting example of environmental factors influencing amelogenesis, seasonal climatic or nutritional changes during pregnancy are unlikely to affect the enamel thickness of permanent molars, which develop after birth.

Fewer studies have examined the effect of specific macro- and micronutrients, rather than generalised dietary preferences, on enamel formation and thickness. The effect of dietary proteins, carbohydrates and fats, as well as calcium, phosphorous, vitamin A and vitamin D, which have all been shown to alter amelogenesis, are outlined below. Although the impact of environmental diseases is not reviewed in this chapter, it should be noted that they can also alter enamel formation and morphology if they disturb the developing tooth germ (Aiello and Dean 1990).

2.5.1. Proteins and calories

As stated previously, proteins have an important role in incorporating calcium phosphate into enamel matrix during mineralization. Simmer e*t al* (2010) described how amorphous calcium phosphate is converted into calcium hydroxyapatite and then arranged into a crystalline structure using enamel proteins. Some proteins, including amelogenins, ameloblastins and enamelins, are particularly important for amelogenesis. In fact, the start of enamel secretion is linked with an up-regulation of amelogenin and the initial release of ameloblastin and enamelin (Simmer *et al.* 2010). The majority (90%) of enamel proteins are amelogenins (Hillson 1996; Diekwisch *et al.* 2009; Kierdorf *et al.* 2014). Ross and Pawlina (2006) stated amelogenins help to establish and maintain the layout of prisms at the start of enamel formation. Both amelogenins and ameloblastins, which are signalling proteins believed to be part of enamel crystal development, are removed during the maturation stage. On the other hand, enamelins are scattered throughout the mineralization front and maintained in mature enamel (Boyde 1989; Ross and Pawlina 2006). If any of these proteins are missing, enamel mineralization may be disrupted. Proteins are synthesised from amino acids and, as Bavetta *et al* (1962) explained, the pathological effects of protein deficiency can occur even when an individual's diet lacks a single amino acid. However, the authors also argued that it can be difficult to determine which is responsible for a deficiency, as protein synthesis is determined by the interrelationships between multiple amino acids.

A steady supply of energy from fats and carbohydrates in the diet is also necessary for healthy enamel formation. Maciejewska and Adamowicz-Klepalska (2000) found ameloblast proliferation was slower in the developing enamel organs of rats who experienced malnutrition in their prenatal life. As a result, they asserted the diet of

pregnant rats needed to include enough fats and carbohydrates as well as protein to support enamel growth, especially the bud stage of amelogenesis, in their developing offspring. Although this study focused on rats, May *et al* (1993) suggested enamel hypoplasia is more prevalent in human groups with low socioeconomic status because of the increased risk of energetic malnutrition. In their study of Guatemalan children with fat- and carbohydrate-deficient diets, the authors discovered increasing calorie consumption through supplementation was linked with a reduction in the number of enamel defects. May *et al* (1993) argued cells in the developing teeth were able to utilise the additional source of energy from the calorie supplement rather than extracting it from protein metabolism. They suggested using protein to supply energy was causing a secondary deficiency in these children, which disrupted amelogenesis and lead to the formation of hypoplastic defects. Thus, fat and carbohydrate levels in childhood diets must be high enough to supply energy for amelogenesis so proteins and amino acids are readily available for enamel mineralization.

Several studies have compared the effects of protein- and calorie-deficient diets on enamel growth. Bavetta *et al* (1962) compared molar development in rats fed diets with limited levels of calories, proteins and tryptophan (an amino acid). They found the teeth of the calorie- and tryptophan-deficient groups were more immature than a control group, which suggested both diets affected the rate of enamel formation. On the other hand, Luke *et al* (1981) found molar development in pigs fed a calorie-deficient diet for their first year of life did not recover as well after rehabilitation as it did in pigs fed a protein-deficient diet. This could be explained by the evidence from May e*t al* (1993), as the calorie-deficient diets could induce a secondary protein deficiency which would also affect enamel formation. This evidence supports the argument that it is often difficult to

determine which nutritional deficiency is negatively affecting growth and development as individuals, specifically children, rarely suffer from only one (May, Goodman and Meindl 1993). Altogether, these studies suggest diets deficient in calories have a greater impact on enamel formation than those with limited protein levels. This could be because the energy necessary for amelogenesis is derived from dietary proteins when children consume a calorie-restricted diet, which causes a secondary deficiency, and so prevents enamel mineralization.

2.5.2. Calcium, phosphorous and vitamin pathways

As stated previously, enamel almost exclusively consists of calcium phosphate in the form of crystalline hydroxyapatite (Boyde 1989). Simmer e*t al* (2010) described how, during the maturation stage of amelogenesis, ameloblasts transport calcium and phosphate ions into enamel matrix whilst removing water. This exchange makes enamel the hardest substance in the human body, which allows it to protect teeth during life and also explains why teeth are frequently preserved in the fossil record. As such, calcium and phosphate ions are essential to enamel structure and mineralization (Ross and Pawlina 2006; Hillson 2014). The levels of calcium and phosphorous available for enamel mineralization is partly determined by dietary intake so, as Mellanby and Mellanby (1948) explained, infant diets during enamel growth should be rich in both of these nutrients.

Dietary deficiencies in calcium and phosphorous during infancy can cause diminished or pathological enamel formation. For example, Ranggård and Norén (1994) induced hypocalcaemia in rats for three weeks and found maxillary incisors were smaller in these animals than in the control group, which was fed a normal, calcium-rich diet. The smallest rat also exhibited enamel hypoplasia with delayed mineralization. Enamel hypoplasia

form during periods of physiological stress that disrupt the functioning ameloblasts and so delay or stop amelogenesis (May, Goodman and Meindl 1993; Larsen 1995).

In humans, the most widely cited example of hypoplasia in response to hypocalcaemia is the neonatal line, which appears to show the onset of calcium deficiency within the first 48 hours after birth (Antoine, Hillson and Dean 2009). As Hillson (2014) explained, the mother supplies the developing infant with calcium through the placenta during gestation. But, once the infant is born, it must provide itself with calcium, either by extracting it from the skeleton or absorbing it from the digestive tract. Hence, it is unsurprising the shift in calcium supplies at birth would lead to enamel hypoplasia as this causes a sudden systemic stress that could disrupt amelogenesis. However, it is important to remember that hypoplasia represent a temporary disruption, not a permanent problem. Thus, even though amelogenesis is more sensitive to nutritional fluctuations than other aspects of dental development, such as tooth morphology or timing of eruption (May, Goodman and Meindl 1993), these disruptions might not cause a significantly altered pattern of enamel formation.

The amount of calcium and phosphorous available for amelogenesis is partly controlled by vitamins D and A. Vitamin D is important for calcium metabolism and consequently the calcification of hard tissues within the body, including dental enamel (Mellanby and Mellanby 1948; Lézot *et al.* 2006; Hillson 2014). Like calcium, newly born infants must supply themselves with vitamin D by absorbing it from sunlight through their skin or their diet through digestion. Vitamin D directly impacts enamel protein levels during amelogenesis, which control enamel matrix mineralization. Lézot *et al* (2006) asserted

that most biomineralization proteins within teeth are controlled by vitamin D, including amelogenins, ameloblastins and enamelins.

Although a link has been established between vitamin D and enamel mineralization, the mechanism or pathway responsible for this regulation is unknown. As Zhang *et al* (2009) outlined, vitamin D systematically manages the concentration levels of calcium and phosphorous by activating and deactivating absorption within the intestines and the kidneys, as well as extracting calcium from mineralized tissues like bones. On a localised level, vitamin D metabolites have been shown to bind with receptors in ameloblasts, which regulate calcium and phosphate ion levels and protein activity (Lézot *et al.* 2006; Żądzińska *et al.* 2013). However, the precise function and effect of specific vitamin D metabolites on enamel formation remains unknown.

Although the mechanisms by which they control amelogenesis are unknown, it is clear that vitamin A and D deficiencies can disrupt enamel mineralization. Vitamin A has a similar systemic effect on calcium and phosphate ion levels as vitamin D (Lézot *et al.* 2006). Boyle (1933) described how vitamin A deficiency can cause the developing enamel organ to atrophy, ameloblasts to degenerate or amelogenesis to cease altogether. In their study of skeletal growth in response to vitamin D deficient diets, Lézot *et al* (2006) found mice developed pathological conditions similar to hereditary rickets in humans, along with hypocalcaemia and hypophosphataemia. The authors also stated vitamin D deficiency can lead to pathological enamel formation similar to amelogenesis imperfecta, as there is an increase in interprismatic areas which causes prism sizes to diminish and so regions of hypomineralization form within the enamel cap. Likewise, Zhang *et al* (2009) described how disruption of the vitamin D pathway, whether from genetics, dietary

deficiencies or other environmental factors, causes inadequate calcium and phosphorous levels in the blood plasma, which can reduce the biomineralization of bone, dentin and enamel. However, Lézot *et al* (2006) discovered if mice fed diets deficient in vitamin D were later given diets rich in calcium and phosphorous, levels within the blood plasma were restored and normal bone development resumed. This suggests biomineralization processes, including amelogenesis, are able to recover from temporary disruptions in the supply of calcium and phosphorous as well as vitamins A and D.

However, enamel formation can be permanently disrupted by prolonged vitamin A and D deficiencies, which is why both must be present in the diets of developing infants. As Mellanby and Mellanby (1948) explained, milk and cod-liver oil both contain high levels of vitamin A and D and, along with tablets, enriched margarine and flour supplemented with calcium carbonate, were supplied to expectant or nursing mothers and infants in Great Britain during World War Two. This meant foods with elevated calcifying properties were provided to infants throughout enamel formation, and so their enamel was more mineralized and resistant to pathological lesions, like caries, than previous generations.

Thus, specific foods, including milk, have been shown to influence enamel formation because they contain high levels of vitamins A and D, calcium and phosphorous. The study by Mellanby and Mellanby (1948) suggests infants with easy access to milk and other foods will have healthier teeth with more mineralized enamel than children whose diets do not include foods with high calcifying properties.

2.6. Applying enamel incremental markings to studies of human life history

Enamel is highly mineralized and does not heal itself or reform throughout life (Ross and Pawlina 2006; Nanci 2008) or during fossilization (Aiello and Dean 1990). This means the incremental markings within enamel are permanently preserved in the archaeological and fossil record. As such, dental anthropologists can use histology (see Methods) to produce two-dimensional cross-sections of enamel (Mahoney 2010) in order to study incremental markings and establish an "independent chronology of crown formation" for fossilised individuals (Antoine, Hillson and Dean 2009, p.53). This chronology is very precise as it is accurate to within days and weeks rather than months and years (Reid and Dean 2006). Since dental eruption and development are considered to be proxies for somatic growth (Smith 1992; Robson and Wood 2008), this timeline also tracks an individual's development from birth to adulthood. Therefore, as Dean (2010) explained, histological analysis of dental enamel provides a unique opportunity to reconstruct the maturation period of ancestral hominins in order to compare them to extant species. These comparisons can highlight trends in the evolution of human growth, nutrition and life history.

2.6.1. Life history theory

Life history theory is the accumulated set of hypotheses employed to explain how evolutionary forces, such as genetic, environmental and energetic constraints, alter an organism's phenotype or observable characteristics, and the consequential relationships between these traits (Hill and Kaplan 1999; Smith 2013). Studies of life history focus on the biological choices an organism makes during the course of its life, including how much energy it allocates to growth, maintenance and reproduction (Smith and Tompkins 1995). Harvey and Clutton-Brock (1985) listed a range of life history variables including the age-

at-weaning, age of first reproduction and maximum lifespan. Duration of dental development is another life history variable. It is currently the principal means of determining the life history patterns of fossil hominins because it is strongly associated with somatic growth (Kelley and Smith 2003). As Robson and Wood (2008) outlined, the microstructure of dentin and enamel, as well as the overall rate of tooth crown and root formation, can be compared between fossils so as to gauge the relative speed of hominin life histories.

2.6.2. Evolution of Human Life History

Life histories are often characterised as fast or slow with regards to the pace of development, rate of reproduction and maximum lifespan. For example, larger organisms usually have slower life histories because of the additional time and energy allocated to growth, and are expected to live longer in order to maximise the benefits of their extended development. Primates, especially great apes, have slow life histories and extended periods of juvenile development that have been linked to a reduced risk of infant mortality from predation (Hillson 2014).

However, humans have a uniquely prolonged life history compared to other great apes (Dean 2006). It also differs from fossil hominins, which exhibit a more rapid ape-like development. Dean *et al* (1993) reconstructed tooth crown formation times and enamel secretion rates for a series of *Homo erectus*, Australopithecine and Neanderthal fossils, and found all three exhibited a faster enamel growth trajectory than modern humans. In a later study, Smith *et al* (2010) counted the Retzius lines present on Neanderthal and ancient *Homo sapiens* dental fossils and discovered that, whilst these species had a similar period of tooth formation, tooth crown growth was more rapid in Neanderthals.

The authors suggested this variation was caused by the thinner enamel, shorter Retzius line periodicities and faster extension rates present in Neanderthals. Both studies support the theory that the extended period of growth in modern human infants, including dental development, evolved relatively recently (Smith *et al.* 2013).

Dental emergence refers to the movement of the tooth crown past the margin of the jaw (alveolar eruption) and gum (gingival eruption) (Smith 2013). In humans, the first deciduous molar emerges after the deciduous incisors, but the first permanent molar emerges before the rest of the permanent dentition (Hillson 2014). This is significant as the emergence of the mandibular first permanent molar is highly correlated with other life history variables in primates. Smith (1989) argued it was strongly associated with neonatal (r= 0.99) and adult (r= 0.98) brain weight as well as age-at-weaning (r> 0.9). Weaning is often viewed as the cessation of suckling, which is the principal method by which mammalian mothers provide nutrition to their newly-born infants. However, as Humphrey (2010) explained, weaning is a process that begins with the first ingestion of non-milk foods and ends when suckling stops, and so it can take place over days, months or even years. Smith e*t al* (2013) discovered infant chimpanzees in Kanyawara, Kibale National Park, Uganda began consuming solid foods at roughly 6 months old, but continued nursing until at least 4 years old. Thus, the correlation between age-at-weaning and emergence of the mandibular first permanent molar appears to be caused by the introduction of solid foods and the subsequent need for a functional dentition (Kelley and Smith 2003).

However, human mandibular first permanent molars emerge much later than other primates (Kelley, Dean and Ross 2009). As Hillson (1996) explained, dental development

in apes takes up to 12 years compared to over 18 years in modern humans, and the first permanent molar erupts between 3-4 years of age in other apes compared to 5-7 years of age in humans. However, the weaning process occurs relatively early in modern humans compared to other extant apes. Smith *et al* (2013) found chimpanzees did not cease suckling until over 4 years of age and Robson and Wood (2008) stated orangutans wean their offspring between 6-8 years of age. In humans, Humphrey (2010) suggested weaning can start when the infant is 5-6 months old, based on the nutritional content of human maternal milk, and may continue until they are approximately 2.4 years old.

Consequently, first permanent molar emergence is not associated with age-at-weaning in modern humans and, as it is unclear when the uniquely early age-at-weaning in humans evolved, the correlation may not have been present in extinct hominins. This means there is currently no method of identifying age-at-weaning from the fossil record. Yet, since enamel incremental markings provide a precise chronology of enamel formation (Reid and Dean 2006; Antoine, Hillson and Dean 2009) and amelogenesis is more sensitive to nutritional changes than dental emergence (May, Goodman and Meindl 1993), it may be possible to use dental histology to identify age-at-weaning and then compare the life history patterns of our hominin ancestors.

Chapter 3: Materials

3.1. Outline

A brief explanation of the human molar samples (Fulbe, Nso and European) analysed during this investigation is provided below. The main methodology applied to these samples was histology. However, SEM was also used to briefly explore the size of enamel prisms.

3.2. Fulbe and Nso

3.2.1. Background

An estimated 20 million Fulbe live across 15 modern nation-states in western African, including northern Cameroon (Walker 1980; Regis 2002; Moritz 2012). Despite the spread of urbanisation and agriculture from colonial occupation, the cultural identity of the Fulbe is rooted in pastoralism. Eguchi (1994) defined pastoralism as a lifestyle centred around cattle, including rearing, herding and milking. For the Fulbe, cattle not only represent a source of subsistence, but are also a symbol of wealth, security, prestige and community (Walker 1980; Eguchi 1994; Moritz, Ritchey and Kari 2011). Moritz asserted "without cattle one cannot live as Fulbe" (2008, p.109).

This cultural focus on pastoralism is evident in the widespread consumption of milk and milk-based products throughout the Fulbe population. Rural communities, referred to as Mbororo'en, raise cattle through nomadic pastoralism and trade their produce for millet and vegetables with urban groups (Walker 1980; Schultz 1984). This includes fresh milk, yoghurt and butter (Walker 1990; Moritz 2008), which can be consumed upon purchase

or as prepared meals such as the milk-based porridge ‘gaari’ (Regis 2002). Thus, milk and its various derivatives form a prominent part of the traditional Fulbe diet.

The Nso are the largest ethnic group (approximately 150,000 people) of the Bamenda Grassfields in the Northwest Region of Cameroon (Chilver and Kaberry 1970; Nsamenang and Lamb 1995; Trudell 2006; Veeramah *et al.* 2008). This area is adjacent to the Adamawa Region, as shown in Fig. 3, where communities of both sedentary and nomadic Fulbe reside. However, unlike their Fulbe neighbours, the Nso live in traditional family-run farming communities and grow crops such as maize, potatoes and beans (Keller, Demuth and Yovsi 2008; Otto, Potinius and Keller 2014). Hence, milk and milk-based products are not culturally significant to the Nso, unlike the Fulbe, and so these foods do not dominate their traditional diet.

Fig. 3- Map of Cameroon
(Cameroon Association for Responsible Tourism (CAMAST) 2016)

3.2.2. Samples

The dental samples examined during this investigation came from clinical extractions conducted at Kumbo, which is in the Northwest Region of Cameroon, as shown in Fig. 3. Anonymous informed consent was granted by each donor. No dental sample could be traced back to an individual. This form of sampling meant an assortment of all three types of mandibular and maxillary permanent molars were available for analysis. As the samples were taken from individuals of known sex, enamel growth and morphology could be compared between males and females as well as before and after the weaning process. The first molar begins to grow before weaning, but the second and third molars usually develop after the cessation of weaning (Smith 1991; Hill and Kaplan 1999; Reid and Dean 2006; Humphrey 2010; Smith *et al.* 2013). Thus, if the weaning process or post-weaning diets of the two groups have any impact on enamel development, it should be most prominent when comparing across the molar row between the agriculturalists and pastoralists. Such evidence could highlight the influence of diet on enamel formation as well as the potential for using histological techniques on hominin fossils to identify the onset of weaning.

The analysis of clinical samples also meant the majority of teeth exhibited pathological damage. Some molars had to be excluded, but only if they could not meet at least one of the following selection criteria. These were designed with the key measurements of the study in mind so as to maximise the reliability of the data collected from the Fulbe and Nso molars. Firstly, the outer enamel surface needed to be intact in order to measure the average thickness or overall area of the enamel cap (see 'Average Enamel Thickness' and 'Crown Size' in section 4.4 of Methods). These measures were not taken from any molar samples with wear or caries along the outer surface of the tooth, and were not based

upon reconstruction of the enamel cap. Secondly, an intact and well-preserved EDJ was needed to measure the dimensions of the enamel cap as well as the average extension rates within it (see 'Enamel Extension Rate' in section 4.4 of Methods). Thus, once the molars were sectioned, those with caries or other pathological damage affecting the clarity of the position of the EDJ were not chosen to measure these variables. Finally, the cuspal enamel, specifically between the dentin horn and the occlusal surface of the tooth, needed to be clear from pathological damage so as to calculate mean extension and secretion rates (see 'Daily Secretion Rate' in section 4.4 of Methods). Hence, these measurements were not taken from any molars with pathological damage that obstructed the view of incremental markings within the cuspal enamel.

It should be noted that, due to the limited number of samples available from the two populations, each molar only needed to meet one of the criteria listed above. For example, extension rates could still be measured in molars with extreme wear if they had a visible EDJ and clear accentuated lines. Also, only a single cusp from each molar needed to meet either one of the criteria in order to measure average enamel thickness, secretion or extension rates. This meant as many measurements were taken from each available sample as possible. Lastly, to maximise the sample sizes, sections were made from every appropriate molar from each individual. As a result, in instances when an individual had donated more than one molar, multiple sections representing multiple molars were made from a single individual's dentition. Thus, as Mahoney (2015) explained, for every type of molar, each histological section represents a single piece of data for each variable within the investigation.

In total, 35 molars from the two African populations were analysed. This included nine Fulbe and 26 Nso molars. Males and females were evenly represented in both samples. However, there was a larger proportion of second molars in both samples compared with the other molar types (see Table 1).

3.3. European

3.3.1. Background

A further sample of 21 first molars from three British archaeological populations were examined and compared to the combined African (Fulbe and Nso) samples. This comparison could highlight any geographic variations in enamel growth and morphology. The comparative European population was collated from the Powell Cotton Museum, St Gregory's Priory and Cemetery, Canterbury (curated in the Human Osteology Lab at the University of Kent), and the Beaker People Project. These collections include individuals from the British Bronze Age and medieval period (Mahoney 2011), recent medieval period (11th to 15th century AD) (Hicks and Hicks 2001), and the early Bronze Age (2500BC to 1500BC) (Parker Pearson *et al.* 2016), respectively. All of the first molar samples within the comparative European population originated from skeletal remains with an unknown age and sex.

3.3.2. Samples

All of the European samples, but not the African sample, had been previously sectioned and mounted onto microscopic slides using the same processes as this investigation. Although these molars were better preserved than the clinically extracted samples from the Fulbe and Nso, they still exhibited some pathological damage. As with the African

specimens, the three selection criteria outlined above were applied to the European samples in order to maximise the reliability of the data collected from this population.

Table 1 below shows the number of first, second and third molars from each population that were examined during this investigation. No distinction was made between mandibular and maxillary molars due to the limited sample sizes present.

Table 1- Frequency of each molar type from the Fulbe, Nso, combined African and European populations examined in this study

	M1	M2	M3	Total
Fulbe	2	5	2	9
Nso	7	11	8	26
Combined African (Fulbe + Nso)	9	16	10	35
European	21			21

Chapter 4: Methods

4.1. Outline

The techniques applied to the Fulbe, Nso and European molars examined during this investigation are explained in detail within this chapter. It is split into four sections for each stage of data collection: histology, microscopy (including scanning electron microscopy or SEM), data recording, and statistical analysis.

4.2. Histology

4.2.1. Sample selection

The primary method used to analyse the morphology of the Fulbe, Nso and European dental samples was histology. As mentioned previously, histology produces a two-dimensional cross-section, which displays the incremental markings that can be quantified to recreate enamel formation (Mahoney 2010). For this investigation, sections were taken from a range of all three mandibular and maxillary molars. Previous investigations (Mahoney 2013; Mahoney 2015) uniformally sectioned molars across the mesial cusps (protoconid and metaconid for mandibular molars and protocone and paracone for maxillary molars). Whilst this approach is preferable in dental histology, the teeth in this investigation were originally taken during clinical extraction, as explained previously, which meant the majority had some pathological damage on one or both cusps. This resulted in a mixture of mesial and distal sections of different cusps being assessed depending on whichever presented the least damage and clearest incremental markings.

To complete histological sectioning safely, powder-free nitrile gloves and laboratory coats were worn at all times. Also, the various chemicals used throughout the process were treated with caution, as many of them are hazardous. Permits were not required but all sectioning adhered to the British Association of Biological Anthropology and Osteoarchaeology Code of Practice (2014).

4.2.2. Embedding

One advantage of studying enamel is that, unlike histological methods used on organic materials, preparation of dental tissues does not require a fixation stage. This is when the metabolic processes occurring within the cell are stopped and pathogenic microbes like bacteria are killed (Hillman 2000; Dusevich, Melander and Eick 2012). Thus, the first stage of dental histology involved embedding the teeth in epoxy resin. Although the majority of the clinical specimens from Cameroon had been previously embedded, or even sectioned, these processes have been described in the methodology below. The resin provided support for the specimens and ensured they were hard enough to cut, grind and then polish down into wafer-thin sections without splintering or damaging the specimen (Hillman 2000; Reed 2005; polyester resin used in Mahoney 2010).

The embedding process began with labelling the selected plane of sectioning. All the teeth were sectioned along a longitudinal plane that stretched from the cusp down to the apex of the root and across from the buccal to lingual side of the tooth. The tip of the cusp and most cervical extension on the transverse side, where the enamel and root converge along the EDJ, were highlighted with a permanent marker to help with orientating the tooth during sectioning (Reid, Beynon and Ramirez Rozzi 1998). It is critical to use these points to orientate the cut because they ensure the section is centred

along the tip of the dentin horn (Hillson 2014; Mahoney 2015). This marks the starting point of enamel formation and so provides the fullest record of incremental markings throughout the enamel cap (Antoine, Hillson and Dean 2009; Kierdorf *et al.* 2014). A label with all of the essential information about the tooth section was made to stick to the microscopic slide later. This included: the code of the site the tooth originated from; identification number of that individual; type of tooth; whether it was the mesial or distal side of the section; and the number of the section.

After the tooth had been marked, releasing agent was wiped around the inside of an embedding tub. This is a petroleum distillate solution used on cold mounting materials, such as epoxy, acrylics and polyester, which contains naphtha (petroleum) and light alkylate trimethylated silica. It lubricates the tub and makes it easier to remove the block of resin later. The tooth was placed into the tub and moved to one side so it was easier to remove excess resin during sectioning.

Next, the epoxy resin and hardener were cured. A four-to-one ratio of EpoxiCure™2 epoxy resin and hardener was mixed together in a plastic cup until the two solutions were combined and streaks were no longer visible. The key ingredients of the epoxy resin are a combination of bisphenol A and epichlorohydrin, whilst the hardener is based on an epoxy polyamine adduct solution. Once the solution was mixed, it was poured into the embedding tub until it covered the tooth and then the tooth, resin and tub were left to dry out and harden for 24 hours. During this time, polymerization occurred (Hillman 2000). Singhrao *et al* (2012) described polymerization as the process by which bonds form between hydrocarbon chains of monomers (molecules) to form a polymer (macromolecule made of multiple repeated units). The authors also noted the relatively

lengthy process of tissue infiltration by epoxy resins is partly caused by the viscosity of the resin mixes. Hillson (2014) explained that the resin must have a low viscosity so that it can enter the microscopic gaps within the tooth before it polymerises into the shape of a hard block that will protect the specimen. This is also why it is important to fill the plastic cup and check there are no bubbles in the solution before it hardens, as these could hinder the polymerization process and so prevent the mould from hardening (Singhrao, Nicholson and Crean 2012).

4.2.3. Sectioning

After the tooth was embedded and the resin was dry, the specimen was then sectioned using a diamond-wafering blade saw (Buehler IsoMet 1000). This machine includes a "slowly rotating metal disc blade coated with industrial diamond", which allows it to cut very thin slices out of hard substances, including resin and enamel (Hillson 2014, p.263). Several key preparations were necessary to use the saw safely. Firstly, water and a small amount of water-soluble IsoCut® Plus Fluid were poured into the cutting draw. This cutting fluid is a coolant that keeps the blade lubricated and revolving smoothly. The blade was attached and secured using flanges that prevented the blade from moving out of alignment. The block of resin was fixed into a flat chuck and aligned with the blade along the previously highlighted plane of section.

Finally, the balancing weight was brought to the top of the gravity tilt so that the embedded tooth rested firmly on the blade. In most cases, two cuts were made, firstly to remove excess resin, and then to section the dental specimen. At the start of cutting, the speed of the blade was set to 100 revolutions per minute (rpms). Shortly after the sawing started, the revolutions were slowly increased to a maximum of 300rpms once the blade

began cutting the enamel. Carefully managing the speed of the saw can help to prevent damaging the surface of the tooth and preserve the detailed incremental structures within the enamel (Silva, Moreira and Alves 2011). After the specimen was sectioned, the two halves were removed and set aside for mounting.

4.2.4. Mounting

The third step of the histological method was mounting the tooth section onto a microscopic slide. Due to the limited number of available samples, both halves of each cleaved tooth were mounted and analysed. To start mounting, the section was washed under tap water and the edge of the freshly-cut surface removed by swiping it along a waterproof silicon carbide paper or grinding pad with a 600 to 1000 grit size. The section was washed again and left to dry. Once it was dry, the section was stuck to a clean microscopic slide using an adhesive, in this case Evo-Stik Express Epoxy Glue. This epoxy medium has the same four-to-one ratio of EpoxiCure™2 epoxy resin and hardener as the mixture used to embed the tooth. A small amount was lathered onto the face of the section and quickly stuck to the slide as the mixture hardens within 60 seconds. The section was gently nudged around the slide to ensure it was firmly attached and would not come off when additional reagents and mechanical processes were applied during later stages (Hillman 2000). The slide was placed on a flat surface that would keep the tooth section stationary as the epoxy was left to dry for a further 24 hours.

Alternative mounting mediums were also considered. The J-B Weld ClearWeld, Quick-Setting Epoxy and MegaFix SLOW Epoxy Adhesive kits were tested on practice sections. The first epoxy resin advertises a five-minute setting time, and a curing time of only one hour. The second kit specifies a slow setting time and allows for repositioning within 30

minutes of application. Like the Evo-Stik Express, both the ClearWeld and the MegaFix dispense equal amounts of resin and hardener, and so can be applied using the same technique. Both kits are suitable for glass. The ClearWeld is designed to cure with a clear, transparent bond when a layer of less than 15mm is applied to a surface, which is essential for histological analysis. Both the ClearWeld and the MegaFix appeared to form a strong bond between the practice section and the slide throughout the histological methodology. The MegaFix was transparent after curing, and incremental lines were clearly visible under the microscope. However, the ClearWeld did not form a transparent bond after curing. When examined under a microscope, little black dots, which appeared to be air bubbles, were present throughout the section. These impurities obstructed the view of the sample and prevented accurate assessment of the incremental lines within the enamel. It is unclear why these bubbles appeared, although it could be connected with the shortened setting and curing time. Although the kit advertises curing after only one hour, it must be left overnight to cure fully. The rapid curing within the first hour could increase the viscosity of the resin, which may trap or slow the movement of air bubbles (Ellis 1993; Hamerton 1996). Therefore, the MegaFix SLOW Epoxy Adhesive kit is a suitable alternative to the Evo-Stik Express Epoxy Glue, but the J-B Weld ClearWeld Quick-Setting Epoxy is not.

4.2.5. Grinding and Polishing

Following the mounting stage, the tooth section was lapped down to its final width. The excess dental tissue was removed by additional sawing using the Buehler IsoMet 1000, although for this stage the mounted specimen was placed into a microscopic chuck with the exposed tooth crown orientated closest to the blade. The two flanges were placed to one side of the blade so it was closer to the slide. A 1.5mm space was left between the

blade and slide, which removed as much of the excess tooth as possible during sawing. The excess tissue removed from the tooth was labelled and stored in a clear plastic bag. The tooth section and slide were washed.

Next, the microscopic slide was placed into a wetted handheld grinder or jig. As Hillson (2014) explained, this jig uses a vacuum to hold the microscopic slide in place and keep it in contact with the grindings pads so that the tooth section can be lapped down in a parallel plane to the slide itself. The section was held against a saturated grinding pad (grit size between 300 and 600) attached to the rotating plate of a Buehler EcoMet 300. During grinding, the long axis of the tooth was aligned with the rotating plate, which was set at a speed of 70 rpms. This prevented the microscopic slide from chipping as the surface of the tooth section was ground down. Each dental sample was initially polished with the coarser, low-graded grinding pads and, when as much of the excess enamel had been removed as possible, the section was manually ground to its final, wafer-thin width using a finer pad (600 to 1200 grit size).

Finally, the section was polished using a cushioned polishing plate in place of the grinding pad on the Buehler EcoMet 300. A teaspoon of 0.3μm aluminium-oxide powder and some water was also applied (Smith, Martin and Leakey 2003; Antoine, Hillson and Dean 2009; Hillson 2014). The handheld grinder and slide were held against the polishing plate using the same technique as during automatic grinding. This step removed scratches from the whole surface of the section.

At the end of this stage, it has been recommended that sections are ground and polished to a width of 100 to 120 μm (Reid, Beynon and Ramirez Rozzi 1998; FitzGerald 1998;

Antoine, Hillson and Dean 2009; Mahoney 2013). These measurements represent the optimum thickness for viewing both Retzius lines, which can only be observed in thicker sections because of the accumulation of visible light scattering through the enamel (Hillson 2014), and cross striations under light microscopy. For this project, each section was reduced to this optimum, although it should be noted that there were variations either within or between sections because of the use of manual preparation techniques (FitzGerald 1998). As the samples were lapped down, the micrometre dial on the jig was used to adjust the distance between the tooth section and the grinding pads. Each quarter turn of the dial corresponded to either an increase or decrease of 0.25µm, and so a process of lapping and adjustment was adopted until the incremental and accentuated lines were clear. Once the section had been fully ground and polished, the slide was washed and left to dry.

4.2.6. Cover Slips

The final histological step was cleaning and applying a cover slip to the microscopic slide and tooth section. The slide was placed in an ultrasonic bath for two minutes to shake off debris (Reid, Beynon and Ramirez Rozzi 1998; Smith, Martin and Leakey 2003). Additional water was added to the surface of the tooth section to wash the debris off. The slide was then left to dry. Next, the section was dehydrated in a series of 95% and 100% alcohol solutions for two minutes apiece. Dehydrating the specimen removes water, water-soluble constituents, lipids and lipid-soluble constituents (Hillman 2000). This prevented dental structures, such as the dentin tubules, from appearing dark when examined microscopically.

After the specimen was dehydrated and dried, it was cleared using Histoclear to remove any impurities still remaining on the section. Histoclear is made from orange terpenes and dissolves paraffin and fats. Hillman (2000) explained that reagents used in clearing, such as xylene, toluene and benzene, increase the refractive index of organic tissues through infiltration, and consequently make them more transparent in light microscopy. Histoclear works similarly to pure xylene, and can be substituted for it with no alteration to the histological procedure. The slide was left to dry completely between each of these chemical steps using a heat lamp. If it was not, the section would not be properly dehydrated or cleaned, and it could also cross-contaminate the chemicals for future use.

Once the slide was dry, a glass cover slip was cemented onto it using DPX, a xylene-based mounting medium (Mahoney, Miszkiewicz, *et al.* 2016). DPX has the same refractive index as the mineral component of enamel and dentin (hydroxyapatite crystals) (Hillson 2014). Other mounting mediums include non-aqueous resins like Canada Balsam and Euparal (Hillman 2000). Singhrao *et al* (2012) recommend using a coverslip with a larger surface area than the tissue section and, if necessary, adding excess DPX to the four edges of the coverslip. The mounting medium seeps under the coverslip through capillary action and so prevents bubbles from appearing and migrating towards the enamel cap during the drying process. Finally, the slide was left on a flat, even surface to seal and dry for 24 hours.

4.3. Microscopy

4.3.1. Transmitted and Polarized Light Microscopy

The incremental markings within the enamel on the Fulbe, Nso and European histological sections were recorded using an Olympus BX51 microscope. This is a bright-field

microscope that emits both transmitted and polarized light. Bright-field microscopes have a condenser lens, which focuses the light source, and an objective lens, which gathers the light as it passes through the specimen, if it is sufficiently thin enough to let the light pass through (Ross and Pawlina 2006). When using transmitted light, a low light between levels two and six was selected. The higher light levels were used as magnification increased because less light was being transmitted through the specimen.

Bancroft and Floyde (2015) explained that, unlike natural light, polarized light only vibrates within a single plane. The Olympus BX51 emits polarized light from a polarizing filter, the polarizer. During data collection, a second polarizing filter, the analyser, was placed above the objective lens (Hillson 1996; Ross and Pawlina 2006). One issue with applying polarizing light to enamel is birefringence. Hillson (2014) defined this an optical property of mineral fibres that causes light beams to be separated into two components, each of which is polarized in a single direction. The analyser filter resolves this problem, and so clear images with distinct contrasts between different mineralization levels in the enamel (such as those surrounding an incremental marking) could be analysed.

Images were taken with the Olympus DP25 digital camera integrated into the Olympus BX51 microscope. The magnification was gradually increased from 2x to 4x, 10x, 20x and finally 40x. At each stage, incremental lines were targeted and brought into focus for observation. Snapshots were manipulated using Olympus Cell D imaging software, such as adjusting the zoom and orientation, in order to gain greater accuracy and precision when recording data.

4.3.2. Scanning Electron Microscopy

A subsample of three molars from both the Fulbe and Nso samples were examined using a Hitachi S-3400N SEM. Schatten (2012) described how SEM microscopes scan the surface of a section using a high-energy focused beam of electrons. As the beam interacts with atoms on the surface, it produces signals on the specimen's topography and surface characteristics. Thus, SEM can produce high resolution images of three-dimensional objects with a greater depth of focus than ordinary light microscopy (Hillson 1996; Reed 2005). This is because, as Ross and Pawlina (2006) explained, the wavelength for the electromagnetic beam is roughly 1/2000 of a light microscope beam, and so the resolution is improved by a factor of 10^3. For this investigation, SEM was applied to the surface of histological sections in order to measure the width of the microscopic prisms within the enamel.

The most widely operated signalling mode for SEM uses secondary electrons (SE) (Dusevich, Melander and Eick 2012). In this mode, electrons are forced from near the surface of the section, collected by an Everhart-Thornley detector and converted into a high-resolution image that almost appears to be three-dimensional (Reed 2005; Ross and Pawlina 2006; Hillson 2014). The other main signalling mode creates images using back-scattered electrons (BSE). Unlike SE, which are emitted from the surface of the section, BSE originate from the high-energy beam, are reflected from the surface by elastic scattering, and then identified by a solid-state detector (Hillson 1996; Dusevich, Melander and Eick 2012; Schatten 2012). The BSE mode was used during this investigation.

Prior to SEM analysis, each molar had been embedded and sectioned but was not mounted onto a microscopic slide. Dusevich *et al* (2012) and Ross and Pawlina (2006)

stated that specimen surfaces must be flat and well-polished to obtain the best results. This is because high-resolution SEM scans the surface of the specimen and anomalies, such as chatter lines from the diamond-wafering saw, appear more pronounced in the resulting images. The surface of each section was polished using cerium oxide powder until there was a light shine. This agent provides high-quality polishing because its chemical properties help with planarisation, when the surface of the section is smoothed through a complex set of mechanical and chemical interactions between the agent, the surface, and the polishing medium (Janoš *et al.* 2016).

Samples were then sputter-coated with a metallic conductive layer. This process, referred to as conductive coating, is used to make biological tissues more conductive so as to prevent insulating specimens from charging under the electron bombardment (Hillman 2000; Buravkov, Chernikov and Buravkova 2011; Schatten 2012) as well as to improve image resolution and contrast (Dusevich, Melander and Eick 2012; Schatten 2012). Reed (2005) described how specimens are placed in a chamber with the chosen metal that will form the conductive layer. Air is removed as argon is injected into the chamber. A high voltage is applied to an electrode at the top of the chamber. This makes the argon ions bombard the metal, which causes the metallic atoms to be removed through 'sputtering' and subsequently deposited onto the outer surface of the specimen. Reed (2005) also stated the thickness of the coating is determined by the current and length of time that the voltage is applied to the electrode. The most common metal used in this process is gold (Smith, Martin and Leakey 2003), although other suitable conducting layers include graphite, platinum, silver and tungsten (Buravkov, Chernikov and Buravkova 2011; Schatten 2012; Hillson 2014). For this investigation, the enamel sections were coated with 20nm of platinum, which was easily wiped off later.

After coating, each section was stuck to an aluminium stub and placed under a high vacuum in the specimen chamber of the SEM. It should be noted that the BSE yield is dependent upon the tilt of the section surface, angle of the electron beam, and position of the detector. However, the Hitachi S-3400N SEM used in this study has a specimen stage that can be moved in five directional planes, which can overcome the negative effect of shadowing (when BSE reflected away from the detector are not identified and so this area of the surface appears dark) (Reed 2005). The majority of images of the enamel section were taken at a low accelerating voltage (5kV) so that fine surface details could be brought into focus. Finally, these images were viewed and the width of visible prisms within the enamel was measured using the INCA software package.

4.4. Measurements

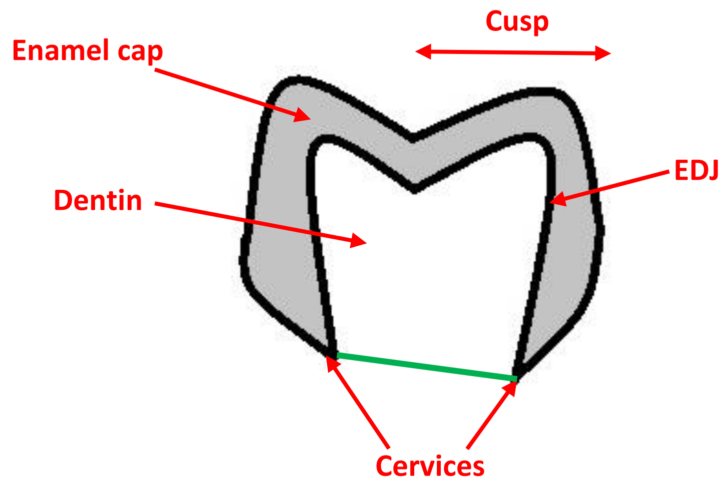
Data for five main variables was collected from the molars examined in this study: average enamel thickness (AET); tooth crown size; daily secretion rate (DSR); enamel extension rate (EER) and prism width. The first four were measured using transmitted and polarized light from the Olympus BX51 bright-field microscope. Prism width was measured using the Hitachi S-3400N SEM.

4.4.1. Average Enamel Thickness

Fig. 4 shows a diagram of a molar section with the enamel cap shaded in blue. The AET of the histological specimens was calculated by dividing the total area of the enamel cap by the total length of the EDJ, which provided an average for the linear distance between the EDJ and outer surface of the enamel (Martin 1983; Martin 1985). The magnification was set at 2x for measuring the entire enamel cap and 4x for individual cusps. The scale was adjusted to millimetres in each image and AET was measured to the nearest 0.01mm.

In specimens with pathological damage, it was not possible to examine the entire enamel cap, and so individual measurements were made of each intact cusp.

Fig. 4 – Diagram of a molar section. The green line between the buccal and lingual cervices shows the cervical limit of the area measured for total crown size



4.4.2. Tooth Crown Size

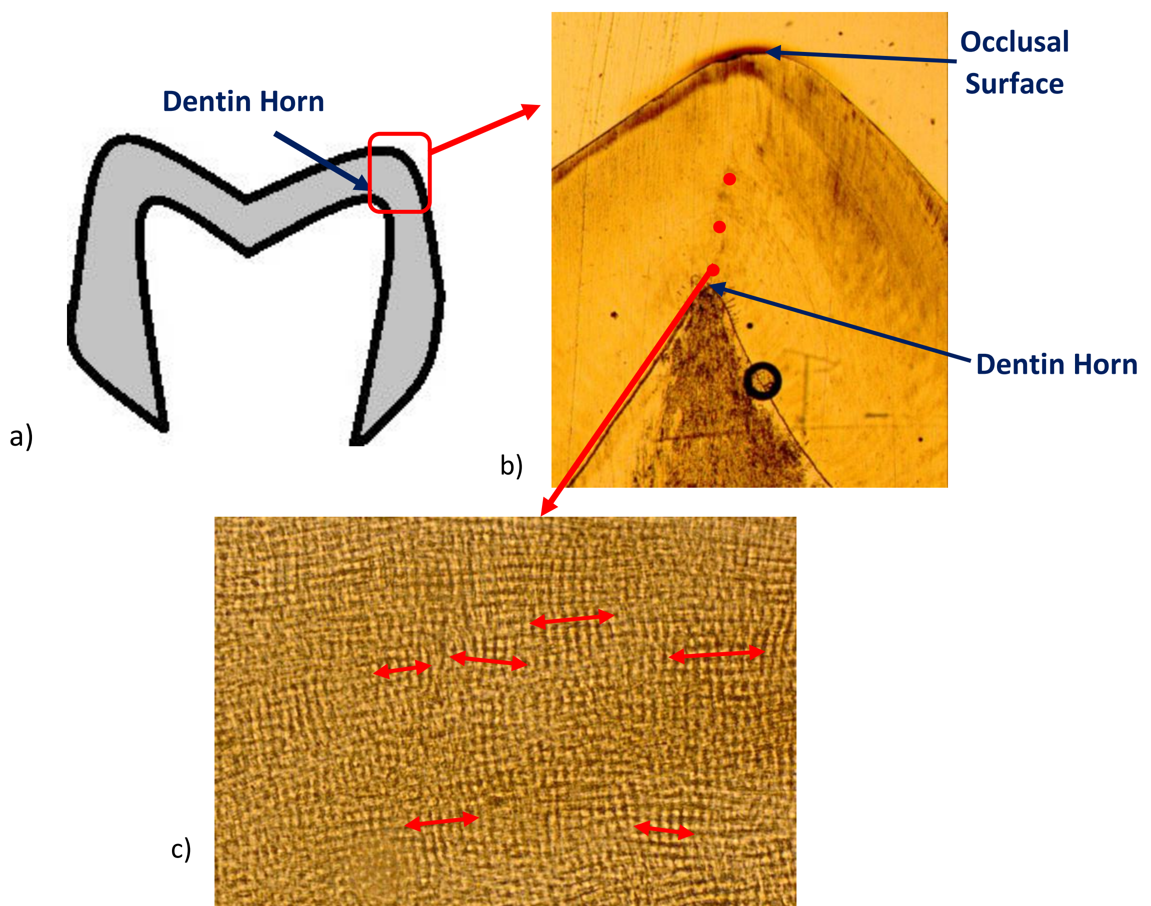
AET does not control for tooth size (Mahoney, Miszkiewicz, *et al.* 2016), and so the crown size of all first, second and third molars examined during this investigation was also measured. The total area of the crown section was measured as a surrogate for tooth crown size. This is the area of all the enamel and dentin defined by the outer limit of the enamel cap and a straight line connecting the buccal and lingual cervices, which is represented by the green line in Fig. 4 (Martin 1983; Grine 2002). Like AET, the microscope magnification was set at 2x and the area of the tooth crown was measured to the nearest 0.01mm^2.

4.4.3. Daily Secretion Rate

DSR is the average 24-hourly rate of enamel formation. This was calculated by measuring the distance along an enamel prism path and dividing it by the number of daily incremental markings or cross striations present (between four and seven cross striations for this project) as shown in Fig. 5. This gave the average amount of daily enamel secreted

by ameloblasts in µm. The transmitted and polarized light microscope was set at 40x magnification and data was rounded to the nearest 0.01 µm. All average DSRs were calculated within the cuspal enamel because the incremental markings between the dentin horn and outer enamel surface provide the most complete record of enamel formation.

Fig. 5 - a) Diagram of a molar section b) Photo of a Nso M^2 taken at 4x. The red dots show where DSRs were measured (200µm, 700µm and 1200µm from dentin horn). c) Photo of the Nso M^2 taken at 40x. The red lines show how DSRs were measured for the inner, mid and outer regions of enamel



The cuspal enamel was split into three regions, as shown by the three red dots in Fig. 5b, which represented the start, continuation and end of enamel deposition in this area respectively: inner enamel closest to the EDJ; mid enamel; outer enamel closest to the surface of the cusp. This separation followed previous methodologies (Beynon, Dean and Reid 1991; Reid, Beynon and Ramirez Rozzi 1998; Dean and Shellis 1998; Smith, Martin

and Leakey 2003; Kierdorf *et al.* 2014). Dean (1998) noted the considerable variation in DSR during enamel formation and thus across the width of the enamel cap could be masked by these broad divisions. Yet, the author also conceded that the best method for studying each tooth section is one which focuses on areas with the most visible incremental markings. Thus, to improve the reliability of this method, at least six average DSR measurements were taken from within each region of enamel (inner, mid and outer). The overall mean DSR and standard deviation for each region was then calculated from these measurements (also outlined by Mahoney 2012).

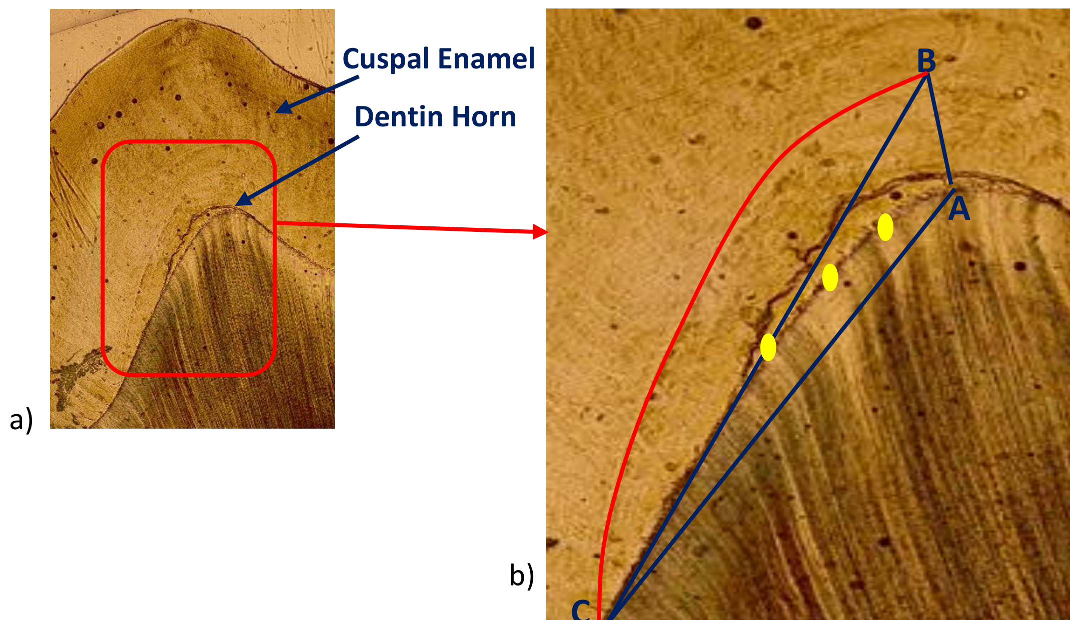
The first set of at least six measurements for the inner enamel region was taken 200µm along the enamel prism path away from the tip of the dentin horn. This was done to avoid the aprismatic enamel and convergence of Retzius lines that can obscure cross striations (Beynon, Dean and Reid 1991; Reid, Beynon and Ramirez Rozzi 1998), although the recommended exclusion area near the dentin horn was doubled because cross striations were consistently clearer at this distance. The second set of measurements was taken 500µm further along the enamel prism path, and the final set another 500µm along the path, close to the outer surface of the tooth crown. DSR was calculated from cusps with the most prominent cross striations. However, if both the buccal and lingual cusps presented clear cross striations, DSR measurements were taken from both cusps and both sets of results were used to calculate the overall average values and standard deviation for that sample.

4.4.4. Enamel Extension Rate

Dental tissue growth occurs both appositionally, as explained previously, and through the extension of ameloblast differentiation. Smith (2008) explained that this extension takes

place along the EDJ, firstly from the dentin horn to the cervix, which is known as coronal extension, and from the cervix to the root apex, which is root extension. EER quantifies coronal extension or the rate of the growth in height, rather than width, of the enamel cap. Calculating the EER of a tooth section involved analysing a single accentuated marking or Retzius line, as shown by the red line in Fig. 6. Each line represents the enamel formation front on a specific day, and so all of the enamel beneath this line, both near the crown and cervix of the tooth, has the same formation time. Due to the small sample sizes of the populations within this study, this measurement was taken from whichever cusp, and whichever side of that cusp, presented the clearest incremental markings.

Fig. 6- a) Photo of the cuspal enamel of a Fulbe M_3 taken at 4x. b) Photo of the Fulbe M_3 taken at 10x. The red line highlights the incremental marking. The blue lines show the method for calculating EER. The yellow dots represent where EER was measured, approximately 200µm, 400µm and 600µm from the dentin horn.



The first step in calculating EER was to establish the formation time of a prism, in days, up to the accentuated marking or Retzius line (Dirks *et al.* 2009). The microscope was set at 40x magnification, and a line was drawn along the enamel prism path from the EDJ to a distinct incremental marking. The blue line between points A and B in Fig. 6 shows this

step of the method using the dentin horn as a starting point. Although it has been recommended that this should be no longer than 200µm (Dean 2009), the difficulties in distinguishing accentuated and incremental markings in some sections meant that, for this investigation, the clearest line between 200 and 250µm was chosen. This also meant it was not possible to count the number of cross striations between the EDJ and incremental marking, which would provide the most accurate estimate of formation time. Instead, it was estimated by dividing the length of the line between these points by the average DSR within that area. For greater accuracy, the line was split into three sections and each one was divided by the average DSR for that section. The DSRs were calculated using the methodology outlined above. Next, the microscope was reset to 20x magnification, and the path of the incremental marking was tracked in a cervical direction until it met the EDJ, as shown by point C in Fig. 6. The distance between this site and the starting point was measured (shown as the length of the line A-C in Fig. 6). Finally, this distance was divided by the estimated formation time to ascertain the EER for this region of the tooth cusp, to the nearest 0.01µm/day.

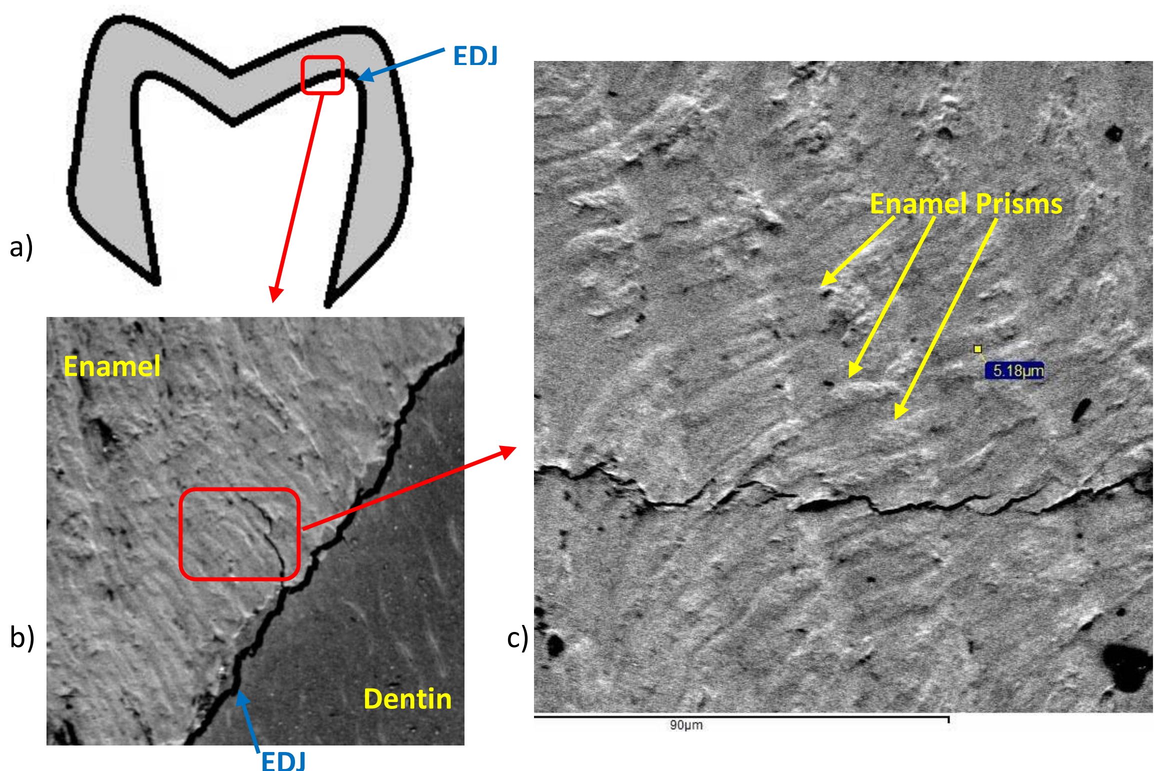
Previous studies have recommended measuring EERs from the dentin horn to the cervix in order to account for variations throughout cuspal and lateral enamel (Shellis 1984; Dean 1998; Dean 2009; Guatelli-Steinberg *et al.* 2012). However, since most of the clinical samples were carious, particularly those from the Fulbe population, only three EER measurements were taken 200µm, 400µm and 600µm from the dentin horn respectively, as shown by the yellow dots in Fig. 6. This meant a standard portion of non-carious cuspal enamel was analysed and compared between the two African populations across the molar row. These comparisons would establish whether extension rates varied between

the Fulbe and Nso and, if so, whether any differences could be linked to their distinct post-weaning diets.

4.4.5. Prism Width

Unlike the previous measurements, the width of prisms within the enamel cap were measured using SEM. Average prism width was calculated from whichever cusp, side and area of that cusp presented the clearest prism boundaries for each of the three molars from the Fulbe and Nso populations. It should be noted that there has been some debate over whether prism diameter is consistent from the EDJ to the outer surface of enamel (Risnes 1998). Several studies have suggested that, at least in cuspal enamel, prism width might increase in the outer enamel region so as to accommodate for the increase in surface area (Dean and Shellis 1998; Jiang, Spears and Macho 2003). For this study, the majority of measurements were taken within the inner enamel region. However, exact area was not standardised.

Fig. 7- a) Diagram of a molar section b) Photo of the cuspal enamel of a Fulbe M_2 taken at 698x c) Photo of Fulbe M_2 taken at 1000x, rotated 90° clockwise. The yellow dots and measurement show how the width of individual prisms was measured.



To measure prism width, the magnification of the SEM was first set at approximately 700x, as shown in Fig. 7. Dean and Shellis (1998) stated that prism packing patterns and shapes are unlikely to be fairly portrayed by two-dimensional prism widths. However, the authors suggested measurements from several prisms could provide a more accurate representation of the true average diameter. Thus, as with calculating average DSR, at least six measurements of the width of individual prisms were taken from across the chosen focal area of each sample, and then an overall mean value was calculated. Several samples presented clear enamel prisms at approximately 700x. However, as shown in Fig. 7, the magnification was increased to approximately 1000x when necessary to gain a clearer view of the prism boundaries.

4.4.6. Intraobserver error

After the first stage of data collection for the combined African and European populations was completed, an analysis of intraobserver error was conducted in order to test the reliability of the results. The author repeated the mean AET, DSR and EER measurements for five samples from each of the Fulbe and Nso groups. All of the prism width measurements for both groups were also repeated. The tooth crown size data was not tested as it was collected later in the project. The intraobserver error was calculated as the average deviation of the intraobserver error measurements from the main dataset (in %). The error values for mean AET, DSR and prism width were all within 5%, but the intraobserver error for average EER was 12.45%. The increased margin of error was likely a result of the multi-stage method for determining extension rates.

4.5. Statistics

The statistical analyses of the data collected during this investigation were split into three objectives. Firstly, the Fulbe (pastoralist) and Nso (agriculturalist) samples were compared. Secondly, the Fulbe and Nso samples were combined into a separate African population so as to compare the combined African first molars to the European first molars. Tooth crown size and average prism width were not included in this comparison as neither of these variables were measured in the European population. Extension rates for the Fulbe and Nso molars were compared to the European samples separately due to reasons outlined below. Finally, values for the combined African and European populations were compared to previous publications. Both Skinner *et al* (2015) and Olejniczak *et al* (2008) published complete datasets with raw AET and crown size values, and so the human data from these papers was included in the statistical analysis. However, the other publications (Beynon, Dean and Reid 1991; Dean 1998; Grine 2002; Lacruz and Bromage 2006; Mahoney 2008; Mahoney 2010) did not, and so only the reported values were compared with the data from this study.

4.5.1. Descriptive Statistics

All three stages of comparisons began with standard descriptive statistics using IBM SPSS 23. Nominal variables were used to identify individual samples: *population, SK* (referring to individual identification number)*, sex, tooth type* (whether the sample was a first, second or third molar), *molar type* (to separate first molars from second and third molars) *cusp (*denoting the cusp from which measurement were taken). The following scale variables were used to input data from all of the measurements listed above: *buccal AET, lingual AET, AET, tooth crown size, DSR inner, DSR mid, DSR outer, DSR, buccal DSR, lingual DSR, EER crown, EER mid, EER cervix, EER, prism width.* For each population, any outlying

values that had a significant effect on the 5% trimmed mean for any of the scale variables was removed. Then, the mean and standard deviation (both rounded to two decimal places) for each variable was calculated. For the Fulbe and Nso populations, comparisons were made between the different sexes, molar types and cusps within these samples.

4.5.2. Inferential Statistics

Three sets of inferential statistical tests were conducted on the Fulbe, Nso, combined African (Fulbe and Nso) and European molar samples examined in this study: correlations, analysis of variance post hoc pairwise comparisons, and regressions. The correlation tests were completed in IBM SPSS 23 and the other two sets of tests were completed using PAST (Hammer, Harper and Ryan 2001). The raw data from the Skinner *et al* (2015) and Olejniczak *et al* (2008) comparative human populations was included in the analysis of variance post hoc pairwise comparison tests.

4.5.2.1. Correlations

Pearson's and Spearman's tests of correlation help to evaluate the strength and direction of the relationship between two variables (Brace, Kemp and Snelgar 2009) and so can be a useful preliminary analysis of new datasets. For example, Guatelli-Steinberg *et al* (2012) applied Pearson's correlations to test EERs against enamel formation time and length of EDJ in order to establish whether EERs varied along the EDJ. In this study, correlation tests were applied to each of the measurements taken from the Fulbe, Nso, combined African and European populations. For the combined African (Fulbe and Nso) population, correlation tests were completed including all three molar types and then after the population had been split by *molar type*. This provided results for just the first molars, which could then be compared with the correlations for the European first molar sample.

The majority of these associations involved a sample of less than 20 individuals, and so the nonparametric Spearman's test was used. In instances where there were at least 20 samples, the parametric Pearson's test was applied. All of the significant results (where $p \leq 0.05$) based on a sample size of at least five individuals, which were more likely to reliably represent an association between two variables, are discussed in the Results chapter. They are presented with an *r*-value, the correlation coefficient, which shows the strength of the positive or negative linear association, as well as the proportion of variance in the dependent variable that can be explained by the independent variable (derived from r^2) (Brace, Kemp and Snelgar 2009).

4.5.2.2. Analysis of Variance post hoc Pairwise Comparisons

The second stage involved testing variations both within and between the Nso, combined African, European and comparative human populations. As there were three or more groups, a one-way analysis of variance (ANOVA) test was used to reveal significant differences ($p \leq 0.05$) in the mean values of different variables. However, an ANOVA does not show exactly which pairs of conditions or populations vary significantly (Brace, Kemp and Snelgar 2009). Thus, post hoc Tukey's-Kramer pairwise comparisons were used to identify which pairs differed. ANOVA and Tukey's-Kramer are both parametric tests, and so were only applied in instances where at least 20 samples were present in each group. The nonparametric equivalents of these analyses, the Kruskal-Wallis *H* and Mann Whitney *U* tests, were used when the sample sizes for each condition were below 20. When enough samples were present for an ANOVA test, Levene's homogeneity-of-variance test was applied to check the equality of variance across the populations. If there was no equality of variance in the samples ($p < 0.05$) then the Kruskal-Wallis test was used instead. These tests have been widely used in previous studies of AET and tooth crown

size (Grine 2002; Smith *et al.* 2006; Mahoney 2010; Smith *et al.* 2012; Skinner *et al.* 2015) as well as DSRs (FitzGerald 1998; Schwartz *et al.* 2005).

For the within-samples analyses, mean AET and DSR variables were contrasted across all of the molars from the Nso and combined African (Fulbe and Nso) populations. The Fulbe sample was too small for any significant result to be reliable, and so it was not analysed independently using these tests. Additional Mann Whitney *U* tests were utilised to compare DSRs between the first and distal molars from the Nso population. In the between-samples analyses, the mean AET values were compared across the combined African (Fulbe and Nso), European, Skinner *et al* (2015) and Olejniczak *et al* (2008) populations. Mann Whitney pairwise comparisons were applied to highlight significant differences in AET, tooth crown size, DSR and EER between all of the molars from the Fulbe and Nso populations. They were also used to test for variations in the DSRs of the combined African and European first molar samples.

4.5.2.3. Regressions

Two types of regression analysis were used: ordinary least squares; reduced major axis (RMA). Least squares regressions were used to test the strength of the associations between AET, DSR, EER and tooth crown size in the Fulbe, Nso, combined African and European populations. They have previously been used to analyse AET and crown sizes (Grine 2002) and EERs (Guatelli-Steinberg *et al.* 2012). Like the correlation tests, least squares regressions can reveal the amount of variance explained by these associations through an r^2-value. Each variable was then log-transformed and RMA regressions were applied to examine the scaling relationships between them. The slope of the regression line depicts the growth ratio between the variables. When a significant relationship ($p\leq$

0.05) is present, as Mahoney *et al* (2016) explained, the slope and 95% confidence intervals can indicate negative allometric (<1), isometric (1) or positive allometric (>1) scaling between the variables.

Chapter 5: Results

5.1. Outline

The results of the statistical analyses are presented in this chapter. They are split into two sections, which will include both descriptive and inferential statistics. The first will review the results for the Fulbe and Nso molar samples. The second will compare the results for the combined African (Fulbe and Nso) population with the European first molar sample examined in this study as well as other comparative modern human datasets from previous publications. All of the results from the statistical analyses, including the non-significant results not discussed in this section, can be found in the Appendix.

5.2. Fulbe and Nso populations

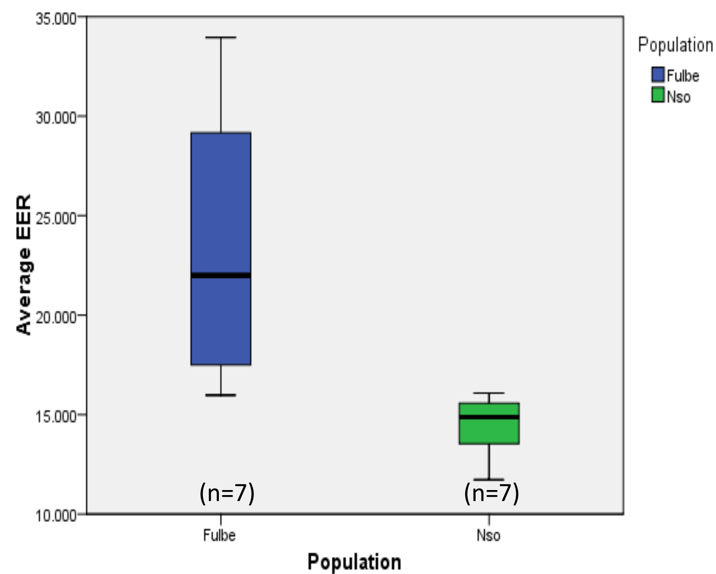
The mean values for AET, tooth crown size, average DSR, average EER, and average prism width of the Fulbe and Nso populations can be seen in Table 2. It should be noted that, as previously explained, the mean values for average DSR, EER and prism width are based upon multiple measurements from different regions within the enamel cap. They are used here to represent the overall average of each variable for all of the Fulbe and Nso molars.

Table 2- Mean values for the five main variables for the Fulbe and Nso populations. All molars combined (+/- 1 SD)

	n	AET Mm	n	Crown Size mm^2	n	DSR µm/day	n	EER µm/day	n	Prism Width µm
Fulbe	7	1.37 (0.30)	5	48.07 (0.86)	9	3.57 (0.27)	7	23.61 (7.51)	3	5.68 (0.45)
Nso	18	1.33 (0.18)	16	54.96 (4.56)	26	3.68 (0.34)	7	14.41 (1.59)	3	5.24 (0.49)

As shown in Table 2, the average prism width and mean EER were larger in the pastoralists. Mean AET was also slightly greater in the Fulbe population. Tooth crown size and average DSRs were greater in the agriculturalists. When the samples were split buccolingually, as shown in Table 3 in the Appendix, lingual cusp AET appeared to be slightly larger than buccal cusp AET in the Fulbe population, but the opposite was true for the Nso population. A Mann Whitney *U* test revealed that mean AET for the Fulbe did not differ significantly when compared to the Nso (*U*= 57.0, *p*= 0.739), and neither did average DSR (*U*= 94.0, *p*= 0.396). However, the larger mean crown size of the Nso molars was shown to be significantly different to the Fulbe (*U*= 1.0, *p*= 0.001), as was the faster average EER of the Fulbe (*U*= 2.0, *p*= 0.005). The difference in overall average EER between the two populations is displayed in Fig. 8 below.

Fig. 8- Graph of average EER (in µm/day) for the Fulbe and Nso



Summary Box 1

Mean AET and prism width was greater in the Fulbe (pastoralist) than the Nso (agriculturalist) population. Tooth crown size was significantly larger in the Nso. EERs were significantly faster in the Fulbe.

Table 4- Mean DSRs (in µm/day) for three regions of cuspal enamel for the Fulbe and Nso populations. All molars combined (+/- 1SD)

	n	Inner	n	Mid	n	Outer
Fulbe	6	3.37 (0.44)	9	3.52 (0.24)	9	3.80 (0.30)
Nso	22	3.28 (0.27)	25	3.57 (0.35)	25	4.05 (0.41)

Table 4 displays the mean DSRs for the two African populations at three distinct regions of the cuspal enamel. These values are based on a set of at least six measurements taken from regions of the enamel that were 200µm, 700µm and 1200µm away from the dentin horn, respectively. As shown in Table 4, both samples displayed the expected increase in DSR from the first measurement at the inner region to the final one toward the outer enamel surface. This increase was greater in the Nso (0.77µm/day) than the Fulbe (0.43µm/day). Table 5 in the Appendix shows the average DSRs for the lingual cusp were slightly greater than the buccal cusp in both populations, although the mean values for both cusps were greater in the Nso sample. Spearman's test revealed a positive correlation between average DSR from the mid enamel region and tooth crown size within the Nso (r= 0.564, n= 15, p= 0.028). Thus, for this population, larger teeth had faster enamel secretion rates in this region of cuspal enamel. No correlation was found in the Fulbe.

The average values for each stage of the EER measurement protocol (see Methods) are shown with the mean EERs for the Fulbe and Nso populations in Table 6. The values are split into four groups, three of which represent measurements taken 200µm, 400µm and 600µm from the dentin horn down towards the cervix of the tooth, respectively. The final group represents the overall mean values for each population. It should be noted that inner DSR does not refer to the mean DSR for the inner region of enamel, as shown

in Table 4. It represents the average DSR between the EDJ and the accentuated marking from which EER was measured. The formation time represents the average number of days it took to form this area of enamel. As explained in Methods, this was calculated by dividing the distance between the EDJ and the accentuated line by the average DSR within this region (inner DSR). The accentuated line length represents the mean distance between the point of measurement on the accentuated line within the cuspal enamel and the site where the line terminated as it rejoined the EDJ towards the cervix of the tooth. The mean EERs were calculated by dividing the length of the accentuated line by the formation time. Thus, Table 6 clearly shows how EERs were calculated from the Fulbe and Nso dental samples.

Table 6- Mean measurement and EER values for three regions of the enamel cap and overall means for the Fulbe and Nso populations. All molars combined (+/- 1SD)

		n	Inner DSR µm/day	Formation Time (~200µm/IDSR) Days	Accentuated Line Length µm	EER (Line/Form. Time) µm/day
Crown (200µm from Dentine Horn)	Fulbe	6	3.18 (0.76)	65.90 (12.60)	1579.74 (517.71)	24.33 (7.97)
	Nso	2	3.05 (0.47)	70.65 (9.06)	1141.85 (56.99)	16.24 (1.28)
Mid (400µm from Dentine Horn)	Fulbe	4	3.47 (0.78)	67.79 (14.15)	1534.37 (833.19)	21.77 (8.96)
	Nso	3	2.90 (0.25)	87.17 (22.36)	1229.23 (497.22)	13.78 (1.98)
Cervix (600µm from Dentine Horn)	Fulbe	5	3.55 (0.16)	64.91 (7.13)	1555.10 (576.67)	23.77 (7.90)
	Nso	7	2.99 (0.18)	83.66 (15.33)	1162.05 (335.52)	13.75 (2.20)
X	Fulbe	7	3.38 (0.48)	68.17 (5.09)	1598.14 (499.03)	23.61 (7.51)
	Nso	7	2.98 (0.21)	85.12 (16.06)	1233.65 (312.63)	14.41 (1.59)

It is clear from Table 6 that EERs were faster in the pastoralists than the agriculturalists for all three regions of cuspal enamel. The greatest variation was in the final measurement region towards the cervix, where rates differed by 10.02μm/day. This trend appears to be linked to slightly faster DSRs near to the EDJ, and consequently shorter formation times, within the Fulbe samples. In fact, the mean formation times for the final measurement region and the overall average values for the two populations fell beyond the range of one standard deviation of each other, which indicates a significant difference between the two populations. The lengths of the accentuated lines were also greater in the Fulbe. There was an overall decrease in EER from the first measurement region at the dentin horn to the final region towards the cervix of the tooth in both populations. However, the initial decrease in EER between the first and second measurement regions within the Fulbe was followed by a slight increase in the final region, although this value was still lower than the first. This trend appears to be linked to a reduced average formation time for the final region, and may explain why the overall decrease in EER was greater in the Nso sample (2.49μm/day) than the Fulbe sample (0.56μm/day).

A Spearman's correlation test showed that the average DSR for the Fulbe was significantly and positively correlated with mean EER from the region of enamel near the crown (r= 0.886, n= 6, p= 0.019), the cervix (r= 0.900, n= 5, p= 0.037), and the overall mean EER (r= 0.929, n= 7, p= 0.003). Thus, faster enamel secretion rates were associated with faster extension rates.

Summary Box 2

Mean DSR was greater in the Nso molars, except for the inner region of cuspal enamel. EER was consistently greater in the Fulbe samples across the enamel cap.

5.2.1. Comparisons across the Molar Row

Table 7 displays the mean values for each variable in the Fulbe and Nso populations, which are subdivided into three groups for the three molar types. The mean value for prism width has been excluded due its small sample size.

Table 7- Mean values for the four main variables for the Fulbe and Nso populations separated by tooth type (+/- 1 SD)

		n	AET mm	n	Crown Size mm^2	n	DSR µm/day	n	EER µm/day
M1	Fulbe	1	1.48 (-)	1	48.10 (-)	2	3.95 (0.13)	1	25.15 (-)
	Nso	6	1.37 (0.19)	6	54.80 (4.91)	5	3.52 (0.08)	3	14.96 (1.07)
M2	Fulbe	4	1.17 (0.21)	4	48.07 (1.00)	3	3.48 (0.03)	4	22.10 (8.36)
	Nso	8	1.26 (0.17)	6	53.88 (4.15)	11	3.80 (0.35)	2	14.61 (2.07)
M3	Fulbe	2	1.70 (0.05)			2	3.35 (0.07)	2	25.84 (10.37)
	Nso	4	1.39 (0.19)	4	56.83 (5.30)	8	3.62 (0.38)	2	13.40 (2.37)

When the Fulbe and Nso data was split by tooth type, a few trends became apparent. Firstly, the mean AET was largest in the third molars and smallest in the second molars of both populations. This variation was larger in the pastoralists (0.53mm) than the agriculturalists (0.13mm). A Kruskal-Wallis test revealed no significant difference in AET between the molar classes of the Nso population (X^2= -2.816, p= 1.000).

Secondly, like the overall average in Table 2, tooth crown size was larger in the Nso population for both the first and second molars. It also appeared to be smaller in the second molars compared to the first molars in both populations, although these were only minor variations. Average crown size was slightly larger in the third molars than the

second molars of the Nso sample, but these values were within one standard deviation of each other.

Furthermore, the average DSRs of the Fulbe and Nso populations appeared to follow two different trends across the molar row. In the Fulbe population, DSRs were lower in the third molars and faster in the first molars, with an overall difference of 0.60µm/day. However, the second molars had a faster DSR than the first molars in the Nso population. Thus, even though the third molars had a slower DSR, just like the Fulbe population, the DSRs of the Nso were larger for both of the distal molars. This trend is more clearly shown in Fig. 9, which displays a graph of mean DSRs for both populations separated by tooth type. Mean DSRs gradually slowed down across the molar row in the pastoralists, but not in the agriculturalists.

Fig. 9- Graph of average DSR (in µm/day) for Fulbe and Nso split by tooth type

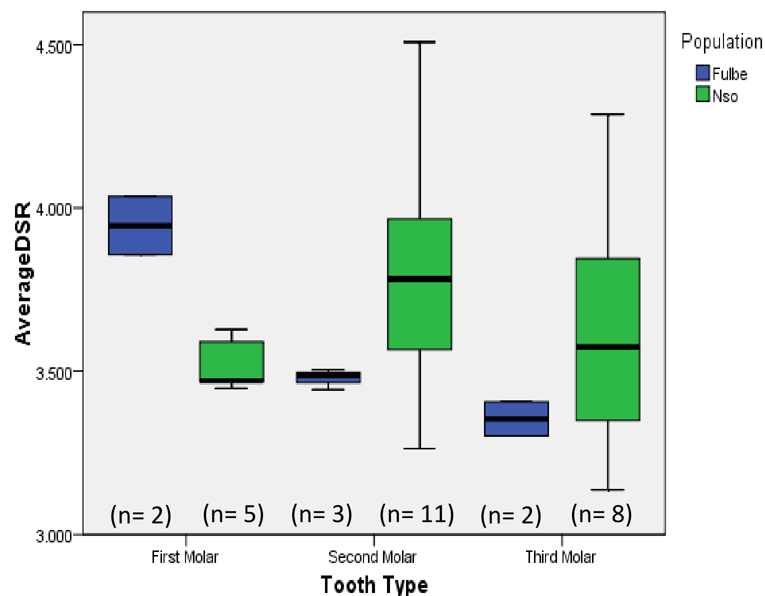


Table 8 below shows the mean DSRs for the three measurement regions of the cuspal enamel from each tooth type in each population. As expected, all of the molars in both populations showed a gradual increase in DSR from the inner to the outer regions. In the

first molars, all three measurements were greater in the Fulbe than Nso. By contrast, average DSR for all three regions within the second and third molars were larger in the Nso population. This appears to be due to the fact that mean DSR was larger in the second molars rather than the first molars for the agriculturalists but the opposite was true for the pastoralists. However, there were no significant differences between the Fulbe and Nso samples in any of the DSR measurement regions (see Table 27 in Appendix). Kruskal-Wallis tests revealed no significant differences in secretion rates from the inner, mid or outer enamel regions between the three molar types.

Table 8- Mean DSRs (in µm/day) for three regions of cuspal enamel for the Fulbe and Nso populations separated by tooth type (+/- 1SD)

		n	Inner[1]	n	Mid[2]	n	Outer
M1	Fulbe	2	3.82 (0.23)	2	3.82 (0.03)	2	4.20 (0.18)
	Nso	6	3.25 (0.22)	5	3.35 (0.07)	6	3.85 (0.35)
M2	Fulbe	2	3.28 (0.47)	4	3.33 (0.03)	5	3.70 (0.24)
	Nso	6	3.32 (0.05)	9	3.60 (0.24)	11	4.09 (0.48)
M3	Fulbe	2	3.00 (0.07)	2	3.47 (0.24)	2	3.65 (0.13)
	Nso	7	3.08 (0.24)	8	3.50 (0.44)	8	4.13 (0.32)

[1] Table 4 DSR at Inner gives Nso n=22. Here SK 23, 50 and 52 removed as outliers. [2]DSR at Mid gives Fulbe n=9 and Nso n=25. Here, SK 34 from Fulbe and SK 1, 3 and 51 from Nso removed as outliers.

Moreover, as shown in Table 7, EER was consistently and distinctly greater in the Fulbe population than the Nso population. The largest difference (12.44µm/day) was between the averages for the third molars. There appeared to be only slight variations between the three tooth types in each population. For example, mean EER was slightly smaller in the second molars of the Fulbe population, and each successive molar in the Nso population had a slightly smaller average than the previous one. These trends are clearly

displayed in Fig. 10, which shows a boxplot graph of the average EER for the Fulbe and Nso subdivided by tooth type.

Fig. 10- Graph of average EER (in µm/day) for Fulbe and Nso split by tooth type

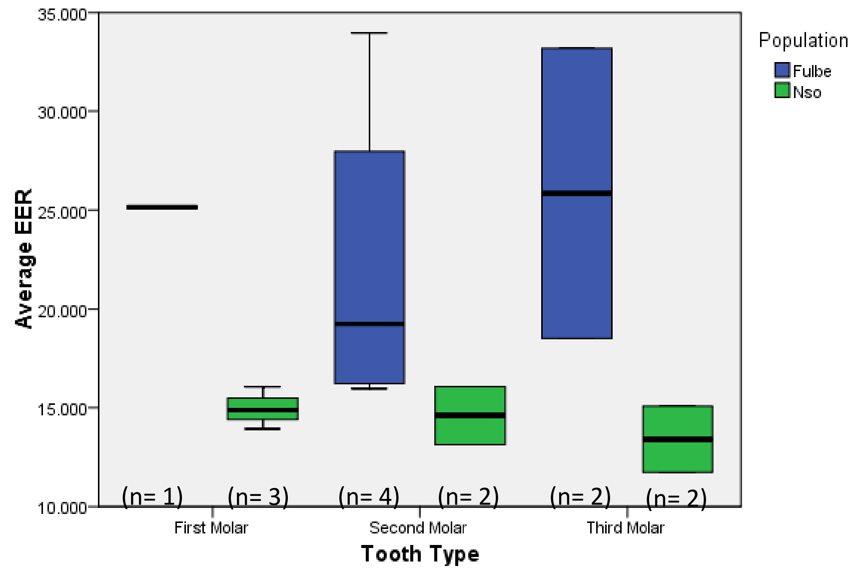


Table 9- Mean EERs (in µm/day) for three regions of enamel cap for Fulbe and Nso populations separated by tooth type (+/- 1SD)

		n	Crown	n	Mid	n	Cervix
M1	Fulbe	1	32.94 (-)	1	20.70 (-)	1	21.80 (-)
	Nso	1	15.34 (-)	1	13.39 (-)	3	14.67 (1.52)
M2	Fulbe	3	18.89 (3.01)	1	16.72 (-)	2	24.00 (14.08)
	Nso	1	17.15 (-)	1	12.02 (-)	2	13.17 (4.12)
M3	Fulbe	2	28.18 (10.34)	2	24.83 (13.97)	2	24.52 (6.81)
	Nso			1	15.93 (-)	2	12.97 (1.76)

Table 9 shows the mean EERs of the three measurement regions for each tooth type from the Fulbe and Nso populations. The mean EER within all three regions was greater in the Fulbe than the Nso for all molars, just like the overall average EERs in Table 7. In the Fulbe population, EERs from the crown and mid were larger in the first and third molars than the second molars. This was also true for the mean EER from the mid enamel

region in the Nso population. EER from the region near the cervix was greater in each successive molar for the Fulbe, but was smaller in each successive molar for the Nso.

Finally, the main measures taken from the Fulbe and Nso samples are displayed in Table 10, just like Table 7. But, in this instance, both groups are split into two groups, one for the first molars and the second for the combined distal (second and third) molars. This grouping was selected to explore enamel growth rates before and after the weaning process because, as explained previously, the first molar starts developing before weaning, whilst both the second and third molars grow after it has ended (Smith 1991; Hill and Kaplan 1999; Reid and Dean 2006; Humphrey 2010; Smith *et al.* 2013). Once again, prism width has been excluded due to small sample sizes.

Table 10- Mean values for the four main variables for the Fulbe and Nso populations divided by molar type (+/- 1SD)

		n	AET mm	n	Crown Size mm^2	n	DSR µm/day	n	EER µm/day
M1	Fulbe	1	1.48 (-)	1	48.10 (-)	2	3.95 (0.13)	1	25.15 (-)
	Nso	6	1.37 (0.19)	6	54.80 (4.91)	5	3.52 (0.08)	3	14.96 (1.07)
(M2+ M3)	Fulbe	6	1.35 (0.32)	4	48.07 (1.00)	6	3.40 (0.10)	6	23.35 (8.20)
	Nso	13	1.30 (0.18)	10	55.06 (4.61)	19	3.72 (0.36)	4	14.00 (1.95)

Mean DSR was faster in the first molars than the combined distal molars in the pastoralists, but sample sizes were very small. The opposite was true for the agriculturalists. Like Tables 7 and 9, Table 10 shows that EERs appeared to be accelerated in the Fulbe compared with the Nso. EERs were also slightly larger in first molars than distal molars in both populations, although these appeared to be only minor variations.

Summary Box 3

In the Fulbe, DSRs and EERs were faster in the first molars, which develop during weaning, compared to the other molar types, which grow after weaning has ended. DSRs for the Nso were faster in the distal molars than the first molars.

5.2.2 Comparisons between the Cusps

Tables 11, 12 and 13 display the mean values for the three main variables for the Fulbe and Nso populations subdivided by the tooth type and cusp from which the measurements were taken. Crown size and prism width were not included as the former is a measure of the overall size of the enamel cap, and the latter was based on a limited sample size. No inferential statistical tests were conducted for this comparison due to the small number of samples from both populations.

Table 11- Mean AET (in mm) for the Fulbe and Nso populations separated by cusp (+/- 1SD)

		n	Mesiobuccal	n	Mesiolingual	n	Distobuccal	n	Distolingual
M1	Fulbe[3]					1	1.35 (-)	1	1.71 (-)
	Nso[4]	3	1.44 (0.16)	3	1.37 (0.29)	1	1.55 (-)	2	1.45 (0.00)
M2	Fulbe[5]	3	1.18 (0.27)	2	1.03 (0.05)	1	1.01 (-)	1	1.31 (-)
	Nso[6]	3	1.41 (0.05)	2	1.53 (0.25)	3	1.36 (0.28)	6	1.30 (0.36)
M3	Fulbe[7]	1	1.69 (-)	1	1.65 (-)	1	1.71 (-)	1	1.43 (-)
	Nso[8]	5	1.50 (0.30)	3	1.53 (0.47)				

[3-8] As explained in Methods, where possible, mean AET was measured from each intact cusp within the molar samples as well as from across the enamel cap. Thus, Table 11 gives higher sample sizes for mean AET for each tooth type in both populations than Tables 7 and 10.

Table 11 shows the mean AET values for the Fulbe and Nso populations separated by tooth type and location of the cusp from which the measurement was taken. When compared to the Fulbe samples, enamel thickness appeared to be significantly larger in

the distal cusps of the Nso first molars and the mesiolingual and distobuccal cusps of the Nso second molars. However, it should be noted these variations, particularly between the first molars, are based upon a limited number of samples. Mean AET was larger in the distolingual cusps than the distobuccal cusps for the first and second molars of the Fulbe population. This variation is similar to the differences found between the buccal and lingual cusps for all of the Fulbe molars shown in Table 3 (see Appendix). Like Table 7, Table 11 shows mean AET was largest in the third molars and smallest in the second molars of the Fulbe population, with the greatest difference being between the distobuccal cusps (0.70mm). The variation seen in the molars from the pastoralists may be linked to the limited number of samples available from this population. This is supported by the lack of variation in mean AET across the molar cusps of the agriculturalists. The greatest variation was between the distobuccal cusps of the first and second molars, which differed by 0.19mm. All of the results for the Nso were taken from a larger number of samples than the Fulbe, except for the distal cusps of the first molars, and were within one standard deviation of each other. As such, there was no significant difference between the molars of this population.

Table 12- Mean DSRs (in µm/day) for the Fulbe and Nso populations separated by cusp (+/- 1SD)

		n	Mesiobuccal	n	Mesiolingual	n	Distobuccal	n	Distolingual
M1	Fulbe			1	3.86 (-)			1	4.04 (-)
	Nso[9]	3	3.72 (0.53)	5	3.55 (0.23)	1	3.47 (-)	1	3.45 (-)
M2	Fulbe[10]	4	3.51 (0.24)			1	3.23 (-)	1	3.78 (-)
	Nso[11]	3	3.88 (0.16)	2	3.70 (0.12)	2	3.74 (0.30)	6	3.82 (0.49)
M3	Fulbe[12]	1	3.59 (-)	1	3.13 (-)	1	3.14 (-)	1	3.46 (-)
	Nso[13]	6	3.56 (0.32)	3	3.54 (0.37)			2	3.90 (0.55)

[9-13] As explained in Methods, if samples included multiple cusps, DSRs were measured in each cusp with visible cross striations and an overall average was calculated for that sample. Thus, Table 12 gives higher DSR sample sizes than Tables 7 & 10 for Fulbe M2 & M3 and all three Nso molars.

The mean DSRs for the Fulbe and Nso populations subdivided by tooth type and cusp are shown in Table 12. Like Table 7, mean DSRs appeared to be faster in the first molars and slower in the distal molars of the pastoralists. The greatest difference was between the mesiolingual cusps of the first and third molars, which varied by 0.73µm/day. In the agriculturalists, mean DSRs increased between the first and distal molars across all cusps except for the mesial cusps of the third molars. However, these variations were within one standard deviation of each other and so did not appear to be significant.

As with the results presented in Tables 7 and 8, the mean DSRs for the mesiolingual and distolingual cusps of the first molars were faster in the Fulbe than the Nso. Conversely, DSRs for all of the comparable cusps across the second and third molars were greater in the Nso than the Fulbe, except for the slightly slower rate for the mesiobuccal cusp of the third molars. These results appear to be linked to the general trends of DSRs increasing in the distal molars of the agriculturalists, but decreasing in the distal molars of the pastoralists. Both populations had faster DSRs in the mesiobuccal cusps compared with the mesiolingual cusps for all three molars. The distolingual cusps had faster DSRs than the distobuccal cusps in both populations, except for the slight variation in the Nso first molars. It is important to note these results come from a small number of samples, which may account for the exceptions to these patterns.

Table 13- Mean EERs (in µm/day) for the Fulbe and Nso populations separated by cusp (+/- 1SD)

		n	Mesiobuccal	n	Mesiolingual	n	Distobuccal	n	Distolingual
M1	Fulbe			1	25.15 (-)				
	Nso	1	13.93 (-)	2	15.47 (0.84)				
M2	Fulbe	2	18.99 (4.25)	2	25.22 (12.35)				
	Nso	1	16.08 (-)					1	13.14 (-)
M3	Fulbe			1	33.18 (-)			1	18.51 (-)
	Nso	1	15.07 (-)	1	11.72 (-)				

Table 13 shows the mean EERs of the Fulbe and Nso populations separated by tooth type and cusp. Like Tables 2, 6 and 7, EERs were greater in the pastoralists than the agriculturalists across all of the comparable cusps from all three molars. The largest difference was between the mesiolingual cusps of the third molars (21.46µm/day) as EERs in the Fulbe sample were almost three times as fast as the Nso sample. When comparing results for specific cusps along the molar row, only minor variations appeared between the mesiobuccal cusps from the Nso population and the mesiolingual cusps from the Fulbe population. Mean EERs were faster in the mesiolingual than the mesiobuccal cusps of the Nso first molars and Fulbe second molars, but the opposite was true for the Nso third molars. The largest variation within the two populations was between the mesiolingual and distolingual cusps of the Fulbe third molars, which differed by 14.67µm/day. It is important to note the data in Table 13 represents a limited number of samples, so it is unclear whether any of these variations are significant.

Summary Box 4

Mean AET, DSR and EER varied more across the molar cusps of the Fulbe than the Nso. Mean DSRs increased between the cusps of the first and distal molars of the Nso but decreased in the Fulbe. DSRs were faster in the buccal cusps of the mesial sections and lingual cusps of the distal sections in both molar samples. The pastoralists displayed greater mean EERs than the agriculturalists, but only slight variations appeared between the cusps for all three molars.

5.2.3. Comparisons between the Sexes

The mean values for the four main measurements taken from the Fulbe and Nso populations were subdivided into two groups, one for males and one for females (Appendix Table 14). The average prism widths were excluded because of the small number of measurements from each population.

There was little difference between the males and females in both Fulbe and Nso populations. In the Fulbe group, the mean values for female AET and EER were slightly greater than their male counterparts, but the mean tooth crown size and DSR was smaller. In the Nso population, the mean values for male crown size and DSR were slightly larger than the female values, but the mean AET and EER were slightly smaller. However, these variations did not appear to be consequential, as the mean values all fell within one standard deviation of each other.

Both the male and female mean AET were slightly larger in the pastoralists, but the average DSR for both sexes was slightly greater in the agriculturalists. These did not appear to be substantial differences. However, mean tooth crown size for both males and

females was larger in the Nso population, and these values were more than one standard deviation apart. The mean EER of both sexes was much faster in the pastoralist population, and these values were also beyond the range of one standard deviation of the agriculturalist values.

Summary Box 5

There were no consistent differences between males and females in either sample.

Average crown size and DSR were greater in the Nso, but mean EER was greater in the Fulbe across both sexes.

5.3. African and comparative populations (first molar and European)

The combined African (Fulbe and Nso) population was compared to the European sample of first molars examined during this investigation as well as datasets from previous publications. These comparisons would place the results from this study in the wider anthropological context.

5.3.1. AET

Table 15- Mean AET (in mm) for the African and European molars examined in this study alongside the values calculated for previously published modern human samples

		M1			M2			M3		
Population	Source	n	*X*	SD	n	*X*	SD	n	*X*	SD
African	This Study[14]	5	1.49	0.04	12	1.23	0.18	6	1.49	0.22
European	This Study	20	1.14	0.15						
European	Skinner *et al* (2015)[15]	9	1.00	0.13	12	1.12	0.07	7	1.21	0.20
European	Olejniczak *et al* (2008)[16]	4	1.04	0.07	13	1.52	0.34	18	1.54	0.20

[14] Fulbe and Nso AET from Table 7 results in n= 7. Here, AET for Nso SK 1 and SK 3 removed as outliers for combined African first molars. [15]Skinner *et al* (2015) used microtomography to measure AET and crown size from two-dimensional mesial sections of recent modern human molars (n= 30). [16]Olejniczak *et al* (2008) used microcomputed tomography to measure three-dimensional AET in a sample of recent modern human molars (n= 39).

Table 15 shows the mean AET for all of the molars from the African and European populations from this study as well as values for the Skinner *et al* (2015) and Olejniczak *et al* (2008) comparative samples. Of the two first molar samples analysed in this investigation, the Africans had a larger AET than the Europeans. It was also larger than both of the comparative modern human first molar samples. However, a Kruskal-Wallis test showed that AET did not differ significantly between these samples of first molars ($X^2 = 5.934$, $p = 0.115$).

Table 15 also shows that the data from the Skinner *et al* (2015) sample had the smallest AET across the second and third molars. The Olejniczak *et al* (2008) sample had the largest mean AET value. There appeared to be an increase in AET between the second and third molars in both of these comparative populations. A Kruskal-Wallis test showed that mean AET differed significantly between the combined African, Skinner *et al* (2015) and Olejniczak *et al* (2008) datasets when all three molar types were included ($X^2 = 9.286$, $p = 0.010$). It should be noted that a one-way ANOVA was not used in this instance because the Levene's test for homogeneity of variance indicated that variance was not equal across these samples (from means, $p < 0.005$). Post hoc Tukey's-Kramer pairwise comparisons revealed significant differences in mean AET between the combined African and Skinner *et al* (2015) datasets when all three molar types were included ($Q = 6.730$, $p < 0.005$).

Summary Box 6

Mean AET of the African first molars was larger than all the comparative, predominantly European human samples. The Skinner *et al* (2015) sample had the smallest AET across all three molar types. These results were found to be significant.

5.3.2. Tooth Crown Size

The crown size values for all three molar types in the African and Skinner *et al* (2015) samples can be seen in Table 16. This table also includes data taken from Grine (2002) and Mahoney (2010). The average crown size for the Skinner *et al* (2015) first molars was slightly larger than the African sample. The mesial and distal samples from Mahoney (2010) were consistent with the African data, and the data from Skinner *et al* (2015). The mean crown sizes for both the mandibular and maxillary first molars from Grine (2002) were larger than all of the other samples.

Table 16- Mean tooth crown size (in mm^2) for all molars from the combined African population examined in this study and comparative samples from previous publications

		M1			M2			M3		
Population	Source	n	*X*	SD	n	*X*	SD	n	*X*	SD
African	This Study[17]	7	53.84	5.14	10	51.55	4.35	5	57.60	4.90
European	Skinner *et al* (2015)[15]	9	56.09	8.04	14	51.90	6.51	6	49.18	6.59
Mixed Ancestry (Maxillary)	Grine (2002)[18]	10	70.10	7.50	10	68.40	8.70	10	62.20	7.80
Mixed Ancestry (Mandibular)	Grine (2002)[18]	10	63.40	6.60	10	58.30	10.70	10	59.20	10.00
European (Mesial)	Mahoney (2010)[19]	26 26	57.28 55.94	6.88 6.63						
European (Distal)	Mahoney (2010)[19]									

[15]See note for Table 15. [17] Fulbe and Nso crown size from Table 2 results in n=21. Here, crown size for Fulbe SK 37 M3, which was removed as an outlier, was included for combined African sample. [18] Grine (2002) measured crown size in modern human deciduous and permanent molars from mixed ancestries and locations (n=80) by analysing histological sections with SEM. [19]Mahoney (2010) used light microscopy to measure crown size in deciduous and permanent mandibular molars. Histological sections were taken from archaeological samples of modern human juveniles (n=69) from England and Scotland.

The combined African sample had the smallest tooth crown size for the second molars and the data from Skinner *et al* (2015) had the smallest for the third molars. The samples from Grine (2002) were much larger than both of these populations. Both of the comparative populations had a slightly smaller crown size in each successive molar. In the African population, the second molars had a slightly smaller crown size than the first molars, but tooth crown size appeared to be slightly larger in the third molars. However, this was only a minor variation.

Summary Box 7

The mixed-ancestry population from Grine (2002) had the largest average tooth crown size across all three molars. In particular, the first molar values were much larger than the combined African (Fulbe and Nso) sample examined in this study. The mean crown sizes of the European comparative human samples did not differ from the combined African population.

5.3.3. DSR

The DSRs of the African and European first molars examined in this study are presented alongside a comparative modern human sample from Mahoney (2008) in Table 17. DSRs

are split into inner, mid and outer to represent the three measurement regions for the cuspal enamel.

Table 17- Mean DSR (in μm/day) for the first molars of the combined African and European samples from this study and a comparative sample from Mahoney (2008) (+/- 1SD)

Population	Source	n	*X*	n	Inner	n	Mid	n	Outer
African	This Study[20]	9	3.64 (0.28)	8	3.39 (0.33)	9	3.58 (0.28)	8	3.94 (0.35)
European	This Study	20	3.81 (0.32)	19	3.61 (0.29)	17	3.96 (0.30)	8	4.14 (0.10)
European	Mahoney (2008)[21]			15	2.97 (0.51)	15	4.15 (0.56)	15	4.55 (0.61)

[20] Fulbe and Nso mean DSR from Table 7 and 10 results in n= 7. Here, mean DSR for Nso SK 1 and SK 6, which were removed as outliers, were included for combined African first molars. Fulbe and Nso mid DSR from Table 7 results in n=7 with Nso SK 1 and SK 3 removed as outliers. Here, values included for combined African first molars. [21]Mahoney (2008) examined DSR in permanent mandibular first molars of archaeological juveniles (n=15) from British Bronze age (2,300-700BC) using polarized light microscopy.

All three populations in Table 17 showed the expected increase in DSR from the inner to the outer regions of cuspal enamel. The European first molars analysed in this investigation had a greater average DSR across the enamel cap than the combined African first molars. A Mann Whitney test revealed there was a significant difference in mean DSR from the mid enamel region between the African and European first molars (U=23.0, p= 0.004). Both samples had greater DSRs from the inner enamel region than the comparative sample from Mahoney (2008). However, the latter sample had faster DSRs in the mid and outer enamel regions.

Table 18- Mean DSRs (in µm/day) for all molars from the combined African population from this study and comparative human samples from previous publications (+/- 1SD)

Population	Source	n	$\bar{X}$	n	Inner	n	Mid	n	Outer
African	This Study[22]	35	3.65 (0.32)	27	3.27 (0.28)	34	3.56 (0.32)	34	3.98 (0.39)
Unknown	Beynon *et al* (1991)[23]			11	2.70 (0.40)	15	4.30 (0.70)	12	5.10 (0.70)
Unknown	Dean (1998)[24]	1	4.00 (1.40)	1	2.66 (0.15)	1	3.44 (0.23)	1	5.50 (0.80)
Unknown	Lacruz & Bromage (2006)[25]			10	2.80 (0.43)	10	4.50 (0.55)	10	5.20 (0.58)

[22]Fulbe and Nso inner DSR from Table 4 results in n= 28. Here, Nso SK 33 removed as outlier for combined African first molars. [23]Beynon *et al* (1991) used polarising light microscopy to analyse sections of *Homo sapiens* premolars and molars (n= 39). No information on origin of samples. [24]Dean (1998) measured DSR in a single *Homo sapiens* second permanent molar using polarized light microscopy. No information on origin of sample. [25]Lacruz and Bromage (2006) applied transmitted light to ground sections of modern human molars (n= 10) to measure DSR. No information on origin of samples.

The DSRs for all of the African molars examined in this study are presented alongside comparative modern human samples published by Beynon *et al* (1991), Dean (1998) and Lacruz and Bromage (2006) in Table 18. Like Table 17, DSRs are split into inner, mid and outer to represent the three measurement regions for the cuspal enamel.

Every comparative human sample in Table 18 displayed the expected increase in DSR across the cuspal enamel. When just the African sample was considered, a Levene's test showed there was equality of variance (p= 0.069) in mean secretion rates across the enamel cap for all three molar types, and so satisfied the assumption for parametric testing. A one-way ANOVA revealed that DSRs differed significantly from the inner, mid, and outer enamel (f [2,92] = 34.19, p< 0.005). Post hoc Tukey's-Kramer pairwise

comparisons showed that DSRs differed significantly between inner and mid (Q= 4.755, p= 0.003), inner and outer (Q= 11.730, p< 0.005) mid and outer (Q= 6.975, p< 0.005).

The combined African population had a greater DSR from the inner region than all three comparative samples, and the difference was greater than one standard deviation of all the mean values. The second permanent molar from Dean (1998) had the smallest mean DSR for the inner and the mid regions. However, it had the largest average for the outer cuspal enamel at the same time as the combined African molars had the smallest. This was the largest difference between all of the populations across all three regions (1.56µm/day).

Summary Box 8

Average DSRs for the European first molars examined in this study were faster than the mean values for the combined African first molars. This difference was present across the enamel cap, and was significant in the middle region. The combined African molars had the greatest DSR from the inner region of enamel, and it was more than one standard deviation faster than all of the other comparative datasets. All of the samples from previous publications had a greater increase in mean DSR from the inner to outer cuspal enamel region.

5.3.4. EER

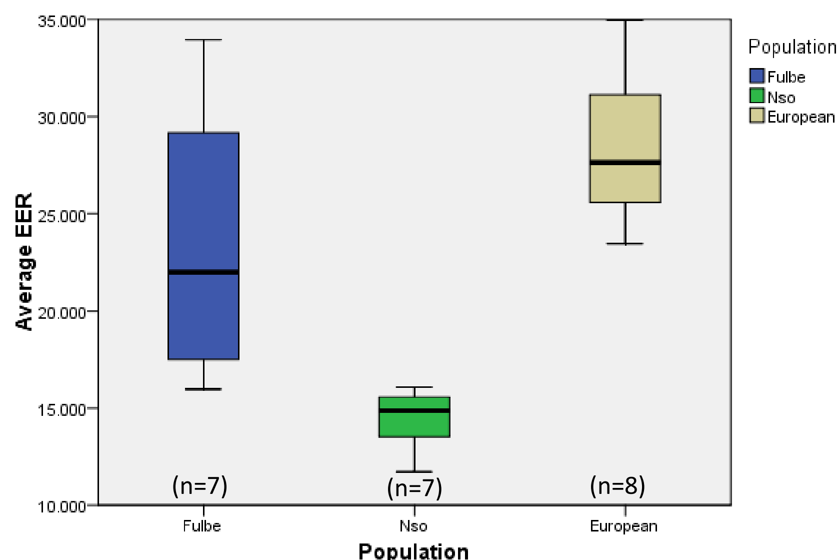
Table 19- Mean values of EER (in µm/day) for the first molars of the Fulbe, Nso and European populations from this study (+/- 1 SD)

	n	*X*	n	Crown	n	Mid	n	Cervix
Fulbe	1	25.15 (-)	1	32.94 (-)	1	20.70 (-)	1	21.80 (-)
Nso	3	14.96 (1.07)	1	15.34 (-)	1	13.39 (-)	3	14.67 (1.52)
European	8	28.39 (3.79)	4	34.83 (7.97)	7	28.72 (3.41)	5	25.31 (4.45)

Since clear differences in average EER were present between the pastoralists and agriculturalists (as outlined in section 5.2 and shown in Tables 2, 6, 7, 9, 10 and 13), the data from these populations has not been combined to contrast with the European comparative sample. Instead, Table 19 shows the mean EER as well as averages for all three measurement regions for the first molars of the Fulbe, Nso and European populations examined in this study.

As expected, all three populations displayed decreasing EERs in each successive measurement region, from the crown down towards the cervix of the tooth. Extension rates were faster in the European first molars than both the Fulbe and Nso samples across the enamel cap. However, the overall mean EER for the Fulbe, as well as the mean values from the regions of enamel towards the crown and cervix, were within one standard deviation of the European values. Hence, there were no significant differences between the Fulbe and European first molars, but there were significant variations between these samples and the Nso first molars. Fig. 11 shows a boxplot for the average EER for all of the molars from the Fulbe, Nso and Europeans. It highlights the similarity between the Fulbe and European samples and the variation between the Nso and European molars.

Fig. 11- Graph of average EER (in µm/day) for all of the Fulbe, Nso and European molars



Summary Box 9

The European first molars had faster EERs than the Nso samples across the enamel cap. Extension rates did not vary between the Fulbe and Europeans.

5.3.5. Correlations and Regressions of combined (Fulbe and Nso) African and European samples

5.3.5.1. Correlations

The significant results for the Pearson's and Spearman's correlations performed on the combined African (Fulbe and Nso) and European molar samples examined in this investigation are discussed below. A full list of results can be found in the Appendix.

When the data for the Fulbe and Nso first molars was combined into one group (the African sample), Spearman's correlations revealed that buccal cusp AET was negatively correlated with average DSR from the outer cuspal enamel (r= -0.900, n= 5, p= 0.037) and overall average DSR (r= -1.000, n= 5, p< 0.005). These associations accounted for 78.9% and 66.0% of the variance in these measures, respectively, and suggested a moderately strong negative association between them in the buccal cusps of the first molars.

Spearman's correlations also showed there was a positive correlation between lingual cusp AET and overall average DSR (r= 0.900, n= 5, p= 0.037) and average DSR taken from the mid cuspal enamel region (r= 0.900, n= 5, p= 0.037). The first association explained 94.3% of the variance, whilst the second explained 88.9%. This suggests whilst there was a negative association between AET and DSR in the buccal cusps, the opposite was true for the lingual cusps of the combined African first molars. However, sample sizes were

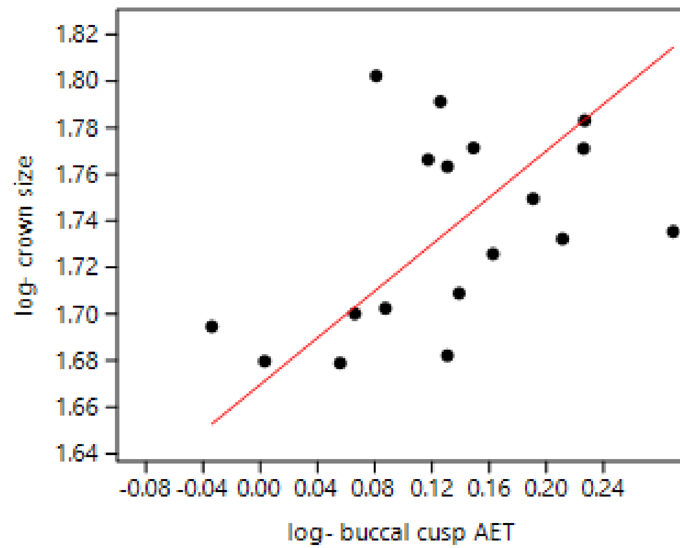
small, which might explain the reversal of this trend between these cusps. There was a positive correlation between mean DSR from the inner region and prism width within all of the molars in the combined African population (r= 0.900, n=5, p= 0.037). This correlation explained 70.0% of the variance.

An additional analysis of the European sample of first molars examined during this investigation revealed a positive correlation between AET of the lingual cusp and average DSR from the outer cuspal enamel (r= 0.857, n= 7, p= 0.014), which explained 61.8% of the variance. This result was similar to the associations found in the combined African (Fulbe and Nso) first molars. There was also a positive association between average DSR from the inner enamel region and EER from the region near the cervix (r= 1.000, n= 5, p< 0.005) which accounted for 88.6% of the variance in these measures.

5.3.5.2. Regressions

Once all three molar types from the Fulbe and Nso populations were combined into an overall African sample, an RMA regression showed that log-buccal cusp and log-crown size were significantly correlated (p= 0.050) across the population, and scaled with negative allometry (r= 0.469; slope= 0.502; 95% CI= 0.239-0.678; intercept= 1.670). Fig. 12 presents a graph of these log-transformed variables plotted against each other for all molars from the combined African population. The RMA regression line has been fitted to the dataset.

Fig. 12- Plot of log-transformed buccal cusp AET against log-transformed tooth crown size for all molars from the combined African (Fulbe and Nso) sample



Least squares and RMA regressions for the Fulbe, Nso, combined African (Fulbe and Nso) and European samples examined during this investigation revealed no other significant associations between the four main variables (AET, tooth crown size, DSR and EER).

Summary Box 10

Both the combined African and European populations analysed in this investigation had a negative correlation between AET and DSR in the lingual cusps. These measures were positively associated in the African buccal cusps. There were also various positive correlations between DSR and EER across the enamel cap of the European samples. Prism width and DSR from the inner enamel region were positively correlated in the African molars.

Chapter 6: Discussion

6.1. Outline

The results of this investigation are discussed below. The discussion is split into three sections, each of which focuses on the three main research questions for this project:

1. *Does enamel formation differ between the Fulbe (pastoralists) and Nso (agriculturalists)?*

   Variations between the first and distal (second and third) molars are discussed in this section.

2. *Does variation in enamel growth between the two African populations correspond with diet after weaning?*

   The impact of these results on studies of hominin life history, particularly identifying the age-at-weaning from fossils, are reviewed in this section.

3. *Does enamel growth and thickness in the African first molars (both Fulbe and Nso combined) differ compared to the European first molars?*

   The variations between the combined African (Fulbe and Nso), European and comparative populations are discussed in this section.

A summary of the main limitations and possible improvements to this study is included, followed by recommendations for future research at the end of this chapter.

6.2. Does enamel formation differ between the Fulbe (pastoralists) and Nso (agriculturalists)?

6.2.1. EER, Prism width and the Stretching Effect model

The most notable difference between the Fulbe and Nso molar samples was the significantly faster cuspal EERs in the pastoralists compared to the agriculturalists. This

trend was present for every cusp measured from all three molar types. As shown in Table 6, whilst both populations showed the expected decrease in EERs from the first measurement region near the dentin horn to the final region towards the cervical enamel, which is in line with previous investigations (Reid *et al.* 1998; Dean 2009; Dean 2010; Guatelli-Steinberg *et al.* 2012), extension rates were consistently faster in the Fulbe molars. There was some variation between the molar cusps for both populations, especially the two lingual cusps of the Fulbe third molars (see Table 13), however it is unclear whether these differences were significant due to the small sample sizes. The average values for the Fulbe were in line with previously recorded EERs taken from human canines and first molars, which ranged between approximately 20-30µm/day (Dean 2009). It should be noted the mix of anterior teeth and molars examined by Dean (2009) may make this an unreliable comparison as EERs have been found to vary between tooth types, although it has been suggested there is limited variation between canines and molars (Mahoney 2015). Nevertheless, the average values for the Nso fell below this range and appeared to be no more than 66.7% as fast as those for the Fulbe (see Tables 2 and 6 in Results).

As mentioned previously, EERs are slower in the lateral enamel than the cuspal enamel. Dean (2009) found an average decrease of between 3-6µm/day between the cuspal and lateral EERs of modern human teeth. The pathological damage present within the Fulbe and Nso molars prevented EERs from being measured in the lateral enamel, which could have helped to establish the full range of variation between these samples. However, it is significant that the cuspal EERs for the Nso molars were similar to lateral values from the anterior teeth of southern African and northern European populations, both of which exhibited maximum values of approximately 16µm/day (see Fig. 6a in Guatelli-Steinberg

et al. 2012). This similarity suggests cuspal EERs in the Nso molars, which were formed at the beginning of amelogenesis and supposed to be at their highest rate, were only as fast as the slowest extension rates for the southern African and northern European populations. Therefore, the difference between the Fulbe and Nso extension rates, and between these samples and previous datasets, suggests cuspal enamel growth may vary significantly between modern human populations.

The higher EERs of the Fulbe molars appear to be linked with faster DSRs near the EDJ, and consequently faster formation times (time to form approximately 200µm of enamel) within this area. Previous studies have linked EERs to overall crown formation times, which have been hypothesised to be 30% quicker in australopithecines (Dean and Reid 2001) and 15% quicker in *H. neanderthalensis* (Ramirez Rozzi and Bermudez de Castro 2004) compared to modern humans. This is significant as the mean formation times for the inner cuspal enamel appeared to be significantly different between the Fulbe and Nso molars, and EERs were almost twice as fast in the pastoralists as the agriculturalists (see Table 6). It is important to note crown formation times were not calculated for the Fulbe and Nso because of the pathological damage present in the molar samples. The formation times for the enamel between the EDJ and accentuated markings were calculated by dividing the distance between these points by the average DSRs within this area. A more accurate formation time could have been gained by counting the number of cross striations along a single enamel prism, like Dean (2009). However, the poor preservation of some samples also meant this method could not be applied to the Fulbe and Nso molars. Yet, the differences in DSRs, formation times and EERs at the beginning of amelogenesis between these neighbouring populations appear to be on the same scale as has been previously attributed to separate hominin species. As a result, they highlight

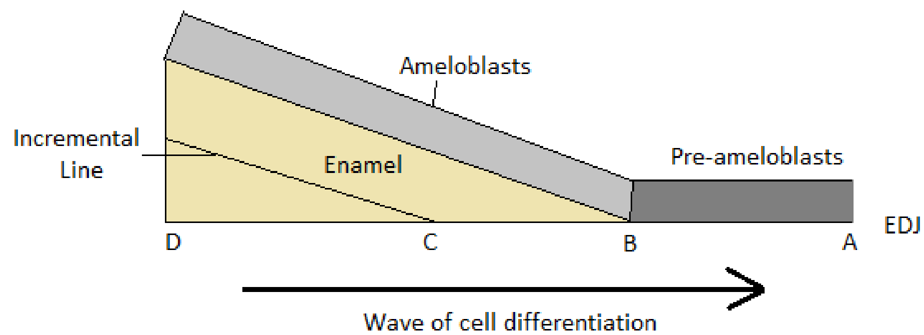
a greater range of variation for cuspal enamel growth in modern human populations than previous revealed.

Average prism width was also found to be larger in the Fulbe than the Nso. It was not possible to conduct inferential statistical tests on these measurements as only a few samples were examined, but the mean values for the two populations were at the extreme ends of their respective standard deviations. Yet, the average widths for both populations were within the expected human range, between 4µm and 7µm (Boyde 1989; Höhling 1989; Risnes 1998; Berkovitz, Holland and Moxham 2002; Ross and Pawlina 2006; Hillson 2014).

Few studies have examined prism widths in fossil hominins or non-human primates because SEM is both destructive and expensive. However, in their study of orangutan, siamang and a single *Proconsul* tooth, Dean and Shellis (1998) discovered prism width remained constant or decreased slightly between the inner and outer region of lateral enamel. Macho *et al* (2003) contended that the opposite was true, and that an increase in prism width was necessary to establish the larger radius of the outer surface of the tooth crown. Dean (2004) rebuked this argument and stated that prism widths only increased in the last 200µm of the outer cuspal enamel, not the lateral enamel. He also stated the reduction in the previous study could be the result of the EDJ being longer than the outer perimeter of the enamel cap. These arguments appear to be more plausible, as although Macho *et al* (2003) appeared to be correct about the function of increased prism width, it should not be applied to lateral enamel because this region does not exhibit the same degree of outward expansion as cuspal enamel. In this study, measurements were only taken within the inner region of cuspal enamel, so it is unclear whether prism width

increased, decreased, or remained constant towards the outer surface of the tooth. However, the pastoralists clearly displayed larger prism widths than the agriculturalists, and these could have increased further in the outer cuspal enamel.

Fig. 13- Diagram of the stretching effect model adapted from Shellis (1984)



Extension rate and prism size could have been greater in the pastoralists because of the stretching effect model outlined by Shellis (1984). As shown in Fig. 13, the author suggested that when the secretory stage of amelogenesis begins, ameloblasts that once occupied the length AB (=BC) must increase their occlusal-cervical width in order to cover the length BD. This stretching effect has a greater impact on teeth with higher rates of ameloblast differentiation (EER) because the EDJ may be longer. Similarly, Mahoney (2015) found initial EERs were correlated with EDJ length, which suggested that teeth with longer EDJs grew proportionally faster than those with shorter EDJs. The deciduous dentition has faster initial DSRs and EERs than the permanent teeth (Macho and Berner 1993), as shown by the fact that Mahoney (2015) recorded extension rates of up to 39.97µm/day for deciduous molars and 52.03µm/day for deciduous incisors. However, Guatelli-Steinberg *et al* (2012) discovered the same relationship was also present in mandibular first permanent molars, as longer EDJs were associated with faster EERs in the cuspal enamel. Shellis (1984) also stated this stretching phenomenon could impact the secretory area of ameloblasts as they migrate through the enamel cap, and so may

alter the size of the prisms they leave in their wake. As such, this theory presents a clear link between EER and prism width. It also provides further support for Dean (2004), as faster EERs may stretch the EDJ and cause increased prism widths in the cuspal enamel, but slower EERs in the lateral and cervical enamel are unlikely to have a prominent effect on prism width.

In this study, EDJ length was not measured and correlated with extension rate, so it remains unclear whether tooth crown height was responsible for faster rates of ameloblast differentiation within the cuspal enamel. The lack of difference in AET, which represents the relationship between enamel cap area and EDJ length (Feeney *et al.* 2010), may indicate that EDJ length did not vary between the Fulbe and Nso. However, the fact that both EER and prism width were greater in the Fulbe could be explained by Shellis' model. Moreover, although these variables were not measured within the lateral enamel and so their relationship throughout amelogenesis remains unclear, a link between them in the cuspal enamel could suggest that the start of enamel growth differed between the pastoralists and agriculturalists. Smith *et al* (2006) stated the level of variation in EDJ shape between modern human populations is unknown. Therefore, the results from these neighbouring populations could provide evidence for an as yet unseen level of variation in modern human enamel development.

6.2.2. Tooth size

Enamel thickness in both the Fulbe and Nso molars was similar to other human samples (Macho and Berner 1993; Smith *et al.* 2006; Smith *et al.* 2008; Smith *et al.* 2012). Mean AET values for both populations were greater than previously published values for *Pan*, but showed some overlap with *Pongo* (Smith *et al.* 2005). These trends are typical of

hominoid variation as chimpanzees have relatively “thin” enamel compared with the “thick” and “intermediate/thick” enamel of humans and orangutans, respectively (Martin 1985, p.261).

There was no significant difference in mean AET between the Fulbe and Nso, although values were slightly greater in the pastoralists. There was also limited variation between the cusps of the Nso samples, although enamel thickness in the first molars was slightly larger in the buccal and distal cusps compared with their lingual and mesial counterparts, respectively. These differences are in line with Mahoney (2010), who found AET to be greater in the buccal cusps of mandibular molars from the UK, and Kono, Suwa and Tanijiri (2002), who recorded larger AET values in the distal cusps of Asian first molars. On the other hand, AET was larger in the distolingual cusps than the distobuccal cusps of the Fulbe first molars, and vice versa for the Fulbe third molars. These patterns appear to contradict Mahoney (2010), and other studies which found enamel to be thicker on the lingual cusps of all three maxillary molars (Macho and Berner 1993; Schwartz 2000a; Kono, Suwa and Tanijiri 2002). However, the mean AET of both the Fulbe first and third molars was based upon a single sample (one mandibular first molar and one maxillary third molar), so it is unclear whether these results were significant or anomalous.

The lack of variation between the Fulbe and Nso was consistent with the study by Górka *et al* (2015), which showed no significant difference in tooth size between hunter-gatherers and agriculturalists for either mandibular or maxillary first molars. It is important to note Górka *et al* (2015) did not analyse histological sections or calculate AET, but instead used digital images to measure the size of the enamel cap, including the

bucco-lingual and mesio-distal crown diameters as well as total area of the occlusal surface. Although these results are not directly analogous to AET, they support the inference that the overall size and thickness of molar enamel did not vary between these two modern human populations with diverse subsistence strategies.

However, average tooth crown size for all three molars combined, which includes the enamel cap and dentin core, was found to be significantly larger in the Nso than the Fulbe. Both populations exhibited smaller average crown sizes than the maxillary and mandibular molars presented in Grine (2005). In his study of mixed-ancestry modern human molars, Grine (2005) argued it is important to include a scaling factor when comparing enamel thickness and tooth crown size between individuals or populations in order to reduce the impact of body or tooth size variations. Hence, for this investigation, the area of the crown enclosed by the outer enamel surface and linear bi-cervical diameter was measured, which Grine (2005) suggested as a suitable scaling factor and proxy for crown size.

Size variations could explain why tooth crown size, which was scaled, was significantly different between the Fulbe and Nso, but AET was not. Any differences in molar size between the two groups may not originate from thicker enamel but may instead be the result of variations in the dentin core size (Mahoney 2010) or EDJ shape (Skinner *et al.* 2008). The length of the EDJ was not measured, however a discrepancy in this factor would be consistent with the arguments outlined above, which suggest that molar crown height may vary between the Fulbe and Nso because they have different EERs and prism widths. Thus, these results could indicate that the Fulbe molars had taller enamel caps but the Nso tooth crowns had a larger area. Since Górka *et al* (2015) found no difference

in crown size between agriculturalists and hunter-gatherers, it appears these morphological differences are most likely a product of modern human population variation rather than differing subsistence strategies.

6.2.3. Enamel secretion and development

The agriculturalists displayed a slightly higher average DSR than the pastoralists, but there was no significant difference between the two populations. Both the Fulbe and Nso molars showed slightly faster secretion rates in the mesiobuccal than the mesiolingual cusps as well as the distolingual compared with the distobuccal cusps. Mahoney (2008) also found DSRs to be accelerated in these cusps during his study of mandibular first molars, although these variations were not present in all three regions of cuspal enamel (see Table 2b). Like AET, DSRs varied more between the molar cusps of the pastoralists than the agriculturalists, which was likely a result of the limited sample size for this population. Nevertheless, positive correlations were found between DSRs and EERs in the Fulbe. These associations show rapid ameloblast differentiation was associated with faster enamel secretion rates in the pastoralists, which Mahoney (2015) also discovered in the deciduous incisors of a medieval British population.

Average DSRs across the enamel cap for the Fulbe and Nso were within the known range of modern human variation (approximately 2-5.5µm/day) recorded in previous studies (Risnes 1986; Beynon and Wood 1987; Boyde 1989; Reid, Beynon and Ramirez Rozzi 1998; Lacruz and Bromage 2006; Birch and Dean 2009; Smith *et al.* 2009). Human DSRs, including those for the Fulbe and Nso, show some overlap with values recorded for the Neanderthals (Smith *et al.* 2007; Smith 2008), but are considerably slower than the average values for *Australopithecus*, which are estimated to be up to 7.3µm/day

(Beynon and Wood 1987). Dean *et al* (2001) explained that human enamel development is slower than other fossil hominins because enamel formed at the start of amelogenesis, near to the EDJ, is secreted in smaller increments over an extended period of time. Thus, it is not surprising that both populations showed an increase in DSR from inner to outer cuspal enamel, which was slightly greater in the Nso.

It is possible the lack of variation in DSR as well as AET between the Fulbe and Nso populations is connected. Several studies have suggested crown enamel thickness is largely determined by secretion rates, which are higher in the thicker outer enamel (Beynon 1992; Macho and Berner 1993; Dean *et al.* 2001; Smith, Martin and Leakey 2003; Ramirez Rozzi and Bermudez de Castro 2004). With this in mind, it is possible mean AET did not differ significantly between the pastoralists and agriculturalists because there were only slight variations in average DSR between the two populations. The lack of variation in DSRs and AET may also be linked with the positive correlation between DSR at the mid region of cuspal enamel and tooth crown size within the Nso molars. In his study of perikymata within a single *Paranthropus* tooth, Dean (2009) discovered enamel surface area was wider towards the cervix of the tooth in this species than in modern humans, which he attributed to faster secretion rates. Since modern humans and *Paranthropus* both have thick enamel (Beynon and Wood 1986; A.J. Olejniczak *et al.* 2008; Smith *et al.* 2012), the author suggested DSRs within this area may alter dental morphology without changing enamel thickness. The correlation between secretion rates and crown size within the Nso molars originates from the mid cuspal enamel, however this theory could explain how slightly faster DSRs might alter the three-dimensional morphology, and subsequently two-dimensional crown size, of the agriculturalist molars without causing significantly larger AET.

Finally, it should be noted that none of the growth or morphology variables showed significant differences between the males and females in either population. Both sexes followed the patterns discussed above (e.g. higher EERs in the Fulbe), but there were no consistent trends and only minor variations between males and females. Although this appears to contradict the assertion that males have larger tooth crowns than females made by Schwartz and Dean (2005), the authors also stated that sexual dimorphism of dental characteristics varies across modern human populations. During their investigation of teeth from South Africa and the UK, the authors found no real difference in third molar AET between males and females (Schwartz and Dean 2005). Górka *et al* (2015) also showed that male and female molars had similar dimensions, including the first molars of agricultural populations from South Africa and the Democratic Republic of Congo. These studies support the evidence that molar crown morphology did not differ between males and females in the pastoralist and agriculturalist populations of Cameroon.

On the other hand, the similarities between the sexes in this study contrasts the findings of Schwartz *et al* (2001), which showed crown formation rates to be faster and linear enamel thickness to be greater in females. This discrepancy is likely caused by sampling differences, as Schwartz *et al* (2001) examined the canines of extant hominoids, including humans. Not only do canines and molars show great morphological variation, but it has also been suggested that the incremental growth of canine enamel may display high levels of sexual dimorphism (Reid and Ferrell 2006). Hence, the lack of significant differences between the male and female molars of the Fulbe and Nso seems to be typical of modern human populations.

6.2.4. First and Distal Molars

When the Fulbe and Nso samples were split by tooth type, no significant differences were revealed between the first, second and third molars in either population. Like the previous comparisons with all three molars combined, tooth crown sizes were larger in the Nso whilst EERs were faster in the Fulbe across the molar row. Both populations displayed a minor increase in AET between the first and third molars, which is typical of the modern human dentition (Macho and Berner 1993; Schwartz 2000b; Smith *et al.* 2005; Mahoney 2010; Mahoney 2013). This trend has been attributed to a relative reduction in the size of the dentin core (Grine 2002; Grine 2005; Smith *et al.* 2006), which also explains the slight decrease in crown size between the first and second molars in both the Fulbe and Nso. There was no further reduction in tooth crown size in the Nso third molars, but this discrepancy could be attributed to the preservation of these samples. It has also been suggested that posterior molars have thicker enamel with an increased bite force as a functional adaptation to cope with prolonged masticatory loading (Macho and Berner 1993; Kono, Suwa and Tanijiri 2002), or it could be the result of a prolonged period of enamel secretion or crown formation (Schwartz 2000b; Smith *et al.* 2005; Mahoney 2010). AET appeared to be significantly larger in the distal cusps as well as the mesiolingual and distobuccal cusps of the Nso first and second molars, respectively. However, these results may be an unreliable representation of enamel thickness in these cusps as a maximum of three samples were measured from both populations.

The DSRs for the pastoralists and agriculturalists followed two opposing patterns, which appeared to be caused by variation between the first molars of the two populations. As shown in Table 12, secretion rates were significantly faster in the mesiolingual and

distolingual cusps of the Fulbe first molars compared to the Nso first molars. However, for all of the comparable cusps measured in the second and third molars, secretions rates were faster in the Nso than the Fulbe. Hence, two distinct trends appeared within the samples from the pastoralists and agriculturalists, as DSRs decreased between the first and second molars of the former but increased in the latter. This pattern could be linked to enamel thickness, as overall mean AET and DSR for the first molars were both greater in the Fulbe than the Nso, and, as explained previously, AET is dependent upon DSR (Beynon 1992; Dean *et al.* 2001; Smith, Martin and Leakey 2003; Ramirez Rozzi and Bermudez de Castro 2004). However, the mean values for these variables were not significantly different between the three types of molar in either population.

Separating the first molars from the combined distal (second and third) molars could reveal variations in enamel growth before and after the weaning process, as first molar development encompasses weaning whilst second and third molars grow after it has ceased (Smith 1991; Hill and Kaplan 1999; Humphrey 2010). Yet, this comparison revealed little difference in enamel formation before and after weaning in either population. AET appeared to decrease slightly between the first and combined distal molars in both populations, as displayed in Table 10, but this is likely the result of the reduced AET of the second molars. EERs for both populations were faster in the first molars than the distal molars, which is consistent with Smith (2008), who highlighted the same trend for coronal extension rates. However, this variation was not significant.

As stated previously, the average DSRs for the Fulbe and Nso samples followed two diverse patterns. The first molars had faster secretion rates than the distal molars for the pastoralists, and vice versa for the agriculturalists. These differences appeared to be

significant when comparing individual cusps between the first and distal molars of both populations. However, when the overall values for each molar type or the first and combined distal molars were compared, only slight variations were present in both populations (see Tables 7 and 10 in Results). This discrepancy is most likely caused by differing sample sizes, as the limited number of molars included in the comparison of DSRs before and after weaning may be exaggerating the level of variation in both populations. Consequently, there was no evidence of any significant difference between enamel growth before and after the weaning process in either the pastoralists or agriculturalists.

6.3. Does variation in enamel growth between the two African populations correspond with diet after weaning?

6.3.1. The impact of pastoralist and agriculturalist diets on amelogenesis

After weaning, the Fulbe continue to consume milk and milk-based products derived from their cattle herds, but the Nso do not. If consuming milk past the age of weaning affected enamel formation, the pastoralists and agriculturalists would exhibit similar growth rates in the first molars, which develop during the weaning process, and varying growth rates in the combined distal (second and third) molars, which grow after weaning (Smith 1991; Hill and Kaplan 1999; Humphrey 2010; Smith *et al.* 2013). Yet, neither population displayed significant differences in enamel formation between the first and combined distal molars. There were some slight variations in mean DSRs, but these were not significant. Thus, diet, or at least one that includes consuming milk after weaning, did not have a significant impact on enamel growth.

The lack of variation after weaning between the pastoralists and agriculturalists could be caused by the nutritional composition of milk. A prominent aspect of the Fulbe diet is cow's milk and its various derivatives, which the United States Department of Agriculture (2016) stated is composed of 3.7% fat, 3.3% protein, 4.7% carbohydrate and 87.7% water. These proportions are similar to those recorded for human milk, which contains 4.4% fat, 1.0% protein, 6.9% carbohydrate and 87.5% water. It is evident from these values that both species express milk which contains a significant amount of water. Hinde and Milligan (2011) outlined how dilute milks are associated with mammalian species in which there is a protracted period of lactation and infants spend the majority of time with their mothers. The authors explained that expressing milk with an elevated water content and low energy density reduces the physiological demand of breastfeeding on the mother. Thus, the cow's milk consumed by the Fulbe mostly consists of water. Since the levels of key macronutrients (fat, protein and sugar) are limited, their presence in the Fulbe diet is unlikely to cause a significant difference in post-weaning enamel formation between the pastoralists and agriculturalists.

However, even though it only constitutes a small proportion of cow's milk, it is surprising that an additional source of protein within the Fulbe diet had no discernible effect on enamel formation. As stated previously, enamel proteins organise the structure of hydroxyapatite crystals during amelogenesis (Ross and Pawlina 2006; Simmer *et al.* 2010; Mahoney 2011). In order to synthesize these proteins, humans must acquire specific amino acids from their diet. This is important, as Bavetta *et al* (1962) explained, because the symptoms of enamel protein deficiency can present themselves even if only one indispensable amino acid is missing from an individual's diet. This is why a full supplement of amino acids is required for protein synthesis to take place. Milk proteins can provide

the amino acids necessary for infant growth, including enamel formation (Hinde and Milligan 2011). With this in mind, it is surprising that cuspal enamel growth rates in the distal molars of the Fulbe and Nso did not differ, as the former had access to an additional, if not substantial, source of the amino acids necessary for enamel protein synthesis.

Similarly, the continued consumption of milk and milk-based products past the age of weaning could provide the pastoralists with an additional source of calcium and phosphorous. Calcium and phosphate ions form the crystalline hydroxyapatite that dominates the enamel tissue (Boyde 1989), and so they are essential to enamel mineralization (Ross and Pawlina 2006; Lézot *et al.* 2006; Zhang *et al.* 2009; Hillson 2014). Most of the protein in cow's milk is casein (Miller *et al.* 2013), which is a phosphoprotein that forms a major source of phosphorous for mammalian infants (Martin and Hine 2008). In total, every 100g of cow's milk contains roughly 119mg of calcium and 93mg of phosphorous (United States Department of Agriculture 2016). These values are made even more substantial by the Fulbe's daily consumption of cow's milk, which means there could be significantly greater levels of these micronutrients in their diet compared to the Nso diet. Thus, it is surprising enamel growth rates were not faster in the distal molars of the pastoralists than the agriculturalists. The lack of variation in post-weaning molar cuspal enamel development between the Fulbe and Nso populations suggests the continued consumption of milk had no effect on amelogenesis.

It is possible cuspal enamel growth rates did not differ between the Fulbe and Nso distal molars because the agriculturalists were able to match the increased levels of protein, calcium and phosphorous provided by cow's milk through other dietary sources. Larsen (1995) described how agricultural societies rely on a small set of domesticated plants with

poor nutritional value, such as maize, which provides a limited supply of amino acids. Yet, in their review of the nutritional properties of sub-Saharan African plants, Uusiku *et al* (2010) stated that leafy vegetables can provide a supplementary source of proteins and micronutrients, including calcium. One example is *Adansonia digitate*, which can be found in northern Cameroon and has a calcium content of 410mg per 100g of plant material. The authors maintained that certain leafy plants "could potentially contribute significantly towards the dietary requirements" of calcium (Uusiku *et al.* 2010, p.504). Furthermore, the Nso also cultivate beans (Keller, Demuth and Yovsi 2008; Otto, Potinius and Keller 2014), which contain relatively more protein than cereals. As Odendo *et al* (2011) explained, a combination of legumes and cereals can provide a complete supply of dietary proteins and essential amino acids. The agriculturalists could also increase their protein and amino acid levels by consuming animal meat, such as sheep and goats (Nurse *et al.* 1994). Thus, the agriculturalists could have access to the same levels of protein, calcium and phosphorous as the pastoralists, which would account for the lack of variation in cuspal enamel growth rates after weaning between these populations. Additional information on the levels of macro- and micronutrients present in the Fulbe and Nso diets is needed to confirm this theory.

Nevertheless, cuspal enamel growth rates post-weaning may not have been faster in the Fulbe than the Nso because amelogenesis does not accelerate exponentially in response to increasing levels of macro- and micronutrients. As explained previously, enamel formation is largely determined by genetics and is relatively resilient towards environmental sources of variation (Tonge and McCance 1973; Smith 1989; Smith 1992). For example, Yoko (2000) examined the crown size of molars extracted from inbred and closed colony (randomly mating within the stock) rats, which had been fed diets with

varying levels of protein and animal fat during early development. The author discovered increasing the amount of protein, fat and carbohydrates consumed during molar growth had a limited effect on the tooth's size. Although this study was conducted on experimental rats with a limited genetic background, it suggests there is a biological threshold for the level of nutrients, such as protein, calcium and phosphorous, necessary for healthy amelogenesis. This could mean that in humans, even if an individual's diet provides levels of nutrients beyond this threshold, enamel growth rates cannot exceed their genetically predetermined levels.

This theory is supported by studies of hypoplastic defects, which form on the surface of teeth during episodes of nutritional deficiencies or pathological stress (May, Goodman and Meindl 1993; Reid and Dean 2000). For example, hypocalcaemia, a temporary calcium deficiency, can disrupt amelogenesis after birth and form a linear hypoplastic defect within the developing enamel (Ranggård and Norén 1994; Antoine, Hillson and Dean 2009; Hillson 2014). Hypoplasia reflects a temporary disruption rather than an on-going problem, because, as May *et al* explained, there is a "threshold level of physiological stress necessary to impair ameloblastic function" (1993, p.46). Similarly, Shaw and Griffiths discovered rats fed a protein-deficient diet could not achieve the "inherited blueprints" for enamel growth (1963, p.140). With this in mind, additional supplies of micro- and macronutrients above the threshold level necessary for normal amelogenesis are unlikely to affect enamel growth rates. Therefore, the distinct diets of the pastoralists and agriculturalists after the age of weaning may not significantly change enamel formation because both include adequate levels of essential nutrients, such as protein, calcium and phosphorous.

Whilst there were no significant differences in cuspal enamel growth rates between the first and distal molars of either population, EERs and prism width were greater in the Fulbe than the Nso. It is unlikely that this pattern was caused by dietary practices alone as it did not change in the distal molars, which develop after the weaning process has ended. Yet, the similarity between the Fulbe and European molars examined in this study (which both exhibited elevated EERs) could be caused by dietary parallels, as both populations have a history of consuming milk (Walker 1980; Witney 1990; Eguchi 1994; Regis 2002; Moritz 2012; see reviews of medieval British childhood diets in Burt 2013; Mahoney, Schmidt, *et al.* 2016). This could indicate an epigenetic link between diet and enamel formation. Townsend *et al* (2011) described epigenetic factors as features that determine gene expression. Epigenetic theories attempt to explain how environmental factors can alter gene expression within an individual and then be passed onto future generations. It is possible a life-long exposure to cow's milk has altered the genetic expression for amelogenesis within the Fulbe and the Europeans, which has caused rapid extension rates across the molar row. Future studies should use genetic techniques alongside chemical analyses to explore the impact of epigenetics on enamel formation.

If an epigenetic link exists, it may be difficult to isolate the specific stimulus for greater extension rates and prism diameter. May *et al* (1993) described how determining the effect of nutritional deficiencies, and in turn excesses, on human amelogenesis is particularly difficult because they rarely occur in isolation, and are connected with several other dietary components. For example, vitamins A and D both play an important role in calcium and phosphorous metabolism, and so consequently are essential for normal enamel mineralization (Boyle 1933; Massler and Schour 1946; Lézot *et al.* 2006; Zhang *et al.* 2009; Hillson 2014). This means it can be difficult to determine whether malformation

of the enamel cap is caused by calcium deficiency alone, or is also the product of vitamin A and/or D deficiency. Further research into the nutritional values of the Fulbe, Nso and European diets is necessary to determine whether prism morphology or enamel growth rates are greater in cultures with a history of consuming milk after weaning.

6.3.2. The impact of the weaning process on enamel formation

Since there were no substantial changes in the main variables between the first and combined distal (second and third) molars, which grow during and after the weaning process, respectively (Smith 1991; Hill and Kaplan 1999; Humphrey 2010; Smith *et al.* 2013) there is also no evidence to suggest that weaning had a significant effect on enamel formation. DSRs exhibited the greatest variation between first and distal molars in both populations. For example, secretions rates in the distal and mesiolingual cusps, and across all three regions of cuspal enamel, were significantly slower in the distal molars than the first molars of the Fulbe sample. The opposing trends of decreasing secretion rates in the Fulbe and increasing secretion rates in the Nso could indicate that the distinct dietary practices of the pastoralists and agriculturalists were affecting enamel growth rates after weaning. However, the variation between individual cusps and measurement regions along the Fulbe molar row is unreliable as it appears to have been exaggerated by limited sample sizes. Moreover, there was no significant difference in average DSR across the enamel cap between the first and combined distal molars from the Nso population. Thus, the results from this study suggest age-at-weaning cannot be identified through the comparative examination of enamel incremental markings.

The most likely explanation for the lack of evidence for age-at-weaning across the Fulbe and Nso molar samples is that weaning is a gradual process, not a sudden conversion.

After birth, human infants are exclusively breastfed. They are then introduced to solid foods during a period of mixed feeding, in which the frequency and duration of nursing is reduced, before breastfeeding finally stops and the infant is entirely dependent on other nutritional sources (Wright and Schwarcz 1998; Humphrey *et al.* 2008; Humphrey 2010). This transition can last for several months or years and varies greatly between human populations because it is dependent upon environmental and social factors as well as the infant's biological needs (Hillson 2014).

Hence, weaning is not simply the end of breastfeeding, but it is the process by which human breast milk is replaced by other foods. This clarification is important as it explains why weaning may not affect enamel formation because it causes a prolonged period of low-intensity rather than immediate stress (Humphrey 2010). There is no 'weaning line' and previous studies have not found evidence of altered or disrupted enamel formation in response to the weaning process. Blakey *et al* (1994) examined enamel hypoplasia in the canines and incisors of 27 African-American skeletons excavated from 18th and 19th century archaeological sites in the US. The authors discovered peak periods for hypoplastic defects occurred between 0.5-3.75 years after the typical age-at-weaning documented for similar African-American populations. Consequently, they rejected the theory that weaning is directly responsible for an increased frequency of linear hypoplasia. The results of this study show weaning does not usually lead to extreme physiological stresses, such as nutritional deprivation, which would cause hypoplastic defects to form more frequently in the dental enamel. Therefore, the weaning process does not appear to affect amelogenesis. In this instance, the unique resistance of enamel formation to environmental factors is detrimental because it is not affected by gradual dietary changes.

Another possible explanation is that enamel growth is able to compensate for the stress of weaning through catch-up growth. Birch and Dean (2009) examined histological sections of 20 modern human mandibular deciduous teeth using transmitted and polarized light microscopy, like this study. They discovered there was a clear reduction in enamel secretion rates within the first 100μm following accentuated markings, particularly the neonatal line. DSRs then increased in the subsequent 100μm to their original level. The authors attributed these variations to catch-up growth by ameloblasts attempting to recover from a systemic stress that disrupted their secretion. Similarly, whilst studying the effects of calorie- and protein-deficient diets on the development of pig teeth, Luke *et al* (1981) found molars were able to recover from both of these nutritional deficiencies, especially protein deficiency, and grow to a similar size after a period of rehabilitation as those from pigs fed unlimited macronutrients. Although the first study focused on deciduous teeth, and the second on molars extracted from pigs, they highlight the possibility that human amelogenesis has adapted to compensate for weaning through periods of increased growth so that any extreme stress experienced during this process may go undetected.

Yet, the phenomenon of catch-up growth in bone is defined as a period of abnormal or above average bone growth following a developmental deficit caused by nutritional or pathological stress (Wit and Boersma 2002; Patwari *et al.* 2005; Reich *et al.* 2008). This suggests the elevated DSRs after stress provided by Birch and Dean (2009) should not be defined as 'catch-up growth' because they were normal enamel growth rates, not bursts of above-average secretion which were compensating for interrupted growth. Either way, the weaning process does not appear to cause enough systemic stress to prohibit cuspal

enamel growth rates, and so it is unlikely that fluctuating DSRs in the enamel would distinguish this event.

The lack of evidence for age-at-weaning within dental enamel contradicts previous assertions that life history events, like weaning, affect hard tissue development and so may be present in the fossil record (Smith and Tompkins 1995). This has direct implications for studying the evolution of human life history. Whilst breastfeeding, human infants have an easy supply of energy for development. However, this supply comes at a cost to the mother, as lactation has a contraceptive effect on her fertility, which is believed to be caused by prolactin (Blakey, Leslie and Reidy 1994; Smith *et al.* 2013), although this has been disputed (Valeggia and Ellison 2004). During weaning, this effect wears off and the mother becomes fertile again, which means she can invest in additional offspring.

Yet, unlike other primates, human infants are not self-sufficient after they are weaned, but instead rely upon other individuals in the community, including their mothers, for food (Hill and Kaplan 1999; Key 2000). Humphrey (2010) suggested this social provisioning is most likely responsible for the evolution of a relatively short period of lactational dependence and reduced age-at-weaning in modern human infants. The uniquely young age-at-weaning and short periods of lactation in modern humans have also been linked with short inter-birth intervals and long juvenile growth periods (Bogin 1999; Key 2000). Thus, identifying weaning in the fossil record could show when these unique life history traits evolved and provide some insight into the relationships between developing infants, their mothers and their local community in our hominin ancestors.

However, as the results of this study suggest that the weaning process has no significant effect on enamel formation, there is still no method of identifying when the uniquely early age-at-weaning in modern humans evolved from the fossil hominin record. The task of establishing a method is made especially challenging by two problems. Firstly, current theories suggest that the shift to a relatively early age-at-weaning was likely the result of a mixture of changes over millions of years (Smith and Tompkins 1995; Kelley and Smith 2003). Variations in nutritional and energetic demand, reproductive lifespan and infant mortality, as well as social structure, are all likely to have altered age-at-weaning across past hominin species (Humphrey 2010). Secondly, these factors are also likely to cause variations in age-at-weaning between individuals. Kelley and Smith (2003) stated humans are adapted to exhibit some plasticity in life history traits, both within and between individuals, in response to environmental and social factors. For example, Smith (2013) explained that age-at-weaning is not a reliable indicator of inter-birth intervals or population growth rates because the contraceptive effect of lactation varies throughout the weaning process, and so it does not have a predictable impact on female birth rates. Therefore, anthropologists cannot track the evolution of human life history in the fossil record without fully understanding the plasticity of age-at-weaning, or how it is connected to other hominin life history traits.

6.4. Does enamel growth and thickness in the African first molars (both Fulbe and Nso combined) differ compared to the European first molars?

6.4.1. African and European first molars

The first molars of the combined African (Fulbe and Nso) population had a larger AET than the comparative European first molars analysed during this investigation. Both populations showed the expected increase in DSRs from the inner to outer cuspal enamel,

but secretion rates were higher in the Europeans across the enamel cap, and significantly so at the mid cuspal enamel. The African and European molars examined in this study had faster DSRs in the inner cuspal enamel than the values reported by Beynon *et al* (1991), Lacruz and Bromage (2006) and Smith (2008), although values for the mid and outer enamel were either equivalent to or lower than these studies. There were positive correlations between lingual cusp AET and DSR in both the combined African and European samples, which support the link between enamel thickness and secretion rates outlined previously (Beynon 1992; Dean *et al.* 2001; Smith, Martin and Leakey 2003; Ramirez Rozzi and Bermudez de Castro 2004). However, the combined African population also showed negative correlations between buccal cusp AET and DSR. Rather than suggesting differing relationships between AET and DSRs across the molar cusps, this discrepancy was most likely the result of the small sample size distorting the relationship between these variables.

Since extension rates were significantly different between the Fulbe and Nso, they were not incorporated to create a mean EER for the combined African population. Comparisons between the two groups and the Europeans showed EERs, like DSRs, were faster in the European first molars than both the Fulbe and Nso first molars. However, as shown in Fig. 11, extension rates appeared to be similar between the Europeans and pastoralists, but considerably different between the Europeans and agriculturalists. As all of the EER values recorded in this study came from small sample sizes, no inferential statistical tests were used to determine whether the differences between these populations were significant.

The similarity between the Fulbe and European populations, and their difference to the Nso sample, presents a new pattern of variation between human populations. This contradicts other studies that have compared enamel growth rates across multiple human populations. Guatelli-Steinberg *et al* (2007) counted perikymata on the surface of the lateral enamel of modern human incisors and canines from Newcastle-upon-Tyne, UK, southern Africa and Alaska. The authors found perikymata packing patterns showed little variation between these populations, which is significant as previous studies have linked the frequency of perikymata, which represent Retzius lines, to EERs (Ramirez Rozzi and Bermudez de Castro 2004). In fact, in a follow-up study, Guatelli-Steinberg e*t al* (2012) inferred EERs for the UK and southern African anterior teeth through dividing the length of the EDJ by formation time, which was the product of the number of perikymata and their periodicity. Extension rates throughout the lateral enamel did not appear to vary between these two samples, which suggests they were also similar in the Alaskan teeth since the distribution of perikymata was consistent between all three populations. These inferences are consistent with the argument made by Reid and Dean (2006) that a small range of variation was a "more realistic picture of worldwide variation in enamel formation" (Reid and Dean 2006, p.329).

Most recently, Modesto-Mata *et al* (2015) suggested EERs shared a common pattern amongst modern human populations. The authors used environmental SEM to examine anterior teeth from archaeological sites in Spain and found extension rates were consistent within European populations, but varied significantly between European and southern African individuals. These results, like those for the Fulbe, Nso and European samples from this study, suggest amelogenesis may differ between modern human populations. Nevertheless, the authors still argued these differences were only minor

when considered in relative terms, specifically compared with Neanderthal extension rates. It should be noted both Guatelli-Steinberg *et al* (2012) and Modesto-Mata *et al* (2015) conducted their studies on the lateral enamel of anterior teeth. They also inferred EERs from the distribution and periodicity of perikymata rather than directly recording them from dental sections. Hence, their methods may not be comparable with the present study, and their conclusions on the lack of variation in enamel formation may not be applicable to the Fulbe and Nso molars. However, the distinct EERs of the pastoralists and agriculturalists from Cameroon challenge the arguments of Reid, Guatelli-Steinberg and colleagues because they suggest there is a wider range of worldwide variation in cuspal enamel growth than previously revealed.

The combined African population exhibited a positive association that linked prism width, which was measured close to the EDJ, with average DSRs for the inner cuspal enamel. DSRs were also found to be positively correlated with EERs in the Fulbe molars. As DSRs at the inner enamel were higher in the combined African population than the comparative sample from Mahoney (2008), these links could indicate growth of the inner cuspal enamel was faster in the individuals from Cameroon, especially the Fulbe, than those from northern England and Scotland. This inference is supported by the fact that Mahoney (2008) used the same histological and measurement techniques to record secretion rates in the British molars as those applied to the Fulbe and Nso molars. However, since DSR at the inner enamel was also positively correlated with EER in the European first molars analysed in this study, and DSRs for this region were also higher than those from Mahoney (2008), it is possible inner cuspal enamel development was also faster in individuals from southern England than northern England and Scotland.

Altogether, these results reveal a greater degree of variation in enamel growth both between and within modern human populations than previously recorded.

In comparison with the datasets from Skinner *et al* (2015) and Olejniczak *et al* (2008), the combined African (Fulbe and Nso) first molars had a larger mean AET than the first molars from both of these populations. The Africans also had a significantly larger mean AET than the Skinner *et al* (2015) sample when all three molar types were analysed together. It should be noted that distinct methods were used to measure enamel thickness in these three samples. In this study, it was directly calculated from histological sections of the Fulbe and Nso molars. On the other hand, Skinner *et al* (2015) used computed tomography to make two-dimensional mesial planes of section from which area of the enamel cap and length of the EDJ were then recorded. Olejniczak *et al* (2008) also utilised a non-destructive and indirect technique. The authors scanned their modern human samples with high-resolution microcomputed tomography and then measured the three-dimensional thickness (volume) of the enamel cap. These differences mean that any contrasts of AET between the combined African and comparative modern human populations may be unreliable. However, these results, along with the elevated AET of the combined African first molars compared with the European first molars examined in this study, may support the inference that AET was larger in the Africans than the Europeans. This contradicts the results of Grine (2004), which suggested European and sub-Saharan African permanent molars were indistinguishable from each other with regards to RET.

However, the values for tooth crown size appear to be consistent with Grine (2004), as they did not differ between the combined African molars and Skinner *et al* (2015) sample,

and the first molars in both populations had similar crown sizes to the northern European first molars from Mahoney (2010). A RMA regression revealed buccal cusp AET and tooth crown size in all three molar types of the combined African population were significantly correlated and scaled with negative allometry. This suggests tooth crown size increases at a relatively lower rate than buccal cusp AET in these samples, which is unsurprising as the area of the crown is also dependent upon the size of the dentin core (Mahoney 2010), which, as stated previously, can vary between molars.

Grine (2002) used scanning electron microscopy to examine molars from a geographically diverse population which included Europeans, sub-Saharan Africans, Native North Americans and people from the Indian subcontinent (Grine 2005). Like this study, the author measured the crown size of these samples as the two-dimensional area of the crown encompassed by the outer enamel surface and the linear bi-cervical diameter of the tooth. When compared to the other datasets, the mixed-ancestry population from Grine (2002) had a larger crown size than the combined African sample from this study as well as both comparative, predominantly European, samples. This could be due to the addition of Asian and North American individuals, as the results from this study indicate the African and European crown sizes would be unlikely to vary. This theory is also supported by the results of Feeney *et al* (2010). Like Skinner *et al* (2015) and Olejniczak *et al* (2008), the authors of this study used microtomography to virtually section dental samples from northern European and southern African individuals. They found there were no significant differences in enamel cap area between the two populations. It should be noted that Feeney *et al* (2010) only examined canines and premolars, so the similarity between these samples does not directly suggest the same lack of variation should exist between the African and European molars from this study. Nevertheless, it

is clear that AET, but not tooth crown size, was larger in the combined African population than the comparative European samples examined here and during previous investigations.

6.4.2. New patterns of modern human variation

The most unexpected result of this investigation was the variation in tooth crown size, EER and prism width between the pastoralists and agriculturalists of Cameroon as well as between these populations and the northern European population examined in this study. Reid and Dean (2006) discovered only slight variations in molar enamel formation between southern Africans and northern Europeans, which were attributed to differences within the cuspal rather than the lateral enamel. Consequently, the authors stated enamel growth in molar samples from populations with relatively similar genetic origins (northern Europeans and North Americans) shows the same level of variation as between those with more diverse genetic backgrounds (southern Africans and northern Europeans). Other studies also found only slight differences between geographically diverse populations (Guatelli-Steinberg, Reid and Bishop 2007; Feeney *et al.* 2010; Guatelli-Steinberg *et al.* 2012). However, the evidence presented in this investigation suggests these studies have not accurately captured the full range of modern human variation. In this study, patterns of variation in molar cuspal enamel growth emerged between both local and geographically diverse populations. Within the African populations, average tooth crown size was larger in the Nso molars, but prism width and EER were greater in the Fulbe molars. Between the combined African (Fulbe and Nso) and European populations, AET was larger in the Africans, in contrast to the results of Grine (2004), and DSRs were higher in the Europeans.

Most significantly, average EERs were faster in the Fulbe than the Nso. This difference was so substantial that the rates for the pastoralists were more similar to those measured in the northern European comparative sample than in the local agriculturalists from Cameroon, although both groups exhibited slower extension rates than the Europeans. This is important on two levels. Firstly, the significant variation in molar cuspal enamel growth between the African and European populations contradicts the lack of variation in molar enamel formation stipulated by Reid and colleagues. Secondly, the results of this study provide new evidence for localised variation in human enamel growth rates. Modesto-Mata *et al* (2015) found EERs did not vary between individuals from Spanish Copper and Bronze Age archaeological sites, or between these samples and other modern human European populations. The authors methods differed from those applied to the Fulbe, Nso and European samples examined in this study as their investigation was conducted on incisors and, like Guatelli-Steinberg *et al* (2007), extension rates were measured from perikymata on the outer enamel surface. However, like the other studies by Reid and colleagues, their conclusions suggest enamel formation is unlikely to vary between neighbouring populations. The Fulbe and Nso results contradict this view and indicate the authors' treatment of multiple "indigenous populations with a mixture of ethnic backgrounds" as a single southern African sample was masking important local variations (Reid and Dean 2006; Guatelli-Steinberg, Reid and Bishop 2007, p.75; Guatelli-Steinberg and Reid 2008, p.238).

An accurate understanding of modern human variation is essential for determining the likely range of variation in fossil hominins (Reid, Guatelli-Steinberg and Walton 2008). Reid and Dean (2006) claimed human geographic populations have a limited range of variation, and this is likely to be similar across all past and present human groups.

Similarly, Feeney *et al* (2010), who examined the same southern African and European samples as Reid and Dean (2006), stated mixed-population samples were suitable for comparisons with other hominin species because of the limited variation in modern human enamel morphology. But, the results from this study showed a broader set of differences both within and between various geographic populations. This means previous comparisons of human and Neanderthal growth rates using the southern African and northern European samples may be inaccurate (Guatelli-Steinberg *et al.* 2005; Guatelli-Steinberg, Reid and Bishop 2007; Guatelli-Steinberg and Reid 2008; Reid, Guatelli-Steinberg and Walton 2008), as it is not possible to contrast different species with confidence if the full range of intra-species variation is not represented. Most of these studies also used enamel growth rates to theorise about Neanderthal somatic growth and life history. This investigation has shown enamel development may not be affected by life history events, like weaning, and could exhibit more variation across modern human populations than previously thought. Thus, the human standards used to recreate fossil hominin development, and the subsequent timelines themselves, may be invalid. Further research into modern human variation is needed before an accurate representation of enamel growth can be compared with non-human primates and fossil hominins.

The cause of the variation between the Fulbe, Nso and European molars analysed in this study is unknown. As stated previously, the dietary practices and life histories of these groups are unlikely to be responsible because there were no significant changes across the molar row. However, the methods applied to the dental samples during this investigation could be a factor. Reid and colleagues found enamel formation was more likely to vary in the cuspal enamel rather than the lateral enamel of the molar teeth. The

lateral enamel was not examined in this study, which is significant as it represents a larger proportion of molar enamel (60-65%) than cuspal enamel (35-40%) (Guatelli-Steinberg and Reid 2008). It could highlight the mechanism responsible for these variations, such as tooth crown height. As previously explained, EERs could have been greater in the pastoralists and Europeans compared to the agriculturalists in response to increased crown heights or EDJ lengths (Shellis 1984). Thus, lateral EERs and tooth crown height need to be measured in order to determine whether the variation between these populations is present throughout amelogenesis, and if it is associated with EDJ length.

However, even if it is possible to determine the underlying mechanism responsible for the differences between the molar samples examined in this study, this mechanism would not explain why the beginning of amelogenesis varies between the Fulbe, Nso and European populations. At this stage, it seems most likely that the differences between these groups are caused by biological variation or genetic drift. Stringer and Andrews (1988) explained that, under the recent African origin model of human evolution, African populations are expected to show the greatest amount of biological variation because of the earlier date of their divergence from our most recent hominin ancestor. In their study of human dentition across 72 major populations and seven geographic populations, Hanihara and Ishida (2005) found sub-Saharan Africans exhibited the greatest intraregional diversity in tooth size, which the authors stated was consistent with genetic data.

A high level of biological diversity in this region could explain the variation in enamel morphology and development between the Fulbe and Nso, especially as Stringer and Andrews (1988) also stated morphological variation is greater than genetic variation. Coia

et al (2005) discovered a significant level of heterogeneity in mitochondrial DNA haplotypes within neighbouring Fulbe populations in Cameroon and Nigeria. Thus, it would be reasonable to expect a large amount of variation in the dental morphology of the neighbouring Fulbe and Nso groups from Cameroon.

Genetic drift could also explain why cuspal EERs were similar between the Fulbe and European molars. Reid *et al* (2008) suggested similarities in enamel formation show a strong affinity between northern Europeans and north-western Africans, which could reflect the recent histories (most likely migration patterns) of these populations. A shared genetic inheritance could lead to similarities in enamel growth, especially as Lewis and Garn (1960) discovered tooth formation was largely gene-determined through their analysis of monozygotic and dizygotic twin pairs. This argument could be undermined by the fact that the majority of the European first molars examined in this study were from medieval British populations, and the Nso and European EERs were substantially different. Further studies into the heritability of enamel formation, particularly with regards to recent human migration patterns, are needed to determine whether the modern human variation presented here is caused by patterns of genetic inheritance.

Nevertheless, the differences in cuspal enamel growth rates between the Fulbe, Nso and European molars examined in this study represent a greater range of modern human population variation than previously reported. The significant localised variation at the start of amelogenesis between the agriculturalists and pastoralists, which could be linked with patterns of genetic diversity, shows the presentation of African and European data by Reid and colleagues may be misleading. This simplistic division represents a false dichotomy based on the assumption that, as Hunley explained, “each local population is

equally diverged from its regional base population, and that each regional population is equally diverged from the base population of the species" (2016, p.561). The results presented here have proven these assumptions to be wrong, as the localised variation was significant enough that Fulbe cuspal EERs were more similar to the Europeans values, a distinct geographic population, than to the cuspal EERs for the Nso, who live in a neighbouring district of Cameroon. Thus, this study challenges the use of geographically diverse populations to represent enamel growth across the entire human species without considering localised variation.

Furthermore, the variation between the Fulbe, Nso and Europeans cuspal enamel growth rates highlights important problems in comparing human enamel formation with fossil hominins and then making deductions on the evolution of human life history. Reid *et al* (2008) suggested molar enamel formation may be more closely related to somatic growth than premolar or anterior enamel development. If so, the results of this study could suggest a broader range of variation in modern human growth than previously revealed. It may be more likely that enamel growth varies at a relatively higher rate than somatic growth. However, Guatelli-Steinberg and Reid (2008) noted human plasticity in enamel formation has not yet been linked to plasticity in somatic development or life history events. Thus, enamel formation cannot be used as a reliable proxy for somatic growth in fossil hominins without further studies into the plasticity of amelogenesis across the human species.

6.5. Limitations

There were some limitations to the assessment of enamel incremental markings within the Fulbe, Nso and European samples examined during this study. Firstly, there were only

a small number of molars in each sample, especially the Fulbe and Nso populations, which limits the reliability of the results. This is especially true for the prism width values, which were based upon only three molars from each of the pastoralist and agriculturalist populations. To increase the reliability of the results, multiple variables were measured from each sample, and values were then compared with datasets from previous publications. These steps helped to highlight atypical patterns that could distort the data. A larger investigation of cuspal enamel growth rates in the Fulbe and Nso populations would support the trends found in this study, although additional use of SEM may be costly.

Secondly, the poor quality of the clinical samples examined during this investigation may have affected the results for the Fulbe and Nso populations. This was compounded by the fact that some molar sections were of better quality and contained more distinctive incremental markings than others. This is a common problem with histology. High-quality sections are rare because cross striations and Retzius lines appear at different enamel thicknesses, and so they can vary across a single section (FitzGerald 1998; Smith 2008). To maximise the quality and reliability of the data, samples with wear and pathological damage were eliminated from this investigation using three criteria (see Materials). In the future, this limitation could be improved upon by obtaining larger sample sizes. Also, non-destructive two-dimensional and three-dimensional methods, such as microtomographic imaging and synchrotron X-ray imaging, could be applied to virtually assess enamel morphology and growth rates (Mahoney 2010; Smith *et al.* 2010).

Furthermore, the assessment of DSRs and EERs was limited by the variable presentation of incremental markings, especially due to prism decussation. As explained previously,

ameloblasts migrate from the EDJ to the outer surface of enamel in a sinusoidal or helicoidal manner which is preserved in the prisms they leave behind (Macho, Jiang and Spears 2003; Dean 2004; Antoine, Hillson and Dean 2009). Dean and Scandrett (1996) found enamel areas with the highest levels of prism decussation had an increased margin of error for cross striation counts, which is most likely due to the difficulty of distinguishing a single line throughout the tissue. For this investigation, the effect of decussation on DSRs was not significant, as shown by the low intraobserver error value for this variable, and only an overall representation of the inner, mid and outer cuspal enamel regions was needed to compare between the Fulbe, Nso and European molar samples. However, it may have had a greater impact on EERs, which could explain the higher intraobserver error score for this measurement. Staining the sections using chemicals like eosin or tetracycline, as recommended by Hillson (1996) and Ross and Pawlina (2006), would help to distinguish individual prisms. Alternatively, EERs could be determined from the angle of intersection between the developing front, represented by the incremental marking under examination, and the EDJ, as outlined by Boyde (1964) and Smith (2008).

Finally, EERs were only measured within the cuspal enamel, specifically within 600µm of the dentin horn. Extension rates were not recorded from the lateral enamel, which has been assessed in previous studies (Shellis 1984; Guatelli-Steinberg, Reid and Bishop 2007; Dean 2009; Guatelli-Steinberg *et al.* 2012). This omission was necessary due to the pathological damage present within the majority of clinical samples. A standard portion of non-carious cuspal enamel was analysed so as to improve the reliability and accuracy of comparisons between the Fulbe, Nso and European datasets. However, it means only a section of the enamel cap representing the start of amelogenesis has been

examined, and the extent to which extension rates vary throughout enamel formation between these groups remains unknown. It also limits the reliability and validity of the comparisons made between these samples and those from previous studies, especially since enamel growth rates are known to vary between the cuspal and lateral enamel (Reid, Beynon and Ramirez Rozzi 1998). Further measurements taken from 600μm down to the cervix of the molars would improve the reliability of the datasets and validity of the comparisons with previous studies. They would also prove whether extension rates differed between the Fulbe and Nso populations throughout amelogenesis, and if this variation was linked to their distinct post-weaning diets.

6.6 Recommendations for Future Research

Future studies should combine histology with methods designed to explore the chemical composition of enamel, such as stable isotope analysis. This method measures isotopes, which are atoms of a single element, such as nitrogen, carbon and oxygen, that have differing masses due to the varied number of neutrons in their nuclei (Howcroft *et al.* 2013). Varying isotopic ratios in plants are passed onto the animals consuming them, including humans, and then incorporated into their bones and teeth during development (Smith 2013). As such, this method could identify varying levels of macronutrients in the Fulbe and Nso as, for example, Wright and Schwarcz (1998) stated carbon isotopes in mammalian milk are mostly derived from its lipid or fat content. In their study of permanent molars from Guatemalan children, the authors found carbon-13 was steadily heavier in the distal teeth, which form at an older age, whilst oxygen-18 was gradually lighter. The former was linked to the introduction and increasing consumption of solid foods and the latter to a prolonged period of lactation. Hence, a combination of histology

and stable isotope analysis could be used to create a timeline for the dietary transitions which occur during an individual's life, including the weaning process.

Another possible method is laser ablation, which is used to identify trace elements on the internal surface of the tooth. Sillen and Smith (1984) described how the strontium and calcium ratios of certain foods occur on a spectrum, with human milk having a very low ratio, followed by animal meats, and then other solid foods, which have a relatively high ratio. Although these categories may seem broad, they could improve upon stable isotope analysis because they differentiate which part of a mammal, whether meat or milk, has been consumed. Sillen and Smith also suggested a "calculated dietary supplementation curve", which would highlight dietary transitions across an individual's lifespan, could be made from calcified bone samples (1984, p.243). This could also be true for samples of tooth enamel. For example, Humphrey *et al* (2008) used laser ablation inductively coupled plasma mass spectrometry [for full details see Kang, Amarasiriwardena and Goodman (2004)] to distinguish dietary patterns between and within first permanent molars from two weanling baboons. The authors discovered an increase in strontium and calcium ratios in one of the baboons at approximately two months old, and then again just before they reached one year of age. They attributed the first change to the introduction of strontium-rich food sources, i.e. solid foods, and the second to the cessation of suckling, and so were able to identify key stages of the weaning process.

An amalgamation of histology, stable isotope analysis and laser ablation, could prove whether the post-weaning diets of the Fulbe and Nso vary on the chemical level, and if this difference is incorporated into the developing molar enamel. Future studies should

also explore the heritability of enamel formation. This would determine whether the varying enamel growth rates of these neighbouring populations is linked to patterns of genetic inheritance. Genetic techniques could be combined with the methods of chemical analysis outlined above to establish whether the cultural practice of consuming milk and milk-based products after weaning has an epigenetic effect on amelogenesis. This could explain the similarity between the Fulbe and European cuspal enamel growth rates. There has been some debate as to whether the methods recommended here should be applied to fossil hominin samples because they are semi-destructive (Smith 2013). However, they could provide direct evidence for the impact of diet on enamel formation, identify the age-at-weaning in fossil hominins, and consequently help to recreate the evolution of human life history.

Chapter 7: Conclusion

In this study, molar enamel morphology and cuspal enamel growth rates for two populations from Cameroon with different post-weaning diets, as well as a comparative northern European sample, were analysed using histology and SEM. The results for these populations were compared in order to answer three main questions. The first was whether enamel formation varied between the Fulbe (pastoralists) and Nso (agriculturalists) from Cameroon. The rate the molar crowns extended in height and the width of enamel prisms were significantly greater in the Fulbe population. On the other hand, the average size of the molar crowns was larger in the Nso population. These results showed the speed of cell differentiation at the start of enamel formation differed between the two neighbouring groups from Cameroon. The combination of extension rate, prism width and tooth crown size variations suggests the difference between these populations is produced by the stretching effect model put forward by Shellis (1984).

Still, the cause of the variation in extension rates, prism width and crown size between the Fulbe and Nso molars remains unclear. This study explored whether any differences in enamel formation between these populations could be attributed to their varying post-weaning diets (the pastoralists have a cultural focus on milk drinking which is not present in the agriculturalists). There was no difference in cuspal enamel growth rates before and after weaning in either population, as shown by the lack of variation between the first and distal (second and third) molars. This suggests the continued consumption of milk past the age of weaning has a limited effect on enamel development. It also means although sudden dietary deficiencies may disturb enamel growth, gradual dietary transitions, like weaning, do not. Thus, the histological analysis of incremental markings in enamel cannot easily identify weaning from the fossil record.

Even though the cause of the difference between the Fulbe and Nso molar samples remains unknown, this study has shown there is more variation in modern human cuspal enamel growth than previously reported. Extension rates were accelerated in the comparative European molars, like the Fulbe. The similarity between these separate geographic populations was contrasted by the varying extension rates of the neighbouring Fulbe and Nso populations. Enamel thickness was larger in the combined African (Fulbe and Nso) sample than the European population. The results presented here contradict Reid and colleagues' assertion that molar enamel formation shows little variation across modern human populations, specifically Africans and Europeans (Reid and Dean 2006; Guatelli-Steinberg and Reid 2008; Reid, Guatelli-Steinberg and Walton 2008; Feeney *et al.* 2010; Guatelli-Steinberg *et al.* 2012). The significant differences between the two neighbouring populations from Cameroon, and the similarity between the Fulbe and European extension rates, showed a more complicated pattern of variation that simply cannot be reduced to comparing entire geographic populations, such as Africans and Europeans, to fossil hominins. Future researchers must consider how enamel formation differs between local populations so as to discover the full range of modern human variation. The similarity between the Fulbe and European samples could be caused by migration patterns. It is also possible the varying cuspal enamel growth rates of the Fulbe, Nso and northern Europeans could be caused by their historical dietary practices.

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Appendix

Table 3- Mean AET (in mm) of buccal and lingual cusps for the Fulbe and Nso populations. All molars combined (+/- 1 SD)

	n	Buccal Cusp	n	Lingual Cusp
Fulbe	7	1.33 (0.32)	6	1.36 (0.30)
Nso	15	1.44 (0.21)	16	1.40 (0.31)

Table 5- Mean DSRs (in µm/day) of buccal and lingual cusps for the Fulbe and Nso populations. All molars combined (+/- 1 SD)

	n	Buccal Cusp	n	Lingual Cusp
Fulbe	7	3.43 (0.24)	5	3.65 (0.36)
Nso	15	3.67 (0.33)	19	3.68 (0.37)

Table 14- Mean values for the four main variables for the Fulbe and Nso populations divided by sex (+/- 1SD)

		n	AET mm	n	Crown Size mm^2	n	DSR µm/day	n	EER µm/day
Male	Fulbe	4	1.33 (0.35)	3	48.45 (0.93)	5	3.66 (0.36)	4	23.52 (7.88)
	Nso	10	1.32 (0.18)	8	57.00 (4.98)	13	3.73 (0.31)	2	14.01 (1.23)
Female	Fulbe	3	1.42 (0.27)	2	47.51 (0.45)	4	3.46 (0.04)	3	23.72 (8.73)
	Nso	8	1.33 (0.20)	8	52.92 (3.19)	13	3.62 (0.37)	5	14.57 (1.82)

Results of the Inferential Statistical Tests

All of the results for the inferential statistical tests are listed in the tables below. The significant results discussed in the main text are in bold.

Table 20- Results of the Spearman's and Pearson's correlations for the Fulbe population

Variables		*r*	n	*p*
Buccal AET	Average DSR	-0.464	7	0.294
	DSR at Inner	-0.100	5	0.873
	DSR at Mid	-0.393	7	0.383
	DSR at Outer	-0.179	7	0.702
	Buccal DSR	-0.429	6	0.397
	Buccal DSR at Inner	-0.800	4	0.200
	Buccal DSR at Mid	-0.429	6	0.397
	Buccal DSR at Outer	-0.200	6	0.704
	Average EER	-0.300	5	0.624
	EER at Crown	0.400	4	0.600
	EER at Mid	-0.500	3	0.667
	EER at Cervix	-0.400	4	0.600
	Prism Width	1.000	3	< 0.005
	Crown Size	-0.400	4	0.600
Lingual AET	Average DSR	0.257	6	0.623
	DSR at Inner	0.300	5	0.624
	DSR at Mid	0.086	6	0.872
	DSR at Outer	0.371	6	0.468
	Lingual DSR	0.200	4	0.800
	Lingual DSR at Inner	0.500	3	0.667
	Lingual DSR at Mid	0.500	3	0.667
	Lingual DSR at Outer	0.200	4	0.800

	Average EER	-0.200	4	0.800
	EER at Crown	1.000	3	< 0.005
	EER at Mid	0.500	3	0.667
	EER at Cervix	-0.200	4	0.800
	Prism Width	1.000	3	< 0.005
	Crown Size	-0.200	4	0.800
Total AET	Average DSR	-0.357	7	0.432
	DSR at Inner	-0.100	5	0.873
	DSR at Mid	-0.286	7	0.535
	DSR at Outer	-0.071	7	0.879
	Buccal DSR	-0.429	6	0.397
	Lingual DSR	-0.600	4	0.400
	Average EER	-0.300	5	0.624
	EER at Crown	0.400	4	0.600
	EER at Mid	-0.500	3	0.667
	EER at Cervix	-0.400	4	0.600
	Prism Width	1.000	3	< 0.005
	Crown Size	-0.400	4	0.600
Average DSR	Average EER	0.536	7	0.215
	EER at Crown	0.371	6	0.468
	EER at Mid	0.600	4	0.400
	EER at Cervix	0.700	5	0.188
	Prism Width	0.500	3	0.667
	Crown Size	0.800	5	0.104
DSR at Inner	Average EER	0.500	5	0.391
	EER at Crown	0.400	4	0.600
	EER at Mid	0.000	4	1.000
	EER at Cervix	0.500	5	0.391
	Prism Width	1.000	2	< 0.005

	Crown Size	0.500	3	0.667
DSR at Mid	Average EER	0.679	7	0.094
	EER at Crown	0.600	6	0.208
	EER at Mid	0.600	4	0.400
	EER at Cervix	0.700	5	0.188
	Prism Width	-0.500	3	0.667
	Crown Size	0.700	5	0.188
DSR at Outer	**Average EER**	**0.929**	**7**	**0.003**
	EER at Crown	**0.886**	**6**	**0.019**
	EER at Mid	0.600	4	0.400
	EER at Cervix	**0.900**	**5**	**0.037**
	Prism Width	0.500	3	0.667
	Crown Size	0.800	5	0.104
Crown Size	Average EER	1.000	3	< 0.005
	EER at Crown	1.000	2	< 0.005
	EER at Cervix	1.000	2	< 0.005
	Prism Width	-0.500	3	0.667

Table 21- Results of the Spearman's and Pearson's correlations for the Nso population

Variables		*r*	n	*p*
Buccal AET	Average DSR	-0.304	15	0.271
	DSR at Inner	-0.228	13	0.453
	DSR at Mid	-0.125	14	0.670
	DSR at Outer	-0.118	15	0.676
	Buccal DSR	-0.098	12	0.762
	Buccal DSR at Inner	0.100	9	0.798
	Buccal DSR at Mid	-0.119	12	0.713
	Buccal DSR at Outer	0.189	12	0.557
	Average EER	1.000	2	< 0.005

	EER at Cervix	-1.000	2	< 0.005
Lingual AET	Crown Size	0.044	13	0.887
	Average DSR	0.191	16	0.478
	DSR at Inner	-0.190	15	0.498
	DSR at Mid	0.086	15	0.761
	DSR at Outer	0.275	15	0.321
	Lingual DSR	-0.011	13	0.972
	Lingual DSR at Inner	-0.479	13	0.098
	Lingual DSR at Mid	-0.225	13	0.459
	Lingual DSR at Outer	0.161	12	0.618
	Average EER	1.000	2	< 0.005
	EER at Cervix	1.000	2	< 0.005
	Prism Width	-0.500	3	0.667
Total AET	Crown Size	0.382	11	0.247
	Average DSR	-0.284	18	0.254
	DSR at Inner	-0.253	16	0.344
	DSR at Mid	-0.375	17	0.138
	DSR at Outer	-0.051	17	0.844
	Buccal DSR	-0.285	10	0.425
	Lingual DSR	-0.363	14	0.203
	Average EER	0.400	4	0.600
	EER at Mid	1.000	2	< 0.005
	EER at Cervix	0.200	4	0.800
	Prism Width	-1.000	2	< 0.005
Average DSR	Crown Size	0.225	13	0.459
	Average EER	0.143	7	0.760
	EER at Crown	1.000	2	< 0.005
	EER at Mid	-1.000	3	< 0.005
	EER at Cervix	0.429	7	0.337

	Prism Width	0.500	3	0.667
	Crown Size	0.391	16	0.134
DSR at Inner	Average EER	0.321	7	0.482
	EER at Crown	1.000	2	< 0.005
	EER at Mid	-1.000	3	< 0.005
	EER at Cervix	0.357	7	0.432
	Prism Width	1.000	3	< 0.005
	Crown Size	-0.027	15	0.924
DSR at Mid	Average EER	-0.071	7	0.879
	EER at Crown	1.000	2	< 0.005
	EER at Mid	-1.000	3	< 0.005
	EER at Cervix	0.250	7	0.589
	Prism Width	1.000	3	< 0.005
	Crown Size	**0.564**	**15**	**0.028**
DSR at Outer	Average EER	0.429	6	0.397
	EER at Crown	-1.000	2	< 0.005
	EER at Mid	1.000	3	< 0.005
	EER at Cervix	0.771	6	0.072
	Prism Width	1.000	2	< 0.005
	Crown Size	0.382	16	0.144
Crown Size	Average EER	1.000	2	< 0.005
	EER at Cervix	1.000	2	< 0.005

Table 22- Results of the Spearman's and Pearson's correlations for the combined African (Fulbe and Nso) population

Variables		*r*	n	*P*
Buccal AET	Average DSR	-0.274	22	0.217
	DSR at Inner	-0.296	18	0.232
	DSR at Mid	-0.173	21	0.454
	DSR at Outer	-0.093	22	0.681

	Buccal DSR	-0.028	18	0.913
	Buccal DSR at Inner	-0.159	13	0.603
	Buccal DSR at Mid	-0.092	18	0.717
	Buccal DSR at Outer	0.156	18	0.537
	Prism Width	-0.200	4	0.800
	Crown Size	**0.480**	**18**	**0.044**
Lingual AET	Average DSR	0.106	22	0.639
	DSR at Inner	-0.067	20	0.779
	DSR at Mid	0.084	21	0.718
	DSR at Outer	0.303	21	0.182
	Lingual DSR	-0.027	17	0.918
	Lingual DSR at Inner	-0.500	15	0.057
	Lingual DSR at Mid	-0.050	15	0.860
	Lingual DSR at Outer	0.150	16	0.579
	Prism Width	0.029	6	0.957
	Crown Size	0.422	18	0.081
Total AET	Average DSR	-0.200	25	0.338
	DSR at Inner	-0.264	21	0.248
	DSR at Mid	-0.260	24	0.221
	DSR at Outer	-0.026	24	0.903
	Buccal DSR	-0.124	16	0.649
	Lingual DSR	-0.370	17	0.144
	Prism Width	0.300	5	0.624
	Crown Size	**0.474**	**18**	**0.047**
Average DSR	Prism Width	0.543	6	0.266
	Crown Size	0.155	22	0.491
DSR at Inner	**Prism Width**	**0.900**	**5**	**0.037**
	Crown Size	-0.173	19	0.479
DSR at Mid	Prism Width	0.371	6	0.468

	Crown Size	0.397	21	0.075
DSR at Outer	Prism Width	0.600	5	0.285
	Crown Size	0.315	22	0.153
Crown Size	Prism Width	-0.800	4	0.200

Table 23- Results of the Spearman's and Pearson's correlations for the combined African (Fulbe and Nso) population (first molars only)

Variables		*r*	n	*P*
Buccal AET	**Average DSR**	**-1.000**	**5**	**< 0.005**
	DSR at Inner	0.200	4	0.800
	DSR at Mid	-0.700	5	0.188
	DSR at Outer	**-0.900**	**5**	**0.037**
	Buccal DSR	-1.000	4	< 0.005
	Buccal DSR at Mid	-1.000	3	< 0.005
	Buccal DSR at Outer	-1.000	3	< 0.005
	Prism Width	-0.200	4	0.800
	Crown Size	-0.400	5	0.505
Lingual AET	**Average DSR**	**0.900**	**5**	**0.037**
	DSR at Inner	1.000	4	< 0.005
	DSR at Mid	**0.900**	**5**	**0.037**
	DSR at Outer	0.700	5	0.188
	Lingual DSR	1.000	4	< 0.005
	Lingual DSR at Inner	1.000	4	< 0.005
	Lingual DSR at Mid	0.400	5	0.600
	Lingual DSR at Outer	0.800	4	0.200
	Prism Width	0.029	6	0.957
	Crown Size	-0.100	5	0.873
Total AET	Average DSR	0.100	5	0.873
	DSR at Inner	0.400	4	0.600

	DSR at Mid	0.500	5	0.391
	DSR at Outer	-0.500	5	0.391
	Buccal DSR	-0.500	3	0.667
	Lingual DSR	0.400	4	0.600
	Prism Width	0.300	5	0.624
	Crown Size	0.100	5	0.873
Crown Size	Average DSR	0.429	7	0.337
	DSR at Inner	-0.314	6	0.544
	DSR at Mid	0.357	7	0.432
	DSR at Outer	0.214	7	0.645

Table 24- Results of the Spearman's and Pearson's correlations for the European population (first molars only)

Variables		*r*	n	*p*
Buccal AET	Average DSR	-0.018	16	0.948
	DSR at Inner	0.075	15	0.791
	DSR at Mid	-0.143	14	0.626
	DSR at Outer	0.452	8	0.260
	Buccal DSR	-0.310	8	0.456
	Buccal DSR at Inner	0.036	7	0.939
	Buccal DSR at Mid	-0.143	6	0.787
	Buccal DSR at Outer	0.500	3	0.667
	Average EER	-0.200	6	0.704
	EER at Crown	-1.000	2	< 0.005
	EER at Mid	-0.600	5	0.285
	EER at Cervix	0.500	3	0.667
Lingual AET	Average DSR	-0.082	18	0.748
	DSR at Inner	-0.203	17	0.434
	DSR at Mid	-0.118	16	0.664
	DSR at Outer	**0.857**	**7**	**0.014**

	Lingual DSR	0.345	10	0.328
	Lingual DSR at Inner	0.115	10	0.751
	Lingual DSR at Mid	0.000	7	1.000
	Lingual DSR at Outer	1.000	3	< 0.005
	Average EER	-0.071	7	0.879
	EER at Crown	-0.500	3	0.667
	EER at Mid	0.086	6	0.872
	EER at Cervix	0.600	4	0.400
Total AET	Average DSR	-0.174	19	0.477
	DSR at Inner	-0.220	18	0.381
	DSR at Mid	-0.213	17	0.411
	DSR at Outer	0.452	8	0.260
	Buccal DSR	-0.476	8	0.233
	Lingual DSR	0.182	11	0.593
	Average EER	-0.143	7	0.760
	EER at Crown	-0.500	3	0.667
	EER at Mid	0.029	6	0.957
	EER at Cervix	0.800	4	0.200
Average DSR	Average EER	0.238	8	0.570
	EER at Crown	0.600	4	0.400
	EER at Mid	-0.071	7	0.879
	EER at Cervix	0.800	5	0.104
DSR at Inner	Average EER	0.476	8	0.233
	EER at Crown	0.600	4	0.400
	EER at Mid	-0.286	7	0.535
	EER at Cervix	**1.000**	**5**	**< 0.005**
DSR at Mid	Average EER	0.257	6	0.623
	EER at Crown	-0.500	3	0.667
	EER at Mid	0.200	5	0.747

	EER at Cervix	0.500	3	0.667
DSR at Outer	Average EER	0.600	4	0.400
	EER at Mid	-0.500	3	0.667
	EER at Cervix	1.000	2	< 0.005

Table 25- Table of ANOVA results for the combined African (Fulbe and Nso) population

ANOVA					*Post-hoc Tukey's*			
Variables		*f*	df, n	*P*	Variables (n)		*Q*	*P*
Average DSR	**Measurement Region**	**34.19**	**2, 95**	**< 0.005**	**DSR at Inner (27)**	**DSR at Mid (34)**	**4.755**	**0.003**
		(Levene's *p*= 0.069)			**DSR at Inner (27)**	**DSR at Outer (34)**	**11.730**	**< 0.005**
					DSR at Mid (34)	**DSR at Outer (34)**	**6.975**	**< 0.005**

Table 26- Table of Kruskal-Wallis results

Variables		X^2	df, n	*P*	*Post-hoc Tukey's* Variables (n)		*Q*	*p*
Tooth Type	Total AET	-2.816	2, 18	1.000				
Tooth Type	Average DSR	5.311	2, 24	0.070				
Tooth Type	DSR at Inner	-4.080	2, 20	1.000				
Tooth Type	DSR at Mid	2.256	2, 23	0.323				
Tooth Type	DSR at Crown	1.825	2, 25	0.401				
Population	**Total AET**	**9.286**	**2, 88**	**0.010**	**African AET (25)**	**Skinner AET (28)**	**6.730**	**< 0.005**
	(Levene's *p*= 0.004 so no ANOVA)				**Skinner AET (28)**	**Olejniczak AET (35)**	**9.110**	**< 0.005**
Population (M1)	Total AET	5.934	3, 38	0.115				

Table 27- Table of Mann Whiney *U* results

Population	Variable	N	*U*	*p*
Combined African (Fulbe+Nso)	Average DSR	M1=5, M2+3=19	27.0	0.155
	DSR at Inner	M1=6, M2+3=13	46.0	0.912
	DSR at Mid	M1=5, M2+3=17	23.5	0.147
	DSR at Outer	M1=6, M2+3=19	36.0	0.192
African	Total AET	Fulbe=7, Nso=18	57.0	0.739
	Crown Size	**Fulbe=5, Nso=16**	**1.0**	**0.001**
	Average DSR	Fulbe=9, Nso=26	94.0	0.396
	DSR at Inner	Fulbe=6, Nso=22	60.0	0.758
	DSR at Mid	Fulbe=9, Nso=25	107.0	0.845
	DSR at Outer	Fulbe=9, Nso=25	67.0	0.079
	Average EER	**Fulbe=7, Nso=7**	**2.0**	**0.005**
Combined African (Fulbe+Nso) and European	Average DSR	African=9, European=20	61.0	0.179
	DSR at Inner	African=8, European=19	45.5	0.111
	DSR at Mid	**African=9, European=17**	**23.0**	**0.004**
	DSR at Outer	African=8, European=8	20.0	0.227

Table 28- Table of Ordinary Least Squares Regression results

Population	Variables		N	r^2	slope	Intercept	*p*
Combined African (Fulbe+Nso)	Buccal AET	Crown Size	18	0.174	8.376	43.098	0.085
	Lingual AET	Crown Size	18	0.154	6.472	44.605	0.107
	Total AET	Crown Size	18	0.134	10.346	40.131	0.136
	Total AET	Average DSR	25	0.040	-0.290	3.997	0.339
	Average DSR	Crown Size	22	0.024	2.523	44.463	0.491
Europeans	Total AET	Average DSR	19	0.031	-0.348	4.240	0.472
	Total AET	Average EER	7	0.096	-13.332	42.476	0.499
Fulbe	Total AET	Average DSR	7	0.057	-0.230	3.861	0.607
	Total AET	Average EER	5	0.030	-4.243	30.730	0.779
Nso	Total AET	Crown Size	13	< 0.005	0.300	54.700	0.978
	Total AET	Average DSR	18	0.032	-0.321	4.058	0.476
	Average DSR	Crown Size	16	0.050	3.125	43.500	0.406

Table 29- Table of log-transformed Reduced Major Axis Regression results

Population	Variables		n	R	Slope	intercept	95% Confidence Intervals	*p*
Combined African (Fulbe+Nso)	**Buccal AET**	**Crown Size**	**18**	**0.469**	**0.502**	**1.670**	**0.239-0.678**	**0.050**
	Lingual AET	Crown Size	18	0.178	0.445	1.664	0.232-0.618	0.081

	Total AET	Crown Size	18	0.383	0.670	1.649	0.393-2.200	0.117
	Total AET	Average DSR	25	0.164	-0.512	0.618	-1.633- -0.284	0.430
	Average DSR	Crown Size	22	0.157	1.108	1.107	0.685-3.428	0.486
Europeans	Total AET	Average DSR	19	0.158	-0.606	0.615	-1.989- -0.335	0.517
	Total AET	Average EER	7	0.319	-1.583	1.478	-6.010- -0.205	0.485
Fulbe	Total AET	Average DSR	7	0.241	-0.350	0.593	-1.345- -0.074	0.602
	Total AET	Average EER	5	0.170	-1.291	1.547	-5.221-0.879	0.783
Nso	Total AET	Crown Size	13	0.029	0.868	1.627	0.683-2.975	0.925
	Total AET	Average DSR	18	0.130	-0.616	0.632	-1.932- -0.241	0.610
	Average DSR	Crown Size	16	0.226	0.937	1.211	0.484-2.791	0.401