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Getting Better All The Time: Re-evaluating Macroscopic Dental Age Estimation Standards In Egypt

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Abstract

This dissertation investigates the use of dental anthropological methods for estimating chronological age-at-death in ancient Egypt, and determines whether these methods can be improved. Tooth calcification, emergence and eruption standards are time honoured in their ability to accurately age subadults though they are compromised by the fact that populations and the sexes vary in their developmental timing. Determining sex in subadults, particularly in the infant and child cohorts, in all populations is not possible, though advances in ancient DNA methods hold promise. This dissertation provides a feasible and ethical model for developing a sex-and region-specific standard for age estimation of subadults for use on ancient Egyptian samples.This method rectifies methodological errors affecting the accuracy of pre-existing standards; and thus, demonstrates that macroscopic subadult dental age estimation methods can be improved.

Moreover, using a photographic sample of occlusal dentition from the Kellis 2 cemetery population in Roman Period Egypt, a new method for adult dental age estimation is designed and tested. To this end, the percentages of exposed occlusal dentine in first and second molars were calculated through photogrammetry, in a technique shown to have little intra- and inter-observer error. These data showed a strong linear correlation with skeletal age estimates, and varied significantly from the popular Brothwell (1963a) standard for age estimation based on dental wear. Dental caries and antemortem tooth loss were similarly tested for correlation with skeletal age, with only antemortem tooth loss showing a strong correlation. As a result, linear regression models were designed and tested for quantified first and second molar wear as well as antemortem tooth loss. Multiple regression models for all combinations of these dental indicators of age were also designed and tested. Although it is also recommended that these models are revised with expanded reference samples, these standards improve the ability to estimate age in individuals from the Kellis 2 cemetery population. It is recommended that these standards are tested and modified for use on geographically- and temporally-diverse populations to determine the boundaries of its application beyond a single population sample.

In summary, this study rejects the null hypothesis (Ho): ‘Current dental age estimation standards cannot be improved’. Consequently, this dissertation serves to encourage the creation of more accurate and precise subadult and adult macroscopic dental age estimation standards.

Keywords

Age estimation, senescence, dental age estimation, dental development, dental attrition, dental wear, primary and permanent teeth, subadult, adult, public oral health, Egypt, North Africa, Middle East, physical anthropology, forensics, archaeology, dentistry, orthodontics, population studies.

Lay Abstract

This dissertation investigates the use of dental anthropological methods for estimating age-at-death in subadult and adult ancient Egyptians, and their potential for improvement. Dental developmental standards are time honoured in their ability to accurately age subadults. Unfortunately, they are compromised by the fact that developmental timing can vary among populations and between the sexes. This dissertation presents an improved method for developing a region- and sex-specific standard for age estimation of subadult Egyptians. This feasible and ethical method rectifies methodological errors affecting the accuracy of pre-existing subadult dental aging standards. This demonstrates potential for improved age estimation in subadults.

Using a photographic sample of occlusal dentition from the Roman Period Kellis 2 cemetery in Egypt, a new method for adult dental age estimation is also designed and tested. To this end, dental wear is quantified through photogrammetry in first and second molars. These data were used to create linear regression models that can be used to predict age based on dental wear. These models vary significantly from the popular Brothwell (1963a) standard for age estimation based on dental wear. Dental caries and antemortem tooth loss were similarly tested for correlation with skeletal age, with only antemortem tooth loss showing a strong correlation. Consequently, linear regression models were also designed and tested for antemortem tooth loss. Multiple regression models were also created to incorporate all combinations of these dental indicators of age in an effort to increase accuracy. Unfortunately, in some cases, the small sample sizes applicable to multiple regression models indicate that these models should be revised with expanded reference samples. Nevertheless, these standards improve the ability to age adults from Kellis 2 cemetery population. In future, it is recommended that these standards are tested and modified for use on geographically- and temporally-diverse populations to test its applicability beyond a single cemetery population.

In summary, this dissertation rejects the null hypothesis (Ho): ‘Current dental age estimation standards cannot be improved’. Consequently, this dissertation serves to encourage the creation of more accurate and precise user-friendly subadult and adult macroscopic dental age estimation standards.

"While we agree that the current status of age estimation in paleodemography is largely that of an "art," we see no apparent reason why we should not strive to make it a science."

~ Lyle Konigsberg and Susan Frankenberg (1992: 253) ~

Dedication

For those who give me strength.

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List of Abbreviations, Symbols, Nomenclature

ADA: American Dental Association
aDNA: ancient deoxyribonucleic acid
AIC: Akaike Information Criterion
AMTL: antemortem tooth loss
ant.: Anterior
Antimere: either of a pair of opposite corresponding symmetrical teeth
Calc: Calculus
CEJ: cemento-enamel junction
dec.: Deciduous
DEJ: dentino-enamel junction
DMTA: dental microwear texture analysis
DNA: deoxyribonucleic acid
e : exponent (xe2 = x^2)
F: Female
HSREB: Health Science Research Ethics Board
Isomere: either of a pair of corresponding teeth in the upper and lower jaws
LEH: Linear Enamel Hypoplasia
LL: Lower Left
LLM1/2/3: Lower left molar 1/2/3 (can also be modified for Incisor (I), Canine (C), or Premolar(P))
LR: Lower Right
LRM1/2/3: Lower right molar 1/2/3 (can also be modified for Incisor (I), Canine (C), or Premolar(P))
LSAMAT: Lingual Surface Attrition of the Maxillary Anterior Teeth
M: Male
MAE: Mean Absolute Error
mand/no mand.: Mandible/no mandible
max/no max.: Maxilla/no maxilla
MSA: Ministry of State for Antiquities
mtDNA: mitochondrial DNA
N/A: Not applicable
OA: osteoarthritis
OHI-S: Simplified oral health index
perio: Periodontitis
perm: Permanent
PMTL: Postmortem tooth loss
RSD: Residual Standard Deviation
SCA: Supreme Council of Antiquities
sign.: significant
UL: Upper Left
ULM1/2/3: Upper left molar 1/2/3 (can also be modified for Incisor (I), Canine (C), or Premolar (P))
UR: Upper Right
URM1/2/3: Upper right molar 1/2/3 (can also be modified for Incisor (I), Canine (C), or Premolar (P))
UWO: University of Western Ontario
WHO: World Health Organization

Selected Definitions

Age: For the purpose of this dissertation, it should be kept in mind that age can be attributed as a result of cultural factors (e.g. the cultural definition of adulthood), chronology (e.g. based on an accepted chronological measure, such as Gregorian calendar years), or biology (e.g. the chronological age estimated through the use of biological indicators such as dental development). This dissertation is focused on the relationship between biological age and chronological age, and the potential for improved estimates of chronological age through the observation of biological age indicators. However, it must be noted that biological age can be affected by many factors and thus, cannot be equated to chronological age.

Population-specific standards: For the purpose of this dissertation, "population-specific standards" refers to methods of age estimation based on, and intended for use on, a specific population within a specific archaeological site. In some cases, population-specific and site-specific can be interchangeable terms.

Universal standards: For the purpose of this dissertation, "universal standards" refers to standards that have been used indiscriminately throughout the world, regardless of whether the original author intended this widespread usage.

Region-specific standards: For the purpose of this dissertation, "region-specific standards" refers to standards created and tested for use on more than one archaeological site or population, but not for universal use. The boundaries of the "region" must be defined through testing of the accuracy of the standard across geographically- and temporally-diverse populations. The intended benefit of "region-specific standards" is that they may be more accurate and precise than "universal standards" but they do not require the creation of new standards for each site, like "population-specific standards".

Preface

Historically, humans have pondered the meaning and processes of aging and death. Philosophers, alchemists, scientists, medical professionals, and religious scholars are among the many that have contributed hypotheses to explain the processes of growth and senescence. Common themes, historically, have also included attempts to slow the biological clock and prolong life (Hayflick 1994). Although medical advances have enabled an increase in average life expectancy and the development of several hypotheses regarding the biological mechanisms of aging, a truly comprehensive understanding of the human aging process remains elusive. In addition to modern scientific views on growth and senescence, there are innumerable non-scientific beliefs regarding the topic of birth, growth, senescence and death. Although an in-depth cross-cultural meta-analysis of perceptions regarding these topics is far beyond the scope of this study, it is worth noting that ancient and modern, religious and secular, scientific and pseudoscientific writings on human aging tend to exclude discussion of the effects of time on the dentition.

A brief survey of the tables of contents of popular anti-aging books reveals a near absence of discussions of the dentition as a whole. This is perplexing, considering the importance of dentition in quality of life and the increased susceptibility to dental disease and tooth loss in older age. This is particularly interesting when considering that some of these age-related dental conditions can contribute to serious illness and possible death. Generally, anti-aging books provide lip service to holistic health care and they largely focus on diets, medications and lifestyle changes that are said to slow systemic aging processes. Unfortunately, these books largely ignore mechanical wear and tear, and other age-related factors that may contribute to the degradation of the dentition. Occasionally, authors briefly discuss issues regarding the impairment of the salivary glands in old age, a relatively easily treated condition that leads to dry mouth and several related dental health problems. Nevertheless, the authors often still manage to avoid in-depth discussion of age-related changes to the dentition itself! This omission of dental aging may be attributed to authors' recognition of the futility in their own anti-aging schemes, as a method for the prevention of dental aging has yet to (and perhaps never will) be discovered!

Knowledge of the relationship between dental development, dental wear, and chronological age has been common for ages. Perhaps since the beginning of pastoralism, farmers have estimated the age of animals by looking at their teeth. This long-held knowledge is evident in the proverb "Don't look a gift horse in the mouth" (meaning: accept gifts gratefully and without judgement), which refers to the ungrateful act of assessing the age (and thus value) of a freely given horse through the observation of its teeth. The earliest evidence of this proverb appears in Latin as 'Noli equi dentes inspicere donati' (Never inspect the teeth of a given horse), in St. Jerome's Latin text, The Letter to the Ephesians, circa AD 400 (Cryer 2010: 195). Similarly, it is believed that because older horses appear to have longer incisors as a result of gum recession, the correlation between equine dentition and chronological age also gave way to the phrase "to look long in the tooth" (meaning: to look aged) (CollinsDictionary.com 2017).

Dental age estimation is still the preferred method for determining the age of animals, particularly horses and cattle, and although the aforementioned sayings are related to the dental age estimation of horses, various dental age estimation methods are applicable to animals and humans alike. The first widespread application of dental age estimation in humans may have been used in the determination of fitness for service in the ancient Roman army. In this case, individuals had to have their second molars fully erupted in order to be eligible for military service (Müller 1990; Willershausen et al. 2001; Manjunatha and Soni 2014).

Coincidentally, the second permanent molar was also used for age determination centuries later. Near the end of the Industrial Revolution, the English government passed the Factory Act of 1833, which restricted child labor to those above 9 years of age, and limited the number of hours children were allowed to work per day. Factories required age certificates for child employees from approved certifying surgeons, and four factory inspectors were responsible for enforcing these laws. Since the Factory Act (1833) was established prior to the Births and Deaths Registration Act (1836), there was no official documentation of the age of child laborers. Instead, the certifying surgeons estimated the age of children through the observation of the developmental stage of the second permanent molars, or so-called 'factory teeth'. These teeth tend to complete eruption between 12-14 years of age, depending whether they are upper or lower molars (AlQahtani 2009) and thus, were used as an indicator that factory workers were of legal working age in England during the 19th century (Saunders 1837).

Since then, much more accurate and specific methods of dental age estimation have been developed for use in humans. However, as you will see in the following dissertation, the work to improve these methods is far from complete. As such, this doctoral research is designed to expand our understanding of the strengths and weaknesses of current methods while suggesting methods for improvement.

Chapter 1

1.1 Introduction

Biological age estimation is central to every study of human remains, and it can be used in sociocultural studies or immigration investigations of living subadults with missing, or suspect, birth data (El-Nofely and İşcan 1989; Maber et al. 2006; Liversidge 2008). In many historic and forensic cases, the estimated age-at-death is a key to identifying individuals (Acsádi and Nemeskéri 1970). Consequently, the accuracy with which we can produce age estimates for the deceased is of supreme importance. Age estimation is also fundamental for reconstructing aspects of individuals' life- and medical- histories, and assembling knowledge of age-related cultural practices and demographics of past populations. It is particularly important for the differential diagnosis of skeletal disease, as age is often used to determine the probability of specific diagnoses. Since teeth can be studied in living patients and are resistant to post-mortem decomposition (Miles 1963; Hillson 1996; Beach et al. 2010; Hollund et al. 2015), they are often used for age estimation in ancient populations and have been shown to have a relatively high accuracy in estimating ages when compared with other age estimation indicators (Lovejoy et al. 1985).

Due to relatively low rates of bias and inaccuracy and little need for specialized equipment, the age of subadults is generally estimated through the use of standards based on the macroscopic analysis of dental development. These standards, relating chronological age to the timing and sequence of dental development, have also served as important indicators of pediatric health, growth and development in the fields of dentistry, orthodontics, and pediatric medicine. Despite the importance of these standards, all of the existing standards were created using North American or European reference populations, with particular emphasis on North American and British reference populations, usually without distinction between male and female developmental timing (cf. Schour and Massler 1940a,b, 1941; Moorrees et al. 1963a,b; Haavikko 1970; Fanning & Brown 1971; Demirjian et al. 1973; Gustafson and Koch 1974; Anderson et al. 1976; Demirjian and Goldstein 1976; Ubelaker 1978; Smith 1991a; AlQahtani et al. 2010b; AlQahtani 2012).

Acsádi and Nemeskéri (1970) noted that the order and chronology of tooth eruption varied slightly between different ethnic groups but dismissed these differences as not significant enough to affect age estimation methods. A recent study, however, revealed statistically significant differences in the timing of dental eruption between males and females and between Egyptian and non-Egyptian subjects (Soliman et al. 2011). El-Nofely and İşcan (1989) also showed that there were variations in dental emergence patterns between Egyptians and North American and European populations. This indicates the possibility for improvement in the estimation of age in Egyptians through region-specific standards. Furthermore, since dental development is still heavily influenced by genetics and many environmental aspects and traditions have long persisted in Egypt, it is probable that modern Egyptian dental developmental standards would produce better estimates of age in ancient Egyptians.

The population specificity of dental development is further indicated by Demirjian et al.'s (1973) study, which demonstrates that French-Canadian children mature more slowly than children in several other parts of the world (e.g. Prahl-Anderson et al. 1979; Proy et al. 1981; Loevy 1983; Nichols et al. 1983; Nystrom et al. 1986, 1988; Kataja et al. 1989; Ocholla 1990; Davis and Hagg 1994; Liversidge et al. 1998). Loevy (1983) and Davis and Hagg (1994) also observed differences in developmental timing between ethnic groups and Molnar (2015) noted that Europeans and Euro-Americans have later eruption times than many other ethnic groups. Harris and McKee (1990) documented the differences in developmental timing between black and white children and, more recently, Blenkin and Taylor (2012) emphasized the need for region-specific dental age estimation standards by presenting a method for the modification of existing standards to render them more suitable for specific populations (in their case, Australians). Moreover, Miles (1962, 1963, 1978) demonstrated that adult dental age estimates could be improved through the consideration of population-specific dental eruption patterns and their effect on dental wear.

In addition to their contributions to the discussion of differences in dental development across populations, Harris and McKee (1990) and Blenken and Taylor (2012) identified significant differences in developmental timing between the sexes. Dental developmental patterns are known to differ between males and females; however, Harris and McKee

(1990) demonstrated that these differences vary significantly across populations, as they found that the average difference between black females and males was twice as large as the average difference between white females and males. Pavlovic et al. (2017) also recently showed that there were sex-specific variations in the accuracy of the relatively new Atlas of Tooth Development and Eruption (AlQahtani et al. 2010b; AlQahtani 2012) when tested on a Portuguese reference sample, and recommended the creation of sex-specific charts.

In addition to subadult dental age estimation methods, several methods have been developed to estimate the age-at-death for adults. The Miles (1962) method is the only existing method which requires the creation of population-specific, or archaeological site-specific, standards for dental age estimation. It creates adult age estimation standards through the observation of dental attrition in the molars, which is calibrated against subadult dental development patterns. Although the Miles method has proven to be one of the most reliable single indicator methods of age estimation (Lovejoy et al. 1985), it is not widely used because of its complexity, reference sample requirements, and time obligations. Consequently, many biological anthropologists disregard the Miles (1962) site-specific method in favour of using Brothwell's (1963a) dental wear atlas. Brothwell's (1963a) atlas standard was based on a study of dental wear in ancient British individuals, however, his methods for its development were never published (Brothwell 1989; Hillson 1996). Brothwell intended for his standard to be used for the estimation of age-at-death in prehistoric to early medieval British specimens. However, this user-friendly atlas-type standard has been adopted for use throughout the world as 'universal' standards (Hillson 1996).

Since rates of dental attrition are highly dependent on environmental and dietary factors, and the Miles (1962) method has proven to be at least as effective as Brothwell's method (Nowell 1978; Lovejoy 1985; Kieser et al. 1983; Lovejoy et al. 1985; Hillson 1996), it is surprising that there has been mass acceptance of universal aging standards based on dental attrition among physical anthropologists. It seems logical to question the accuracy and universality of these 'universal' standards and to test the efficacy of these standards under the influence of different variables such as genetic proximity, geographic proximity, environment, diet, health, dental morphology, dental hygiene etc.

Theoretically, region-, time-, and sex-specific standards should better represent environmental and genetic variation age-related dental changes than ‘universal’ dental aging standards, while eliminating the need for repetitive and time-consuming work required for the development of site-specific aging standards. I hypothesize that the development of (time-,) region- and sex-specific dental aging standards developed through the use of innovative methods for data collection and statistical analysis will provide more accurate and specific, though still user-friendly, methods for estimating age-at-death.

This dissertation focuses on age estimation in Egyptian dentition because of my familiarity with the region, its history, and the local dialect. This region was also selected as a result of the popularity of bioarchaeological work in Egypt, and thus the need for accurate methods for age estimation. This dissertation delves into the question of whether macroscopic dental age estimation standards can be improved. Following the declaration of this study’s *Research Objectives and Hypotheses*, this dissertation is organized in four chapters:

Chapter II: The Significance of Dental Age Estimation

This chapter begins with a brief overview of the techniques used within the field of dental anthropology and examples of their significant contributions to our collective knowledge in fields such as bioarchaeology, paleoanthropology, forensic anthropology, and human biology. This is followed by an abbreviated preliminary meta-analysis of the relative accuracy and bias of some of selected skeletal and dental age estimation methods. Results show the significance of dental age estimation methods among some of the most popular macroscopic age estimate indicators.

Chapter III: Creating an Improved Subadult Age Estimation Standard Based on Dental Development

In this chapter, following a review of the existing macroscopic subadult dental age estimation standards, a new method for the creation of subadult dental age estimation standards is presented and demonstrated to be feasible. This method integrates bioarchaeological and public oral health research methods, allowing for much more specific and accurate reference material. The demonstrable gaps in previous dental age estimation methods, and the newly recommended use of Bayesian statistics, indicate that there are significant improvements to be made in the development of subadult dental age estimation standards, and thus in the accuracy and specificity of dental age estimates. A meta-analysis comparing the methodological errors of all existing macroscopic subadult dental age estimation standards and the newly proposed method further demonstrates the possibility for improved standards.

Chapter IV: Creating an Improved Adult Age Estimation Standard Based on Dental Attrition

This chapter presents a new macroscopic dental age estimation standard based on photogrammetrically quantified dental attrition in Egyptian adults from the Kellis 2 cemetery, Dakhleh Oasis, Egypt. The "FIJI (is just ImageJ)" image analysis software is used to quantify the area of the occlusal surface and areas of exposed dentine. The percentages of exposed dentine to the associated area of the occlusal surface were then calculated for first and second molars in the Kellis 2 cemetery population. This method of quantification is tested for intra- and inter-observer error and reveals a very high intra-class correlation for both tests.

Following these tests, comparisons are made between dental wear in first molar isomeres and antimeres. These data indicate that there is more severe wear in the lower dentition and a statistically significant asymmetry in first molar antimeres. Dental pathology is investigated in the individuals with the largest differences in wear between antimeres. Perhaps due in part to the small sample size, there is no observable correlation between asymmetrical dental wear and one-sided dental pathology.

Caries and antemortem tooth loss are then investigated in relation to skeletal age, revealing a positive correlation only between antemortem tooth loss and skeletal age. Consequently, linear and polynomial regression models are designed and tested for age estimation in males, females, and individuals with unknown sex. These tests reveal strong predictive value in all models.

The quantified dental wear data for first and second molars are then determined to have a strong positive correlation with skeletal age, and linear regression models are similarly designed and tested. These models show relatively high predictive value and small confidence intervals. As a result, multiple regression models were designed for all combinations of antemortem tooth loss and first and second molar wear. Although small reference sample sizes appear to have an effect on the accuracy of the models that incorporate male first molar wear data, all other multiple regression models demonstrate the potential for significant improvement in age estimation based on dental wear. A comparison of the Brothwell standard to the new linear regression models for first and second molar wear confirms that the linear regression models are more specific and accurate than the Brothwell standard.

Chapter V: Dissertation Summary

This chapter summarizes the conclusions of the previous sections and draws upon them to make an argument for the continued improvement of macroscopic dental age estimation standards, with a particular focus on the development of sex-, region, and time-specific standards. Ultimately, I conclude that the null hypothesis: "Current dental age estimation standards cannot be improved" can be rejected given the proven potential for improvement in subadult standards and the presentation of new and improved adult standards.

1.2 Research Objectives and Null Hypothesis

The objectives of this study are to re-evaluate macroscopic dental age estimation standards and the methods used in their development. To this end, new and improved methods are presented for the creation of (time-,) sex- and region-specific dental age estimation standards through the use of innovative techniques for data collection and statistical analysis. This research project aims to test the null hypothesis (H_o): "Current dental age estimation standards cannot be improved". The results of this study can also be used to address the related research questions: “Is there room for improvement in macroscopic dental age estimation methods?” and “How can macroscopic dental age estimation methods be improved?”

This study includes critical reviews of the different macroscopic dental age estimation standards, their methodologies, and the reference collections used in their creation. New methods for the creation of (time-,) sex- and region-specific dental age estimation standards are presented for subadults (based on the macroscopic analysis of dental development) and adults (based on the macroscopic photogrammetric analysis of dental attrition). Theoretical and practical discussions of the potential for improved subadult dental age estimation standards are considered in this study; and results from tests of a newly created sex-, region-, and time-specific adult age estimation standard are presented and compared to those of the most popular ‘universal’ age estimation standard based on dental wear (Brothwell 1963a).

This project tests whether the current universal dental aging standards can be improved through the development of (time-,) sex- and region-specific dental age estimation standards. It is important to continually re-evaluate and test all age estimation methods so that we may improve accuracy and decrease bias of all age estimates. Improved methods for dental age estimation contribute to the accuracy of all subsequently related studies of ancient or modern people.

Chapter 2

2 The Significance of Dental Age Estimation

2.1 The Significance of Dental Anthropology

The study of dental anthropology, bioarchaeology and paleoanthropology are inextricably linked through their coevolution within the broader field of biological anthropology and their mutual contributions to our collective knowledge of the past. The term "dental anthropology" first appeared in the title of an article published in 1900 by George Buschan (Scott and Turner 1988). However, the growth of the field really began during the first half of the 20th century with dental studies being published by pioneers in physical anthropology such as Hooton, Hrdlicka, and Dahlberg. It was not until 1953 that Klatsky and Fisher published a formal introduction to the study of dental anthropology. The term was later solidified in the professional vernacular through its use by Don Brothwell during a meeting held in London, England in 1958 (Brothwell 1963b). Dental anthropology is now defined as the "study of people (and their close relatives) from the evidence provided by teeth" (Hillson 1996: 1). As indicated in this definition, dental anthropology continues to have applications in the fields of paleoanthropology and primatology, in addition to the study of populations of anatomically modern *Homo sapiens*.

Dental anthropology is an essential and important contributor to the understanding of human and hominid evolution, behaviour and adaptation. Teeth are an especially important source of information about the past, in part, because they often survive better than bones in the archaeological record (Hollund et al. 2015). As such, dental analyses have played a large role in the study of extinct hominoids and tooth bearing vertebrates. The following are just some examples of the contributions that dental anthropological methods have made in a variety of fields of research, including dentistry, forensic anthropology, bioarcheology, primatology, and paleoanthropology. Given the vastness of the collection of literature related to the following topics, references are limited in selection with preference given to seminal articles, recent publications, and publications that have made significant impacts.

2.1.1 Comparative Anatomy

Comparative anatomical studies can be used to identify different species, as well as differences in tooth use, in archaeological contexts. Beginning with the broad strokes, most mammals, including humans, are diphyodonts, meaning that they have two sets of teeth (deciduous and permanent) in their lifetime. This is in contrast to monophyodonts, who have only one set of teeth (e.g. beluga whales), and polyphyodonts, whose teeth are continually replaced (e.g. sharks) (Irish 2016). In addition to being diphyodonts, most mammals including humans are also heterodonts, meaning that they have a variety of tooth types or classes within their dentition. This category is differentiated from homodonts, such as many fish and reptiles, who have similarly shaped teeth throughout their dentition (Irish 2016). Heterodonty is believed to have initially evolved from the more primitive reptilian conical teeth in Tertiary Period mammals with the tritubercular molar (Osborn 1888; MacCord 2017).

Despite this shared morphological change, which eventually led to most mammals being heterodontic diphyodonts, there is still significant variety in the number and types of teeth included in mammalian dentition. Dental formulae (counts of tooth types within a single quadrant of the dentition) vary widely across different species. In hominids, the deciduous dental formula is made up of two incisors, one canine, and two premolars (2/1/2/0), and the dental formula for the permanent dentition consists of two incisors, one canine, two premolars, and three molars (2/1/2/3). Although these formulae are applied to *Homo sapiens* as a group, there is variation among individuals due to anomalous agenesis or supernumerary dental development. Third molars have the highest prevalence of agenesis or impaction in modern humans, which has been hypothesized to be a recent biological change on an evolutionary time scale, although the mechanisms driving this change are debatable (Carter and Worthington 2015; Brothwell 1963b; Lavelle and Moore 1973; Bermudez de Castro 1989). Proponents of this hypothesis compare trends in 3rd molar agenesis to the loss of the first two premolars in the Catarrhini branch of the primate evolutionary tree from the primitive mammalian dental formula in which there are 4 premolars in each dental quadrant (Swindler 2002). Thus, the two premolars noted in the human dental formulae are actually the retained third and fourth mammalian premolars.

Consequently, dental anthropologists variably refer to these teeth as P1 and P2, or P3 and P4 (Hillson 1996; Irish 2016).

The variety in the expression of tooth types seen in primates, and the associated cranial morphology, is a reflection of the wide range of dietary niches occupied by different primate species. Related cranial traits such as the shape of the dental arcade, TMJ morphology, and masticatory musculature can also be used to identify dietary preferences and help differentiate between species. In fact, studies of comparative cranial and dental anatomy in primates have a demonstrated ability to distinguish between fruit, nectar, or exudate eating primates with relative accuracy (Dumont 1997). The dentition of primate species that are primarily insectivorous or folivorous are also readily identifiable.

Through the observation of dental and related cranial traits, extrapolated patterns of diet and behaviour in hominoids may also give a better idea of how, and from where and from when, modern humans evolved. More specifically, the reconstruction of ancient diets enables a limited reconstruction of the ancient ecology, with regard to the history of consumed flora and fauna (Dupras 1999). For example, some early middle Miocene hominoids are characterized by thickened molar enamel, enlarged incisors and massive jaws, which is said to reflect a shift from a predominantly frugivorous diet to a diet with more variety and harder foods. (Andrews and Martin 1991; Alba et al. 2010).

A comparison of dentition in early and modern *Homo sapiens* indicates a marked difference in the size of the dental arch, teeth and masticatory muscles, with the larger teeth of early humans being well-adapted for heavy attrition resulting from the consumption of fibrous, uncooked foods (y'Edynak 1992; Brace 1991). The strong mandibular muscles developed through the mastication of tough foods caused increased gonial eversion, wide zygomatic arches and defined temporal ridges in early *Homo sapiens* (Loth and Henneberg 2000). The temporalis muscles are so robust in some great apes and early hominins that the attachment sites are significantly higher on the skull and more pronounced, resulting in the development of a mid-sagittal crest (Cartmill and Smith 2009). These characteristics differ greatly from the relatively gracile bones, muscles, and teeth of modern *Homo sapiens,* who have become accustomed to softer foods following the advent of various food processing methods, including cooking (Wragham et al. 1999).

2.1.2 Odontometrics

Odontometry, the study of tooth size and dimensions, has frequently been used in research regarding human evolution. Data are usually generated through the use of needle point sliding calipers. Dental crown diameters have been recorded widely and compared across all types of hominins. Through the use of dental crown metrics, a trend toward permanent molar crown reduction over time has been documented, as seen in comparative studies of early *Homo,* through *H. erectus*, archaic *H. sapiens*, and *H. neanderthalensis* to modern *H. sapiens* (Brace and Mahler 1971; LeBlanc and Black 1974; y'Edynak 1989; Frayer 1978; Brace et al. 1987; Calcagno 1986; Quam et al. 2009). This trend toward permanent molar crown reduction is also seen throughout the history of *H. sapiens* as seen above *(*See Section 2.1.1). Despite the thorough documentation of this phenomenon, the evolutionary reason for this trend in molar crown reduction remains debatable. One hypothesis suggests that tooth size decreased in association with a decrease in body size resulting from increased population density (Macchiarelli and Bondioli 1986). Another attributes the dental reduction to the advent of advanced hunting technology, as energy efficiency of the body would have become more desirable than robustness (Frayer 1978, 1980, 1984). Alternative hypotheses have focused on the jaws and dentition alone, specifically relating to the effect of jaw and dental arcade size on dental crown size. One such hypothesis posits that jaw size reduced as a result of decreased functional stress following the introduction of softer foods, resulting in decreased tooth size (Carlson and van Gerven 1977). A variation of this hypothesis suggests that malocclusion and dental disease may have increased following this reduction in jaw size, which resulted in selective pressure for smaller tooth crown size (y'Edynak 1978, 1989, 1992; y'Edynak and Fleisch 1983). Perhaps the most controversial hypothesis attempting to explain the trend toward dental crown reduction is the “Probable Mutation Effect” (Brace 1964, 1987; Brace and Mahler 1971; Brace and Ryan 1980). This hypothesis posits that in earlier hominids there was selective pressure for large tooth size, which was de-emphasized with increased tool use and food preparation techniques, leaving the dentition vulnerable to changes resulting from random mutations. Of course, this hypothesis is improbable as the evolutionary model assumes that all characteristics are subject to selective pressure and that selective pressure, not the lack thereof, is the driving factor in directional evolutionary changes on a large scale (Bailit and Friedlander 1966; Hillson 1996).

More recently, Bermudez de Castro and Nicolas (1995) have challenged these hypotheses in their odontometric study of the Atapuercan hominids, who had smaller molar sizes comparable to anatomically modern humans, despite being older than Neanderthals on an evolutionary scale. Given the lack of evidence for cooking, reduced body size, reduced jaw space, malocclusion or dental disease in this population, arguments were made against all of the aforementioned hypotheses on dental size reduction. The authors instead proposed a new hypothesis in which Middle Pleistocene European hominids differed with regard to dental size as a result of long periods of isolation and genetic drift. They note that there is evidence that a decrease in the rate of cell proliferation over time in individuals with longer developmental periods could result in crown size reduction, particularly in the later-forming molars, as seen in the Atapuercan hominids. As hominid periods of growth and development have extended over the last two million years (Bromage and Dean 1985; Bromage 1987; Smith 1986, 1991b; Dean 1987), this would account for the reduction in molar crown size on an evolutionary scale. Assuming that the Atapuercan hominins are ancestral to modern humans, this decreased rate of cell proliferation over time may even help to explain third molar agenesis in anatomically modern humans (Bermudez de Castro and Nicolas 1995).

Although net tooth size has been used to identify and distinguish between different human populations (Peck and Peck 1975; Brace and Hinton 1981; Brace et al. 1987), it has been shown to be a poor indicator in this regard (Hanihara 1977, 1979; Harris and Nweeia 1980; Kirveskari 1978; Moorrees 1957; Perzigian 1984; Hillson 1996). Perzigian (1984) demonstrated the lack of correlation between net tooth size and different populations in a large scale odontometric study. In this study, he created a dendrogram based on size distances, which showed clusters of geographically and genetically distant individuals and the wide dispersal of some closely related individuals. Nevertheless, Harris and Rathbun (1991) have since demonstrated that through the use of discriminant analysis, it was possible to classify half of the 90 studied individuals from around the world correctly. In a study of Pakistani and Indian individuals using three odontometric methods, Hemphill (2015) found that more than 50% of individuals could be correctly associated with their locality and more than 80% could be attributed to a region. He also found that principle components analysis was more useful for the analysis of within-group variation, while canonical variates analysis was more useful for the assessment of variation between groups.

In addition to variation across populations, differences in tooth size have also been attributed to sexual dimorphism in humans (Garn et al. 1964), although human sexual dimorphism is far less than that observed in some non-human primates (Swindler 2002; Hillson 2005). Nevertheless, dental size has been used with population-specific discriminant function formulae to estimate sex in modern (Garn et al. 1964, 1977; Margetts and Brown 1978; Potter et al. 1981; Garcia Godoy et al. 1985; Kieser et al. 1985a,b; De Vito and Saunders 1990) and archaeological populations (Lunt 1969; Ditch and Rose 1972; Owsley and Webb 1983; Beyer-Olsen and Alexanderson 1995; Teschler-Nicola and Prossinger 1998). However, it has been argued that discriminate functions, because they are population- or site-specific, are more descriptive of the population than predictive in nature (Feeney 2005). Consequently, the resulting data are not well suited for age estimation. Furthermore, dental attrition impedes accurate dental measurement (Teschler-Nicola and Prossinger 1998) and there is little size differentiation between the sexes relative to the rates of observer error (Hillson 1996: 82). Thus, traditional odontometric analysis is not recommended as a sole indicator of sex, but can be useful as one of multiple indicators for sex in human remains (Joseph et al. 2013; Angadi et al. 2013). However, in cases where bones have succumbed to taphonomic processes and the teeth are the only remaining indicator of sex, odontometrics may present the only clue to the sex of the individual (Joseph et al. 2013; Banerjee et al. 2016).

Beyond species-, population-, and sex-specific differences in tooth size, odontometry is also used in the observation of differences in tooth size resulting from environmental stress. Fluctuating odontometric asymmetry is the variation in size of dental antimeres (tooth pairs on opposing sides of the dentition). Under ideal conditions, dental antimeres develop at the same rate and have very similar measurements upon completion of growth. However, this metric symmetry is based on epigenetics, and is thus vulnerable to environmental and genetic factors such as prenatal exposure to teratogens, inbreeding, and malnutrition (Groeneveld and Kieser 1991). Consequently, some researchers have used the asymmetry between dental antimeres to try to identify differences in environmental stress and developmental stability among populations (Groeneveld and Kieser 1991).

In addition to the aforementioned post-mortem applications of odontometry, it also has value in the field of orthodontics. In modern humans, there is a demonstrated correlation between tooth size and malocclusion, particularly with regard to tooth spacing or crowding (Puri et al. 2007; Bugaighis and Elorfi 2013). Accordingly, tooth size is often compared to arch length to help plan orthodontic treatments (Bugaighis and Elorfi 2013). It is important that clinical intervention in orthodontics includes the assessment of the potential changes in dental growth.

Although there are many practical uses for the study of odontometry, it is complicated by issues of unspecified nomenclature, the inability to standardize datum points and the effects of anthropogenic and unintentional dental modification of the size of the tooth (Foley and Cruwys 1986). Consequently, odontometrics are often used only as supporting evidence or in cases with few other options for analysis. Nevertheless, the benefits of odontometric study still outweigh the associated challenges and dental anthropologists continue to work toward mitigating these limitations.

2.1.3 Odontoscopic Morphology

Odontoscopic morphology refers to the observation and analysis of genetically-linked nonmetric dental variations. Morphological traits within the dentition are known to be inherited, and their study reveals important information about familial and ancestral relations. The first publication relating to dental morphology was that describing Carabelli's tubercle in 1842 (Hoffman-Axthelm 1981), however, it was not until the early 20th century that morphological research began in earnest (Campbell 1925; Weidenreich 1937; Pedersen 1949; Robinson 1956; Moorrees 1957). These studies only noted the presence or absence of discrete non-metric traits until Hrdlicka (1920) introduced a standardized gradient for scoring shovel shaped incisors while also demonstrating geographically distinct variation in the expression of this trait. Prior to the discovery of DNA, traits that showed strong links to specific ancestral lineages, like shovel shaped incisors in Native Americans and Asians, acted as proof of the heritability of these traits and spurred further investigations into dental morphology. This genetic link could not be independently established until the introduction of dental genetic studies in the second half of the 20th century, which also clarified the significant role of environment in the epigenetics of dental morphology (cf. Goose and Lee 1971; Harris 1977; Harris and Bailit 1980; Scott 1973; Paynter and Grainger 1956; Kruger 1966). As is now well known, the heritability coefficients developed through the study of these traits can now inform us about how much the variation in nonmetric traits is under genetic control not the presence of the trait per se (Sjøvold 1984).

Starting in the 1940s, Dahlberg expanded upon Hrdlicka's idea to standardize the documentation of dental trait variants by creating the first standardized dental morphology reference plaques for 16 traits (Dahlberg 1956). This prompted Hanihara (1963) to develop a similar set for deciduous dental morphological variants, and Turner (1970) later developed the first two plaques for the adult crown and root morphological grading system, which would eventually be called The Arizona State University Dental Anthropology System (ASUDAS). Through the efforts of Dr. G. Richard Scott, Dr. Christy Turner II and colleagues, 42 non-metric adult dental traits have been identified to date. Only 29 of these traits are included in the ASUDAS, chosen because of their relative independence from each other and their lower vulnerability to dental wear (Scott and Irish 2017). The ASUDAS plaques were revised and re-released by Scott and colleagues in 2018 and a related web-based application, called rASUDAS, has also been released to facilitate the ancestry estimation for individuals (Scott et al. 2018).

In Egyptological research, Greene (1972) and Berry (1976) utilized many of the ASUDAS variants in their investigations into the origins and biological relationships of the ancient Egyptians. More recently, Haddow (2012) has used the ASUDAS variants to examine the biological relationships and affinities of the Kellis 2 population sample from Dakhleh Egypt. A large part of the photographic sample of occlusal dentition used in this dissertation was derived from Haddow's study.

In addition to the ASUDAS variants, an alternate method was developed in Russia and is called the odontoglyphic method (cf. Zubov 1977; Hillson 1996). This method concentrates on "reading" the molar furrows on the occlusal surface. This odontoglyphic research has had little use outside of Russia but can be compared to studies based on the ASUDAS through equivalence systems (cf. Haeussler et al. 1988; Haeussler and Turner 1992).

In addition to these two methods of dental morphological analysis, some research has been done regarding the measurement of these genetically linked traits. Early studies were based on macroscopic (cf. Corruccini 1977a,b; Aas 1983), microscopic (cf. Suwa 1986; Suwa et al. 1994), or photogrammetric (cf. Biggerstaff 1969, 1975, 1976) measurement of morphological traits. While these methods continue to be used, more recent advances in imaging technology have allowed for some interesting metric analysis of morphological traits through the use of computed tomography (e.g. Zhang et al. 2011), micro CT (e.g. Plotino et al. 2006), laser scanning (e.g. Ungar 2004), and geographic information systems (e.g. Zucotti et al. 1998; Ungar 2004).

Regardless of the methods used, dental morphology has been fundamental to research in dental anthropology. Given the strong genetic association of dental traits, morphological studies have contributed significantly to the phylogenetics of Hominidae, as in Irish's (2013) study on the dental morphology of *Australopithecus sediba*. This study resulted in the assertion that *A. sediba* is distinct from east African australopiths, but is close to *A. africanus*, resulting in the formation of a southern African australopith clade with links to the genus *Homo*. Dental morphology has also made a significant impact on the debate regarding the peopling of the Americas. In this case, Turner (1971) noted the differences in 3 rooted mandibular first molar frequency throughout the Americas and presented the "Three wave" model of human migration from the Old World. In more recent archaeological studies, dental morphology has been used to identify the ancestry of human remains and track migration and, on a smaller scale, familial relationships for individuals buried at a particular site. In addition to these applications, teeth can also be used to indicate a minimum number of individuals in a mass burial, or dental morphology and spacing can be compared to dental records and radiographs, for the individuation of human remains

(Pretty and Sweet 2001). This last application for comparative morphological analysis is also considered to be a forensic odontological method (See Section 2.1.9).

2.1.4 Enamel Thickness and Microstructure

Expanding on the previous discussion of phylogenetics in the family Hominidae, the relative thickness of enamel is considered to be a defining characteristic of the family Hominidae with the exception of *Pan* and *Gorilla* (Pilbeam 1979; Schwartz 2000a). Following comparative studies of living and extinct hominoids, Martin (1983, 1985) asserted that thin, fast-formed enamel was the ancestral form of the taxonomic superfamily Hominoidea, and that through evolution, slower-formed thick enamel was the primitive form for the taxonomic family Hominidae and the fossil hominoid, *Sivapithecus*. In this study, he argued that thin enameled hominoids had fast-forming ‘pattern 3’ enamel prism microstructures, while thin enameled African apes had the slower-forming ‘pattern 1’ enamel prism microstructure. Since the thick enameled hominids also showed a ‘pattern 3’ enamel prism microstructure, Martin (1983, 1985) argued that the thin enamel found in African apes represented a secondary decline in the rate of enamel secretion. A more recent study by Beynon et al (1991) rejected Martin’s (1983, 1985) hypothesis. Their study posits that fast-formed thin enamel may have been the primitive condition for hominoids and hominids, from which the relatively thick enamel seen in humans and *Sivapithecus* is derived, having evolved independently on more than one occasion (Beynon et al. 1991). Schwartz (2000a) has also hypothesized that enamel thickness in *Homo* and *Pongo* may be independently derived, though further research is needed.

Furthermore, although enamel prism microstructure was once used to identify members of the Hominidae family, as they were believed to have a ‘keyhole’, or ‘pattern 3’, prismatic enamel microstructure (Gantt et al. 1977), it was later discovered that prismatic patterns differ within individuals and even within teeth, depending on the depth of the sample taken within the enamel (Vrba and Grine 1978; Boyde and Martin 1984). It is now known that enamel prism patterns 1, 2, and 3 may be found in any human or ape tooth crown, and that irregularities and intermediate forms of prismatic patterns can also be observed (Hillson 1996). Nevertheless, there remain differences in the thickness of the outer layer of prism-free and Pattern 1 enamel between the hominids, gibbons, and *Sivapithecus* (10% of dental enamel), orangutans (20%), and gorillas and chimpanzees (>40%) (Boyde and Martin 1984; Martin and Boyde 1984; Martin 1985; Martin et al. 1988). In addition to the differences in enamel prism patterns, the orientation of enamel surface in relation to the tooth's surface prisms is indicative of the area of the tooth from which the sample is derived.

Spacing of the brown striae of Retzius, another enamel microstructure, can also be used to determine the area of the tooth from which a sample is derived. These growth-related microstructures, as well as perikymata and enamel prism cross-striations are, however, detailed in Section 2.1.11 on age determination methods. Perikymata are also discussed briefly in the following section on enamel defects.

2.1.5 Enamel Defects

Enamel defects can be used to identify pathological conditions, periods of biological stress, and for the consumption of some specific chemicals. For example, the excess consumption of tetracycline or doxycycline is known to result in dental staining in developing teeth (Sanchez et al. 2004) as well as fluorochrome labelling, which is detectable in the archaeological record (cf. Bassett et al. 1980; Cook et al. 1989). The evidence of tetracycline labelling has been identified in individuals interred in the Ain Tirghi (Cook et al. 1989) and Kellis 2 (Maggiano et al. 2003) cemeteries in Dakhleh Oasis, Egypt. The source of the tetracycline is hypothesized to originate from streptomycetes bacteria growing within grain stores, as hypothesized in an earlier study of Nubian individuals (Bassett et al. 1980). Based on the incremental nature of the fluorochrome labelling in the teeth and bones from Dakhleh Oasis, it was determined that tetracycline was consumed occasionally; perhaps seasonally as grain stores ran low, and more of the Streptomycetes infested grains were collected from the bottom of the store. In addition to revealing information about grain storage and consumption, the discovery of fluorochrome labelling at Dakhleh Oasis also presented a possible reason for the relatively low rates of infection seen in the cemetery population (Cook et al. 1989).

Excessive consumption of fluoride, otherwise known as fluorosis, is also known to produce enamel mottling and discolouration, and has been seen in archaeological populations (cf. Yoshimura et al. 2006; Petrone et al. 2013). Since fluoride concentrations differ geographically, an individual with evidence of fluorosis found within an area without endemic fluorosis may indicate that the affected individual was a migrant.

Mercury is another chemical that left lasting effects on the dentition of past peoples. Mercurial defects are identifiable as “large expanses of deficient enamel that [are] rugged and pitted, ultimately producing an appearance of a dirty grey honeycombed tooth” (Iaonnou et al. 2016: 617), at times with large areas of occlusal enamel entirely missing, exposing the dentine (Iaonnou et al. 2016). Mercurial defects are often seen in association with dental defects pathognomonic for congenital syphilis (i.e. Hutchinson’s incisors, Moon’s molars, and Fournier’s molars); the ailment most commonly treated with mercury in the past. Since syphilis does not always present with dental abnormalities and mercury was rarely used in significant amounts for any other infant ailment, it has been proposed that in some circumstances dental deformities attributable to mercury may be interpreted as a sign of treatment for syphilis in skeletal remains even without paleopathological evidence of treponemal infection (Iaonnou et al. 2016).

Congenital syphilis is not the only disease to result in the development of dental enamel defects. Some congenital malformations and genetic disorders can also result in abnormal dental appearance. For example, congenital erythropoietic porphyria can produce red-purple discolouration of the dentition (Brown et al. 2014). *Amelogenesis imperfecta* is a collection of congenital conditions that affect the structure and/or appearance of dental enamel in all, or almost all, of the teeth (Aldred et al. 2003).

More commonly, enamel defects are seen in the form of enamel hypoplasia, which is the result of a disruption of the development of enamel matrix by ameloblasts resulting from systemic stress, such as febrile illness or starvation (Antoine 2000). These defects can be manifest in the form of isolated pits, rows of pits, furrows, prominent steps, or plane-like defects. In the case of plane-like defects, also known as linear enamel hypoplasia (LEH), the production of the perikymata (the microscopic lines on the surface of dental enamel resulting from the layers of enamel that are deposited to form a tooth, also known as striae of Retzius) become varied in spacing and prominence and the result is an enamel surface which appears wavy (Hillson and Bond 1997). These defects can be caused by hereditary factors, trauma or any periods of severe (Selyean) physiological stress, such as febrile illness or starvation during the formation of the tooth (Selye 1956). Enamel hypoplasias are also sometimes attributed to physiological stress associated with birth and weaning (Blakey et al. 1994; Goodman and Martin 2002; Seow 2015). Consequently, enamel defects can provide a host of information about the lives of past peoples, particularly with regard to their early years during dental development. However, caution must be taken in making conclusions from macroscopic analysis of enamel hypoplasia alone as some hypoplastic defects can only be seen microscopically (which is not necessarily a reflection of the severity of physiological stress) and the enamel surface can be affected by dental erosion and microwear (Hillson and Bond 1997).

2.1.6 Oral Pathology

Just as the study of enamel defects can provide information about aspects of subadult disease and diet, the study of oral pathology can provide information about dietary norms in past populations. The population prevalence of dental caries lesions, commonly known as dental cavities, is a good indicator of dietary norms. Carious decay is the result of demineralization of the dental tissue from organic acids released through the bacterial fermentation of dietary carbohydrates (Sheiham and James 2015). Increases in carbohydrate consumption (like that resulting from the switch to intensive agriculturalism) are reflected in higher rates of dental caries (Armelagos 1969; Turner 1979; Cohen and Armelagos 1984; Milner 1984; Patterson 1984; Larsen et al. 1991; Lukacs 1992; Larsen 1995). This increase in caries associated with the introduction of agriculturalism may also be attributable to the fact that the grains of domesticated cereals such as wheat and maize contain more carbohydrate and relatively less protein than those of wild grasses (Tayles et al. 2000). Despite this high carbohydrate ratio, it has been revealed that, for reasons that remain unclear, this rise in dental caries rates does not appear to be associated with the introduction of rice-based agriculture (Tayles et al. 2000, 2009; Temple and Larsen 2007; Halcrow et al. 2013).

An increased prevalence of dental caries may also be attributable to the introduction of cooking, which occurred prior to the introduction of agriculture, as it allowed various fibrous foods to be rendered softer and more prone to sticking to the dentition, allowing carbohydrates to remain in contact with cariogenic bacteria for longer periods of time (Powell 1985). Furthermore, cooking reduces the abrasiveness of fibrous foods. In the absence of regular dental hygiene, high rates of dental abrasion resulting from the consumption of abrasive foods or dental attrition from tooth-on-tooth contact can create a hostile environment for the growth of caries, sweeping away adherent food particles and grinding down sites of decay before they can spread (Maat and Van der Velde 1987; Milner 1984; Powell 1985). However, Meiklejohn et al. (1992) advocate for caution in the negative correlation of dental wear and caries as they argue that they are independent factors and, as can be seen in some populations, are not mutually exclusive.

In addition to dental wear, other factors that should be considered in the study of population caries rates include: fluoride consumption, dental hygiene, anthropogenic dental modification, antemortem tooth loss or extraction, post-mortem tooth loss, host resistance, hormones (sometimes related to sex), salivary excretion (sometimes related to age), and cariogenic bacterial load (Goodman and Martin 2002; Larsen 1995; Lukacs 1995, 1996; Lukacs et al. 2006). Despite these complicating factors, studies of worldwide prevalence of caries have demonstrated that hunters and gatherers usually have a caries rate of less than 2%, mixed economies generally have a caries rate around 5%, and agricultural economies have a caries rate from 2% to 25%, usually resting around 10% or more (Scott and Turner 1988; Goodman and Martin 2002). A study of a Greenlandic Inuit society with a diet based predominantly on meat and fish, with practically no carbohydrates, also showed less than 5% of individuals, and 0.3-0.6% of permanent teeth, affected by carious lesions (Pedersen 1938, 1947; Davies and Pedersen 1955; Hillson 2001). Consequently, it may be possible to estimate the subsistence strategies of past populations through an examination of their dentition and consideration of their oral pathology. This strategy was applied in the current author’s Master’s thesis in which it was determined that the caries rates observed in the paleolithic population from Site 117 (widely considered to be the world’s earliest evidence of mass violence and the earliest Nubian cemetery) were consistent with rates usually observed in populations subsisting on a traditional huntergatherer diet (cf. Kirkpatrick 2009). Although population studies of caries can be informative, it must also be considered that differences in subsistence between the sexes and/or different socioeconomic groups may also be reflected in intra-population variations of dental caries prevalence (e.g. Lukacs and Largaespada 2006; Stranska et al. 2015). Consequently, it is important that oral pathology is considered in relation to age, sex, and socioeconomic status, wherever possible.

In addition to its consideration in the study of dental caries, dental wear can be informative in its own right. Dental wear is a term that encompasses dental attrition, abrasion, abfraction, and erosion; all of which can be affected by diet (See Section 4.2 for detailed information on the biomechanics of dental wear and its contributing factors). Macroscopic studies of occlusal dental wear are widely used for adult age estimation (See Section 4.3 for more information on age estimation methods based on dental attrition) and severe wear can result in alveolar bone loss and be a contributing factor in the development of periodontal infection, abscess and/or antemortem tooth loss. Anthropogenic dental wear can also provide information about: the use of teeth as tools, early dental interventions, toothpicking, pipe use, oral jewelry, and culturally prescribed practices (such as dental filing). Both macroscopic and microscopic studies of dental wear are also useful for reconstructing chewing cycles, jaw movements, and dental biotribology in primates (e.g. Ryan 1979; Gordon 1984; Walker 1984). These methods may be of particular interest to those researching evolutionary changes in the orofacial skeleton and dentition.

More specific dietary information can be gleaned from the microscopic study of dietary-derived pits and scratches on enamel surfaces, also known as abrasion, though this microwear, particularly on the occlusal surfaces, may only truly represent abrasion from a short period of time before death (Grine 1986; Teaford and Oyen 1989; Perez-Perez et al. 1994). Microwear textural analysis based on factors such as anisotropy, complexity, textural fill volume, and heterogeneity can contribute to dietary inferences for modern and ancient species with preserved enamel (Scott et al. 2005; Scott et al. 2012; Schmidt et al. 2015). Furthermore, microwear may also be useful in the identification of specific foods in the diet as different phytoliths may create differently shaped microscopic grooves on the tooth surface (Gugel et al. 2001). Silica phytoliths (Lucas et al. 2013), calcium oxylate phytoliths (Danielson and Reinhard 1998) and spheres of amorphous silicon dioxide (Xia et al. 2015) are all known to leave microscopically visible grooves on enamel. In some cases, these phytoliths have even been found embedded in the enamel at the end of these grooves (See Section 4.2 for further details on the biomechanics of dental wear and its contributing factors; Ciochon et al. 1990; Lalueza-Fox et al. 1994, 1996).

Both attrition and abrasion may be affected by a third form of dental wear: enamel erosion (Barbour and Rees 2006). This type of wear involves the acidic softening or dissolution of enamel surfaces, usually associated with a diet that is high in acidity, though it may also be affected by chemical, behavioural, or biological factors (Lussi et al. 2004). Certain medical conditions, such as acid reflux, bulimia, or pregnancy-related ‘morning sickness’, can also be positively correlated with enamel erosion on the lingual surface of the anterior teeth, as these conditions cause this dental enamel to be coated by regurgitated stomach acids (See Section 4.2.4 for further details on the biomechanics of dental erosion and its contributing factors; Valena and Young 2002). Enamel erosion has had a variable impact on past populations, however indicators of enamel erosion are still debated and knowledge of erosive factors are needed to support physical evidence (Coupal and Soltysiak 2017; See section 4.4.1 for discussion of the impact of erosion on ancient Egyptian dental wear).

Just as dental attrition can be affected by, and altered by, caries and vice versa, both dental wear and dental caries may also affect the prevalence of abscesses and/or antemortem tooth loss. Periapical abscesses are strongly linked to dental infection, which often results from the exposure of the dental pulp through large carious lesions or severe attrition, and periapical abscesses can, in turn, result in the loss of the associated tooth (Molnar 2008; Grant et al. 1988; Alt et al. 1998). If periapical abscesses are left unresolved, particularly in the maxillary dentition, then it may become a threat to the life of the individual as the infection may spread, in some cases to the brain (Li et al. 1999). Lingual tooth dislocation (or tilting) can also result from a combination of dental wear, continual eruption, and destruction of the buccal alveolar bone resulting from periodontitis, periapical abscess, or alveolar fenestration (Clarke and Hirsch 1991). Dental abfraction has also been associated with high bite forces, dental attrition and dental erosion (See Section 4.2.3 for more on abfracture; Rees 2002). Many of the aforementioned conditions, as well as osteoarthritis of the temporomandibular joint, are positively correlated with chronological age. Other pathological conditions (e.g. cleft palate, oral cysts, granuloma, tumors, etc.) may contribute to an understanding of the life history of an individual.

Periodontitis is another oral pathology that is regularly documented in the study of dental anthropology as it can lead to painful infections, alveolar abscess and ante-mortem tooth loss (Podzorski 1990). In skeletal materials it is characterized by alveolar bone loss as indicated more than 1.5mm space between the tooth's crown and the alveolar bone (Lavigne and Molto 1995). This disease can be localized or widespread throughout the dentition and is often a result of poor hygiene, localized infection, embedded foreign materials, excessive attrition (Greene et al. 1967), compromised immunity (Hajishengallis 2015), and/or the build-up of calculus along the gum-line (White 1997). Severe periodontitis can result in antemortem tooth loss and can make dental roots more vulnerable to caries and infection through exposure (Ravald and Johansson 2012).

Dental calculus can, in itself, provide information about the diet of an individual through microscopic analysis of dental calculus inclusions such as phytoliths or starch granules (e.g. Fox et al. 1996; Hardy et al. 2009). As further discussed in section 2.1.7, dental calculus can also preserve information about the oral microbiome of the individual. In an effort to document any correlations between dental calculus and diet or other oral pathology (e.g. caries, attrition, or periodontitis), dental calculus is often reported in terms of the amount of buildup found on teeth in the archaeological record. These recordings are also useful for identifying significant calculus samples for biomolecular study. With all of these pathological conditions in mind, it is clear that the study of oral pathology can inform our collective understanding of: dietary links to oral pathology, co-morbidity in the oral cavity, dietary customs in past populations, and aspects of individual life histories that may have affected behaviour and quality of life.

2.1.7 Biomolecular Analysis

Samples of dental calculus and whole teeth are often collected for the biomolecular analysis of archaeological remains as they tend to preserve well, are easy to collect in small samples, and provide a certain amount of protection from contamination to the interior of the sample.

As a result, a growing number of biomolecular methods have been used in the analysis of teeth and dental calculus. For example, ancient DNA (aDNA) extracted from dental materials has enabled the reconstruction of some familial, social and evolutionary relationships (e.g. Cappellini et al. 2004; Simon et al. 2011). The sex of individuals has also been determined through DNA analysis (e.g. Cappellini et al. 2004; Vaňharová and Drozdová 2008). The complete human mitochondrial DNA (mtDNA) genome has also been reconstructed from dental calculus, providing a method for mtDNA extraction that is not destructive to the human remains (Black et al. 2011; Ozga et al. 2016). Genetic analysis of dental calculus can also help to identify starch granules or plant microfossils, enabling the collection of important information on heritage plant species (Weyrich et al. 2015). Furthermore, aDNA analysis from tooth and calculus materials can help to identify some pathological conditions, though this research is still blossoming (e.g. Drancourt et al. 1998; Raoult et al. 2000; Drancourt et al. 2005; Papagrigorakis et al. 2006; Bedarida et al. 2011). Bacterial aDNA derived from dental calculus can also provide information about past oral microbiomes and microbiotic changes within a population perhaps resulting from dietary and/or environmental changes (e.g. Eisenhofer et al. 2017; Adler et al. 2013; Warinner et al. 2015). Scanning electron microscopy, fluorescence microscopy and transmission electron microscopy have similarly been used to identify bacteria in ancient dental calculus (Linossier et al. 1996; Preus et al. 2011). These methods of detection, and particularly genetic analyses, of these pathogens can contribute to the evolutionary understanding of these pathogenic species and their co-evolution with early hominins and humans (Harkins and Stone 2015).

Ancient pathogens and host immunological responses can also be detected through paleoproteomic analysis of dental pulp (e.g. Barbieri et al. 2017; Drancourt et al. 2018; Wasinger et al. 2019). Paleoproteomic methods represent a tremendous potential for improved understandings of disease and host co-evolution that can inform the field of evolutionary medicine. Proteomic analysis can also be used for dietary research as it has revealed the whey protein, β-lactoglobulin, in the dental calculus of Greenlanders from the Bronze Age until the present, indicating their consumption of milk (Warinner et al. 2014).

More popularly used for dietary research is stable isotope analysis. In this respect, isotopic analysis can serve to determine the types of foods consumed by an individual, and in what relative quantities (e.g. Van der Merwe et al. 2003; Beaumont and Montgomery 2016; Scott and Poulson 2012), giving a better idea of the lifestyle of studied populations. In bioarchaeological studies, differences in diet within a population can also indicate economic and social inequalities or ‘structural violence’ (Klaus 2012). Less wealthy individuals or those belonging to marginalized groups often have reduced access to prized nutritional resources, resulting in observable differences in health in the archaeological record (Reitsema and Vercellotti 2012). Differences in food consumption may also be related to cultural beliefs about food, or food taboos. As Foster and Anderson stated, “No group, even under conditions of extreme starvation, utilizes all available nutritional substances as food” (Foster and Anderson 1978, as cited in Helman 2000: 33). Isotopic analyses of dental tissues and dental calculus have also contributed to our understanding of individual migration by indicating the range of ecologies occupied by an individual (e.g. Schweissing and Grupe 2003; Budd et al. 2004; Schroeder et al. 2009; Shaw et al. 2016). In an innovative approach, isotopic studies of dentine have even been used to narrow down samples to specific time periods within an individual’s life, opening up great potential for the observation of isotopic changes through an individual’s life relating to dietary changes such as those related to weaning, famine, or migration (e.g. Beaumont et al. 2013; Sandberg et al. 2014; Beaumont and Montgomery 2016).

In addition to the analysis of isotopes, elemental analysis through methods such as mass spectrometry, gas chromatography, X-ray Fluorescence (XRF), and x-ray micro-fluorescence using synchrotron radiation have been used to detect tooth labeling from materials as diverse as lead (e.g. Budd et al. 2000), coca (e.g. Ubelaker and Stothert 2006), and betel nut (e.g. Hocart and Frankhauser 1996). These methods can also be used to detect normal dietary elements and isotopes (e.g. Dolphin et al. 2005; Anjos et al. 2004). All of these studies further inform our understanding of the diets, environment, pathology and lifestyles of past people.

2.1.8 Anthropogenic Dental Modification

At times, it can be difficult to distinguish between pathological changes (e.g. caries, attrition, tooth loss), developmental defects, and anthropogenic modifications of the dentition. Anthropogenic dental modification may be done intentionally (for reasons of status, ethnicity, identification or aesthetics) or unintentionally (through the use of the teeth as tools, or the use of pipes, toothpicks, labrets, etc.). Both types of modification can reveal information about the individual and sometimes about their society.

Intentional dental modifications found in archaeological remains include dental ablation, dental filing, drilling and shaping, medical intervention, the use of dental appliances, the placement of inlays, and staining (Hillson 1996; Alt and Pichler 1998; Barnes 2010; Burnett and Irish 2017). Many of these modifications are done as a point of pride, marking life milestones or identifying the individual's cultural affiliation or social status. However, some modifications are done solely for aesthetic purposes and may be limited to higher socio-economic strata. An example of aesthetic modification that is also linked with gender and marital status is a practice called *ohaguro*, in which early- to pre-20th century married Japanese women stained their teeth black, using an acidic mixture containing iron compounds (Ai et al. 1965; Oyamada et al. 2017).

In addition to the staining of teeth, there exist more destructive intentional dental modifications such as dental filing, drilling, breaking, chipping, shaping, piercing, movement and ablation. Germectomy, or surgical removal of the tooth germ, has also been documented in some populations (Benedix 1998; Alt and Pichler 1998; Barnes 2010; Gonzalez et al. 2010). These methods of dental modification can be a result of aesthetic preference, a sign of status or social belonging, or have religious or ritual meaning (Gonzalez et al. 2010). As such, these elements of the dentition can be critical to individuation. The placement of inlays may have similar reasoning; however, inlays may also be used in medical interventions of dental decay. For example, Mayans have been discovered with jade, turquoise, or pyrite inlays (Vukovic 2009). Modern examples of medical inlays include the use of amalgam or composite material fillings. Similarly, the use of dental appliances such as crowns, bridges, braces, or dentures may also be considered as intentional dental modifications and can provide information about the status of an individual and the dental expertise in a society (Alt and Pichler 1998). In modern contexts, these interventions can be compared to dental records for forensic odontology (See Section 2.1.9 for more information on forensic odontology).

Unintentional dental modifications can inform dental anthropologists of the use of teeth as tools, such as in the case of sinew or leather processing methods (e.g. Brown and Molnar 1990; Lous 1970). Lingual and transversal occlusal grooves have also been attributed to the use of the teeth as tools in the processing of cordage for cordage or basketry (Larsen 1985; Alt and Pichler 1998). Wood shaping and the gripping of hard objects (e.g. nails, needles, torches) can also leave characteristic dental wear (Hylander 1977a; Alt and Pichler 1998) Another abnormal pattern of dental wear, known as lingual surface attrition of the maxillary anterior teeth (LSAMAT) has been attributed to the processing and/or consumption of manioc or tubers, or the chewing of sugar cane (Turner and Machado 1983; Irish and Turner 1987, 1997; Turner et al. 1991; Berbesque et al. 2012). Many of these examples of unintended dental modification have shown differences in wear prevalence according to sex, reflecting differences in occupations or habits between the sexes. A more recent example of unintentional occupation-related dental modification would be the development of "phossy jaw" (a deadly affliction involving dental infections, fistulae, abscesses, tooth loss, and bone necrosis) in early industrial era matchstick factory workers exposed to white phosphorous vapour (Roberts et al. 2016). Discoloured dentition can also be associated with some occupations—most notably workers in the metal industry, where a variety of staining has been reported (Schour and Sarnat 1942; Gupta 1990; Brown et al. 2014). The recreational chewing of the psychostimulant, betel nut, has also led to tooth staining in populations in the Western Pacific Basin and South Asia (Fitzpatrick et al. 2003; Gupta and Ray 2004; Zumbroich 2008; Brown et al. 2014).

Unusual dental wear patterns can also indicate if the individual was adorned with labrets (e.g. Cybulski 2010) or if they habitually used pipes (e.g. Ubelaker 1996). Habits of dental hygiene, such as tooth brushing and the use of chewing sticks, have also been suspected in the unintentional modification of teeth though these effects are still debated and can be difficult to distinguish from dental erosion or dental abfraction (Slop 1986; Turp 1990;

Grippo et al. 2004; Oginni et al. 2003; Michael et al. 2009; Senna et al. 2012). It has also been argued that some dental grooving seen in ancient dentition is the result of the use of flossing with abrasive materials or probes for picking between teeth (Formicola, 1988; Turner 1988; Brown and Molnar 1990). This may have been for hygienic reasons but it is more likely that it was done to relieve discomfort from interproximal caries or other pathological irritations (Formicola, 1988). In any case, all of these anthropogenic dental modifications give us clues to the life histories of past people.

2.1.9 Forensic Odontology

Just as some forms of anthropogenic dental modification can be used as identifiers, modern forensic odontology can be used to individuate unknown persons. Although forensic odontology represents an overlap between the dental and legal professions (Pretty and Sweet 2001), it is still a study of human dentition and can be categorized as a form of dental anthropology. Forensic odontology is usually used in cases where it is necessary to identify otherwise unrecognizable individuals, often from mass disasters or forensic cases. When possible in these cases, the dentition of the human remains and their radiographs are compared to modern dental records of the missing people in order to find a match. These matches are usually reliant on dental developmental stage, dental morphology, dental pathology, dental appliances, or dental interventions (Pretty and Sweet 2001). Bite marks, lip prints (cheiloscopy), and palatal rugae (palatoscopy) have also been used in the identification of both victims and perpetrators (Rai and Kaur 2013). Despite its role in bringing the infamous Ted Bundy to justice, bite mark analysis has proven to be unreliable in proficiency testing with error rates as high as 64% (Saks and Koehler 2005). In addition to these applications, forensic odontology can be used to build legal cases for abuse and neglect, or dental malpractice and negligence (Rai and Kaur 2013). In addition to the aforementioned goals, forensic odontologists may also create a post-mortem dental profile for the individual that may include age and sex estimates, information on dental morphological indicators of ancestry, as well as indicators of socio-economic status, diet, occupation, habitual behaviour and pathology (Pretty and Sweet 2001). Many of the methods used in the creation of a post-mortem dental profile are also used in bioarchaeology, however, there are some methods that, so far, have only proven useful in the recently deceased. An example of this is sex determination through the observation of sex chromatin Barr bodies and F bodies in pulp tissues; elements that have been deemed reliable only up to a year after death, depending on circumstances (Duffy et al. 1991; Rai and Kaur 2013). This information can be used to build a profile of an individual that cannot be matched through dental records. Forensic odontology can play an important part in civil and criminal law cases, bringing justice to the guilty and closure to the victims and/or their families.

2.1.10 Dental Sex Estimation

For purposes ranging from the identification of missing people to reconstruction of ancient population demographics, sex estimation is a crucial part of the study of human remains. Although sex estimation is usually conducted through the examination of selected bones, sexual dimorphism in the dentition can also be considered, particularly in cases of limited preservation. In these cases, odontometric analysis of the mandibular canines is often used due to their relatively high level of sexual dimorphism (e.g. Rao et al. 1989; Khangura et al. 2011; Tardivo et al. 2011; Acharya and Mainali 2007). However, studies of bucco-lingual and mesio-distal measurements of other teeth have also proven useful (e.g. Ditch and Rose 1972; Acharya and Mainali 2008; Narang et al. 2015). Although these methods have shown great success in some studies, odontometrics are not commonly consulted for the estimation of sex due to their vulnerability to change resulting from dental wear or breakage, and the differences in odontometrics and sexual dimorphism between populations (Teschler-Nicola and Prossinger 1998; Prossinger 1998; Zorba et al. 2011). However, relative area or volume measurements of dental crown tissues have shown some promise for the estimation of sex in teeth subjected to significant dental wear (Schwartz and Dean 2005; Saunders et al. 2007; Tardivo et al. 2011, 2015; De Angelis et al. 2015; Garcia-Campos et al. 2018). Sexual dimorphism in dental root length, dimensions and relative measurements have also been studied and have shown potential for sex estimation in human remains (Zorba et al. 2014; Kazzazi and Kranioti 2017; Gouveia et al. 2017).

Differences in dental developmental timing have also been suggested for use in sex estimation in subadults (Hunt and Gleiser 1955). However, this method assumes accurate knowledge of the age of the individual, which is not usually possible in bioarchaeological contexts (Teschler-Nicola and Prossinger 1998).

In addition to sexual dimorphism in the dentition, some skeletal elements relating to the oral cavity have been used in the estimation of sex. For example, mandibular morphology can differ between adult males and females. The prominence of the mental eminence is one such characteristic which is commonly considered along with other cranial elements in the estimation of sex according to the Buikstra and Ubelaker standards (1994). Multiple elements of mandibular morphometrics have also been observed to differ in relation to sex in some populations (e.g. Loth and Henneberg 1996; Hu et al. 2006; Franklin et al. 2007; Indira et al. 2012; Vinay et al. 2013; de Oliveira Gamba et al. 2014). Investigations in the patterns of palatal rugae have also been conducted with a view to distinguish between male and female (e.g. Bharath et al. 2011; Thabitha et al. 2015). Although this method may have some utility in forensic odontology, its usefulness has not yet been demonstrated in mummified human remains where the connective tissues of the palatal rugae may be preserved but almost certainly altered.

In addition to the above, teeth are remarkable storage units for aDNA and proteins. Analysis of sex-linked DNA sequences amplified from dental pulp have shown utility in the determination of sex (Hanaoka and Minaguchi 1996; Rai and Kaur 2013). For example, in cases of significant deterioration of DNA, sex-linked amelogenin genes, located on the X- and Y- chromosomes, may be useful in the determination of sex (Hanaoka and Minaguchi 1996; Rai and Kaur 2013). A major problem for this method is the fact that it is a nuclear DNA locus and does not have the mitochondrial advantage of high copy numbers per cell (Chahal et al. 2007). Thus, it is more likely to suffer from taphonomic influences. X- and Y-chromatins can also be identified through immunocytochemical staining of cells from the dental pulp, depending on the time since death and the diagenesis of the pulp tissues (Duffy 1989; Galdames et al. 2010; Muñoz et al. 2012).

The numerous methods available for the estimation of sex in dental remains reinforces the importance of dental anthropology in the study of human remains. Since teeth are often the best preserved tissue, many studies have been conducted to identify reliable methods for sex estimation using teeth. From these studies, several promising methods have shown potential for future use and, as a result, dental sex estimation may be used in the identification of persons in modern forensic cases or in bioarchaeological contexts. Sex estimation in bioarchaeology is of extreme importance as it contributes to the individuation of unknown remains, the reconstruction of paleodemographics and family structure, and the identification of sex-specific dietary differences, occupations, socio-economic statuses and burial treatments. It also helps in the construction of differential diagnoses for paleopathology as some conditions are more or less prevalent according to sex. Although some methods of dental sex estimation require further research and refinement, they still represent an important contribution to forensics, bioarchaeology, and paleopathology. With the ongoing advances and movement toward affordability in biomolecular methods and the significance of teeth as units of storage for uncontaminated aDNA, the importance of dental sex estimation will likely continue to increase with time.

2.1.11 Dental Age Estimation

Just as sex estimation is crucial to the study of human remains, the same can be said for age estimation. Like sex estimates, age estimates are invaluable for the identification or individuation of unknown persons, the analysis of paleodemography and paleoepidemiology, the differential diagnosis of paleopathology, and the reconstruction of life histories.

Life history studies include the estimation of age with respect to biological, environmental, dietary, pathological, cultural, and social changes or events. For example, infant weaning is a life history event that can be tracked relative to chronological age, as determined through dental analysis. The change in diet can be recognized through isotopic, elemental or chemical analyses of dental tissues from teeth with different developmental timing (e.g. Wright and Schwarcz 1998; Dupras and Tocheri 2007; Prowse et al. 2008). Not only can these biomolecular changes be tracked through sequentially developing teeth, they can be identified throughout life in accordance with incrementally growing dental structures found in cementum, enamel, or dentine (e.g. Smith and Tafforeau 2008; Beaumont et al. 2013; Austin et al. 2013). The study of biomolecular changes across incremental dental structures is particularly powerful as the incremental microstructures of enamel and dentine reflect circadian (enamel prism cross-striations and Von Ebner's lines in dentine) and approximate circaseptan (Andresen lines in dentine and striae of Retzius and perikymata in enamel) rhythms and are thus extremely accurate in the estimation of age (Dean et al. 1993a; Dean 2000a; Antoine 2000; Wittwer-Backofen et al. 2004; Antoine et al. 2009; Huffman and Antoine 2010; Gupta et al. 2014). The physiological stress of birth and the related environmental change is also recorded in neonatal lines in the enamel and dentine, providing solid reference points for age estimation and allowing for comparison of pre- and post-parturition biomolecular changes resulting from environmental, biological and dietary changes (e.g. Arora et al 2006; Goodman et al. 2003; Humphrey et al. 2007; Austin et al. 2013). With these histological methods of dental age estimation, isotopic analysis can also be used to identify migration events throughout life (e.g. Schweissing and Grupe 2003; Evans et al. 2006; Prowse et al. 2007). This process can provide information about travel habits, marriage patterns, ethnicity, trade, pilgrimage, exile, and many other topics relating to life history and sociocultural norms. In addition to the analysis of common dietary molecules, elemental analysis across incremental structures (or across sequentially erupting teeth) may reveal exposure to other elements, such as heavy metals, in relation to the individual's age(s) of exposure (e.g. Montgomery 2002; Arora et al. 2014; Martin et al. 2004).

Of course, the analysis of dental incremental structures is not limited in function to the study of biomolecular changes. These methods have proven to be useful in the determination of age-at-death in forensic cases, primate studies and bioarchaeological studies. Determination of age-at-death in bioarchaeological studies is often used in the differential diagnosis of paleopathology as well as the study of paleo-epidemiology and paleodemography (Hoppa and Vaupel 2002; Dewitte 2010; Milner and Boldsen 2012). Dental age estimation methods are extremely important for the development of differential diagnoses in individuals, since many illnesses are more prevalent in, or limited to, certain age groups and some illnesses are strongly correlated with age (Ortner 2003; Hillson 2005; Milner and Boldsen 2012). Consequently, all paleopathological and pathological analyses for humans and non-humans should be conducted in relation to known or estimated age (Hillson 2005).

Linear enamel hypoplasia (LEH) has also been used to identify periods of physiological stress in relation to normal incrementally formed perikymata. It has been argued that the use of LEH for this purpose is flawed unless conducted through microscopy as many hypoplastic enamel defects are not visible to the human eye and dental erosion can further complicate macroscopic analyses (Hillson and Bond 1997). The study of LEH is also challenged by its imprecise aetiology and misunderstandings of the nature of physiological stress (Hillson, personal communication). Furthermore, it has not yet been determined how quickly an aetiological event will be reflected in LEH, nor how long the hypoplasia takes to stop following the event. Moreover, enamel furrows will differ significantly in size based on their location on the crown (Hillson and Bond 1997). Lastly, it is not yet known if there are differences in the size of the LEH or the bodily reactions resulting in hypoplasia among individuals or according to different biological events. Nevertheless, LEH may still be useful in indicating periods of hypoplastic dental growth in the imbricational zone and may be associated with chronological age through the microscopic study of perikymata or striae of Retzius, bearing the aforementioned caveats in mind.

Despite the high accuracy and precision of subadult age estimates produced through dental incremental structure analysis, these methods are not regularly used as they are very time-, resource-, and work-intensive, not to mention destructive in nature. As such, subadult dental age estimation is usually reliant on standards relating chronological age to macroscopic characteristics of dental development and eruption. In addition to providing an easy and accessible method of age estimation in subadults, the macroscopic study of dental development on an evolutionary scale has been revealing in itself, as different primates and early hominins have demonstrated differences in their time to maturation (e.g. Simpson et al. 1990; Tompkins 1996; Dean 2000b). This information is significant in the understanding of reproductive strategy, birth spacing, subadult dependency, family structure and life span (e.g. grandmother hypothesis, Williams 1957), and the evolutionary adaptations responsible for the development of the long period of human life we know as childhood.

Knowledge of these developmental differences across species has also been applied to correct age estimates in paleoanthropological research. For example, early researchers applied subadult dental aging standards derived from modern human and non-human primates to fossil hominins, in an attempt to estimate their age-at-death (Dart 1925; Mann 1975). The type specimen for *Australopithecus africanus*, also known as the 'Taung child', was estimated to be six years of age using a human dental aging standard (Dart 1925). It was later shown that most early hominids have a more ape-like pattern of dental development, apart from *Australopithecus robustus*, who differed significantly from apes and humans (Smith 1986). Smith's (1986) study led to a lengthy dispute regarding the nature of dental development in early hominids. Consequently, Dean et al. (1993b) estimated the age of one of the disputed *Australopithecus robustus* specimens through the histological analysis of enamel prism cross-striations. As previously mentioned, these cross-striations are known for their circadian rhythm of amelogenesis which provides an extremely accurate and precise indicator of age in subadult remains (Antoine 2000; Antoine et al. 2009). Dean et al. (1993b) concluded that the cross-striation analysis indicated a pattern of development that most closely resembled the developmental timing of ape dentition. It should be noted that the correlation demonstrated by Dean et al. (1993b) does not necessarily indicate that dental age estimation standards derived from apes should be used for *Australopithecus robustus*. Furthermore, the *Australopithecus robustus* specimen observed represents only a moment within the long developmental schedule of a single individual and growth rates and developmental sequence are known to vary in extant species (cf. Winkler 1996; Godfrey et al. 2005; Braga and Heuze 2007). As such, macroscopic dental aging standards are not an ideal tool for age estimation for individuals from extinct species although they do confer the benefit of being non-destructive in nature and may be useful for relative aging or biological aging, rather than chronological age estimation. Although the destructive processes involved in dental histology are generally avoided for rare and important specimens, it remains the most accurate and specific method of age estimation applicable to extinct hominins (Hillson 2005).

Besides its use in subadult dental age estimation in ancient individuals, dental development has played an important part in the biological study of growth in modern populations (Hillson 1996) and growth studies have recently seen a new surge of interest since it has been shown that conditions of early life may affect health later in life, called the 'developmental origins of health and disease (DOHaD)' (Gluckman et. al. 2007). As previously mentioned, dental age estimation is also extremely important in cases of forensic identification in modern populations. This is particularly true for subadults, as dental development is among the fastest and most accurate indicators for skeletal individuation through macroscopic analysis. Another use for dental aging methods in modern populations is to estimate the age of immigrants, young offenders, and orphans up for adoption, where birth data are suspect or missing (Maber et al. 2006; Cameriere et al. 2012). Similarly, dental age estimation methods may be used by socio-cultural anthropologists to estimate the ages of their study participants when alternative information about birth data are unavailable or unreliable. As a result, subadult dental age estimation plays extremely important, sometimes crucial, roles in the study of past and present humans and our primate relatives.

Just as teeth can be used to estimate age in subadult remains, they can be used for age estimation in older individuals. Of course, beyond the point of dental maturity, the aforementioned methods based on odontogenesis are not applicable. However, there are a number of other methods for estimating age from mature dentition. Perhaps the most popular dental indicator of adult age is dental wear, though there are many alternative methods (See Section 4.3 for more information on age estimation through dental wear). For example, the histological count of the concentric annular lines of Salter in root cementum can reflect the number of years since dental root formation. This enables relatively reliable year-by-year age estimates in biologically mature individuals (e.g. Stott et al. 1982; Naylor et al. 1985; Condon et al. 1986; Charles et al. 1986; Kvaal and Solheim 1995; Kagerer and Grupe 2001; Hillson and Antoine 2003; Wittwer-Backofen et al. 2004; Aggarwal et al. 2008; Avadhani et al. 2009; Gupta et al. 2014). However, difficulties in differentiating cementum lines or reproducing line counts throughout the root have cast some doubt on this method (Renz and Radlanski 2006; Huffman and Antoine 2010; Le Cabec et al. 2019), particularly as diagenesis can sometimes mimic cementum lines (Stutz 2002). Irregular cementum apposition and calcification, pathology and/or trauma can result in the resorption of areas of the root cementum. As these areas accumulate over time, it results in an increase in root surface roughness. This change in root surface roughness, however, has shown little promise as an independent indicator of age (Solheim and Kvaal 1993; Rosing and Kvaal 1998).

In addition to these root surface changes, the colour of the root surface is known to change at a rate that is positively correlated with chronological age (Solheim 1993; Pudil and Pilin 2000; Lackovic and Wood 2000; Laskarin et al. 2006; Pilin et al. 2007; Devos et al. 2009), perhaps as a result of the cementum apposition or changes in the underlying dentine (Ten Cate et al. 1977; Mincer et al. 1993; Laskarin et al. 2006). However, applicability of this method is limited in archaeological contexts as a result of taphonomic change. Dental enamel has also shown age-related changes in colouration and translucency (Solheim 1998; Odioso and Reno 2001; Devos et al. 2009), however, the colour of dental enamel may be affected by staining, acid erosion, enamel water loss, and absorption of trace elements, complicating attempts to correlate age with tooth shades (Solheim 1998). Tooth colour correlates with changes on a deeper level as blue and red fluorescence is known to increase in dentine with age (Kvaal and Solheim 1989; Solheim 1998).

Following closure of the dental root apex, secondary dentine begins to line the pulp chamber at a fairly stable rate, allowing for the measurement of the secondary dentine or pulp cavity to estimate age (e.g. Drusini et al. 1997; Cameriere et al. 2007; Paewinsky et al. 2005; Someda et al. 2009; Singaraju and Sharada 2009). Peritubular dentine similarly grows inward over time, narrowing the dentine tubules and changing the refractive index of the dentine, resulting in increasing translucency, at a regular rate that can be used to predict age at death (Gustafson 1950; Nalbandian et al. 1960; Bang and Ramm 1970; Solheim 1989, 1998; Lamendin et al. 1992; Prince and Ubelaker 2002; Prince 2004; Singhal et al. 2010). Unfortunately, diagenetic changes in dental tissues recovered from archaeological contexts can reduce the accuracy of all of these methods of age estimation (Lucy et al. 1995; Chandler and Fyfe 1997; Sengupta et al. 1999; Vlcek and Mrklas 1975).

In addition to these age-related physiological changes in dentition, periodontal recession (e.g. Loe et al. 1992; Grossi et al. 1995), number of teeth (e.g. Hoefig 1964; Endris 1979; Lindemaier et al. 1989; Rosing and Kvaal 1998), and dental wear (see Sections 4.2 and 4.3 for a detailed description of dental wear and its related standards for age estimation) have all shown correlations with chronological age. Biomolecular analyses of dental tissues have also shown promise for the estimation of age-at-death. For example, amino acid racemization (changes from L- to D-aspartic acid) in dentine has been proven to be highly correlated with chronological age (Ohtani and Yamamoto 1991; Yekkala et al. 2006; Meissner and Ritz-Timme 2010). However, use of this method is restricted to living individuals and some forensic cases as changes in temperature and pH affect the rate of racemization (Masters 1986; Ohtani 1995; Torres et al. 2014). Advanced glycation endproducts (AGE) have also been investigated in relation to age estimation and have demonstrated very accurate age estimates from pentosidine or furosine levels in dentine from non-diabetic individuals (e.g. Greis et al. 2018; Valenzuela et al. 2018). However, these studies are vulnerable to biases resulting from heat exposure or chemical changes related to caries or diabetes mellitus (Greis et al. 2018). DNA analyses are also vulnerable to taphonomic variables such as heat. Nevertheless, aDNA analytical methods continue to improve. Recently, some attention has turned to the relationship between chronological age and the shortening of telomeres recovered from dental pulp DNA (e.g. Takasaki et al. 2003; Marquez-Ruiz et al. 2018). Although telomere shortening was shown to correlate with chronological age, Marquez-Ruiz and colleagues (2018) noted inaccuracy in age estimates based on this biomolecular indicator and recommended its use only in a complementary role in a multifactorial age estimation method. Lastly, DNA methylation and epigenetic analysis of DNA from dental tissues are also beginning to show potential as indicators of age (Bekaert et al. 2015; Giuliani et al. 2016; Hanghøj and Orlando 2018). Many of the aforementioned biomolecular methods are still in their infancy and require further research to determine their true value with regard to age estimation, particularly in dental specimens recovered from archaeological contexts.

Like subadult dental age estimation, adult dental age estimation methods are essential for the placement of life events within a chronological context. For example, dental aging methods can be used to determine the minimum and maximum age-at-death for soldiers interred at a military cemetery. This would give an idea of the socially acceptable ages for people to serve in the military. A study like this may give an indication of the target population’s ideas about childhood and adulthood, since the definition of childhood varies greatly among populations and it is not necessarily dependent on biological factors (Helman 2000). Furthermore, the incremental apposition of cementum may even allow for the identification of migration, dietary change, or elemental exposure during adulthood (e.g. Martin et al. 2004; Martin et al. 2007; Martin et al. 2010; Lefever 2010; Dean et al. 2018).

On a larger scale, dental age estimation is useful for reconstructing population paleodemographics and conducting paleoepidemiologic analyses involving the mapping of disease transmission and the identification of at-risk demographic groups within the population. Keeping the osteological paradox (Wood et al. 1992) in mind, paleopathologists must also calibrate their pathological findings against their population age distribution when comparing their data to pathological findings at another site (Waldron 2009). Comparisons of pathological distribution in different populations may also give indications about possible aetiologies for the disease that are biologically or culturally linked to chronological age. As such, the ongoing advancement of dental age estimation methods are of the utmost importance.

2.1.12 Conclusion

Since dental tissues resist taphonomic processes more than bone, teeth have contributed immensely to our current understanding of the evolutionary history of hominins, hominoids, primates, mammals, and all other living things with teeth. On a narrower scale, dental anthropology has made a significant impact on a variety of disciplines including (but not limited to): human and primate biology, evolutionary biology, biological anthropology, anatomy, comparative anatomy, physiology, pathology, paleopathology, paleoepidemiology, odontology, dentistry, orthodontics, forensics, law, archaeology, and socio-cultural anthropology. Dental anthropology embraces techniques spanning from macroscopic to biomolecular analysis and provides invaluable information about topics such as ancestry, migration, familial structure, disease, development, senescence, diet, evolution, and social structure in past and present populations.

Dental age estimation methods are perhaps the most significant contribution that dental anthropology has made due to its broad applicability and usefulness in living and deceased individuals. Age estimation is one of the most important elements considered in the forensic identification of unknown persons, the creation of osteobiographies, the differential diagnosis of (paleo)pathology, and the study of paleodemography or paleoepidemiology. Dental age estimation methods are often used for these purposes due to their relative accuracy. Also, since teeth are more resistant to taphonomic change than soft tissues or bone, in many cases researchers must rely on the dentition for estimates of age. Given the supreme importance of dental age estimation, it is incumbent on dental anthropologists to regularly evaluate and improve the existing methods while investigating and integrating new techniques and technologies.

2.2 The Significance of Dental Age Estimation Methods: A Preliminary Meta-analysis

2.2.1 Introduction

Age estimates determined from dental analysis are widely considered to be the best macroscopic means for determining the age of a skeleton (White et al. 2011; Senn and Weems 2013; Schmidt 2016). The accuracy and reliability of dental age estimation methods have been tested and compared to other skeletal age indicators by several researchers, however, none have included both dental wear methods and dental development methods in the same study. In an effort to fill this gap, and to explore the value of dental age estimation standards, a preliminary meta-analysis was conducted to compare selected tests of the accuracy of skeletal and dental age estimation standards. Although this meta-analysis is used here to provide an idea of the relative accuracy and bias of popular macroscopic age estimation methods, it is not comprehensive and a more thorough study comparing these methods is recommended.

2.2.2 Materials and Methods

This exploratory meta-analysis focused on a selection of popular macroscopic dental and skeletal age estimation methods for subadults and adults. More specifically, it is a comparison of tests of the accuracy and bias of: 1) subadult age estimates based on dental development, and 2) adult age estimates based on the pubic symphysis and auricular surface morphology, suture fusion, and dental wear. Articles were primarily selected for inclusion based on their experimental and statistical methods. All of the included studies examined known age individuals from populations not used for the development of the standard being tested, and the level of accuracy was determined through the comparison of the estimated age with the known age. In all of the articles, results were presented in the form of inaccuracy, bias, correlation with known age using Pearson's r, or a combination of more than one of these statistical forms. These parameters for sample selection ensured that the data were methodologically and statistically comparable. Finally, the articles selected for inclusion in this meta-analysis were limited to studies that compared accuracy tests for multiple methods of age estimation. This selection criterion was used to minimize inter-observer error and systematic bias due to minor differences in methodology. From this defined pool of studies, three articles were chosen for inclusion in this meta-analysis due to their tests of the multiple methods of interest with limited overlap. This meta-analysis was limited in size due to the vast quantity of published age estimation accuracy studies and the limited scope of this dissertation, within which this meta-analysis is tangential to the main arguments. Given that the studies selected for inclusion in this meta-analysis represent a fraction of the published tests of skeletal and dental age estimation standards, this meta-analysis should be considered a preliminary assessment of the relative reliability of the selected age estimation methods. As such, a much larger meta-analysis is recommended for a broader understanding of the relative reliability of age estimation methods.

Returning to sample selection, data from the selected studies included in this meta-analysis were restricted to tests of age estimation methods used to recognize a range of ages. As such, tests of single age threshold indicators (e.g. accuracy tests based on an epiphyseal point of fusion associated with a single age of completion) were excluded from this meta-analysis. In an effort to minimize bias due to small sample size, data based on studies of less than 50 individuals were also excluded from this analysis.

The first article from which data were extracted is Aiello and Molleson's (1993) *Are Microscopic Aging Techniques more Accurate than Macroscopic Aging Techniques?* This article compares rates of inaccuracy for histological aging methods to methods of age estimation through macroscopic analyses of the pubic symphysis, which is said to be the most reliable macroscopic skeletal aging indicator particularly for individuals under the age of 40 (Aiello and Molleson 1993). In this study, cortical histological aging techniques were compared to the Todd/Brooks (T/B) (Todd 1920, 1921; Brooks 1955), McKern, Stewart and Gilbert (M/S/G) (McKern and Stewart 1957; Gilbert and McKern 1973), and Acsádi and Nemeskéri (A/N) (1970) techniques for aging the pubic symphysis in 387 individuals with known sex and age from the crypt of Christ Church, Spitalfields, London.

Although histological methods are not included in the following meta-analysis, Aiello and Molleson (1993) found that cortical histological methods were significantly more effective than deep tissue histological methods, and histological aging techniques provided similar levels of inaccuracy as aging techniques based on the morphology of the pubic symphysis. Although the A/N technique based on pubic morphology provided the most accurate results among the tested methods, it was noted that the accuracy of the A/N method may be a result of the demographic similarity between the Spitalfields sample and the British reference sample on which the A/N technique was developed (Aiello and Molleson 1993).

The second article selected for inclusion in the meta-analysis is Lovejoy et al.'s (1985) *Multifactorial Determination of Skeletal Age at Death: A Method and Blind Tests of its Accuracy*. This article tested several single-indicator macroscopic aging methods and compared them to multifactorial summary methods with regard to their rates of inaccuracy and bias. The independent indicators included in Lovejoy et al.'s (1985) study are: the auricular surface, the pubic symphyseal surface, radiographs of the proximal femur, suture closure and dental wear. The multifactorial summary method incorporated all of these independent factors to develop an age estimate. Lovejoy et al. (1985) also included a "clinical" method in which a selection of single indicator aging methods were added to the multifactorial summary method in an effort to improve accuracy. Two tests were conducted on known age individuals from the Hamann-Todd Skeletal Collection; Test I used the original techniques, and Test II used revised versions of the original techniques for all methods except the dental wear and proximal femur aging techniques.

The results of this study showed that the multifactorial "clinical" and summary methods produced age estimates that most strongly correlated with the known ages. The auricular surface standards and the dental wear standards followed closely with low rates of inaccuracy and bias. Moreover, the Miles (1962) dental wear standard showed significantly lower bias than all single-indicator and multifactorial methods in test I. On a subsequent test of the methods on the Libben archaeological skeletal population, the dental wear method also showed the highest correlation with the multifactorial summary age estimates, which have been shown to have the highest rates of accuracy and lowest rates of bias (Lovejoy et al. 1985). These results prompted Lovejoy et al. (1985) to declare the Miles (1962) dental wear aging method to be the most accurate and unbiased single macroscopic indicator of age-at-death tested in the archaeological population. Despite the inherent value of the data gleaned from the "clinical" method, these data were not included in the meta-analysis because there was no clear indication of the specific methods used for age estimation.

The third article considered in the following meta-analysis is Smith's (2005) *A Test of Ubelaker's Method of Estimating Subadult Age from the Dentition*. This thesis tested the accuracy of the Schour and Massler (1944) and Ubelaker (1989) methods for determining subadult age through the analysis of dental development on modern individuals of known age and sex from European ancestry. It was found that both methods had low rates of inaccuracy and bias, however, the Schour and Massler (1944) confidence intervals were found to be too narrow and some later developmental stages showed significant differences between the sexes (Smith 2005). Since the standards were already widely distributed and used prior to Smith's (2005) study, it was recommended that instead of revising the development charts, wider confidence intervals could be used along with the original charts for the affected developmental stages. However, it was also noted that the new confidence intervals suggested by Smith may not be applicable to populations from different time periods or genetic backgrounds and, consequently, should be used with caution (Smith 2005).

2.2.3 Results

Table 2.1. Inaccuracy and Bias in Skeletal and Dental Age Estimation Methods

Indicator	N	Correlation between Known and Estimated Ages (Pearson's r)	Inaccuracy (Mean Difference between Known and Estimated Ages)	Bias
A/N P.S.	64		10.8	
T/B P.S.	64		18.2	
M/S/G P.S.	65		22.5	
T/B/MS. P.S.*	96	0.57	10.5	-7.5
Rev. T. P.S.**	109	0.78	6.5	-0.4
Aur. S.*	98	0.72	7.8	-3.7
Rev. Aur. S.**	108	0.71	7.3	-0.5
Suture*	118	0.65	10.1	-5.9
Rev. Suture**	117	0.53	9.9	-4.8
Summ. Age*	130	0.83	7.5	-5.4
Rev. Summ. Age**	131	0.80	5.2	1.7
Miles Dent. Wear*	114	0.70	9.5	-3.6
Miles Dent. Wear**	117	0.71	7.9	1.0
U. Dent. Dev.	419		1.05	-0.71
S/M. Dent. Dev.	419		1.03	-0.66

Legend:

*****Lovejoy et al. (1985) Test I ****** Lovejoy et al. (1985) Test II

A/N – Acsádi and Nemeskéri (1970); **T/B** – Todd (1920, 1921)/Brooks (1955); **M/S/G** – McKern & Stewart (1957)/Gilbert & McKern (1973); **T/B/MS** – Todd (1920, 1921)/Brooks (1955)/McKern & Stewart (1957); **U.** – Ubelaker (1978, 1989); **S/M** – Schour/Massler (1944); **P.S.** – Pubic Symphysis; **Aur. S.** – Auricular Surface; **Rev.** – Revised; **Summ.** – Summary; **Dent.** – Dental; **Dev.** - Development

Through this preliminary meta-analysis, it is clear that the most accurate methods for aging individuals are those based on the assessment of dental development. They also rank among the lowest for bias. Smith's (2005) study showed that Schour and Massler's (S/M. Dent. Dev.) (1944) standards performed slightly better than Ubelaker's (U. Dent. Dev.) (1978; 1989) standards, which is surprising given that the Ubelaker standards were derived from the Schour and Massler standards. Unfortunately, these age estimation methods based on dental development could not be compared to a skeletal standard that assesses age over the span of the subadult age range since most macroscopic subadult aging methods are based on single age threshold indicators. Nevertheless, the results of this meta-analysis indicate that the tested standards for age estimation based on dental development far outperform skeletal and dental age estimation standards for adults.

Aiello and Molleson's (1993) tests for aging of the pubic symphysis [A/N – Acsádi and Nemeskéri (1970), T/B – Todd (1920, 1921) and Brooks (1955), M/S/G – McKern and Stewart (1957) and Gilbert and McKern (1973)] revealed the highest rates of inaccuracy in the meta-analysis. The remaining data were derived from two separate tests by Lovejoy et al. (1985) on two independent skeletal samples from the Hamann-Todd Collection. For this meta-analysis, results for the Miles (1962) dental wear method were compared to results for other methods using the median of the test results for each skeletal indicator (i.e. pubic symphysis, auricular surface, cranial sutures, multifactorial summary), due to the small sample sizes. Comparisons of bias were made using the absolute values of the bias results in order to emphasize the distance from the real age, rather than the directionality.

It is evident from the Lovejoy et al. (1985) tests that age estimates from multifactorial summary aging methods (Summ. Age; Rev. Summ. Age) have the strongest correlation with the known age of adult individuals. Results also showed significant differences between tests I and II. In the first test, the Miles (1962) dental wear method had the median rate of inaccuracy among all Test I methods, surpassed only by the summary age method and the auricular surface method. The Miles (1962) dental wear method did, however, present the lowest absolute rate of bias among all of the methods in Test I. In Test II, although the dental wear method showed improvement in accuracy and bias, its accuracy fell below the median of the test results for all skeletal and multifactorial indicators and its rate of bias rose to the median. In this test, dental wear was also tied with the revised auricular surface method for the second weakest correlation between known and estimated ages.

2.2.4 Discussion

The pubic symphyseal age estimation standards tested by Aiello and Molleson (1993) presented the highest rates of inaccuracy in the meta-analysis. These results highly conflict with Aiello and Molleson's claims that analyses of pubic symphyseal morphology provide the most accurate indicator of age in skeletal samples for individuals less than 40 years of age (Aiello and Molleson 1993). Through comparative study, it becomes apparent that almost all of the adult age estimation methods tested by Lovejoy et al. (1985) present lower rates of inaccuracy than those tested by Aiello and Molleson (1993). While questions of inter-observer bias may arise as a result of this discrepancy, the original Todd pubic symphyseal age estimation standards tested by Lovejoy et al. (1985) also presented relatively high levels of inaccuracy in comparison with the other methods tested. Nevertheless, the mean difference between known and estimated ages presented by Aiello and Molleson (1993) for the Todd-Brooks pubic symphyseal method remains almost 8 years higher than Lovejoy et al.'s (1985) test of the similar Todd method. These data indicate that there may be a significant inter-observer error between the Aiello and Molleson (1993) and Lovejoy et al. (1985) studies, though this difference may be a result of differences between the Todd and Todd-Brooks methods tested.

In this study, the Miles (1962) method for age estimation based on dental wear followed closely behind the multifactorial summary age estimation method and the auricular surface indicator with relatively low rates of inaccuracy. Furthermore, in Lovejoy et al.'s (1985) test I, this dental wear method presented a significantly lower rate of bias than any of the age estimation methods. Despite these statistics, Lovejoy et al. (1985) suspected that mixed ancestries, nationalities and individual histories of the Hamann-Todd collection, the lack of subadults for scaling reference, the differences in dental health and tooth loss, and the lower rates of tooth wear in the modern skeletons may have posed an inordinate challenge for dental wear analysis (Lovejoy et al. 1985). Consequently, they conducted another test on an archaeological population from the Libben site in Ohio. Although these results could not be included in the meta-analysis due to differences in experimental and statistical methods, it was apparent that the accuracy of the Miles (1962) method may have improved greatly in an archaeological context, as it had the highest correlation with the summary method, which in the previous tests had proven to be the most accurate method. It can, therefore, be suggested that the Miles (1962) dental wear method for estimating age in adult human skeletal remains is among the most accurate macroscopic single indicator methods for the analysis of the Libben archaeological population. Consequently, it may be hypothesized that the Miles (1962) dental wear aging method may be particularly well-suited for skeletal age estimation in other archaeological populations, although this would require further testing.

Unfortunately, there are currently no tests of the accuracy and bias of the Brothwell (1963a) standard for age estimation through dental wear based on a known age population. Nevertheless, this atlas-style method is believed to be less accurate and more biased than the Miles method based on the subjectivity of its atlas style and its generalized use despite being developed, and intended for use, on an ancient British population. Ubelaker has discouraged the universal use of the Brothwell standard, stating that it is specific to the diet and culture of the reference population (Rose and Ungar 1998) – the details of which were never published. The Brothwell (1963a) method is also less specific than the Miles (1962) method, due to the relatively large age ranges provided in the atlas-style standard. Despite these facts, the Brothwell (1963a) method is the most commonly used standard for age estimation based on dental wear due to its user-friendly nature. Encouragingly, this comparison of the attributes of these dental wear age estimation standards suggests that a middle ground may be found in a new method that is more user-friendly than the Miles (1962) method and more accurate and specific than the Brothwell (1963a) method.

The age estimation methods that showed the lowest rates of inaccuracy and bias in the meta-analysis overall were those based on dental development in subadults. Although these methods seem to present little room for improvement, it may be possible to improve accuracy by developing dental age estimation standards that are sex- and region-specific, since: 1) epigenetic factors may impact the timing and sequence of dental development and, 2) there are known differences in dental development between the sexes in later dental stages (Smith 2005). These standards would need to be developed using a sample of known age individuals since sex cannot be determined in archaeological subadult specimens. The resulting standards would give two possible age ranges depending on whether the subadult was male or female. Aside from improving the accuracy of the age ranges, sex-specific age estimations may become very useful if there are further improvements to sex determination methods through the analysis of ancient DNA (Smith 2005). Regarding dental developmental age estimation techniques, it is interesting to note that Schour and Massler's

(1944) original standards provided slightly lower rates of inaccuracy and bias than the revised version of this dental development standard by Ubelaker (1978, 1989, 1999). This may support the hypothesis that the accuracy of these universally-applied age estimation standards may differ depending on the similarity between the reference population and the population being analyzed. Smith (2005) noted that evidence has been found for differences in the rates of tooth eruption dependent on socioeconomic conditions, nutritional status and ancestral groups. Consequently, the results of this meta-analysis may lend further credence to the hypothesis that dental development may differ slightly in accordance with epigenetics. This conclusion may support the development of sex- and region-specific age estimation standards based on dental development instead of making new attempts to improve universal standards. Similarly, the dental wear age estimation methods would also benefit from revision, especially with regards to the development of time-, sex-, and region-specific standards since dental attrition is largely dependent on the food types and preparation methods available for use by a population.

2.2.5 Conclusion

This preliminary meta-analysis demonstrates the great value of dental age estimation techniques and their reliability in comparison with other macroscopic age estimation techniques. Subadult age estimation through the analysis of dental development was shown to have significantly lower rates of inaccuracy than all of the adult age estimation techniques in this meta-analysis. They also had among the lowest absolute rates of bias. The Miles (1962) dental wear aging method rested at, or slightly below, the median rate for accuracy among the adult aging methods in this study, and its rate of bias varied from the lowest rate to the median rate within the same group of results. Nevertheless, it was determined that dental wear may actually be the best single adult age indicator for archaeological skeletal populations. This conclusion was reached as a result of Miles' (1962) age estimates having the highest correlation with the summary age estimates in Lovejoy et al.'s (1985) analysis of the Libben archaeological population. Unfortunately, there were no suitable known-age accuracy tests for the atlas-style Brothwell (1963a) standard for age estimation through dental wear. Nevertheless, a comparison of the characteristics of the Miles (1962) and Brothwell (1963a) standards indicates that the

Brothwell (1963a) method is less accurate, less specific and more biased than the Miles (1962) method. Despite this, the Brothwell (1963a) method is the most commonly used standard for age estimation based on dental wear due to its user-friendly nature. As such, the creation of a new method that is more user-friendly than the Miles (1962) method and more accurate and specific than the Brothwell (1963a) method is recommended. Although it still remains favourable to use multifactorial summary age estimation techniques in adult archaeological skeletons when possible, the analysis of dental wear will remain important due to its inclusion in these multifactorial methods and the relatively high survivorship of dental tissues compared to bone as a result of dental resistance to taphonomic processes.

Since the value of dental age estimation standards has been clearly demonstrated through this study and little work has been done to revise these methods, it is suggested that regional, sex-specific standards are developed and tested in an attempt to further improve the accuracy of these important macroscopic single indicator age estimation methods. With regard to the future testing of age estimation techniques, it is also recommended that the presentation of results be standardized in order to facilitate further cross-comparisons and meta-analyses. An extension of this meta-analysis is also recommended for a broader comparison of the accuracy and bias of macroscopic skeletal and dental age estimation methods and to verify these preliminary results.

Chapter 3

3 Creating an Improved Subadult Age Estimation Standard Based on Dental Development

3.1 Research Objectives and Null Hypotheses

Given the paramount importance of dental age estimation, it is surprising that macroscopic age estimation methods based on dental development have not been tested on more populations. Perhaps this is a result of the relatively small rates of inaccuracy and bias reported in the published tests of these methods; however, given that age estimates are not 100% accurate, there may still be room for improvement. To investigate this possibility, in reflection of the broader scope of this project, the original null hypotheses must be revisited with a focus on dental development for this section. Thus, the null hypothesis for this part of the dissertation is (H_o): “Current dental age estimation standards based on dental development cannot be improved”.

In an effort to test this null hypothesis, research was conducted to investigate the methods used to create the existing age estimation standards based on dental development, enabling the identification of areas of weakness that may be contributing to inaccuracy and bias in subadult dental age estimates. These weaknesses are addressed in a thought experiment and feasibility study in which it is demonstrated that it is possible to ethically create new sex- and region-specific subadult dental age estimation standards using more rigorous methods. Time-specificity in dental developmental age estimation standards would require a large scale histological study of dental microstructures to determine if dental developmental timing has changed over time. Since this type of study would require a significant amoung of time and may face many practical challenges (e.g. equipment accessibility/affordability, destructive sampling permissions), it has not been included in this feasibility study.

This feasibility study is composed of the preparations taken to organize a research project originally meant to be carried out by the author, but unforeseen circumstances prevented the planned data collection. As a result, details within the research plan are specific to the permissions and resources available in Egypt and the ethical considerations required by the

University of Western Ontario. Of course, this plan may be adapted for use in other regions. It is still hoped that the originally proposed project will be completed in Egypt in the future.

3.2 Literature Review: The biology of dental development

3.2.1 The Biology of Dental Development

Development of the human oral cavity commences at approximately 4 weeks gestational age in the formation of a lacuna lined with epithelium over an ectomesenchymal layer. The primary epithelial band is formed around a week later when the epithelial layer thickens along the maxillary and mandibular processes in the shape of horseshoes (Osborn and Ten Cate 1983; Ten Cate 1998; Hillson 2014). Soon thereafter, the dental lamina is formed as the primary epithelial band grows into the underlying ectomesenchyme. This dental lamina persists until after the permanent teeth are fully erupted, at which point the dental lamina breaks down and regresses (Buchtova et al. 2012).

Tooth germs are created within the dental lamina as the epithelium thickens at intervals, proceeding from the midline to the distal ends of the arches. Creation of these tooth germs is the ‘bud stage’, and the first step in the development of the deciduous dentition through the primary dental lamina. Vestibular lamina for eventual permanent tooth development are formed alongside the dental lamina for deciduous teeth. Tooth germs for the permanent molars, however, are created through the same thickening of the primary dental lamina at 14 weeks gestational age. The permanent molars also have vestibular lamina, but they are absorbed. These differences in the formation of the permanent molars indicate that they are actually a part of the primary dentition, but that they simply develop later in life (Hillson 2014).

Each tooth germ transitions into the ‘cap stage’ when an indentation alters its surface. Upon growth of the edges of this ‘cap’, development of an apical point or multiple cusp apices, and deepening of the indentation, the tooth germ enters the ‘bell stage’. In the ‘bell stage’, the tooth germ begins to resemble teeth as we know them. The tooth germ develops a dental, or enamel, organ as the apical cells on the internal surface stop dividing, layer by layer from the apex to the edge, and the enamel epithelium folds in on itself, creating the tooth germ's bell shape. Ectomesenchyme fills the bell-shaped tooth germ, becoming the dental papilla, and surrounds the tooth germ, becoming the dental follicle (Hillson 2014).

Later in the 'bell stage', the enamel epithelial cells are differentiated into ameloblasts, which trigger the differentiation of the superficial cells of the dental papilla into odontoblasts. The odontoblasts then secrete the first layer of dentine matrix, which triggers the ameloblasts to begin amelogenesis (Hillson 2014).

For simplicity, the following steps of odontogenesis will be described with regard to the formation of the hard tissues of a single cusped tooth (e.g. the canine). The formation of enamel within the tooth germ begins with a small group of ameloblast cells in the internal enamel epithelium at the superior point of the conical layer, which is the foundation for the formation of the cusp tip. These ameloblasts secrete proteins that contribute to the formation of the enamel matrix. Successively, more ameloblasts are differentiated and begin protein secretion in subjacent concentric rings following the structure of the inner enamel epithelium toward the cervical loops. In teeth with multiple cusps, this process begins at the tip of each cusp and continues on the occlusal surface until the cusps are joined (Hillson 2014).

Apart from areas of more complex structures of gnarled enamel, enamel prisms grow from the dentino-enamel junction (DEJ) toward the eventual coronal surface. Within each prism, daily incremental secretion is recorded in enamel prism cross-striations. Preliminary crystal formation within the enamel matrix occurs shortly after matrix secretion (Fincham et al. 1999). Brown striae of Retzius are formed at 6-12 day intervals, dividing groups of ameloblasts into bands. These bands of ameloblasts cease amelogenesis at the same time resulting in the creation of perikymata, the microscopic furrows on the enamel surface at the ends of the striae of Retzius. Given that cuspal enamel is gnarled and not linear toward the enamel surface, perikymata do not appear at the occlusal point of the cusp (Hillson 2014). Following completion of the secretory phase of amelogenesis, the enamel matrix undergoes maturation in which the organic tissues are further resorbed and mineralization is completed (Fincham et al. 1999). When amelogenesis reaches the cervical loop, the enamel organ develops a tapering tube-like extension known as Hertwig's sheath, which acts as a template for root shape (Hillson 1996, 2014).

As previously mentioned, the differentiation of enamel epithelial cells into ameloblasts triggers the differentiation of dental papilla cells lining the newly formed ameloblasts into odontoblasts. These odontoblasts immediately begin secreting predentine (unmineralized dentine matrix) which is later mineralized, forming true dentine. The first layer of dentine does not have dentine tubules and is known as mantle dentine. Following the secretion of the mantle dentine, Tomes' fibers develop in odontoblasts and anchor themselves in the mantle dentine. As the odontoblasts continue to form predentine, the Tomes' fibers grow, providing a structure around which dentinal tubules are formed. Odontoblasts lay down dentine matrix while moving toward the eventual pulp chamber border where they come to rest (Hillson 2014).

Although the specifics of radicular development are controversial and the development of dental roots varies according to tooth type and area within the tooth, the following is a basic summary of the developmental process according to Li and colleagues (2017). In the development of root dentine, Hertwig's sheath sends induction signals to differentiate odontoblasts from the ectomesenchymal cells lining the sheath. These newly formed radicular odontoblasts form a layer of radicular mantle dentine before again forming Tomes' fibers and secreting tubule-containing circumpulpal dentine (Li et al. 2017). As Hertwig's sheath extends apically, it becomes perforated through localized apoptosis or an epithelial-to-mesenchyme process. The latter may result in the formation of cementoblasts and the former allows the apical mantle dentine to interact with the dental follicle, which also induces the differentiation and migration of cementoblasts and fibroblasts to the root surface. Fibroblasts then secrete collagen fibers, extending from the root surface to the surrounding bone wherein these fibers thicken and become organized to form periodontal ligaments. The cementoblasts secrete cementum surrounding these fibers. These cementoblasts remain embedded in the matrix at the apical region of the root, forming cellular cementum, while the remaining cementum becomes acellular (Li et al. 2017).

Generally, dental development occurs at around the same time in antimeres, and mandibular teeth develop slightly earlier than maxillary teeth. Although some teeth take longer than others to develop, dental eruption sequence is relatively predictable. Deciduous teeth commonly emerge in the following order: I1, I2, M1, C, M2 (Hillson 2014; Liversidge 2016). Later, the permanent mandibular teeth generally emerge as follows: M_1, I_1, I_2, C, P_1, P_2, M_2, M_3. Meanwhile, permanent maxillary teeth usually emerge in a slightly different order: M^1, I^1, I^2, P^1, C, P^2, M^2, M^3 (Smith and Garn 1987). Developmental timing is also fairly predictable but it does vary in response to a number of factors.

3.2.2 Factors affecting dental developmental timing

Although dental development is normally characterized by specific developmental sequences and timing, variants are far from rare. In fact, there are two sequential variants that occur just as often as the traditionally accepted developmental sequence (Smith and Garn 1987). These variants are the eruption of I_1 (often in the mandible) prior to the eruption of M_1, and the eruption of the mandibular canine after the eruption of P_1 (Smith and Garn 1987). Although these developmental sequential variants are the most common, there exist a number of other possible variations from the accepted developmental sequence (Schmidt 2016). Given that a difference in canine eruption sequence has been found to be linked to ancestry (Schmidt 2016), it is likely that these differences in developmental and eruption sequence are related to genetics.

Although the genetics and molecular signaling factors contributing to variation in dental developmental timing are not well understood, it is clear that dental developmental timing is influenced by environmental factors as well as genes. The genetic component of dental developmental timing is illustrated by the amelogenin gene locus on the sex chromosomes and the gene's tendency to differ in sequence depending on their association with X or Y chromosomes. The amplicon sizes on the Y and X chromosomes are respectively 112 and 106 bases (Sullivan et al. 1993). Amplifying this gene for sex determination is a standard procedure in forensic DNA analysis (Butler 2009). Its use in ancient DNA research has been limited because it is a nuclear DNA variant with large amplicon sizes which presents problems in amplification (Hildebrandt 2003). However, recent ancient DNA research using next generation sequencing (NGS) has resulted in the complete amplification of the mtDNA genome from a Roman period bone sample from the Kellis 2 cemetery in Roman Period Egypt, which shows promise for the amplification and study of nuclear DNA (Molto et al. 2017). This would greatly enhance sex determination in subadults as we know there are significant differences in developmental timing noted between the sexes in many populations (e.g. Schour and Massler 1941; Gleiser and Hunt 1955; Garn et al. 1958, 1973; Lewis and Garn 1960; Glister et al. 1964; Hillson 1996, 2014; Ubelaker 1978; Liversidge et al. 1998; Smith 1991a,b, 2005, 2010).

Although differences in dental developmental timing might be attributed to sex-linked hormonal differences, they begin far before puberty and show no evidence of significantly increased differences in dental development during puberty as might be expected (Garn et al. 1958). Moreover, the genetic role in dental developmental timing may also be evident in the earlier development of mandibular dentition than the maxillary dentition (Gaur et al. 2011). As Sperber (2004) noted, different genetic codes are responsible for dental morphogenesis in the upper and lower dentition. Thus, it might be reasonable to assume that the timing of odontogenesis in the upper and lower dentition may also be controlled by separate genes. Further demonstrating the role of genetics in developmental timing are congenital pathologies that are linked to delayed dental development, such as Down syndrome (Diz et al. 2011).

Although sexual hormones may not play a large role in human dental developmental timing, hormones may still have a regulatory role in the process. This is evident in an experimental study in which methyl testosterone injections in a castrated rhesus monkey significantly accelerated canine eruption (Garn et al. 1958). The abnormally low levels of specific hormones resulting from hypopituitarism or hypothyroidism in children also result in delayed dental development (Garn et al. 1965; Edler 1977). Furthermore, children with Type-I diabetes, a disorder characterized by a reduction or lack of insulin hormone, have accelerated dental development in early childhood and decelerated dental development in later years (Adler 1973). Although it is clear that hormones can impact dental developmental timing, more research must be done to ascertain their specific roles.

Environmental factors also have an impact on dental developmental timing. In particular, Selyean stress, resulting from febrile illness or severe malnutrition, is known to interfere with amelogenesis during crown formation. Selyean stress is also known to affect hormonal secretion. Although little is known about the hormones related to dental development, it may be reasonable to assume that some hormones may be repressed during the exhaustion phase of Selye's general adaptation syndrome (Selye 1946), given that the body must conserve energy. Furthermore, it may be a function of environmental factors that dental developmental timing becomes more variable in teeth developed later in life. Dental eruption timing and sequence have also been known to vary in relation to malocclusion, premature loss or extended retention of deciduous teeth, dental ankyloses, or even dental caries (Sierra 1987; Diz et al. 2011).

Multiple factors may contribute to dental developmental timing. It is perhaps for this reason that it continues to be difficult to untangle the relationships between epigenetic factors and the developmental process. Nevertheless, it is clear that dental developmental timing and sequence are variable given its possible vulnerability to genetic, hormonal, and environmental factors. As such, it follows that dental developmental timing differs between some populations (e.g. Garn et al 1973; Owsley and Jantz 1983; Tompkins 1996).

3.3 Literature Review: Subadult Age Estimation through the Macroscopic Assessment of Dental Development and Eruption

The relationship between gingival emergence and chronological age was most famously noted by Saunders (1837) in his publication urging the use of second permanent molar eruption as an age estimation method for the prevention of illegal child labour. The examination of childrens' 'factory molars', as they became known, was used extensively following the Industrial Revolution in Britain as factory inspectors were required to verify that individuals were of legal age to work, often without documentation (Althorp's Act 1833).

Thomas Dwight, the father of forensic anthropology in the United States, was the first to popularize skeletal and dental age estimation methods for forensic identification (Stewart

1979; Ubelaker 2010). Although Dwight (1878) did not provide a dental age estimation standard, he cautioned against the use of third molars for age estimation due to their variable timing, and encouraged the study of large samples to identify possible correlations between dental emergence and chronological age. Following Dwight's lead, several macroscopic studies of dental eruption and emergence were completed (Peirce 1887; Legros and Magitot 1880, 1893; Black 1908; Bean 1914; Spier 1918). After these efforts to demonstrate the association between chronological and dental age, forensic anthropologists Wilder and Wentworth (1918) encouraged further advances in age estimation based on dental eruption. This led to numerous macroscopic, histologic and radiographic studies of subadult dental growth and development (e.g. Brady 1924; Boas 1927; Orban 1928; Cohen 1928; Cattell 1928).

In the late 1920s, William H.G. Logan, an Oral Surgeon, was interested in the repair of cleft palates and attempted to gain a more thorough understanding of dental development in subadults as an aid in the planning of reconstructive surgeries and the assessment of recovery. Logan teamed up with Rudolph Kronfeld to develop a dental age estimation standard based on dental calcification and eruption (Logan and Kronfeld 1933). Their dental age estimation standard was the first to be developed through detailed observations using radiographic and histological methods. The standard showed a significant difference in developmental timing and sequence when compared to the previous standards of Peirce (1887), Legros and Magitot (1893), Black (1908), and Brady (1924). Kronfeld also published a related study of the resorption of deciduous teeth (Kronfeld 1932) prior to the joint publication (Logan and Kronfeld 1933).

Since Logan and Kronfeld's research, innumerable studies have incorporated age estimates based on the observation of dental development. The Logan and Kronfeld (1933) method has also been revised or incorporated into new age estimation standards by Kronfeld (1935), Kronfeld and Schour (1939), Schour and Massler (1940a,b, 1941, 1944, 1958), Lysell et al. (1962), Nomata (1964), Kraus and Jordan (1965), Lunt and Law (1974) and Ubelaker (1978, 1989, 1999). Adaptations of the Logan-Kronfeld (1933) standard, first published after 1991, also include modifications of the calcification and eruption schedules that reflect the findings of Smith (1991a) (Chandra et al. 2004).

Moorrees et al. (1963a,b) created dental developmental timing standards via observation of radiographic data collected through longitudinal studies of modern subadults in Yellow Springs, Ohio (Fels Institute study directed by Dr. Stanley M. Garn) and Boston, Massachusetts (Forsyth Dental Infirmary and Harvard University study directed by Dr. Harold C. Stuart and radiographed by Dr. Arthur B. Lewis). This study is also known as the MFH standard. Data were gathered from the permanent mandibular canines, premolars and molars from healthy middle-class Caucasian subadults in Ohio (the biannual radiographs of 136 boys and 110 girls were selected for this study). Maxillary cheek teeth could not be studied due to overlap in lateral radiographs (Moorrees et al. 1963a,b). Data for the permanent incisors were gathered from the radiographs of subadults in Boston (from a sample of 134 children, the radiographs of 48 males and 51 females were selected for this study due to radiographic quality) (Moorrees et al. 1963a,b). Moorrees et al. (1963a,b) did not discuss the frequency with which radiographs were taken in the Boston study. However, Gleiser and Hunt (1955), who had used the same radiographic data for their study of the permanent first molar, indicated that, in most cases, radiographs were taken every 3 months for the first 18 months of life, and every 6 months between the ages of 18 months and 10 years old. There is no indication that either radiographic study made an effort to collect radiographs in close proximity to the patients' birthdays, nor were the participant recruitment and selection processes discussed beyond stating the age range and ethnic background of participants.

In the Moorees et al. (1963a,b) study, teeth were scored according to stages of development modified by Fanning (1961) from those used by Gleiser and Hunt (1955), Demisch and Wartmann (1956), Garn et al. (1959), and Nolla (1960). For each developmental stage, children were divided by sex and a cumulative percentage frequency was calculated by dividing the number of children that reached or passed each developmental stage by the total number of children studied within the sex-specific group. The mean ages for each developmental stage were then derived and, in units of logarithmic conceptional age, were transformed into chronologic age to create the existing dental age estimation standard (Moorrees et al. 1963a,b). This method for finding correlations between continuous dental development and discontinuous age ranges through the use of cumulative distribution functions is believed to be the best suited for the creation of age estimation standards without checking developmental progress at more specific intervals aligned with date of birth (Smith 1991a; Hillson 1996), though Bayesian statistical methods had yet to be tested.

All existing subadult dental age estimation standards, including the Moorrees et al. (1963a,b) standard, have been based on individuals of known age. However, most of the existing subadult dental age estimation standards fail to include information regarding the methods and statistics used to determine the developmental stages said to be representative of specific age groups. Dental growth is continuous and growth rates are variable within the growth period. Consequently, maximum accuracy cannot be attained through retroactive calculations that reduce an entire year of growth to a single, static dental development stage, as this would equate a child one day away from their birthday to a child 364 days younger than them (Molto, personal communication). Systemic error can be reduced, and accuracy and precision increased, by limiting age attribution to subadults within one month of their birthdate. This will result in systematic error rates of less than 10% and it will reduce bias. The proposed method for the creation of a sex- and region-specific subadult dental age estimation standard is the first to use this method for limiting age attribution to a specific time period around the patient's birthday.

Despite the possibility for methodological improvements, the Moorrees et al. (1963a,b) method consistently produced error rates of half a year when tested for accuracy on individuals of known age (Liversidge 1994; Saunders et al. 1993). Saunders et al. (1993) attribute what they consider to be a relatively small error rate to the comparable developmental schedules of the white North American MFH reference population and the North American test subjects with known British, Irish, and Western European ancestry. Since Liversidge (1994) also tested the MFH method on a British population of known age, similarities in genetic ancestry might also explain the concordant error rates seen in her study. Although Saunders et al. (1993) consider error rates of a half year to be low, it can be argued that this error rate is substantive for age estimation in infants, toddlers and young adults, resulting in relatively unreliable age estimates.

Smith (1991a) statistically reworked the Moorrees et al. (1963a,b) data to create charts for "Mean Age of Attainment of Developmental Stages (Permanent Mandibular Teeth)"

(Smith 1991a: 160) and “Values for Predicting Age from Stages of Permanent Mandibular Tooth Formation” (Smith 1991a: 161). Smith indicated that the charts were limited to mandibular teeth because, as noted, only mandibular teeth were used. In fact, data for maxillary teeth are rare in all dental developmental studies (Smith 1991a) because maxillary cheek teeth are often obscured by overlap in lateral x-rays (Moorrees et al. 1963a,b), a problem that can be avoided through the use of panoramic x-rays.

Smith (1991a) produced separate charts for males and females but recommended the average estimation of male and female estimates when working with subadult human remains of unknown sex. Through testing, it was shown that Smith’s (1991a) standard was more accurate in age prediction than the Anderson et al. (1976) standard when applied to the reference collection on which Anderson et al.’s (1976) method was based. This improvement was attributed to the use of cumulative distribution functions in the creation of the Smith standards (Smith 1991a). As a result, this statistical method has since been recommended for the development of any new age estimation standards (Hillson 1996; Smith 1991a). Although Smith’s (1991a) standard has gained acceptance in the bioarchaeological community, like the MFH method, it still lacks data for maxillary teeth (Smith 1991a) and the time of dental eruption, or alveolar emergence. The inclusion of dental eruption timing in dental age estimation standards is very important to bioarchaeologists conducting macroscopic assessments of human remains without access to radiographic equipment (which includes the majority of bioarchaeologists, especially those in the field).

Two widely accepted age estimation standards have been developed with indicators for the timing of dental eruption. The first standard was developed by Schour and Massler (1940a,b). This standard was actually created prior to the MFH (1963) standard. Although Schour and Massler (1940a,b) give no indication of the origin of the data, Kraus (1959) suggests that the chart may be partly based on observations of partial jaws of 30 chronically-ill subadults (ages 0-15yrs) studied by Logan and Kronfeld (1933) and Kronfeld (1935). Smith (1991a) proposes that Schour and Massler combined their own data with data from Logan and Kronfeld (1933), Kronfeld (1935), and Kronfeld and Schour (1939) and possibly from other older sources since fetal development is included in the

Kronfeld (1935) standard despite Kronfeld never having previously described fetal dentition. Hillson (1996) notes that there is still some confusion and debate over the origin of the reference data but says that academics generally agree that the data were based on a small sample of known-age, terminally ill children. Aside from problems stemming from small sample sizes, ongoing biological stress associated with chronic illness may affect dental developmental timing in deceased subadults, especially if the illnesses were febrile (Garn et al. 1959; Miles 1963; Ubelaker 1987). This is further complicated by the 'Osteological Paradox' (Wood et al. 1992), as most subadults succumbed to acute disease in the past, which would not have significantly affected dental development.

Schour and Massler (1941, 1944) modified their original standard twice in an effort to improve accuracy, and despite the methodological problems and the potential for inaccuracy, the 1944 standard, the only version that included error ranges, was adopted by the American Dental Association for use as a wall-sized chart. It is now found in dental offices internationally (Smith 1991a; Smith 2010). Unfortunately, the calculations used to create the error ranges are still unknown (Smith 2010). Schour and Massler released a final modified version in 1958, which was also published by the American Dental Association, but it was never fully embraced by academics (Smith 2010).

Many years later, Ubelaker (1978, 1989, 1999) revised the Schour and Massler (1944) dental age estimation standard for the analysis of Native American human remains. During this revision, Ubelaker also incorporated data from Robinow et al. (1942), Steggerda and Hill (1942), Meredith (1946), Hurme (1948), Demisch and Wartmann (1956), Dahlberg and Menegaz-Bock (1958), Kraus (1959), Nolla (1960), Moorrees et al. (1963a,b), Glister et al. (1964), Moorrees (1965), Banerjee and Christensen and Kraus (1965), Coughlin and Christensen (1966), Mukherjee (1967), Lunt and Law (1974), Anderson et al. (1976) (Hillson 1996, 2014; Smith 2010; Ubelaker 2018). In addition to revising the standard, Ubelaker also provided different error ranges than the Schour and Massler (1944) standard. The error ranges produced by Ubelaker represent an expression of variability found in the pre-existing literature but he notes that they may be inaccurate by as much as 5 years, especially in older cohorts (Ubelaker 1978, 1989, 1999).

Ubelaker modified Schour and Massler’s (1944) standard because it constantly overestimated age-at-death for Native Americans (Merchant and Ubelaker 1977). Despite indications that there are differences in rates of dental development, eruption and emergence between Native Americans and North Americans of European ancestry (Dahlberg and Menegaz-Bock 1958; Mayhall et al. 1978; Trodden 1982), the Ubelaker (1978, 1989, 1999) standards were quickly embraced by academics for the analysis of all ancestral groups (Smith 2010). In his last version, Ubelaker (1999) stated that his standards are most effectively applied to the analysis of non-white, prehistoric and contemporary subadults.

Although the Schour and Massler (1940a,b, 1941, 1944, 1958) and Ubelaker (1978, 1989, 1999) standards indicate eruption times for both mandibular and maxillary teeth, neither is sex-specific. There are significant differences between the dentition of males and females in particular developmental stages (Schour and Massler 1941; Gleiser and Hunt 1955; Garn et al. 1958, 1973; Lewis and Garn 1960; Glister et al. 1964; Hillson 1996, 2014; Ubelaker 1978; Liversidge et al. 1998; Smith 1991a,b, 2005, 2010), so it is preferable to calculate male and female age estimations separately. Smith (1991a) recommends taking the mean of the male and female values for age determination in subadults of unknown sex, but it may be prudent for academics to also provide both male and female age estimates for subadults. As noted, advances in the determination of sex in subadults from archaeological samples may provide more accurate sex estimates in the future, in which case it may be useful to have the sex-specific age estimates in addition to the age estimates for unknown sex.

A comparative investigation of the rates of inaccuracy and bias in the Schour and Massler (1941) and Ubelaker (1978) standards showed that the original Schour and Massler (1941) standards were more accurate and less biased than Ubelaker’s (1978) standard (Smith 2005, 2010). This may have been a result of the test population (North Americans of European ancestry) more closely resembling the Schour and Massler (1941) reference population. If so, this lends further support for the use of Ubelaker’s standards on specified populations and indicates the potential for improved accuracy through the use of region-specific dental age estimation standards.

Another subadult dental age estimation standard was published by Demirjian et al. (1973). This sex-specific panoramic radiographic analysis was conducted on a large sample of French-Canadian children aged 3 to 17. Despite access to the entire dentition through the panoramic radiographs, this study focused only on 7 left mandibular teeth (M3 was excluded). In this study, one of eight developmental stages was assigned to each tooth according to the amount of root growth, pulp chamber changes, and crown calcification. Inter-observer error associated with the assignment of these scores was estimated to affect up to 10% of tooth scores (Demirjian et al. 1973) and the method has shown relatively low rates of intra- and inter-observer error when compared to other scoring methods for dental development (Hagg and Matsson 1985). Scores for the seven teeth were then summed and the resulting dental maturity score was converted to a chronological age through the use of a table of standards (Demirjian et al. 1973). This table of standards for age estimation was later revised to incorporate a larger sample size and age range, 2.5 to 17 years, and to give two alternative standards based on differing combinations of 4 teeth (Demirjian and Goldstein 1976). These standards have been widely used and tested and have been incorporated into other studies of sex-specific dental development (e.g. Demirjian and Levesque 1980) and the relationship between dental age and physiologic age (e.g. Demirjian et al. 1985). Subsequently, software (CD ROM) incorporating the Demirjian data was developed (Demirjian 1994). All of these standards have been subjected to numerous tests for accuracy (e.g. Chaillet and Demirjian 2004; Liversidge et al. 1999, 2006; Nykanen et al. 1998; Willems et al. 2001), often showing inconsistent results in unrelated populations, further supporting the need for region-specific standards.

In 1974, Gustafson and Koch published a dental developmental age estimation standard based on data gathered from previous publications (i.e. Rose 1909; Cohen 1928; Logan and Kronfeld 1933; Klein et al. 1937; Schour and Massler 1941; Robinow et al. 1942; Kranz 1946; Dahlberg and Maunsbach 1948; Stones et al. 1951; Clements et al. 1953; Godeny 1955; Orban 1957; Tegzes 1959; Nolla 1960; Fanning 1961; Sjoberg 1961; Carr 1962; Moyers 1963; Lysell et al. 1964; Haavikko 1970). In this standard each tooth is assigned one of four developmental scores indicating the commencement of mineralization, the completion of crown calcification, gingival emergence, or the completion of root growth (Gustafson and Koch 1974). The distance between these stages allows for a lower chance of observational error, but it does so while sacrificing specificity. Perhaps as a result of this scoring method in conjunction with the merging of data from different sexes and ethnic backgrounds, gathered using various observational techniques, the age ranges presented by Gustafson and Koch (1974) are fairly accurate but not very specific.

While developing her sex-specific subadult dental age estimation standard for Inuits and North American Indians, Trodden (1982) attempted to improve accuracy and precision through the development of a new scoring system. Trodden's (1982) scoring system is based on a combination of the scoring systems of Nolla (1960), Nevile (1973), Schour and Massler (1941), Fanning (1961), Gleiser and Hunt (1955) and Moorrees et al. (1963a,b). This scoring system is well-defined, presenting scores for stages of crypt formation, calcification, resorption, and eruption as well as scores for unobservable or abnormal teeth. Trodden's (1982) scoring method was tested to ensure low rates of inter- and intra-observer error, however, it relied on relative tooth measurements, which are necessarily subjective as researchers must approximate the final length of the crown and root. The statistical treatment employed by Trodden for estimation of age may have also led to biased age estimates, as demonstrated through relatively late achievement of developmental milestones in the Inuit and North American data when compared to those of Europeans (Smith 1991a,b). This comparison indicated a bias in the standard because stages of dental development in Inuits and North American Indians are actually known to be reached earlier than in whites (Dahlberg and Menegaz-Bock 1958; Mayhall et al. 1978; Smith 1991a,b). This bias was later attributed to the age structure of the reference population used by Trodden (Smith 1991a). It has been demonstrated that the estimated age of crown completion in the first molar (for example) is highly correlated with the age of the youngest child in the reference sample (Smith 1991a). Age distribution within reference samples can, therefore, have a significant impact on the efficacy of a dental age estimation standard when applied to independent populations with a different age distribution pattern (Smith 1991a; Hoppa and Vaupel 2002; Holman et al. 2002). However, this type of statistical bias can be mitigated through the use of Bayesian statistics (See Section 3.4.10).

The most recent atlas connecting dental development to chronological age was designed by AlQahtani (2012; AlQahtani et al. 2010b). This online reference tool was created through a cross-sectional study of dental development and alveolar eruption in dental radiographs from living individuals and known age-at-death skeletal remains. The resulting atlas presents developmental stages for children from 28 weeks *in utero* to 23 years of age. Although developmental data for the ages 2 to 23 years were based on uniform age and sex distribution within the sample, age attribution was not restricted to a specific time frame within the year, creating the potential for significant bias in the data, especially with regards to the youngest age categories.

For the creation of the London Atlas (AlQahtani et al. 2010b; AlQahtani 2012), data were collected from developing teeth from 72 prenatal and 104 postnatal skeletal remains of known age-at-death white individuals (Males: 91, Females: 72, Unknown sex: 13) in the Royal College of Surgeons of England's skeletal collection and the Christ Church Spitalfields Skeletal Collection at the Natural History Museum in London (AlQahtani 2012; AlQahtani et al. 2010a). Additional data for ages 2 to 23 were collected from an archive of dental radiographs taken on living individuals (Males: 264, Females: 264), around half of which were 'White' and half 'Bangladeshi' children living in England (AlQahtani et al. 2010a). Tooth development was scored according to the system of Moorrees, Fanning and Hunt (1963a,b). The median stage for tooth development and eruption for all age categories was then used to construct the atlas (AlQahtani 2012; AlQahtani et al. 2010a).

Using only the right side of the dentition, dental scores were determined "for males, females, and combined sex for each of the following age groups: the seventh, eighth, and ninth month of gestation; birth at midpoint of 2 weeks; the first, second, third, and fourth 3 months of life; and for each chronological year over the age of 1 up to the age of 23 years." (AlQahtani et al. 2010a: 482). This atlas does not include gingival emergence but the author suggests that "[allowances] should be made for gingival eruption when using this atlas in the presence of oral soft tissues" (AlQahtani et al. 2010a: 490). Unfortunately, as no amendment is given, the reader is pointed to median ages of alveolar eruption in relation to clinical/gingival emergence for each tooth type (AlQahtani et al. 2010a). These comparisons are of limited use, since the clinical data regarding gingival emergence were collected from separate populations than the data collected for alveolar eruption and full eruption. Furthermore, the recommended references are for separate unrelated studies of deciduous and permanent eruption and emergence (i.e. Lysell et al. 1962 for deciduous teeth; Haavikko 1970 for permanent teeth).

The London Atlas assumes universal applicability without comparison to a region-specific standard and assumes that the 'White' and Bangladeshi reference population is developmentally diverse enough to account for global variation in dental development. This assumption may be especially questionable as the Bangladeshi participants also lived in London, England and, therefore, may not have been exposed to significantly different environmental factors that may affect dental development. The reliability of the London Atlas is further compromised by its rigidity and reliance on subjective identification of the stage deemed to be closest to the dentition of the individual in question. Nevertheless, this atlas has gained popularity, in part because it is sex-specific and because it is available through online open-access and user-friendly interactive software.

3.4 Feasibility Study for the Ethical Creation of Region-specific Subadult Dental Age Estimation Standards

As previously mentioned, this research plan was originally meant to be carried out by the author, but unforeseen circumstances prevented the planned data collection. As a result, details within the research plan are specific to the permissions and equipment available in Egypt and the ethical considerations required by the University of Western Ontario. It is presented here as a thought experiment and feasibility study for the ethical development of region-specific subadult dental age estimation standards using more rigorous methods. As such, it is written as a hypothetical research proposal, outlining the methods that would be used in the creation of a region-specific and sex-specific macroscopic subadult dental age estimation standard. Although it is designed around details specific to research in Egypt, it may be adapted for use in other regions. It is still hoped that this proposed project will be completed in Egypt in the future.

Data Collection Methods: Procedural Overview

During the participant recruitment process for the proposed project, potential participants would be given letters of information, consent and assent forms, and a participant questionnaire (See Appendix 1) to be completed by the participants and/or their guardians. Following the return of the completed consent and assent forms and the verification of the participants' birthdates, appointments would be made for dental examinations and x-rays of qualifying participants on study dates at the Radwania Scan Radiological Laboratory in Luxor. At this facility, volunteering participants would receive a dental panoramic x-ray and be examined by one of two dentists. Small children awaiting an x-ray will be educationally entertained about teeth and toothbrushing by a research assistant who will introduce the children to the freezing game, which may be used during the x-ray procedure to help the child stay still. Following clinical data collection, panoramic x-rays will be scored to collect additional data on dental development. Following this procedure, patients will be given a copy of their panoramic x-ray and an educational pamphlet on preventative oral health care on which the dentist can include patient notes (Appendix 4). Details regarding participant recruitment, the questionnaire, dental examinations, radiographic examination and radiographic data collection can be found below, followed by a feasibility statement.

Sample Selection

The proposed research would be conducted on volunteering subadult participants from rural public schools on the west bank of Luxor; a location chosen, in part, due to the primary investigator's intimate knowledge of the area, culture, and history, though this study model can be applied elsewhere. This location was also chosen because of its proximity to a laboratory equipped with a panoramic dental x-ray machine, and experienced dentists and radiologists who expressed an interest in participating in this study. Additionally, this population was chosen because it would provide insight into the standard of dental health and hygiene in a population with a low socioeconomic status, which may help to identify dental health inequalities and opportunities for the improvement of dental health care within Egypt. The lower socioeconomic status and traditional diets and lifestyles generally characteristic of this rural population would also more closely approximate environmental factors that may have affected dental developmental timing and sequence in ancient populations, than a city-dwelling population with a higher socioeconomic status. Participation in this study would not be restricted according to socio-economic status or diet, so comparisons would be made between the developmental patterns of participants associated with the lowest and highest socio-economic statuses, as indicated by information provided in the participant questionnaire. Depending on the scope of socio-economic variation in the reference sample, this comparison may allow for the preliminary identification of socio-economic class-related differences in dental development and oral health. For a broader scope of public health research and a deeper understanding of epigenetic effects on the dentition, a follow-up study of dental health and development in Lower Egypt (northern Egypt) would be appropriate for further comparison of dental development and oral health across geographical locations and socio-economic classes.

For this study, 30 Egyptian males and 30 Egyptian females would be accepted to form representative samples for each year of age (from 1 to 17 years old). In total, 1020 voluntary participants would take part in this study. Although a sample of at least 30 individuals per cohort is the gold standard, for this research design, it must be noted that an increased sample size would help to avoid issues of sample size when cohorts are broken down during epidemiological analysis. However, since the primary goal of this study is to develop age estimation standards, samples of 30 individuals per cohort will suffice. Participants accepted for this study would necessarily have birthdates within one month of the available study dates, with preference given to participants with birthdates closer to the study dates, in order to minimize systemic error. Previous studies of dental development have attributed children to an annual age cohort until the day before the transition to the next annual age cohort (typically on their birthday). This contributes to systemic bias and inaccuracy in the resulting standards since children one day away from their birthday are still considered to be the same age as children that are a full 364 days younger. The recording of dental development based on birth month should give a “sufficient number [of stages] to quantify variability, while maintaining reliability [since] too many stages decrease reliability, while too few compromise sensitivity” (Liversidge 2008: 237). Participants’ birthdates would be verified through each participant's school or official documents with the understanding that official documents in Egypt do not always record the actual date of birth.

3.4.1 Participant Recruitment

Participants would be recruited through school meetings and the distribution of 'information, consent and assent' forms, and 'participant questionnaires' at public schools on the west bank of Luxor (See Appendix 1 and Appendix 2) Within these small villages from which participants would be recruited, teachers are very familiar with students' families and they would be asked to inform recruiters if assistance might be needed for the explanation and completion of forms for students with families not proficient in reading and/or writing, to ensure that guardians have been properly informed about the study. Arabic speaking volunteers would assist in these explanations and in filling out the questionnaire. Through the cooperation of the participating schools, eligibility for participation in this study would also be verified through the use of school records indicating students' birthdates.

Participating dentists would be allowed to invite qualifying patients in his/her practice that do not attend school to participate in this study using the translated version of the adapted school recruitment script (Appendix 3). Referrals would be allowed since the participating dentists would not benefit financially and the patients would benefit from the services that this study offers (i.e. coverage of costs for a dental examination and a panoramic x-ray, dental health and hygiene advice, etc.). Participants (and their guardians) would also be welcome to inform the dentists, researcher or research assistants of any eligible out-of-school individuals that might be interested in participating in this study.

3.4.2 Inclusion Criteria

Egyptian subadults ranging in age from two to 17 years old living on the west bank of Luxor would be included in this study. All volunteers would require consent from their mother, father, or primary guardian prior to participation in this study, along with a completed and returned questionnaire. Participation would be limited to individuals with birthdates within one month of the available study dates. Birthdates would be verified through each participant's school or official documents with the understanding that official documents in Egypt do not always record the actual date of birth.

If demand for participation is high, qualified applicants would be accepted following a lottery for each age/sex cohort. Otherwise, participants would be accepted upon verification of their birthdate and eligibility, following receipt of their completed questionnaire and consent/assent forms, until the number of participants necessary for each cohort is satisfied.

3.4.3 Exclusion Criteria

Applicants would be excluded from this study if they had significant prior exposure to ionizing radiation or if they were pregnant. Participants would also be excluded from this study if their birthdates could not be verified to fall within one month of the available study dates, or if all positions within the age/sex cohort relevant to the applicant were filled. Applicants would be excluded from this study if they had any dental interventions that significantly affected normal dental development. Alternatively, participants with naturally abnormal dental development would be examined for comparison with normal dentition and additional participants would be accepted to fill the relevant age/sex cohort with children representing relatively normal dental development. In this case the Health Science Research Ethics Board would be consulted regarding the change in number of participants

3.4.4 Consent and Assent

All participants would require the completion of a consent form (See Appendix 1) by a parent or legal guardian following the reading, or oral explanation, of the letter of information (See Appendix 1). Children aged 13-17 would also be required to sign a consent form (See Appendix 1) at their own free will for eligibility, following a reading and/or oral explanation of the letter of information. Children aged 7-12 would be required to complete the assent form (See Appendix 1) following an oral reading and/or explanation of the form. It would be made clear to all participants and guardians that consent and/or assent can be withdrawn at any point. The necessary forms in AppendicesAppendix 1 and Appendix 2 were translated by a volunteering Egyptian law school graduate, Ahmed Mokhtar, and are available upon request. Indications of participants' unwillingness to participate in this study, demonstrated by verbal and/or physical cues, would also be respected and the examination or procedure would be discontinued immediately.

3.4.5 Feasibility of the Proposed Participant Recruitment Process

During this feasibility study, Dr. Tarek El Mokkadem, the Head of the Department of Dentistry in the Egyptian Ministry of Health and Population, and Dr. Safah Abu el Fadl, the Superintendent for Schools in Luxor, pledged their intention to respectively support and assist in the recruitment of participants for the proposed project. Three local native Arabic speaking colleagues also agreed to help with the participant recruitment process by assisting with information dissemination and helping those not proficient in reading and writing with form comprehension and completion. The Superintendent for Schools in Luxor also confirmed that the birthdates of students could be provided from the school records, although caution would need to be taken as Egyptian birth records are sometimes inaccurate. As a result, it was determined that the proposed plan for patient/participant recruitment was feasible, however a limitation was identified with regard to the consultation of Egyptian birth records.

3.4.6 Data Collection: Questionnaire

3.4.6.1 Equipment

The questionnaire designed for this study had a primary goal to collect data relevant to the creation of an age estimation standard based on dental development, and information that might give further insight into epigenetic factors leading to differences in dental developmental timing and sequence (Appendix 1). The questionnaire also includes the WHO Oral Health Assessment Form for Children (2013) to collect health and hygiene data for inclusion in a public dental health report and the WHO/Malmo University Oral Health Country/Area Profile Database (WHO/Malmo 2011). Given the large scale of the proposed study, it is important to embrace the opportunity to collect data relevant to public oral health as studies of this type are costly and rare in Egypt, despite their obvious benefit to the population. Questions regarding respondents' opinions on issues of dental health and hygiene were also included in the questionnaire in an effort to give the public some input regarding future dental health promotion programs, as recommended by Scutchfield et al. (2004).

Quantitative and qualitative data collected from participant questionnaires would be compared to data collected from dental examinations and radiographs in an effort to identify factors that might have significantly affected dental development and dental health. Information collected through the questionnaire would also be used to give a broad description of the reference population in this study.

This questionnaire was designed for participant understanding and interviewer follow-ability (Sanchez 1992) to ensure comprehension regardless of whether the participant completed the questionnaire independently or accepted assistance from a local research assistant. The conversational dynamics within the questionnaire were scrutinized and improved in order to identify and avoid systematic biases resulting from the wording, order, format or answer options of the questionnaire (Knauper and Turner 2003). Following translation to Arabic by an Egyptian law school graduate, Ahmed Mokhtar, the questionnaire was also reviewed by local individuals to ensure cultural sensitivity and comprehensibility in the local dialect.

3.4.6.2 Procedure

Questionnaires would be distributed at participating schools for children to take home, and explanatory meetings for parents would be hosted. It would be requested that the applicant’s mother, father or primary guardian complete the questionnaire along with the consent form, following the reading and comprehension of the letter of information about the proposed study. Assistance in understanding and completing these forms would be offered to any family desiring assistance so that all eligible individuals would have an opportunity to participate in this study. Upon collection of the required forms, qualifying applicant birthdates would be verified through school records before booking eligible children for dental x-ray and examination appointments.

3.4.7 Data Collection: Clinical Dental Examination

3.4.7.1 Training and Calibration of Examiners (Determining Inter- and Intra-Observer Error)

Participating dentists would be trained by the primary investigator and/or World Health Organization personnel to identify and record oral health indicator data according to the World Health Organization's standards. These dentists would also be trained to accurately identify and record dental development scores in accordance with the given scoring system (See section 3.4.9). Methods for educating children and their guardians regarding pediatric health and hygiene would also be reviewed along with techniques for conducting trauma-free pediatric dental examinations. World Health Organization affiliates may help in the training and calibration of the examining dentists, though photographic indices and written descriptions for each scoring system are provided for reference during dental examinations (See Appendix 4).

As recommended by the World Health Organization (WHO 2013a), following training, the dental examiners would be required to independently examine the same 25 individuals. Results of these examinations would be immediately compared so that discrepancies in scoring could be discussed and reviewed in the patient before agreeing on a score. This exercise would serve to determine inter-observer error while allowing for calibration in diagnostics. This exercise would also be repeated near the middle of the study to ensure that the dental examiners' diagnostic methods have remained consistent and calibrated throughout the study.

As recommended by the World Health Organization (WHO 2013a), another two groups of 25 individuals would be examined on two separate occasions. These participants would be asked to return within a week of the initial examination to be re-examined by the same dentist. The examining dentist would not be informed that they would be conducting a duplicate exam until the duplicate exams were complete. At this point, duplicate data would be compared and discussed with the examining dentist. This exercise would serve to determine the rate of intra-observer error. If the intra-observer error rate was not satisfactory according to the World Health Organization's standards (i.e. between 85-95%)

(WHO 2013a), options for improvement would be discussed with the dental examiner and this exercise would be repeated.

3.4.7.2 Equipment

The clinical examination portion of this study would require the use of dental plane mirrors, periodontal probes matching the WHO specifications, gauze, tweezers, cleaning solution, an autoclave and recording materials. These materials were found to be readily available in medical supply stores in Luxor and Cairo, and an autoclave could be borrowed from the practice of a participating dentist.

The clinical examination forms are made up of an expanded version of the World Health Organization (WHO) Child Oral Health Assessment Form (WHO 2013b) and a dental development data collection form designed specifically for this study (See Appendix 4). The decision to use the WHO Child Oral Health Assessment Form was based on its ability to collect data that would be comparable to data collected from both modern and ancient populations. The ability to contribute the collected data to the WHO/Malmo University Oral Health Country/Area Profile Database (WHO/Malmo 2011), and the widely accepted and standardized methods associated with the WHO, ensure that the data collected would benefit the local population. The coding system used in the WHO Child Oral Health Assessment Form (WHO 2013b) also enables quick and easy data entry and analysis, for which the WHO also offers support and guidance. Additional benefits include the willingness of the WHO to sometimes offer financial, material and academic support to researchers conducting oral health surveys, and their occasional willingness to train and calibrate the study's dental examiners. This training would leave the local community's specialists better equipped to conduct their own oral health surveys while ensuring the academic excellence of the proposed study.

A minor adjustment was made to the WHO Child Oral Health Assessment Form (WHO 2013b) as further data were desired for the purpose of comparing the dental disease prevalence with prevalence rates observed in ancient populations. As a result, the instructions were modified so that if a deciduous tooth and an erupted permanent tooth occupy the same space on the dental arcade, both teeth would be scored and recorded in the same box, as opposed to just the permanent tooth (See Appendix 4).

The examining dentists would also be required to include detailed documentation of dental decay, missing teeth due to dental caries and carious lesions resolved through the application of fillings. This information would be analyzed in relation to variations in dental developmental timing and sequence within the sample. Documentation of decay, missing teeth due to decay, and fillings make up a standardized "DMF" documentation method for oral health analysis in a population (dental caries frequency in a population), allowing for the comparison of epidemiological profiles with others throughout the world (WHO 2013a). With the collection of detailed DMF data, comparisons might also be made with less detailed "point prevalence" studies, which indicate the number of individuals in a sample population that are, or have been, affected by dental caries (WHO 2013a). In accordance with the WHO recommendations for the standardization of dental health surveys, dental caries would only be identified if there were a macroscopically visible cavity. Pre-carious lesions would not be included in this study due to the difficulties associated with inter- and intra-observer error, and the possibility that these pre-carious lesions might be resolved before a cavity is formed.

The dental development data collection form (Appendix 4) was created using permanent and deciduous dental diagrams derived from the standardized forms used by Canadian Dentist, Dr. Henry Choi DDS (the author's former employer), with scoring boxes placed adjacent to each tooth. Examiners would use these forms to draw in any carious lesions or developmental anomalies, such as dental enamel defects. A legend is provided in the form with the choices of developmental scores (from Trodden 1982) that examiners would use to fill out every box adjacent to the dental diagrams. Following this, the form includes two questions requiring: 1) the description of any macroscopically visible developmental or pathological anomalies; and 2) the description of any conditions that were observable through the dental radiograph that were not initially observed through the clinical examination.

An additional form was created for the examination of children aged 15 and over to assess oral hygiene. This form was created for the observation of dental plaque and dental calculus buildup, along with periodontal loss of attachment (See Appendix 4). All of these analyses would be completed only on selected index teeth for the sake of brevity. Data would be collected in accordance with the instructions given by the World Health Organization (WHO 2013a) for the periodontal loss of attachment indicator. For this score, the first and second molars in each quadrant would be paired for recording and, if one was missing, there would be no replacement. If no index tooth was present in a participant qualifying for examination, all the teeth that were present in that participant would be examined and the highest score recorded as the score for the individual (WHO 2013a).

The Simplified Oral Hygiene Index (OHI-S, Greene and Vermillion 1964) instructions would be followed for the documentation methods for plaque and calculus health indicators. If one of the four first molars could not be observed due to absence, the adjacent (second) molar would be scored in its place. If the second molar was also missing, no score would be assigned to cheek teeth in this quadrant of the dental arcade. If either of the anterior indicatory teeth were missing, a score would not be recorded for that tooth and an alternative would not be observed.

A study of dental morphology in the older cohorts of the study participants might also be considered for a preliminary comparison of ancient and modern Upper Egyptian dental morphology. This type of study could provide insight into the genetic relationships between modern and ancient populations in Egypt. Furthermore, the examination of dental morphology in relation to dental developmental timing and sequence would also be interesting, as it may reveal a relationship between certain morphological aspects and the timing of dental development, alveolar eruption, and/or gingival emergence. However, for the purposes of this study, it was determined that the analysis of dental morphology by examining dentists who are not familiar with the identification of dental morphological traits could present inter- and intra-observer bias and inaccuracy in the documentation of dental morphology. Additionally, it would significantly prolong the clinical dental examinations and introduce the possibility of patient discomfort while the dentist attempts to identify all of the dental characteristics using only a dental mirror. As such, dental morphological study was not deemed feasible for this specific study.

3.4.7.3 Procedure

All participants would recieve a standard pain-free and non-invasive dental examination, designed to avoid stress and trauma. During the dental examination, all procedures would be explained using the ‘show and tell’ (Iannucci and Howerton 2012) method, where the procedures are explained in child-friendly terms, demonstrated and then completed on the participant. This method would ensure that the participant understands the procedure and would dissuade any fears, enabling the patient to stay calm and still for the procedure (Iannucci and Howerton 2012). In the dental examination of young children, the participating dentist would observe the teeth and enthusiastically count them out loud while touching them with a periodontal probe, introduced to young children as a ‘tooth tickler’. This procedure is meant to give the child a friendly (and somewhat entertaining) introduction to the dental office and it would allow the child to become comfortable with the dentist and the use of dental instruments. Dentists would document all participants' gingival emergence status, oral health and hygiene, and anything that might seem pertinent to the study of dental development using a standardized clinical dental form (See Appendix 4). Standard health, safety and sterilization precautions would be taken and enforced during, and between, all examinations.

During the examination, the dentist would also educate the volunteering patient, and their guardian (in the cases of the youngest participants), about the importance of dental health and hygiene in both the deciduous and permanent dentition. The panoramic radiograph would be used as an educational tool to show the participant their current state of dental development and dental health. The dentist would also briefly teach all volunteering patients ideal techniques and habits for dental flossing and toothbrushing. Following the dental examination, all participating children would be given a small package including dental floss, a toothbrush and toothpaste and an informative dental health and hygiene brochure filled out with the dentist’s notes on the results of the dental examination. The provision of dental examination notes and a copy of the panoramic dental x-ray is important as it would ensure that parents and/or guardians are informed of the child’s dental health and hygiene status and the dentist's recommendations. The provision of the dental notes and the dental x-ray might also save the patient from having to endure (and pay for) a second dental examination and x-ray if a follow-up appointment and/or dental intervention were required. Furthermore, in Egypt it is common for patients to maintain their own medical records and bring them to dental appointments.

3.4.8 Data Collection: Panoramic Dental Radiography

3.4.8.1 Why Panoramic Radiography?

The use of panoramic x-rays and clinical examination of living patients allows for the recording of both left and right sides of the maxillary and mandibular dentition, as well as both alveolar eruption and gingival emergence. As noted, several methods have been used to create dental age estimation standards in the past, such as macroscopic observation, histological analyses and dental radiography. Macroscopic observation of gingival emergence timing and sequence is a popular method of study because it is an inexpensive method that can be used on living populations without the need for special equipment. As this method is based on the presence or absence of erupted teeth and their stage of eruption, it is vulnerable to inaccuracies due to dental agenesis, dental impaction, variation in developmental timing, premature tooth loss, extraction, or post-mortem tooth loss. It has also been shown that gingival emergence is significantly more variable than alveolar eruption (Demirjian et al. 1973), complicating comparisons between living and deceased population studies, as gingival emergence cannot be accurately assessed in human remains (Haavikko 1970).

Histological analyses of dental calcification and eruption have played a large role in the understanding of dental development. These studies allow for the observation of dental growth, rather than just dental eruption and emergence. Histological analyses are also more accurate than radiographic analyses because they enable observation of newly formed structures that have not been sufficiently mineralized to appear on radiographs (Logan and Kronfeld 1933). Unfortunately, histological research is very tedious, time consuming and destructive, and are thus usually limited to small reference samples made up of extracted teeth or the teeth of deceased individuals. Additionally, these destructive methods are not applicable to living or, in many cases, ancient individuals and standards of dental growth based on these histological analysis of mineralized and unmineralized tooth structures will produce biased age estimates if applied to radiographic analyses of mineralized tooth structures alone.

Radiographic methods are ideal for the creation of age estimation standards based on dental development because they can be conducted on a living reference population, allowing for a large reference sample of known age and sex individuals. Age estimation standards based on radiography are also applicable to living individuals as well as individuals recovered from forensic or archaeological contexts.

Panoramic dental radiography is essential for the development of a new macroscopic subadult dental age estimation standard. Several of the existing standards are based on lateral x-rays, which obscure the view of maxillary cheek teeth due to overlap within the x-rays (e.g. Moorrees et al. 1963a,b). Consequently, these standards must assume similar patterns in maxillary and mandibular eruption timing, an assumption which has been proven to be false in Logan and Kronfeld's (1933) pioneering study of dental eruption patterns. Alternatively, the resulting standard must be based only on the mandibular teeth, making age estimation for a specimen with an incomplete dentition difficult, if not impossible.

Panoramic dental radiography allows dentists and researchers to see an individual's entire dentition at one glance. It is often used to monitor growth and development in subadults because erupted teeth and their occlusion can be seen, in addition to any un-erupted teeth in the mandibular and maxillary bones. Other options for capturing the entire dentition through dental radiography include the collection of a full mouth series of bite-wing and periapical x-rays (18-21 x-rays per series), lateral cephalometric radiography (e.g. Moorrees et al. 1963a,b; Demirjian et al. 1973), or CT scanning (e.g. Graham et al. 2010; Maret et al. 2011). There are obvious organizational, practical, ethical, and analytical difficulties involved with the collection of up to 21 x-rays per patient for a study of growth and development. Bite-wing x-rays do not show the complete roots of each tooth and show a limited number of teeth in occlusion. Periapical x-rays show the entire tooth root but they show even fewer teeth and do not show the details of the dental occlusion. Panoramic x-rays enable the dentist or researcher to see the relationships between teeth clearly without the difficulties involved in piecing together a series of 18 to 21 x-rays. They are also less costly and more accessible than lateral cephalometric radiography and computed tomography (CT), and they expose patients to significantly less radiation than all of the other radiological methods (Iannucci and Howerton 2012).

As previously mentioned, it might be argued that radiographs present a source of error in dental age estimation standards due to their inability to show enamel or dentine prior to sufficient mineralization (Logan and Kronfeld 1933). Although the actual developmental stage will always be slightly ahead of the developmental stage seen through radiographs, dental radiography continues to be the best method of monitoring growth and development in subadults, particularly in situations where destructive analysis of a large sample of known age skeletal remains is not possible (Hillson 1996, 2005). Furthermore, limitations regarding the invisibility of unmineralized dental tissues in radiographs are only relevant if radiographic standards are applied to non-radiographic studies. If, however, radiography is used to analyze the dentition, the radiographic standards will accurately represent the state of mineralized dental development for unbiased age estimation.

Another challenge associated with radiography is that all radiographs are magnified to some extent due to the nature of the x-ray beam and the position of the specimen in relation to the x-ray tube and plate or film (Iannucci and Howerton 2012). For this reason it may be advisable to use relative measurements of dental structures rather than actual measurements, though actual measurements have been used in prior studies (e.g. Mays et al. 1995; Liversidge and Molleson 1999a,b; Liversidge et al. 1993, 2003). Inaccuracy and bias may also be avoided through the use of an appropriate scoring system that has a "sufficient number [of stages] to quantify variability, while maintaining reliability. Too many stages [will] decrease reliability, while too few [will] compromise sensitivity" (Liversidge 2008: 237). Despite the known magnification issue inherent in radiographic methods, it should be noted that panoramic x-rays present the most standardized visualizations of the dentition due to their positioning method (Sassouni 1963). However, it is important to know the specifics of a panoramic radiographic machine, particularly if actual measurements are used, since different machines have different focal troughs that approximate the elliptical shape of the dental arch through the use of different centres of rotation (e.g. 2 centres, 3 centres, a continually moving centre, or a combination of 3 stationary centres and a moving centre) (Whaites and Drage 2013). As a result, not all panoramic radiographs are created equally, nor do they all represent the same degree of magnification between radiographic methods and within each radiograph: a fact that has not been taken into account in prior panoramic radiographic metric studies. In an effort to avoid these complications of metric radiographic analysis, the proposed study would score dental calcification according to the scoring methods of Trodden (1982) and Demirjian (1973) (See Table 3.1). Dental eruption would be scored according to the standards presented by Trodden (1982; See Table 3.2).

3.4.8.2 Equipment

The radiographic equipment that was investigated for use in the proposed study was owned and operated by Radwania Scan Medical Center on El-Mostafa Street in Luxor, Egypt. This medical center operates a digital panoramic dental radiography machine which typically takes 45 seconds to complete the x-ray procedure. Digital panoramic x-ray machines typically emit less radiation than the older analog machines and all panoramic machines emit significantly less radiation than the alternative full mouth series of bitewing and periapical x-rays. The x-ray machine was located in an enclosed room with a window for the operator to observe the patient (who would wear a protective lead apron) and the progress of the machine. The radiologist in charge was consulted regarding machine maintenance, safety precautions, facility layout, interest in participating in the proposed study, and cost for the proposed number of radiographs. It was determined that the machine maintenance procedures, safety precautions, and facility layout were ideal to host the proposed study. The chief radiologist was eager to have the business participate in the study and offered a significant discount on the proposed number of panoramic radiographs. As a result, this aspect of the study was determined to be feasible.

3.4.8.3 Procedure

Pediatric radiography requires special skills to keep young children calm, happy and still. In the clinical examination, patients would have all procedures, including the x-ray, explained using the 'show and tell' method (Iannucci and Howerton 2012). In this method, the procedure is explained in child-friendly terms, demonstrated (without radiation or by viewing a prior patient from the observatory), and then completed on the patient. During this demonstration, young participants that seem receptive to game-playing might be encouraged to stay still by playing the "freeze" game, where the child pretends that they are frozen in place during the x-ray. These methods would ensure that each participant understands the procedure and that their fears are dissuaded, enabling the participant to stay calm and still for the procedure (Iannucci and Howerton 2012). The panoramic x-ray machine investigated for the proposed study operates quite quickly with a 45 second run, which would minimize the risk of x-ray blurring caused by patient movement (Iannucci and Howerton 2012). If it were necessary and feasible in the radiographic laboratory, a proven distraction method such as a television (Alexander 2012), or a tablet playing cartoons, might also be used to keep young patients still during the radiographic procedure.

Upon completion of scanning, x-rays would be immediately saved onto a CD for data collection. The provision of a film copy of the x-ray would also be provided for the participant at no additional cost or increase in radiation exposure.

If it was determined that the resulting radiograph was not clear enough for the purposes of the proposed study, it would be decided if another radiograph should be taken or whether the child and their guardian would like to postpone the x-ray for another day (as recommended by Iannucci and Howerton 2012). If deemed necessary, the x-ray procedure would be repeated only once on a given patient, regardless of the clarity of the final x-ray. If it was decided that repetition of the x-ray procedure was not advisable, a new patient would be chosen to fill the data void in the relevant age group.

3.4.9 Data Collection: Scoring Dental Developmental

There are several methods for the scoring of dental calcification from radiographs and each has benefits and challenges (cf. Hess et al. 1932; Gleiser and Hunt 1955; Garn et al. 1958;

Nolla 1960; Moorrees et al. 1963a,b; Fanning 1971; Liliequist and Lundberg 1971; Demirjian et al. 1973; Gustafson and Koch 1974; Trodden 1982). For the proposed study, a composite scoring method is presented using elements from the scoring standards of Trodden (1982) and Demirjian et al. (1973), both of which were created for use on panoramic radiographs (See Table 3.1. A composite dental calcification scoring method merging Trodden's (1982) numerical standard and Demirjian et al.'s (1973) alphabetical standard).

The dental developmental scoring method presented by Demirjian et al. (1973) is based on developmental criteria visible through panoramic radiography. This method limits subjectivity as it does not rely on the prediction of crown or root lengths, which can vary significantly between individuals and tooth types (Demirjian et al. 1973; Demirjian 1978). Consequently, the Demirjian et al. (1973) scoring method has been demonstrated to have relatively low rates of inter- and intra-observer error when compared to other scoring methods for dental development (Hagg and Matsson 1985). This scoring system was originally developed in association with a weighted scale for age estimation. These calculations were created through a study of a French Canadian population sample and require the presence of seven teeth (I1 to M2). Although this study would not use the weighted age estimation function of the Demirjian et al. (1973) method, the original alphabetical scores would be retained for ease of comparison with other studies of dental development using the Demirjian et al. (1973) method. Retention of these alphabetical scores also enables reference to the Demirjian et al. (1973) pictorial and radiographic representations of developmental stages in each tooth type.

Trodden's (1982) scoring system is based on a combination of the calcification scoring systems created by Nolla (1960), Nevile (1973) and Fanning (1961), and the dental eruption scoring system of Schour and Massler (1941). Trodden's system has demonstrated relatively low inter- and intra-observer error, even in the face of patient positioning errors of up to 20 degrees vertically and/or horizontally (Trodden 1982). However, its reliance on relative measurements based on predicted crown and root lengths makes this scoring method quite subjective. Nevertheless, unlike Demirjian et al. (1973), Trodden's (1982) scoring system includes stages for no bony changes, development of the crypt, and missing data as a result of abnormal tooth inclination or faulty film, and gives a basic notation method for cleft formation. It also includes scores for missing teeth through stages such as: tooth exfoliation, premature extraction, tooth agenesis, and teeth that are missing for unknown reasons. The documentation of missing teeth is important due to the impact that missing teeth can have on the timing of subsequent dental growth (Ruiz-Mealin et al. 2012). Although Trodden (1982) did not account for this interaction, the relationship between missing teeth and the timing of subsequent dental growth would be considered during the proposed study. Questions regarding missing teeth and extraction were incorporated into the patient intake/health history information collection stage of the dental checkup for this purpose. Given the value of these elements of Trodden's (1982) dental calcification scoring method, they have been combined with scores from Demirjian and colleagues' (1973) standard for use in the proposed study (See Table 3.1).

Table 3.1. A composite dental calcification scoring method merging Trodden's (1982) numerical standard and Demirjian et al.'s (1973) alphabetical standard

Score	Definition
0	No change in bone density, and no crypt visible. This category is the same as that used by Nolla. (1960)
1	The crypt is clearly visible, but there is no evidence of calcification. This category is the same as that used by several authors.
A	In both uniradicular and multiradicular teeth, a beginning of calcification is seen at the superior level of the crypt in the form of an inverted cone or cones. There is no fusion of these calcified points.
B	Fusion of the calcified points forms one or several cusps which unite to give a regularly outlined occlusal surface
C	a. Enamel formation is complete at the occlusal surface. Its extension and convergence towards the cervical region is seen. b. The beginning of a dentinal deposit is seen. c. The outline of the pulp chamber has a curved shape at the occlusal border
D	a. The crown formation is completed down to the cementoenamel junction. b. The superior border of the pulp chamber in the uniradicular teeth has a definite curved form, being concave towards the cervical region. The projection of the pulp horns if present, gives an outline shaped like an umbrella top. In molars the pulp chamber has a trapezoidal form, c. Beginning of root formation is seen in the form of a spicule
E	*Uniradicular teeth:* a. The walls of the pulp chamber now form straight lines, whose continuity is broken by the presence of the pulp horn, which is larger than in the previous stage. b. The root length is less than the crown height. *Molars:* a. Initial formation of the radicular bifurcation is seen in the form of either a calcified point or a semi-lunar shape.

	b. The root length is still less than the crown height
F	*Uniradicular teeth:* a. The walls of the pulp chamber now more or less form an isosceles triangle. The apex ends in a funnel shape. b. The root length is equal to or greater than the crown height. *Molars:* a. The calcified region of the bifurcation has developed further down from its semi-lunar stage to give the roots a more definite and distinct outline with funnel shaped endings. b. The root length is equal to or greater than the crown height.
G	a. The walls of the root canal are now parallel and its apical end is still partially open (Distal root in molars)
H	a. The apical end of the root canal is completely closed. (Distal root in molars). b. The periodontal membrane has a uniform width around the root and the apex.
17	Extraction of the permanent or deciduous tooth. This category is used when evidence exists that the deciduous or permanent tooth has been extracted prematurely.
18	This category is used when evidence exists that a tooth is congenitally missing. Third molars are scored as “0” unless the child is over the age of 12 years, by which time the third molars should have begun calcification.
19	This category is used for missing permanent teeth where the reason is unknown. The tooth may have been extracted or is congenitally missing.
98	This category is used if the tooth is obviously present but an accurate determination of development is not possible due to an abnormal inclination of the tooth.
99	This category is used if the film is faulty, so that the presence or absence of the tooth was unknown.
V	Cleft formation is recorded separately for the permanent molars.

When using this scoring method, each tooth is assigned a score, and if a tooth’s developmental stage falls between two scores, the earlier/lower stage will be recorded. In cases where scores have more than one defining character (listed as a,b,c), one out of two, or two out of three characteristics must be met to qualify for the relevant stage (Demirjian et al. 1973; Demirjian 1978).

Like the Demirjian et al. (1973) method, the proposed study would not include third molars in the age estimation standard because they vary greatly in their developmental timing and the reference sample would be limited to individuals that have not attained full occlusion of the third molars. However, if this reference population was extended to include older individuals and the study of third molars, Trodden (1982) noted that population distribution bias could be avoided by excluding third molar scores of ‘0’.

In addition to the aforementioned dental calcification scoring system, Trodden (1982) provided a second scoring system specifically for dental eruption, including initial eruption, alveolar eruption, gingival emergence, and occlusion (See Table 3.2). The division of alveolar eruption into two stages is beneficial because it helps to clear up the definition of alveolar ‘eruption’ and it distinguishes between two events during the eruption period that are often used as sole indicators of alveolar eruption. The age of alveolar eruption is important for archaeologists who often do not have access to radiographic technology in the field.

Similarly, the inclusion of gingival eruption is beneficial for age estimation in living subadults and the recently deceased seen in forensic cases. Gingival emergence timing does not benefit archaeologists unless soft tissue has been preserved (and even then, tissue shrinkage and post-mortem damage may prevent an accurate analysis). Unfortunately, soft tissue is not always visible in radiographs. In an attempt to circumvent this problem in the creation of the new standard, clinical analysis of gingival emergence will be documented during the dental checkup on each patient. This will ensure that the new dental age estimation standard includes accurate soft tissue data, enabling specific age estimations in a broad range of circumstances. The last stage included in Trodden’s (1982) eruption scoring system is “attainment of occlusal level” (Trodden 1982: 43). Unfortunately, during panoramic x-rays, the patient’s position must be stabilized by holding a bite block between their teeth. Given that the maxillary and mandibular teeth are held slightly apart by the bite-block, it may be difficult to differentiate between teeth that are approaching occlusion or have recently attained first occlusal contact through the x-ray, particularly if adjacent teeth are missing or malaligned (Trodden 1982). Consequently, the occlusal status of teeth would also be noted during the proposed dental examination to ensure precision when creating the new standard.

Table 3.2. Trodden's (1982: 41-43, 131) Eruption Stages

Score	Definition
0	**Not erupted**: Tooth is congenitally missing or has not reached the calcification score of 6
I	**Initial eruption (Schour and Massler (1941), Stages I and II)**: Initial stages of tooth movement. Every tooth that has reached the calcification score of 6 but has not pierced the alveolar crypt. The crypt margin (lamina dura) appears unbroken, or there is evidence of the crypt occlusal to the tooth crown.
II	**Alveolar emergence**: The elevation of the cusps, or incisal ridge, about the margin of the alveolar crypt, and if the occlusal margin of the crypt appears to be broken.
III	**Gingival emergence (Schour and Massler (1941), Stage III)**: The emergence of any portion of the tooth crown through the gingiva. No evidence of soft tissue occlusal to the tooth crown.
IV	**Attainment of the occlusal level (Schour and Massler (1941), Stage V)**: Full occlusal contact as determined by the overall line of occlusion (Curve of Spee), and the approximation of the contact areas through macroscopic and radiographic observation. If a tooth is malaligned, only a portion of the occlusal (incisal) surface must be in occlusion.
V	**Missing teeth**: If the tooth has been exfoliated (score 16), extracted (score 17), probably extracted (score 19), or if the presence of absence of the tooth is unknown (score 99).

It is imperative that scoring methods are reproducible, particularly for application of an age estimation standard. As such, during the proposed study, inter- and intra-observer tests would be conducted to determine the reliability of the dental calcification and dental eruption scoring methods.

3.4.10 Data Analysis: Statistical Analysis

The search for an accurate, specific, and unbiased dental age estimation method has led some paleodemographers and bioarchaeologists to scrutinize more closely the statistical methods being applied to the reference data when creating new standards. Smith (1991a) notes that eight categories of methods have been used in the association of chronological age and dental age in past studies. The categories that Smith (1991a) identified are: cumulative distribution functions, average age at first appearance less half an interval between examinations, mean age of subject in a stage, alternative methods, mean formation stage for subject age group, maturity scales, pictorial charts and atlases, and miscellaneous methods. For age estimation standards, the use of 'cumulative distribution functions', which are loosely defined as "several methods (probit analysis, other methods of locating the mean or median in a cumulative distribution function)" (Smith 1991a: 151), was recommended.

In 1999, a paleodemography workshop was hosted in Rostock, Germany, where statistical methods for age estimation were evaluated, which resulted in the acceptance of a theoretical approach, aptly named the 'Rostock Manifesto' (Hoppa and Vaupel 2002). This manifesto calls for better reference collections with validated reports of chronological age, better osteological methods (i.e. improved observation and scoring systems for age-related changes), assessments of age distribution in target populations, and the use of Bayes' theorem (Hoppa and Vaupel 2002). The Bayes theorem is a statistical tool that allows one to estimate the probability of age [a] conditional on developmental stage [c] with regards to the prior distribution of that age in the target population [$f_T(a)$], when the probability of the developmental stage [c] occurring at age [a] with relation to the prior age distribution of the reference population [$f_R(a)$] is known. In other words, Bayes' theorem allows for the determination of $P(a|c)$, when $P(c|a)$ and the reference [$f_R(a)$] and target [$f_T(a)$] population age distribution patterns are known (Hoppa and Vaupel 2002).

In the proposed study, if 30 radiographs are collected for each sex-specific age category, as planned, the target sample age distribution should be estimated independent of the reference (Konigsberg and Frankenberg 1992; Bocquet-Appel 1986). However, since the reference sample would not begin when the first tooth begins to develop, age estimates might still be biased by the truncation of the age distribution pattern resulting in 'age mimicry' (Mensforth 1990; Smith 1991a; Konigsberg and Frankenberg 1992; Hoppa and Vaupel 2002). Furthermore, despite the narrowing of age attribution to within one month of patients' birthdates in the reference population, there would still be some variability in age attribution that might cause bias.

Several methods have been proposed to eliminate the biases inherent in age estimations calculated with Bayes' theorem. Konigsberg and Frankenberg (1992) demonstrated the efficacy of a multivariate probit method (also known as the maximum likelihood of the age distribution), a dependent Bayesian method (Heuze and Cardoso 2008), for use with a small number of age indicators. This method requires a large sample and is very numerically intensive, requiring the calculation of means, variances, and covariances between indicators. It has been stated that "multivariate integration over more than about five dimensions takes a great deal of computing time, even using very fast computers… If we wanted to use 20 indicators (for example, by observing the emergence of all the deciduous teeth), we would need to estimate 230 parameters!" (Holman et al. 2002: 16-17). To get around this difficulty, one of several methods of stochastic integration could be used, such as the Gibbs sampler or the Markov Chain Monte Carlo (MCMC) method (cf. Konigsberg and Holman 1999; Konigsberg and Hermann 2002). These methods make it feasible to integrate multivariate integrals to fairly high dimensions" (Holman et al. 2002: 16). However, the requirement for a large sample size grows with the number of indicators used because age predictions can only be made for developmental mineralization sequences found within the reference population (Heuze and Cardoso 2008). This means that if 48 indicators were used (one for each deciduous and permanent tooth excluding the wisdom teeth) with multiple ordinal categories of growth, the required reference population size would exceed the sample being collected in this study.

Boldsen et al. (2002) proposed the transition analysis method, an independent Bayesian method, which requires fewer calculations and does not require a large reference sample, because it assesses the developmental stages of teeth independently, rather than as blocks of dental mineralization sequences (Heuze and Cardoso 2008). This method is also ideal for its ability to assess dentitions with missing teeth (Heuze and Cardoso 2008). It does, however, require the assumption of independence between indicators once conditioned on chronological age (Holman et al. 2002). This assumption of independence between teeth is not entirely reflective of reality since teeth are "topographically, developmentally, and functionally associated to each other, and essentially grow as a unit (Heuze 2004; Braga et al. 2005)" (Heuze and Cardoso 2008: 277).

The latent trait method, proposed by Holman et al. (2002), can also assess incomplete dentition and is perhaps the most suitable for application to developmental dental age estimation standards. It does not require a very large sample size, it does not require the assumption of independence between indicators, and the calculations are more manageable for a study using a large number of indicators (i.e. 48 teeth) (Holman et al. 2002). The latent (or concealed) trait method assumes that each child has an individual unknown growth rate (*z*) which affects the time required to meet each developmental stage (Holman et al. 2002). Lower growth rates result in the passing of more time between developmental stages, while higher growth rates will result in faster development (Holman et al. 2002). Growth rates (*z*) can be different for each tooth and the strength of the effect of '*z*' on the age indicator '*i*' is variable (Holman et al. 2002). The strength of association between the growth rate (or latent trait) '*z*', and the age indicator '*i*' can be described parametrically 'β_{zi}' as it is assumed that the distribution of the growth rate (*z*) follows an unknown parametric distribution within the population (Holman et al. 2002). The effects of '*z*' can be estimated using proportional hazards models, assuming that '*z*' "increases or decreases the hazard of making the [developmental] transition at each age" (Holman et al. 2002: 212). The new parameter 'β_{zi}' showing the strength of association between the growth rate '*z*' and age indicator '*i*' is then incorporated into the distribution formulae for each age indicator. These formulae and the assumed function describing the variation of '*z*' among individuals is then incorporated into an independent multivariate Bayesian equation for estimation of the parameters of the reference distribution (Holman et al. 2002). The equation for the estimation of age-at-death distribution in the target population is similarly integrated over the distribution of '*z*' (Holman et al. 2002). This statistical method can sometimes result in relatively large standard errors despite the similarities found between estimated and known age-at-death distributions. These standard errors may be reduced through the use of stochastic methods of integration, such as the Markov Chain Monte Carlo method (Holman et al. 2002). The transition analysis method is a promising method for the application of dental developmental age estimation standards.

Following data collection for the proposed study, the Akaike Information Criterion (AIC) (Akaike 1973, 1998; Anderson and Burnham 1998) would be used to determine the relative dependence of age indicators to determine whether to use the Multivariate Independent

Bayesian Method or the Latent-Trait Method for the most accurate results (Holman et al. 2002). A recent statistical analysis of the permanent molars in a large sample of panoramic x-rays scored according to Moorrees et al.'s (1963a,b) method indicated that assumptions of conditional independence or complete dependence of molar development are not warranted (Liversidge and Konigsberg 2016).

3.4.11 Data Application: Statistical Software for Age Estimation

With respect to the method of application for the new standard, it has been determined that statistical formulae are more specific and accurate than atlas and graph methods. This is because statistical formulae prevent the observer error that may result from trying to match the dentition of an individual to figures of average developmental stages shown in atlases and graphs. So, rather than creating another dental atlas, the proposed study would integrate the collected data and the selected Bayesian statistical method into a computer software program to facilitate the calculation of age estimates upon entry of an individual's dental developmental data. Following in the footsteps of Demirjian (1994) and AlQahtani et al. (2010b), this software would be made available to download from the internet, enabling people working in a laboratory or in the field to easily calculate estimated age based on an Egyptian dental development standard.

3.4.12 Data Application: Public Dental Health Report and WHO Global Oral Health Database

Data collected from the proposed study that are relevant to public dental health would be contributed to the WHO Global Oral Health Database and included in a public dental health report. This report would be published and presented to the Egyptian Ministry of Health and the World Health Organization. Copies of the report would also be provided to the principals of each participating school for inclusion in the school library. Dental public health researchers have a social responsibility to advocate for the improvement and equality of the dental health and hygiene in the studied population (WHO 2013a). This study would highlight issues of dental health, hygiene, and service accessibility in a generally lower socioeconomic class of subadults in Upper Egypt. The report would also be used to argue for better preventative oral health care and public oral health education, which has the potential to affect change on a larger scale if reparative health care costs and illness-related loss of labour hours can be minimized. At the time of this feasibility study, the proposed research was embraced by Dr. Tarek El Mokkadem, the Head of the Department of Dentistry in the Egyptian Ministry of Health and Population, as the Egyptian government was renewing efforts to improve social, educational and medical programs.

3.4.13 Testing the new Standards

The subadult age estimation standard(s) developed through this study would be tested on populations that are independent of the reference population to explore its applicability to Egyptians outside of the reference population. The newly created region-specific subadult dental age estimation standards would be tested against the existing ‘universal’ dental age estimation standards on independent samples of radiographs from known sex and age Egyptian subadults from a variety of locations in Egypt. Pre-existing panoramic radiographs with information regarding sex and date of birth could be used in the testing stage for ease of access and reduced cost. Alternatively, the new dental age estimation standard could be tested on data collected through dental examinations of new population samples or, if necessary, on the original data through a statistical bootstrapping method without replacement. These tests would help to determine the applicability of the new standard to modern subadults throughout Egypt.

Provided the appropriate permissions are granted, a preliminary study of this standard’s applicability to ancient human remains would also be conducted. The new region-specific standard and the ‘universal’ standards would be applied to excavated human remains from the early Roman Period in Upper and Lower Egypt (Kellis 2 and Fag El-Gamous cemeteries, respectively). With permission from the Egyptian Ministry of State for Antiquities (MSA) and the Supreme Council of Antiquities (SCA), the Dakhleh Oasis collection would be made accessible through Dr. El Molto, Dr. Peter Sheldrick and the Dakhleh Oasis Project, and the Fag El-Gamous collection would be made accessible through Dr. Kerry Muhlestein and the Brigham Young University Egypt Excavation Project. By using two geographically distinct archaeological samples from the same time period, it is hoped that the effects of time and space could be assessed in relation to the efficacy of the age estimation standard. A multifactorial skeletal age estimation method, shown to be the most accurate and unbiased (Lovejoy et al. 1985), would also be applied to the selected subadult remains to determine relative rates of accuracy and bias for the old and new subadult dental age estimation standards.

Another possible method for testing the new standard in ancient populations would be to compare age estimates from the new standard to age estimates derived from the histological analysis of dental tissues (e.g. counts of enamel cross-striations, striae of Retzius, perikymata, Von Ebner lines, Anderson lines, or cementum annulation; root translucency; relative pulp width). These methods are known to be particularly specific and accurate indicators of age (Antoine 2000; Antoine et al. 2009). As such, a comparison of age estimates from the new region-specific standards to age estimates obtained through histological analysis would be especially powerful for the determination of accuracy, bias, and specificity of the new standard.

Although dental morphology has remained fairly constant in Neolithic to Post-dynastic Egyptians (Irish 2006), and there is evidence of little change in diet since the Pharaonic Period in rural areas of Egypt (Wood 1988), a recent study has demonstrated secular change in the dental development of Dutch children over only four decades (Vucic et al. 2014). Secular change can be attributed to changes in environment, diet, health, hygiene, socioeconomic status, and/or lifestyle (Eveleth 2001). Consequently, further testing would be required to determine the accuracy and specificity of the new standard to other ancient populations. Ideally tests of the new region-specific subadult dental age estimation standards would be conducted at a wide variety of geographically and temporally diverse archaeological sites throughout Egypt to ascertain the scope of its applicability and, if necessary, create correction intervals for improvement. These tests could also be extended to other countries in the Middle East and Africa to determine the regional boundaries for this ‘region-specific’ standard.

3.4.14 Feasibility of the Proposed Methods

During this feasibility study, several dentists, a radiological laboratory, and multiple local volunteer research assistants were contacted and recruited for participating in this study. Through an investigation of the Radwania Scan Radiological Laboratory, it was determined that the facilities were an appropriate location for the clinical dental examinations as well as the radiographic exams. The owner and chief radiologist at Radwania Scan also indicated that the facility could be made available in its entirety during the daytime and offered a discounted price for the proposed number of panoramic x-rays. The interested dentists volunteered to bring dental mirrors and periodontal probes and one of the dentists volunteered to bring their autoclave for use in sterilizing dental instruments. A medical supplies store with the needed disposable instruments was also located. The questionnaire and an educational brochure were also designed for use in the proposed study. In addition to the medical professionals and research assistants contacted, Dr. Tarek El Mokkadem, the Head of the Department for Dentistry in the Egyptian Ministry of Health and Population, and Dr. Safah Abu el Fadl, the Superintendent for all schools in Luxor also expressed interest in the proposed study, further lending credence to the feasibility of the proposed study. Moreover, as the proposed test for the new method at the Kellis 2 and Seila archaeological sites does not require any special permissions beyond the normal bioarchaeological study permissions, the proposed tests for the new method are also feasible when provided access to the sites.

3.4.15 Limitations

This proposed method for the development of sex- and region-specific subadult dental age estimation standards may be limited by the ability to accurately determine the date of birth of participants. In Egypt, where and for whom this plan was developed, it is not uncommon for families and/or delivery doctors to register the birth after the actual birth date. Since it is illegal to retroactively register a birth, official records will often indicate a later birthdate than the actual date of birth. In some cases, this discrepancy is only a matter of days, but in my research I have found cases in which rural families delayed travel to register the births of their children until after all planned children were born, causing a delay of several years in the birth registration of a number of individuals. Although this situation is far from ideal for research purposes, it may be prevalent in developing countries. As such, it is necessary to consider this as a possible limitation in developing a more accurate age estimation standard. Furthermore, it would be prudent to inquire about local norms for birth registration and, when necessary, ask patients or their caregivers if official documents reflect their true date of birth.

Another consideration and possible limitation in the implementation of this proposed study relates to cost and accessibility of dental professionals. In the original planning of this project, this challenge was circumvented by attaining grant funding, utilising the current author’s familiarity with Egypt and Arabic to make arrangements with dentists and the Egyptian Ministry of Health, and using the current author’s professional skills as a dental assistant to reduce costs. Given the clear benefit to the participating medical professionals and patients as well as the Egyptian economy, there was overwhelming support from the Egyptian Ministry of Health, the dentists, and the radiologist approached for participation in the proposed study.

3.4.16 Research Ethics

In the development of the proposed study, all possible precautions were taken to ensure that volunteers would understand the potential risks of taking part in the study and provide informed consent; that they were not coerced into participating in the study; and that the risks associated with the study were minimized and justified by the potential knowledge gained and benefits to the patient and society. Research ethics board approval would be required prior to the launch of the proposed study; however, this project has been designed to comply with modern medical ethical standards.

3.4.16.1 Ionising Radiation Dosimetry and Biomedical Ethics

Although there is a greater chance of developing cancer spontaneously (3300 in 1 million) than from dental radiographs (3 in 1 million) (Iannucci and Howerton 2012), it must be acknowledged that x-radiation can cause some biologic damage (Iannucci and Howerton 2012; ADA/FDA 2012). It is for this reason that conventional precautions would be taken to protect the patient and the radiographer from unnecessary radiation, or radiation scatter (ADA/FDA 2012).

It is recommended that x-rays are only taken if the benefit to the patient outweighs the risk, or if the taking of an x-ray will have a likely effect on the course of treatment (Iannucci and Howerton 2012). Panoramic x-rays can help to diagnose occult diseases in patients and assess their oral health as well as dental development (Sassouni 1963; Iannucci and Howerton 2012; ADA/FDA 2012). In addition to assessing growth and development and identifying dental and skeletal abnormalities, in the proposed study the panoramic radiograph would be used for educational purposes while teaching children and their guardians about dental hygiene. This education is a necessary part of preventative care and it is a recommended function of panoramic x-rays (Iannucci and Howerton 2012).

Young children in Upper Egypt are at a high-risk for dental disease due to their socioeconomic status, their diet and a lack of dental health resources, including fluoridated water (ADA 2011; ADA/FDA 2012). Participants in the proposed study would benefit by receiving a copy and analysis of their x-ray, dental hygiene/health education and a clinical dental checkup, a routine service which is unaffordable to many in Egypt. The benefits and risks associated with participation in this study would be explained completely and clearly to patients and their guardians, and written consent would be required before participation in this study.

The amount of radiation received from a digital panoramic dental x-ray machine is extremely small (0.01mSv in 200 film speed model) and equivalent to a little over half a day of the average daily individual effective dose from natural sources worldwide (2.4mSv/year) (Cohnen et al. 2002; nuclearsafety.gc.ca 2014). Since the proposed study is cross-sectional, it would require only one panoramic x-ray per patient (with the possibility of one retake, if absolutely necessary for x-ray interpretation); thus requiring minimal radiation exposure. Given the terrible oral health and hygiene problems and limited access to diagnostic dentistry (some government subsidized programs will only offer free or discounted dental extraction) in some developing nations such as Egypt, it is believed that the benefits to the participants of the proposed study would far outweigh the risks.

3.4.16.2 Benefits to Participants and Society

The proposed research project would challenge and test the efficacy of current age estimation standards based on dental development in subadults. This project would also result in the creation of a sex- and region-specific dental age estimation standard based on the most controlled and statistically sound methods used for the creation of macroscopic dental age estimation standards to date. If proven effective, the proposed model for the creation of age estimation standards would be useful for the creation of other sex- and region-specific dental age estimation standards. These dental age estimation standards have applications to dentistry, orthodontics, forensics, archaeology, sociocultural studies, law and immigration.

The education of participating children and their parents regarding dental hygiene, dental health and dental disease prevention would raise public awareness and lower rates of dental disease in the target population. The population investigated in this feasibility study has a high risk of developing caries due to socioeconomic status, unfluoridated water, a high carbohydrate diet and irregular access to dental professionals, making it both an ideal population for the proposed study and a population that would most benefit from dental examinations and oral health education. If permitted by the Egyptian Ministry of Health, the collected data would also be used for the creation of a public dental health report that would attempt to identify areas for improvement of access to dental services and education about oral health and hygiene in rural Egypt.

Participants would benefit from this study by receiving a free dental examination from a local dentist. Much of the Egyptian population cannot afford regular dental examinations and many Egyptians are at high risk for developing dental caries for the aforementioned reasons. Government subsidized dental health programs in Luxor generally focus on dental extraction rather than diagnosis, prevention, or repairs. In addition to the free dental examination, participants would receive free education regarding dental hygiene techniques, and would gain a better understanding of the importance of dental hygiene. Participants would also receive a free panoramic dental x-ray to be used as an educational and diagnostic tool during the examination. A copy will be provided to participants, which may be useful for follow-up appointments. Young participants would have a child-friendly introduction to the dentist. All participants would receive dental hygiene products such as toothbrushes, floss and toothpaste samples, and young children would receive a small toy or treat, which is common practice in pediatric dental offices. These giveaways would not be advertised or mentioned prior to participation as they are not meant to act as incentives to participate, but are meant to encourage oral health care at home and to provide a lasting positive impression of the dentist for young children.

3.4.16.3 Possible Risks or Inconveniences to Participants

Participants would be exposed to a small amount of ionizing radiation, which in large amounts has been known to contribute to the development of cancer. However, as previously mentioned, the amount of radiation received from a digital panoramic dental x-ray machine is extremely small compared to other radiological procedures as the effective dose of an older model with a film speed of 200 is 0.01mSv (Cohnen et al. 2002). This is equivalent to a little over half a day of the average daily individual effective dose from natural sources worldwide according to the 2.4mSv/year estimate reported by the Canadian Nuclear Safety Commission (nuclearsafety.gc.ca 2014). The proposed study is cross-sectional and so it would require only one panoramic x-ray per patient (with the possibility of one retake, if absolutely necessary for x-ray interpretation); thus requiring minimal radiation exposure.

All dental instruments would be sterilized and disposables would be discarded safely. Standard precautions would be used to protect participants from receiving unnecessary radiation and the panoramic x-ray machine will be set to minimize radiation exposure without sacrificing the quality of the radiograph. The proposed study would adhere to the golden rule of medical ethics: namely, the benefits to the participants must outweigh the risks to participants.

With parental and school permission, older children participating in the proposed study may be required to attend a dental appointment with chaperones during school hours for which transportation would be provided (and parents invited). This is due to the fact that dental offices and radiological laboratories in Egypt are usually open after regular working hours, so it is preferable to conduct this study during the daytime in an effort to avoid negatively affecting access to dental health care for the general population during normal clinic hours. As a result, a missed day (or less) of school is a possible, and likely, inconvenience to participants in this study. However, this minor inconvenience would be mitigated by the benefits to the participants. Younger children participating in the study would be required to attend an appointment with a parent or legal guardian. These appointments would be scheduled to fit with the guardian’s schedule with preference given to daytime appointments.

Some participants may also be asked to return for a follow-up examination to ensure that dentists are maintaining consistent recording methods. During these follow-up examinations, x-rays would not be taken as they would not provide any additional benefit to the participant.

3.4.16.4 Project Funding

All expenses associated with the implementation of the proposed research would necessarily be covered without a conflict of interest.

3.4.16.5 Data Security and Confidentiality

For the proposed study, all participants would be assigned a participant number, which would appear on every document relevant to that participant (i.e. consent forms, questionnaires, clinical dental records, x-rays). X-ray technicians would use the participant number in place of the patient name on the x-ray. An encrypted master list would be created to link participant numbers to individuals and their contact information. This master list and the consent forms containing identifying data would remain confidential and accessible only to the primary researcher.

The assigned participant numbers, birthdates and sex of the participants would be included on the clinical dental reports, questionnaires, and x-rays. Data would be uploaded (i.e. digital x-rays) or scanned (i.e. consent forms, questionnaires, clinical dental records), then encrypted and saved onto a laptop and redundantly on an external hard drive. All documents would be encrypted on the laptop and on the external hard drive using Windows 7 Encrypting File System (EFS). EFS scrambles the contents of encrypted files so that they can be read only by someone who has the appropriate encryption key to unscramble it. Original documents would be shredded and discarded securely, apart from the original x-rays, which would be given to participants for their personal records. Unidentifiable data would be made available for academic study through a digital archive at a later date.

Data resulting from this research would be stored in an online database or archive. The online archive would be password protected (with periodic changes to the password to ensure security) and it would be made available to scholars upon approval of a research proposal. The home page for the digital archive would describe the types of data contained within the archive and the data collection methods so that scholars could assess the suitability of the collection for their proposed topic of study, or adjust their topic of study to suit the available data. Unidentifiable data collected using the World Health Organization’s standardized methods would also be posted on the World Health Organization’s database for public health research, if permitted by the Egyptian Ministry of Health.

The primary researcher for the proposed study would keep the collected data indefinitely and make it available for future study through an open access digital archive. It is important to make these data available to the public as it would be the first panoramic radiographic collection in the world with such narrow age attributions and well-planned and documented collection methods. This radiographic archive would provide important information for scholars interested in the study of growth and development, dental disease, occult dental and skeletal disease, dental health and oral hygiene in Upper Egypt, and the relationship of all these things (and more) with the environmental, cultural, familial and dietary data provided in the digital data archive.

3.4.16.6 Proposal for Research Dissemination and Participant Feedback

Participants and their guardians would receive immediate verbal feedback regarding their oral health and hygiene, as is customary during a dental examination, and a brief written oral health report from the examining dentist. Participants and guardians would also be educated about oral health care and would be given a copy of their panoramic x-ray.

Contact information for the primary researcher would be provided in the letter of information so that participants can request a copy of the final results of the study.

Participants demonstrating interest in seeing the final product of this study would be directed to a copy of the study in a local library or sent a link to an online copy via email. Results from the proposed study would be publicized through a monograph, peer-reviewed publications, and academic presentations. Any age estimation software resulting from this study would also be made available to academics upon completion.

As per the World Health Organization's (WHO 2013a) recommendations, a report of the study's public health results would be given to the principal of each participating school for viewing in the school library. The data regarding public health would be also be submitted for inclusion in the WHO Global Oral Health Database, if permitted, and a public dental health report would be published and presented to the Egyptian Ministry of Health and the World Health Organization.

3.4.17 Discussion and Conclusion

This thought experiment resulted in the development of a new method for the creation of macroscopic subadult dental age estimation standards based on dental development. The proposed method was developed in a manner that was deemed to be feasible in a practical sense. All elements of the proposed method were investigated, including the availability and affordability of necessary facilities, equipment, and professional services, the cooperation of all necessary Egyptian authorities and professionals, and public interest in participation. This method was also created in accordance with biomedical ethical standards, with all possible ethical issues having been addressed through the detailed planning for: fair and responsible participant recruitment, informed assent/consent, a standardized ethically sound questionnaire, examination and radiographic protocols, equipment safety and hygiene protocols, public health education and advocacy, knowledge dissemination, and data security protocols. As a result of this planning and the paucity of affordable dental services in the selected population, the benefits to participants far outweigh any risks associated with the proposed study, satisfying the golden rule of medical research ethics.

In future, it is hoped that the proposed plan for the creation of a region-specific, sex-specific subadult macroscopic dental age estimation method will be implemented in Egypt as it would contribute significantly to the collective understanding of dental development and its variability worldwide, as well as to many aspects of oral health and hygiene in Egypt. This project would be beneficial to the local community by providing dental health care and oral health education and the data would be used to advocate for improved public dental health strategies in Egypt. Additionally, the information gleaned through the proposed study and the resulting subadult dental age estimation standard would have applications for dentistry, orthodontics, biology, health sciences, public health policy, pediatric medicine, evolutionary medicine, socio-cultural studies, forensics, immigration, law, and bioarchaeology in Egypt

The proposed method for the creation of a new macroscopic subadult dental age estimation standard was compared to the methods used in the creation of pre-existing subadult dental standards. The resulting meta-analysis revealed that all of the identified methodological errors that may contribute to inaccuracy or bias in the pre-existing standards can be resolved through the use of the proposed method (See Table 3.3). Consequently, it is probable that the use of the proposed method for creating a new subadult dental aging standard would further limit sources of inaccuracy and bias, while increasing specificity. As a result, it can be deduced that there is room for improvement in macroscopic methods of subadult dental age estimation. Given that all aspects of the proposed method for the creation of enhanced macroscopic subadult dental age estimation standards were determined to be feasible and ethically sound, the null hypothesis for this part of the dissertation, (H_o): "Current dental age estimation standards based on dental development cannot be improved", can be rejected.

Table 3.3. Comparison of methodological errors in dental developmental age estimation standards

Date of Publication	Author(s)	Missing patient recruitment and selection info	Study based on chronically ill patients	Imprecise age attribution methods	Missing description of sample population	Small sample size	Missing methodology	Does not focus on full dentition	Maxillary eruption patterns not included	Assumes mand. & max. eruption patterns same	Based on eruption or emergence times only	Missing data on eruption and/or emergence times	Not tested on an independent population	Missing statistical analysis	No error ranges included	Not sex-specific	Uses mean of male and female values	Standard not useful for subjects with missing data	Based on E.U., U.K., or N. American reference pop.	Assumes universality of development patterns	Based on actual tooth length	Based on literature only
1887	Peirce	x		x	x	x	x	x	x	x	x		x	x	x	x	x		x	x		
1933	Logan and Kronfeld		x	x	x	x							x	x	x	x			x	x		
1935	Kronfeld		x	x	x	x							x	x	x	x			x	x		
1939	Kronfeld and Schour		x	x	x	x							x	x	x	x			x	x		
1940	Schour and Massler	x	x	x	x	x	x						x	x	x	x	x		x	x		
1941	Schour and Massler	x	x	x	x	x	x						x	x	x	x	x		x	x		
1942	Steggarda and Hill			x							x	x							x			
1944	Schour and Massler	x	x	x	x	x	x						x	x		x	x		x	x		
1948	Hurme	x		x	x	x		x			x	x	x			x	x	x	x	x		x
1960	Nolla	x		x		x		x	x	x		x	x	x	x			x	x			
1961	Fanning	x		x			x	x	x	x		x	x					x	x	x		
1962	Miles			x		x		x	x	x								x				
1963	Miles			x		x		x	x	x								x				
1963	Moorrees et al.	x		x			x	x	x	x		x	x					x	x	x		
1970	Haavikko			x							x								x	x		
1971	Fanning and Brown	x		x			x	x	x	x		x	x						x	x		
1973	Demirjian et al.			x				x	x	x		x	x		x			x	x	x		
1974	Gustafson and Koch	x	x	x	x		x	x				x			x	x	x		x	x		x
1976	Demirjian and Goldstein			x				x	x	x		x	x		x			x	x	x		
1976	Anderson et al.			x								x							x	x		
1978	Ubelaker	x	x	x	x		x						x	x		x	x		x	x		x
1982	Trodden	x		x	x								x						x			
1987	Ubelaker	x	x	x	x		x						x	x		x	x		x	x		x
1989	Ubelaker	x	x	x	x		x						x	x		x	x		x	x		x
1991	Smith	x		x		x	x	x	x	x	x							x	x	x		
1998	Liversidge et al.			x				x	x	x		x	x			x	x		x	x	x	
1999	Ubelaker	x	x	x	x		x						x	x		x	x		x			x
2004	Liversidge and Molleson	x		x				x	x	x	x	x	x			x	x		x	x	x	
2006	Molnar	x	x	x	x	x	x				x	x	x	x		x	x		x	x		x
2011	Soliman et al			x							x	x	x									
2012	AlQatahni et al.	x		x		x						x	x						x	x		
?	Standard created through Kirkpatrick's method																					

Chapter 4

4 Creating an Improved Adult Age Estimation Standard Based on Dental Attrition

4.1 Introduction

This chapter details the complex intrinsic and extrinsic factors that result in dental wear in human dentition. These factors are overviewed both to illustrate the multiplicity of interacting etiological agents in dental attrition and to provide a detailed resource for future researchers considering this aspect of dental bioanthropology. Following this, a study of dental wear in Roman Period Egyptians from the Kellis 2 cemetery is described and a new method and region-specific standard for adult age estimation is presented.

4.2 Literature Review: Dental Wear in Human Permanent Molar Crowns

The term 'dental wear' was first coined in 1778 by the preeminent Scottish anatomist, surgeon, and dental scientist, John Hunter (Hunter 1778; Grippo et al. 2004; Zhou and Zheng 2008), who recognized three mechanisms of wear: attrition, abrasion and erosion. Since this definition of dental wear, another mechanism of wear has been identified: namely, abfraction (Grippo 1991). Grippo (1991) describes abfraction as a type of non-carious cervical lesion caused by high occlusal loading, which causes flexure and failure of enamel and dentine, and ultimately tissue loss, usually in the labial or buccal cervical region of the tooth. These processes are interdependent in "an extremely complex process that involves mechanical, thermal, and chemical reactions" (Zhou et al. 2013: 43), ultimately resulting in dental tissue loss. Here I describe these mechanisms, their expressions in permanent coronal dental tissues, and the many factors that can affect dental wear rates and patterns in human permanent molar crowns.

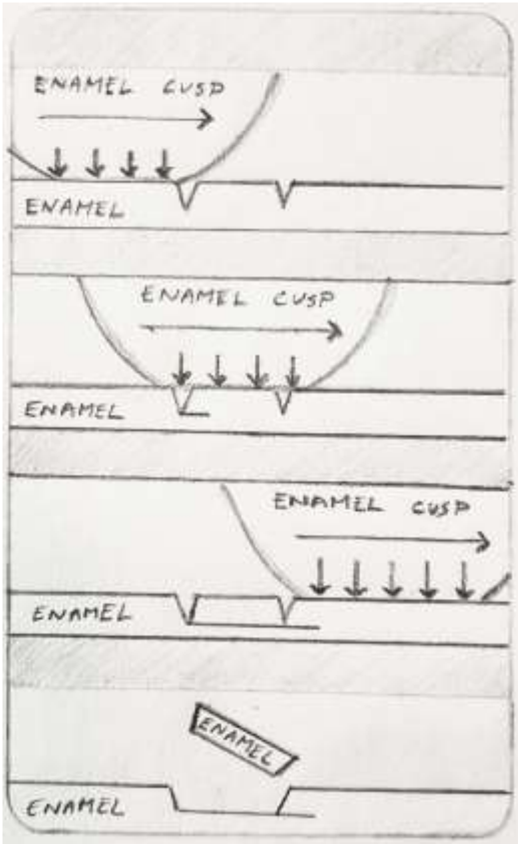
4.2.1 Dental Attrition (Two-body Abrasion)

Dental attrition, also known as two-body abrasion in tribology, is the gradual wear of a tooth's surface as a result of tooth-on-tooth contact, whether it is a result of mastication or bruxism (Hillson 1996). Dental attrition results in the formation of distinct facets at the area of contact as well as tensile stress-related microscopic subsurface fracture (Arsecularatne and Hoffman 2012). Although they are often overlooked, interproximal dental wear facets are a type of dental attrition, resulting from the mesial tilting of teeth accommodating tough occlusal loads (Wolpoff 1971). Although interproximal wear has shown little potential for use in age estimation, particularly among individuals with high rates of occlusal wear, occlusal attrition (in reality, occlusal wear as a whole) has become a staple of adult age estimation methods (Sarig et al., 2015; Deter 2012; See section 4.3).

In the case of occlusal attrition, and in the absence of other mechanisms of wear, these facets continue to grow as the enamel surface is worn down until they are joined together forming a flat occlusal surface with no dentine cupping (Kaidonis 2008). Through focused ion beam (FIB) and field emission scanning electron microscope (FESEM) analysis, Arsecularatne and Hoffman (2010) have demonstrated that subsurface cracking occurs during wear of enamel under nominally elastic contact conditions. They likened the process to that of abrasion in ceramics as the surface roughness and specific wear rate in severe dental attrition is similar to those in ceramics. In this model of dental wear, occlusal forces perpendicular to the surface create or exacerbate surface cracking, while tensile stress from the movement of the occluding surface produces microfractures parallel to the surface, resulting in the separation of microfragments (See Figure 4.1).

Zhou et al. (2013) have proposed that there are two stages in the attrition of the occlusal surface. The first (running-in) stage involves significant delamination of the surface enamel, resulting in a rough surface which is quickly ground down to produce a wear-particle layer between the occluding teeth. This stage generally lasts for approximately two years based on clinical observations. Further dental wear with this wear-particle layer advances more slowly and steadily, representing the second (steady-state) stage of dental attrition. In this model of dental wear, Zhou et al. (2013) hypothesize that even two-body abrasion (aka attrition) turns into three-body abrasion (aka abrasion) as a result of the formation of a layer microscopic dental particles that coat the occluding surface.

Figure 4.1. Subsurface cracking from enamel wear under nominally elastic contact conditions [modified from Kato and Adachi 2001]



4.2.2 Dental Abrasion (Three-body Abrasion)

Dental abrasion (or ‘three-body abrasion’ in tribology; Zhou et al. 2013) is the wear produced by foreign objects and particles forced across the dental surface. This mechanism of wear can result in the progressive creation of occlusal facets, fine polishing of dental crown surfaces, the dulling of perikymata (external manifestations of Lines of Retzius resulting in microscopic grooves), the creation of microscopic scratches and pits, and microscopic surface fragmentation (Arsecularatne and Hoffman 2012; Kaidonis 2008). More obvious examples of dental abrasion seen in the archaeological record can include interproximal grooving, deliberate dental mutilation, and abnormal wear resulting from regular contact with foreign materials (e.g. labrets, pipe stems, coarse plant fibers or sinews, etc.). Although the latter types of dental abrasion can produce fascinating information about diet and habitual tooth use, they are much less common than the regular dental abrasion resulting from mastication.

Human mastication has two cycles (Krueger et al. 2008). The first is a puncture/crushing process in which there is no interdental contact and a larger bolus or hard food is broken down into smaller fragments. The puncture/crushing action typically results in dental wear at the tips of cups (Krueger et al. 2008). The second cycle, commonly called the shearing or power stroke, is a movement in two phases which is designed to grind foods into a slurry of fine particles and saliva, and results in phase-specific wear facets with differing microwear textures. Phase II facet microwear texture has been shown to better distinguish between diets of different primate taxa than microwear texture on phase I facets (Krueger et al. 2008). Fine particles in these slurries include substances like sand, dirt, grit, bone, exoskeleton fragments, and perhaps most interestingly, siliceous orcalcium oxalate particles (otherwise known as plant phytoliths). Although it has been argued that phytoliths are not hard enough to cause dental tissue loss (Lucas et al. 2013), there is evidence to indicate that the mastication of phytoliths causes true dental tissue loss, and thus dental wear. In addition to finding phytoliths embedded in the enamel at the end of microwear grooves (Fox et al. 1994), *in vitro* nanoscale and microscale studies conducted by Xia et al. (2015) have demonstrated enamel loss through friction with amorphous silicon dioxide (SiO_2) and other particles softer than enamel (aluminum and brass spheres). Given this, Xia et al. (2015) concluded that enamel wear results from exceeding the binding force of the proteins holding together enamel hydroxyapatite crystallites rather than being directly related to relative material hardness. This concept is supported by a tribological study of various materials which demonstrated that "hardness of the wearing material affects the penetration depth of abrasive particles but… it fails in predicting abrasive wear resistance of different materials" (Zum Gahr 1998: 589).

Dental microwear texture analysis (DMTA) of dental surfaces has provided significant information regarding the nature of individuals' and species' diets as differences in the enamel pit-to-scratch ratio will indicate differences in the hardness and fibrous nature of consumed foods (e.g. Merceron et al. 2010; Scott et al. 2012; Karriger et al. 2016). Patterns of microwear have also been used in the study of masticatory jaw movement, leading to a better understanding of the ways in which foods are orally processed (e.g. Gordon 1984; Morel et al. 1991; Teaford 1994; Mahoney 2006; Krueger et al. 2008; Hua et al. 2015). Behavioural inferences, such as incisal preparation or non-dietary tooth use, have also been made and verified through the use of dental microwear analysis (e.g. Puech and Albertini 1984; Krueger and Ungar 2012). More recently, Gugel et al. (2001) have demonstrated the potential for identifying specific plant phytolith consumption through the examination of morphologically characteristic enamel pitting, though further research is required. Of course, it must be noted that microwear analyses are limited by the processes of enamel remineralization and acid erosion that can dull these markings, as well as the continuous obliteration of old microwear with new microwear, termed the “Last Supper Effect” (Grine 1986). As such, it has been suggested that occlusal microwear analysis may only reliably reveal information about tooth use and meals consumed in the weeks, days, hours, or even minutes before death, depending on conditions (Grine 1986; Teaford and Oyen 1989; Teaford and Lytle 1996). Although this is a limiting factor, this high turnover rate may be useful in the identification of seasonal dietary changes, and the slower microwear turnover seen in buccal tooth surfaces may help preserve evidence of earlier consumption of more abrasive foods (Romero et al. 2012; Sanchez-Hernandez et al. 2016).

4.2.3 Dental Abfraction (or Fatigue Wear)

As noted, dental abfraction is a type of non-carious cervical lesion caused by high occlusal loading, which causes flexure and failure of enamel and dentine, and ultimately tissue loss, usually in the labial or buccal cervical region of the tooth. Abfractions typically present as sharp-edged wedge-shaped lesions at or near the CEJ. Maxillary and mandibular first and second premolars are most frequently affected by abfraction (Bernhardt et al. 2006) and the size, depth, and number of abfraction lesions are positively correlated with age (Levitch et al. 1994). It is believed that abfraction is a result of the lateral flexure of tooth cusps by way of lateral bulging resulting from vertical pressures and lateral loading from horizontal grinding pressures (Rees 2006). Oblique forces exerted on the inner inclines of buccal or lingual cusps in second premolars have also been shown to result in abfraction (Rees 2002). Through these biomechanical pressures, it is believed that the repeated subjection to large shear and tensile stresses at the occlusal surface results in a breakdown of the bonds between enamel crystallites in a structurally vulnerable region of the tooth (Bernhardt et al. 2006; Rees 2006). Although the biomechanics of abfraction are still debated, the contribution of occlusal loading to abfraction has been supported by several studies, including the study of German soldiers in high stress and low stress conditions, which demonstrated five times more abfraction in conditions of high stress, likely related to stress-related bruxism (Dawid et al. 1994; Bernhardt et al. 2006).

Although occlusal load certainly contributes significantly to abfraction, there is ample evidence to support a multifactorial aetiology, especially as abfracture predominantly occurs on the labial/buccal surfaces despite seemingly equal vestibular and lingual occlusal loads (Rees 2006). Some of these proposed aetiological factors include erosion, abrasion, salivary flow, and dental morphology (Bader et al. 1996; Lussi and Schaffner 2000; Rees 2006). Stress corrosion, the interaction of acid erosion and mechanical stress resulting in significantly higher wear rates than either of these mechanisms alone, may also contribute to abfraction or abfraction-like lesions, as indicated in a study by (Grippo and Masi 1991). However, more research is necessary regarding the distinction between different non-carious cervical lesions as their similarities in appearance make it difficult to tease apart these lesions, their mechanisms of development and their aetiologies (Sarode and Sarode 2013).

4.2.4 Dental Erosion (or Corrosion)

Dental erosion (or ‘corrosion’ in biotribology; Grippo et al. 2004; Zhou et al. 2013) is a chemical mechanism of dental wear resulting from an acidic oral environment that dissolves the mineral aspect of the tooth resulting in tissue loss. Acid erosion of the teeth will progress more quickly if the teeth are continually subjected to acidic foods and drinks without time for recovery and in the absence of dietary phosphate and calcium. These elements have been shown to prevent or limit dental erosion, as in the case of low pH, high calcium and phosphate yogurt (Lussi and Jaeggi 2006). If the dentition is given a significant break from acidic ingestibles after a limited time in acidic conditions, the saliva will also help the oral environment return to neutral pH balance and minerals in the saliva may help to remineralize the enamel and restore some of its hardness (Zhou et al. 2013). Erosive changes in the absence of dietary phosphate and calcium have been reported to reach depths up to 3μm from the surface of the enamel, resulting in both enamel softening and tissue loss (Amaechi and Higham 2001; Eisenburger et al. 2001; Wiegand et al. 2007; Cheng et al. 2009; Lussi et al. 2011). Following softening of the dental enamel, the tooth is more vulnerable to abrasion and attrition, contributing to more severe dental wear (Zheng et al. 2011; Zhou et al. 2013). Acid erosion usually results in the creation of smooth, shiny enamel surfaces with rounded cusps, smoothed edges and the scooped out/cupped appearance of exposed dentine (Lussi et al. 2011). This dentine cupping results from a lower tolerance of, or resistance to, acid attack in dentine than in enamel. Dental abrasion can also result in dentine cupping but it is usually shallower than dentine cupping caused by erosion. As a result, depth-to-breadth ratios of dentine exposures have been used to identify dental abrasion and acid erosion in archaeological teeth (Bell et al. 1998). In a study of this method compared to SEM methods of identifying erosion vs. abrasion, Kieser et al. (2001) determined that Bell's depth-to-breadth method was relatively accurate. However, it is still preferable to conduct SEM analysis, as erosion can be positively identified by the honeycomb-like pattern resulting from the faster dissolution of enamel rods than interrod enamel or the observation of "a mat of erosion products together with exposed dentinal tubules" (Kieser et al. 2001: 208). A new method for identifying dental erosion by quantifying shifts in the microstructure of the tooth surface through laser speckle image estimation analysis has also been proposed by Koshoji et al. (2015), though it has not yet been tested on archaeological remains.

4.2.5 Physiological Factors Affecting Dental Wear

As a result of the various mechanisms at work on dental surfaces, occlusal dental wear is a complex process that requires further investigation. This is particularly true with regard to factors that may affect rates of wear as they may impact the accuracy of related age estimates. Here I discuss the anatomy of the permanent molar crowns and the many physiological factors that have the potential to affect dental wear rates and pattern in human teeth, from macroscopic- to nanoscale-levels.

On a macroscopic-level, patterns of dental occlusion can have a significant impact on macroscopic dental wear patterns. Differences in dental occlusion can be seen early in life as a result of genetic, epigenetic, environmental and individual ontogenetic differences in orofacial and dental development. This may be apparent in populations such as the Yuenmendu Aboriginal people of Australia, whose occlusal pattern has a tendency toward 'alternating intercuspation', often starting early in life. This occlusal relationship is considered to be a form of malocclusion in orthodontics as the maxillary arch is wider than the mandibular arch, preventing alignment in a manner that allows maximal intercuspation simultaneously on both sides of the dentition (Barrett 1953; Molnar and Molnar 1990; Brown et al. 1987; Oxilia et al. 2018). This pattern of occlusion requires broader lateral chewing motions to most efficiently break down foods, resulting in a horizontal wear pattern (Brown et al. 1987; Molnar and Molnar 1990). This horizontal wear pattern is also said to be associated with hypsiloid-shaped maxillae, which similarly require broad lateral motions for efficient mastication against parabola-shaped mandibulae (Molnar and Molnar 1990). Alternatively, it may result in the use of a preferred side for mastication, thus resulting in asymmetrical dental wear across the dental arches (Oxilia et al. 2018). In contrast, hyperbolic- or parabolic-shaped maxillae more closely match the shape of the parabolic mandible and thus tend to result in oblique wear patterns (Molnar and Molnar 1990). However, as previously mentioned, the occlusal relationship between maxilla and mandible is governed by more than just genes, and is still poorly understood. This is evident in studies of the transition from edge-to-edge bite to overbite seen across a single generation in Australian Aboriginal and Inuit families who switched to softer, less abrasive diets (D'Amico 1961; Molnar and Molnar 1990). However, genetic and environmental influence on dental occlusion likely varies across populations, as Boyd (1972) reported the retention of an edge-to-edge bite in New Guinea Eastern Highlanders who subsist on a soft diet.

Dental occlusion can be characterized as group function type (where there are multiple contacts between the upper and lower during lateral movement) or canine-guided type (where the overlap of the canine teeth disengages the posterior teeth during lateral movement) (Abduo et al. 2015). In addition to the aforementioned differences in wear associated with dental occlusion, a clinical study has also shown a six-fold increased risk for abfraction in dentitions with a group function type of occlusion, as opposed to a canine-guided occlusion in which the posterior teeth do not occlude during the lateral excursion of the masticatory power stroke (Heymann 1998; Rees 2006). Increased risk of abfraction is also demonstrably correlated with tooth tilting or positional change, occlusal change, and inlay restorations, which may represent further evidence of the role of occlusal forces in abfraction (Braem et al. 1992; Rees 2002, 2006; Bernhardt et al. 2006).

It should be noted that despite their strong genetic links, dental occlusion patterns are not static. In fact, the size and shape of dental arches, as well as the inclination of the teeth, are known to change over time as a result of multiple forces from various masticatory muscles. For example, mandibular molars erupt with a mesiolingual tilt, which becomes more vertical over time (Van der Linden 1986; Kasai and Kawamura 2001). This change in inclination has been attributed to the effects of occlusal bite force and significant masticatory pressure on the buccal side of mandibular molars resulting from normal occlusion (Ishida and Soma 1993; Kasai and Kawamura 2001). Kasai and Kawamura (2001) have demonstrated that differences in diet, and thus bite force, can result in population-scale changes in dental inclination, which would affect dental wear pattern on a large scale. Tongue posture and movement have also been implicated in the change in dental inclination over time. It has been proposed that mandibular molars become tilted more vertically through lingual forces (tongue muscles), buccal forces (buccinator and masseter muscles), and occlusal forces (aka bite force) (Koc et al. 2010; Masumoto et al. 2001; Janson et al. 2004; Oxilia et al. 2018). Lingual tipping of the mandibular and maxillary incisors has also been observed in association with age and dental wear and has been attributed by some to be a result of asymmetrical forces exerted by the lips against the teeth (Selmer-Olsen 1937; Lysell 1958; Hylander 1977b; d'Incau et al. 2012). Similarly, it has been suggested that pressure exerted on the palate by the tongue may result in morphological change of the maxillary, vomer and sphenoid bones and thus changes in maxillary dental inclination (Brodie 1946; Fishman 1969; Kapoor et al. 1979; Rakosi 1978; Proffit 1978; Oxilia et al. 2018). All of these muscular pressures can result in either relatively symmetrical or asymmetrical changes in teeth and in bone, which in turn affect the pattern of dental occlusion and the dental wear plane, consequently changing patterns of dental macrowear (Oxilia et al. 2018; Kasai & Kawamura 2001).

In addition to changes in dental inclination, incorrect tongue posture or pressure (e.g. pathological tongue thrust [a common orofacial myofunctional disorder]), can cause tooth migration, prevent dental eruption, produce orofacial bone asymmetry, and/or result in occlusal loading asymmetry, further affecting dental wear patterns (Alghadir et al. 2015; Hiiemae and Palmer 2003; Hori et al. 2013; Palmer et al. 1997; Matsuo and Palmer 2008; Van Dyck et al. 2016; Oxilia et al. 2018). Bite force has also been shown to affect the dental occlusion in other ways, particularly with regard to dental arch form (Lundstrom 1925). In an experimental study of animals, Beecher and Corruccini (1981) observed a decrease in dental arch width in conditions of reduced masticatory function. In humans, Murphy (1964) described a positive correlation between dental arch width and dental wear over time, which was echoed by Smith and Bailit (1977) who noted the greatest increases in arch width at the molar teeth. Harris (1997) confirmed these findings and observed that the arch length decreased as the arch width increased. As previously mentioned, these changes and variations in arch shape, size and occlusion are associated with differing macroscopic wear patterns (Molnar and Molnar 1990; Kasia and Kawamura 2001; Oxilia et al. 2018). Other morphological variants and normal physiological forces acting on the dental occlusion patterns, and by extension on dental wear patterns, include: dental agenesis, hypodontia, supernumerary teeth, antemortem tooth loss, dental impaction, dental ankylosis, retention of primary teeth, orofacial morphology, diastema, continuous eruption, mesial shifting of the teeth, and dental crowding/spacing.

Dental crowding is associated with cumulative mesiodistal tooth dimensions exceeding the dental arch length. Efforts to pinpoint the morphological cause of this relative change have been inconclusive; some studies pointing to a shortened dental arch (e.g. Howe et al. 1983; Radnzic 1988; Niedzielska 2005), some pointing to larger mesiodistal tooth size (e.g. Puri et al. 2007; Poosti and Jalali 2007; Agenter 2008), and others indicating both tooth and arch size differentials between dentitions with and without dental crowding (e.g. Chang et al. 1986; Melo et al. 2001). Dental size discrepancy between the mandible and maxilla, otherwise known as the Bolton tooth size discrepancy, has also been hypothesized to have a role in malocclusion (Bolton 1958). However, these discrepancies have been seen to vary between the anterior dentition and the total dentition, and increased discrepancies are not always associated with malocclusion (e.g. Agenter 2008; Paredes et al. 2006; Fattahi et al. 2006). Tooth size discrepancies are still not well understood and have also shown differences between sexes and populations, further complicating matters (e.g. Uysal et al. 2005).

The contribution of dental metrics to dental wear rate and pattern extends beyond the aforementioned impacts on dental occlusion. Walker et al. (1991) argue that teeth with larger occlusal surfaces wear more slowly than those with smaller occlusal surfaces. Odontometric studies have shown significant differences in occlusal surface size between some populations and sometimes between males and females of the same population (cf. Schwartz and Dean 2005; Hanihara and Ishida 2005). Population-scale differences in tooth size have been attributed to genetic differences as well as environmental factors and functional morphology (Dempsey and Townsend 2001). Sex-linked genes and the resulting hormonal differences have similarly been indicated as possible factors affecting variation in tooth size between the sexes (Schwartz and Dean 2005; Guatelli-Steinberg et al. 2008). Generally, male permanent molars and canines are larger than their female counterparts in modern human populations (e.g., Garn et al. 1964, 1967; Alvesalo 1971; Townsend 1979; Harris and Bailit 1987; Harris and Hicks 1998; Mayhall and Kanazawa 1989; Kieser 1990). This difference in tooth size has been variously attributed to larger proportions of dentine (Harris and Hicks 1998; Stroud et al. 1994, 1998) or enamel (Moss and Moss-Salentijn 1977; Moss 1978; Alvesalo et al. 1987).

The first line of defence against dental attrition is the enamel cap, which protects the dentine and, ultimately, the pulp. Humans are known to have relatively thick enamel compared to other non-hominin primates (Molnar and Gantt 1977; Martin 1985; Shellis et al. 1998). Observations of modern and fossil *H. sapiens* dentitions show that a reduction of dental size over time has resulted in a greater reduction of the coronal dentine compared with the enamel. As a result, modern *H. sapiens* have relatively thicker enamel than fossil *H. sapiens* (Martin 1985; Olejniczak et al. 2008; Suwa et al. 2009; Smith et al. 2012). This disproportionate reduction of coronal dentine along with tooth size can also be used to explain the increasing enamel thickness from first to the third maxillary molars in humans (Grine 2002, 2005; Smith et al. 2005, 2006, 2012), although some researchers believe it to be a result of functional morphology accommodating for increasing bite force toward the posterior of the dental arcade (Mansour and Reynick 1975a,b; Molnar and Gantt 1977;

Molnar and Ward 1977; Ward and Molnar 1980; Osborn and Baragar 1985; Koolstra et al. 1988; Macho and Berner 1993, 1994; Schwartz 2000a; Smith et al. 2008, 2012; Mahoney 2010, 2013). However, this hypothesis has been called into question since mandibular molars do not show the same significant increase in enamel thickness from M1 to M3 (Schwartz 2000a; Kono et al. 2002). Following the observation that enamel thickness appears to correlate with the developmental timing of molar cusps and DEJ shape (which is related to the timing of cusp initiation), it has been argued that differences in enamel thickness across the molar crown are affected both by morphogenetics and functional morphology (Kono et al. 2002).

The effects of functional morphology can perhaps be seen in differences in enamel thickness related to dietary differences among extant hominoids (Molnar and Gantt 1977; Martin 1985; Schwartz 2000a; Smith et al. 2008, 2012). Several researchers have noted that thin enamel tends to be associated with diets composed of relatively soft foods, while thicker enamel tends to be associated with diets made up of more abrasive, hard or fibrous foods (Jolly 1970; Molnar and Gantt 1977; Dumont 1995; Lucas et al. 2008; Pampush et al. 2013). However, like the aforementioned aetiology of differences in enamel thickness across molar positions, the relationship between diet and enamel thickness is complex and not yet fully understood (Dumont 1995; Maas and Dumont 1999; Schwartz 2000a,b; Teaford 2007; Le Luyer et al. 2014). Consequently, observed differences in enamel thickness between populations and between sexes within populations are likely a product of epigenetic factors, though these specific factors and their relative effects are still under investigation. Differences in enamel thickness have also been observed between individuals' upper and lower molars with greater enamel thickness usually seen in the maxillary molars (Grine 2002; Smith et al. 2006).

In addition to variations across species, dental arches, and molar positions, variations in enamel thickness across individual dental crowns have also been found to positively correlate with the mechanical forces to which they are subject. Given the intercuspal occlusal pattern often seen in the centric position of the dentition, it is not surprising that lingual maxillary molar cusps generally have thicker enamel than buccal maxillary cusps, and lateral mandibular cusps generally have thicker enamel than buccal mandibular cusps

(Molnar and Gantt 1977; Molnar and Ward 1977; Macho and Thackeray 1992; Beynon and Wood 1986; Macho and Berner 1993, 1994; Schwartz 2000a,b; Grine 2005; Mahoney 2010; Shillingburg and Grace 1973; Martin 1983; Kono et al. 2002). It is hypothesized that this variation in enamel thickness across the dental surface is an adaptation to severe dental wear on an evolutionary scale, allowing for further protection of the dental pulp in positions of maximum occlusal contact and force (Hlusko et al. 2004; Kelley and Swanson 2008; Pampush et al. 2013; Le Luyer et al. 2014).

As crown surfaces are not flat, dental morphology or topography may also be considered in relation to dental wear rates and patterns. On basic inspection, it is obvious that dental crown morphology will affect the pattern of occlusion and mastication early in life or in individuals with low levels of wear. This is apparent in the development of different dental wear facets for phases I and II of the masticatory power stroke, which show distinct differences in microtexture (Krueger et al. 2008). Given that the puncture and crushing process of mastication results in wear of the cusp tips (Krueger et al. 2008), the pattern of wear resulting from this type of mastication will also be dependent on crown morphology. The structural integrity of the tooth is also affected by dental morphology as the various cusps and grooves and fissures help to dissipate occlusal loads and tensile stress (Benazzi et al. 2013). This was demonstrated by Benazzi et al. (2013) as they found that the inclination of cusps fragmented and distributed occlusal forces, creating tensile stress in the concave regions between the cusps and reducing tensile stress at the thin-enamelled cervix. They also observed that the protostylid played a disproportionately significant role in this process and hypothesized that the expression of the protostylid would have a direct effect on the distribution of forces within the tooth, and thus the tooth's vulnerability to fracture (Benazzi et al. 2013). The direction of occlusal force on cusps has also been shown to produce differences in stress distribution throughout the tooth, as oblique forces acting on cusps result in significantly higher tensile stress at the CEJ than vertical forces, and are likely linked to abfracture (Rees 2006).

One of the more complex patterns of dental wear often observed with severe attrition and/or abrasion, is the so-called helicoidal wear pattern (Ackerman 1953; Richards and Brown 1986). This pattern is characterized by a change in the slope of dental wear from M1 to

M3. Differences in occlusal alignment at each molar have been suggested as contributors to helicoidal wear (Campbell 1925; Richards and Brown 1986), as well as the axial tilt of the molars (Smith 1986). Dental developmental pattern has also been indicated as a factor that may contribute to helicoidal wear as the first molar is worn severely in a direction opposite to the natural curve of Monson, the second molar is less severely worn resulting in a flat plane of wear, and the third molars retain their natural curve of Monson (Macho and Berner 1994). Of course, dental developmental timing and sequence can vary between sexes and populations, and consequently may cause variation in dental wear patterns (Miles 1962). Differences in enamel thickness across the dental arches and across dental crowns have also been implicated in the aetiology of the helicoidal pattern of wear (Macho and Berner 1994; Schwartz 2000b).

Another macroscopic physiological factor that affects dental wear is salivary flow. Salivary flow has been demonstrated to have an extremely important role in dental wear as a liquid lubricant. In experimental studies comparing simulated mastication with and without a liquid lubricant, dental wear was shown to progress at a greatly increased speed even at low occlusal loading forces without liquid lubricant (Li and Zhou 2001; Zhou et al. 2013). This liquid lubricant is said to have a role in both friction reduction as well as thermoregulation at the dental surface, and it is also known to regulate the oral pH level (Li and Zhou 2001; Zhou et al. 2013). As such, the amount of saliva produced by an individual, which is known to vary throughout the day and across the dentition, may have an impact on dental wear rates and patterns. In fact, the lower salivary flow associated with nighttime (or specifically during sleep) is believed to contribute to the significant wear seen in nocturnal bruxists (Li and Zhou 2001; Zhou et al. 2013).

Moving from the macroscale to the microscale and nanoscale, it is necessary to consider the wear-resistance of dental microstructures and their potential impact on dental wear rates and patterns. Beginning with the basic microstructure of dental enamel, we first consider the orientation of enamel rods (or prisms) on dental wear resistance. Although enamel rods tend to abut the dentino-enamel junction approximately perpendicularly, this relationship varies slightly in relation to their proximity to the cervico-enamel junction, as the angle between rods and the DEJ become more acute as they approach the CEJ (Simmons et al.

2011). Apart from this gradient in dental rod orientation across the crown, the ends of the rods at the DEJ and the enamel surface are generally perpendicular to these respective surfaces and provide wear resistance through this orientation. The middle of each rod, however, varies greatly as most rods take on wave-like patterns. The most complex rod morphology is seen in so-called 'gnarled enamel' in cuspal areas, where enamel rods seemingly intertwine, resulting in higher wear resistance (Simmons et al. 2011; Zhou et al. 2013). In contrast to the strong cusp enamel, subsurface enamel at the DEJ has been observed to have poorly organized, shortened rod structures contributing to very little gnarled enamel (Poole et al. 1981; Rees 2002). This enamel also generally has a lower mineral content, larger pores, and higher protein content, resulting in lower wear resistance compared to occlusal enamel. Subsurface enamel within 0.4mm of the CEJ has also been shown to be lacking enamel rods, and instead made up with morphologically indistinct crystallites (Kodaka et al. 1991; Poole et al. 1981; Rees 2006). Furthermore, the DEJ is poorly developed and weakened by a paucity of scalloping in the area of the CEJ (Spir 1988; Rees 2006). As such, enamel approaching the CEJ is distinctly less resistant to wear and fracture, as seen in abfracture, while enamel near the occlusal surface, particularly in cusps, is known to be more wear-resistant. However, minor differences in the positional relationship between dental wear and enamel rod orientation can result in differences in wear resistance as well as surface friction, and thus wear behaviour (Kaidonis 1995; Cuy et al. 2002; Zhou et al. 2013). Hypomineralized areas of enamel, such as enamel tufts or enamel lamellae, represent areas of less wear-resistant enamel (Cuy et al. 2002). These areas may be more vulnerable to microfracture under stress and erosion when exposed (Amizuka et al. 1992; Cuy et al. 2002). They may also contribute to additional surface friction potential, once exposed through dental wear, if not covered by secondary dentine.

The aforementioned enamel rods are long keyhole-shaped collections of tens of thousands of crystallites divided by interstitial (multiple) grain boundaries (Gordon and Joester 2015). The circular part of the keyhole, known as the 'head', is composed of the most densely packed highly organized crystallites, while the adjacent trapezoid-like 'tail' of the enamel rod is composed similarly but with less densely packed crystallites that rest at an approximately 60 degree angle to the head of the rod (Habelitz et al. 2001; Fincham et al. 1999). Surrounding these enamel rod structures is the enamel rod sheath, which is composed of slightly more disorganized crystallites (Habelitz et al. 2001; Fincham et al. 1999). Finally, these enamel rod sheaths are separated by interrod enamel with the most disorganized crystallites and more organic matter compared to the enamel rod (Habelitz et al. 2001; Fincham et al. 1999). Since dental erosion largely results in the dissolution of inorganic matter, differences in the rate of erosion are observed between enamel rods and interrod enamel. Specifically, the enamel rods erode at a faster rate, leaving a microscopic honeycomb-like structure of interred enamel on the enamel surface. This characteristic pattern of wear is perhaps the most reliable indicator of dental erosion (Meurman and Ten Cate 1996; Imfeld 1996).

Looking even closer at the composition of dental enamel, it is well understood that enamel is made up of 98% hydroxyapatite by weight, with the remaining 2% made up of organic molecules and water (Eastoe, 1960; Gordon and Joester 2015). The typical formula for hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$, however, this formula is vulnerable to elemental substitutions; particularly substitutions of Na^+ and Mg^{2+} for Ca^{2+} , Cl^- and F^- for OH^- , and (CO_3^{2-}) for hydroxyl or phosphate ions (Pan and Fleet, 2002; Gordon and Joester 2015). All of these elemental substitutions, apart from fluoride, result in increased enamel solubility and there is variability in enamel composition throughout the dentition. For example, in one study, it was found that the surface enamel contained over 96% mineral, while enamel abutting the DEJ contained less than 84% mineral (Robinson et al. 1995). As such, the enamel’s elastic modulus and hardness values also exist on a gradient with the highest values at the enamel surface and the lowest values at the dentino-enamel junction (DEJ; Meredith et al. 1996). These variations in the organic content of enamel rods have been shown to affect the fracture-resistance of the enamel (Simmons et al. 2011).

Through atom probe tomography (APT) on mouse incisors, Gordon and Joester (2015) have found that elemental substitutions of Mg, in particular, tend to accumulate at significantly higher concentrations within some multiple grain boundaries (aka the intersections of tissues separating crystallites within enamel rods). Gordon and Joester (2015) hypothesized that the elevated organic and carbonate content within this tissue, as well as the water that may allow for proton transport and removal of dissolved ions, may also contribute to an increase in solubility in the multiple boundaries. This finding explains the observed higher rate of erosion of multiple grain boundaries compared to that of crystallites in enamel rods (Gordon et al. 2015). Although the implications of this finding with regard to dental wear remains to be fully discovered or replicated in human dentition, many researchers have noted the importance of the interfaces between crystallites with regard to the mechanical and wear properties of enamel at the nanoscale (He and Swain 2008; Ang et al. 2010; Arsecularatne and Hoffman 2012; Yilmaz et al. 2013; Gordon and Joester 2015).

The composition, structure, and biomechanics of dental enamel have received most of the attention, because it is the first line of defense against dental wear and is assumed to set the pace of tooth wear due to its relative hardness. However, existing information about the dentino-enamel junction (DEJ) and dentine wear resistance suggests that the relationship between dentine and enamel is more complex than is obvious. The DEJ is a narrow but complex interface separating the enamel from the dentine with a multi-level microstructure made up of 25–100 µm scallops with 2–5 µm micro-scallops located within each scallop, and another nanoscale structure found within each micro-scallop (Hayashi 1992; Lin et al. 1993; Marshall Jr. et al. 2001; Marshall et al. 2003; Chan et al. 2011). Along this interface, collagen I fibres from dentine penetrate into enamel (Lin et al. 1993; Marshall et al. 2003; White et al. 2000; Chan et al. 2011). Likewise, amelogenins from enamel cross the DEJ to penetrate the dentine (Nanci et al. 1994; Chan et al. 2011). This interaction between enamel and dentine at the DEJ provides reinforcement for the junction and prevents the delamination of enamel from dentine on a nanoscale (Chan et al. 2011). It also results in a higher flexural strength of the DEJ than that in dentine, and only slightly lower than the flexural strength of the adjacent enamel (Chan et al 2011). Consequently, the DEJ provides an enamel-like protective barrier for the dentine that absorbs and redistributes occlusal forces (White et al. 2000; Chan et al. 2011; Rees 2006). The slight difference between the flexural strength of the DEJ and enamel, however, sometimes results in the initiation of microfractures spreading from the DEJ through the enamel (Bai et al. 2007). However, both DEJ and dentine fracture resistance are highly related to the hydration of the collagen, with experimental studies showing that well-hydrated collagen often stops the spread of fractures within the DEJ and dentine (Chan et al. 2011). Dentino-enamel junction (DEJ) shape has demonstrated significant variation among populations, particularly with regard to dentine horn height (Smith et al. 2006). These differences may significantly impact the structural stability of the tooth and could result in differences in the timing of dentine exposure through dental wear, if enamel thickness of the cusps is not proportionate to the height of the dentine horns.

Dentine is composed of around 70% mineral, 20% organic matrix and 10% water by weight on average (Xu and Wang 2012). The mineral content largely consists of carbonate-substituted hydroxylapatite. The organic matrix is made up of around 90% fibrous proteins (mostly type I collagen) and the remaining 10% is comprised of lipids and non-collagenous matrix proteins (Nanci 2003; Xu and Wang 2012). The layer of dentine adjacent to the DEJ is known as the mantle dentine and is characterized by the (near) perpendicular relationship between the dentine tubules and the DEJ, the relatively lower number of dentine tubules compared to the remaining dentine, and the high prevalence of canalicular branching from tubules (Mjor and Nordahl 1996; Goldberg et al. 2011). These branches fall into three categories. The terminal ends of the dentine tubule and acutely angled offshoots are considered to be major branches. Fine branches extend from the dentine tubule at a 45 degree angle at 1-2 μm spacing along the tubule. Finally, the smallest branches, microtubules, meet dentine tubules at a 90 degree angle (Mjor and Nordahl 1996; Goldberg et al. 2011). The mantle dentine is also characterized by lower mineral content, and is therefore more elastic in nature than the remaining dentine, facilitating the absorption and dissipation of occlusal forces and reducing the risk of enamel fracture and/or delamination from the DEJ (White et al. 2000; Marshall et al. 2001, 2003; Goldberg et al. 2011). Separating the mantle dentine and the circumpulpal dentine is the globular layer of dentine, which is the result of primary and secondary mineralization of dentine with complete crystalline fusion. This layer of dentine is peppered with interglobular dentine; arc-shaped areas of incompletely fused dentine that were only subject to primary mineralization (Chan et al. 2011; Goldberg et al. 2011). The effect of this layer of dentine on dental structure and wear, if any, has yet to be explored.

Circumpulpal dentine comprises the bulk of human tooth dentine and is characterized by a highly-organized structure and a higher mineral content than the mantle dentine or the predentine. The basic microscopic structures of coronal circumpulpal dentine are the s-curved dentine tubules, the surrounding peritubular dentine and the interstitial intertubular dentine. Coronal dentine tubules extend into the mantle dentine layer as less mineralized branches and into the predentine layer as newly-formed, more densely packed unmineralized structures with only microtubule branches (Fosse et al. 1992; Garberoglio and Brannstrom 1976; Ten Cate 1998). Root dentine tubules vary from coronal dentine tubules as they are less densely packed, terminate in a loop adjacent to the cementum and are associated with copious branches of all kinds throughout the diameter of the dentine. These differences in branch prevalence between root and coronal dentine dissipate near the CEJ as coronal circumpulpal dentine presents with significantly more fine branches in this transitional area (Mjor and Nordahl 1996).

Peritubular dentine is composed of carbonate apatite crystals and a small amount of collagen (Xu and Wang 2012). This highly-mineralized matrix is believed to materialize through the aid of an odontoblast process (a cytoplasmic extension of an odontoblast that remains at the boundary of the dental pulp) and dentinal fluid containing albumin, transferrin, tenascin, and proteoglycans (Linde and Goldberg 1993). As peritubular dentine is deposited along the internal surface of the tubule it results in the progressive decrease, and sometimes complete obliteration, of the tubule diameter over time (Nalbandian et al. 1960; Weber 1974). This age-related change in the structure of dentine tubules has not yet been investigated in relation to dental attrition or abrasion.

Intertubular dentine is composed of a dense network of collagen fibrils coated by non-collagenous proteins and plate-like crystallites, which align parallel to the collagen fibril axis. Crystallites are also found unattached to collagen fibrils with no preferred orientation (Wang and Weiner 1998; Goldberg et al. 2011). The elemental composition of intertubular dentine is believed to be similar to peritubular dentine but with a larger percentage of collagen and the association of carbonate apatite crystals with the collagen matrix (Weiner et al. 1999; Xu and Wang 2012). Given the higher mineral content of peritubular dentine, it is known to be extremely vulnerable to acid erosion. As such, acid erosion results in the dissolution of the peritubular dentine and restoration of the original diameter of the dentine tubule, while the intertubular dentine maintains its network of collagen fibrils (Kvaal et al. 1994; Wiegand et al. 2007; Xu and Wang 2012). Given the higher prevalence of tubules, and thus peritubular dentine, approaching the pulp, dentine closer to the pulp is more vulnerable to erosion than dentine near the DEJ.

Although peritubular dentine hardness has been found to be independent of location within the tooth, the hardness of intertubular dentine varies, with decreasing hardness approaching the dental pulp. Despite this gradient of hardness, intertubular dentine is consistently softer than peritubular dentine (Kinney et al. 1996). Studies of dentine stiffness have, however, produced varying results, but show that dentine generally has more flexural strength than enamel (Angker and Swain 2006; Chan et al. 2011; Ryou et al. 2011).

With regard to dental wear in dentine, an experimental study by Burak et al. (1999) demonstrated that although attrition/abrasion results in faster dentine loss than enamel loss at low occlusal loads, the rates of dentine loss are similar to enamel loss under higher occlusal loads. It is believed that the higher organic content and relative softness of dentine compared to enamel contributes to the difference in rates of wear at low occlusal loads, while the brittle nature of enamel results in disproportionately higher tissue loss through fracture at higher occlusal loads, more closely matching the tissue loss seen in dentine (Boyde 1984; Beynon et al. 1991; Khera et al. 1990; Macho and Berner 1993; Burak et al. 1999).

In addition to differences in attritive, abrasive and erosive tissue loss, Braden and colleagues (1966) have demonstrated piezoelectric properties in dentine resulting from dentine's high collagen content. This pressure induced electric charge is not seen in enamel. Grippo and Masi (1991) found a surface voltage of 0.4 on exposed dentine, which is enough to cause enamel mineralization, in an individual with severe bruxism. Although this phenomenon is not clearly understood, it has been hypothesized that organic acids may be attracted to, and repelled from, the surface, perhaps resulting in erosion of the dentine, if this voltage is found to cyclically increase and decrease (Rees 2006).

Following establishment of occlusion, secondary dentine is produced in the predentine layer by odontoblasts at the boundary of the pulp. Initially, a layer of 10 micrometers thick is deposited through continual secondary dentine production. Thereafter, production is reduced to 4 micrometers per day. This secondary dentine differs from primary dentine only in the S-curve of the tubules, as these curves become more accentuated in the relatively cramped space available adjacent to the pulp. Over time, however, the deposition of this dentine slowly reduces the diameter of the pulp chamber (Goldberg et al. 2011), thus increasing the dentinal area subject to dental wear, and perhaps slightly affecting the rate of overall wear. Dental pulp is usually unmineralized and composed of soft connective tissues, nerves and a vascular network (Goldberg et al. 2011; Linde and Goldberg 1993). As such, despite the possibility for a slight increase in the rate of wear at tooth levels including the pulp, its effect on the rate and pattern of dental wear is likely considered to be negligible and has consequently not been studied in this respect.

4.2.6 Pathological Factors Affecting Dental Wear

There are a number of pathological conditions that can significantly impact dental wear rates. For example, congenital dental defects such as *amelogenesis imperfecta* or *dentinogenesis imperfecta* dramatically increase the vulnerability of teeth to any type of dental wear. Malocclusion and/or abnormal temporomandibular joint form may also produce differences in the rate and pattern of wear (Owen et al. 1991). Dental crowding, dental morphology and dental agenesis may also affect dental wear rates and patterns. Derived dental defects, such as mulberry molars, fluorosis or enamel hypoplasia, as well as dental defects resulting from medication (e.g. mercury), may also cause variations in the enamel structure, compromise the structural integrity, and provide more friction potential (e.g. Ioannou et al. 2016). Pain and discomfort derived from injury or disease in teeth, jaw bones, masticatory muscles, or the temporomandibular joint also have the potential to affect the pattern of wear and the rate of wear. Conditions sometimes resulting from dental wear, such as tooth tilting, dental dislocation, infection and abscess (resulting from dental wear at a rate higher than the production of tertiary dentine), periodontitis, and antemortem tooth loss, may also contribute to changes in dental wear rate and pattern (Fiorenza and Kullmer 2013, 2015). Many of these conditions promote change in bite force or chewing side preference to avoid pain or discomfort, or to compensate for a loss of occlusal surface. Bruxism, also known as tooth clenching or grinding, may also be considered pathological and, although the aetiology is debated, may arise as a result of occlusal, physiological, genetic, or stress factors (Lavigne et al. 2008; Zhou et al. 2013; Deo et al. 2017).

Individuals with bruxism can apply mechanical loads of around 1000N for 30 minutes to 3 hours a day, as opposed to normal biting loads of 100-500N for 10 minutes a day. As a result, individuals with bruxism can have up to three or four times the vertical dental loss as individuals without bruxism (Gregory-Head et al. 2000; Zhou et al. 2013). Gastrointestinal disturbances such as habitual vomiting and acid reflux can also produce acidic conditions in the oral cavity resulting in the softening and more rapid wear of the dentition (Bartlett et al. 1996; Robb et al. 1991). In addition to these conditions, xerostomia, a condition defined by a lack of salivary lubrication, significantly raises the friction potential during mastication and bruxism and resulting in rapid dental wear. Xerostomia is often found in individuals of advanced age as well as individuals with salivary gland tumors, or those undergoing radiation therapy (Zhou et al. 2013). Also in seniors, temporomandibular joint degeneration or osteoarthritis can result in complex differences in dental wear relating to pain during mastication and/or a change in the occlusion (Richards 1990).

4.2.7 Other Factors Affecting Dental Wear

In addition to physiological and pathological factors, it is necessary to consider the many environmental and sociocultural aspects that can have an impact on dental wear rates and patterns. These include socioeconomic and culturally-prescribed differences in food accessibility, including differences relating to individual sex, age, or status. Work-related dental wear may also be apparent in individuals specializing in work requiring use of the teeth as tools (e.g. processing of sinews, to hold pipes, etc.) or those exposed to chemicals that may erode dental tissues (e.g. Ubelaker 1996; Kim et al. 2006; Molnar 2011). Purposeful bodily modifications, such as the use of labrets, can result in characteristic patterns of dental wear (e.g.Torres-Rouff 2003; Cybulski 2010; Molnar 2011). Likewise, some culturally-prescribed dental modifications created through dental wear can provide information about social status or ethnicity (e.g. Romero 1958; Burnett and Irish 2017). Of course, some forms of medical dental intervention, such as palliative tooth picking, also result in forms of dental wear and/or can contribute to differing patterns and rates of wear (e.g. Siffre 1911; Formicola 1988; Lukacs and Pastoro 1988).

In addition to these culturally-linked forms of dental wear, differences in food processing methods can have a significant impact on the rates and patterns of wear seen in the dentition (e.g. Chattah and Smith 2006; Watson 2008; Fiorenza et al. 2018). Dietary composition, a large contributor to dental wear, is also subject to limitations imposed by the environment, socio-economic status, and cultural taboos. The grit and acidity of accessible processed foods can thus vary greatly within and between populations, resulting in differences in dental wear (Brace 1962; Dahlberg 1963; Greene et al. 1967; Molnar 1971; Molnar et al. 1972; Turner 1979; Hinton 1982; Smith 1984; Macchiarelli 1989; El Zaatari 2008, 2010; Deter 2006, 2009; El Zaatari et al. 2011; Fiorenza et al. 2011; El Zaatari and Hublin 2014; Fiorenza 2015). Although oblique molar wear has been attributed to occlusal pattern (Molnar and Molnar 1990), early researchers related oblique molar wear to soft, less abrasive diets characteristic of agriculturalists (e.g., Brace 1962; Smith 1984; cf. Macchiarelli, 1989) and noted a paucity of this pattern of wear in hunter-gatherers with hard, abrasive diets (e.g., Dahlberg 1963; Molnar 1971; Smith 1984; Kaifu 1999; Deter 2006, 2009). More recently, this difference has been explained as a difference between predominantly attrition-related dental wear (in the case of soft foods where there is more tooth-to-tooth contact while chewing), and predominantly abrasion-related dental wear during chewing (in the case of gritty, hard and abrasive foods (Kaidonis 2008; Burnett et al. 2013; Le Luyer et al. 2014). Additionally, as a consequence of dietary differences and related masticatory patterns and timing, agriculturalists tend to wear their molar crowns more rapidly than their anterior teeth while the opposite has been observed in hunter-gatherers (Hinton 1981; Molnar and Molnar 1990).

The use of masticatories such as betel nut or papyrus can also result in dental wear-related changes to the dentition, as can certain dental hygiene habits such as tooth brushing or tooth picking, which can produce characteristic signs of wear (Alt and Pichler 1998; Burnett and Irish 2017). Consumption of significant amounts of mercury, tetracycline or fluoride, either through purposeful ingestion or through naturally higher prevalence in accessible foods, can also manifest in dental defects, rendering teeth more prone to mechanical and chemical wear (Zhou and Zheng 2008). See Figure 4.2 for a summary list of factors that affect dental wear. This list is not exclusive since our collective understanding of dental wear processes and their aetiologies continues to advance.

Figure 4.2. Factors Affecting Dental Wear

Physiological and Pathological Factors
- age (time subjected to dental attrition, abrasion and erosion)
- enamel thickness
- enamel defects due to physiological stress
- enamel defects due to congenital disease/disorder
- tooth size
- dental morphology
- tooth inclination
- elemental composition of enamel and dentine
- enamel rod or dentinal tubule orientation
- dentine tubule diameter, peritubular dentine thickness, open tubules
- dental eruption timing, sequence, or abnormalities (e.g. impaction, ankyloses, retention of primary teeth)
- hypodontia, dental agenesis or supernumerary teeth
- continuous eruption
- mesial shifting
- tooth dislocation/tilting due to severe wear and/or abscess
- (mal)occlusion
- dental arch size and shape
- dental crowding or spacing, including diastema
- bruxism
- paramasticatory functions
- salivary flow (often decreases with age)
- biteforce
- TMJ size and shape? (Owen et al 1991)
- TMJ degeneration? (Richards and Brown 1981a; Richards 1990)
- orofacial morphology (e.g. gonial angle, palatal clefting)
- position of the mental foramen in relation to the teeth? (Green and Darvell 1988)
- antemortem tooth loss
- dental/oral trauma
- pain/discomfort/disease in the orofacial bones, muscles, or dentition
- acid reflux, bulimia, pregnancy, or other conditions that lead to increased oral acidity
- arbitrary preference for a chewing side

Environmental and Dietary Factors
- toughness of diet (e.g. phytoliths)
- gritty inclusions from stone grinding grains
- gritty non-organic inclusions used to facilitate grain grinding
- wind-blown dust/sand
- enamel erosion (due to acidic foods/drinks, contact with acidic materials, certain medications)
- medications affecting salivary flow
- enamel defects due to fluoride/mercury/tetracycline consumption

Social and Behavioural Factors
- use of teeth as tools
- non-dietary masticatory habits (e.g. papyrus, betel nut, or tobacco chewing)
- abrasive dental hygiene habits
- medical interventions or use of prosthetics, implants, or inlays
- dietary taboos (sometimes age- or sex-specific)
- dietary differences according to socio-economic status and affordability
- sex-specific work specializations affecting dental wear
- structural violence
- abnormal wear caused by labrets or other jewellery
- abnormal wear caused by habitual pipe use
- abnormal wear caused by tooth picking
- abnormal wear caused by intentional filing, cutting, or drilling

4.3 Adult Age Estimation Methods through the Assessment of Dental Wear: Literature review

In 1870, Mummery was the first to publish a manuscript noting a relationship between dental caries and dental wear. He also conducted a cross-cultural study of modern crania, concluding that sand and grit included in foods from processing and preparation methods positively corresponded with the amount of dental wear, and deduced that this was likely the reason for higher rates of dental wear in ancient and 'primitive' populations. Furthermore, he attributed oblique wear to particular hard dietary staples, which piqued academic interest in the possibility of dietary reconstruction from dental wear analysis (Mummery 1870). Nine years after his seminal discussion of dental wear, Broca (1879) developed the first dental wear scoring protocol to facilitate and standardize further research into dental wear. This simple scale was embraced for the documentation of dental wear and used for comparative studies. Although he did not cite it, Ales Hrdlicka (1908, 1909) used this standard for the documentation of dental wear in his osteological appendices on ancient Native Americans (Rose and Burke 2006; Rose and Ungar 1998). Throughout his studies, Hrdlicka paid little attention to the contributions of dental wear to oral pathology or dietary reconstruction. However, he was the first to consider dental wear in the estimation of age-at-death, publishing age estimates to match Broca's dental wear levels in his laboratory guide (Hrdlicka 1939). He noted that this method of age estimation was only relevant to ancient Native Americans and recommended the use of recalibrated systems for other populations (Rose and Ungar 1998).

In 1950, Gustafson's (1950) multivariate dental method for adult age estimation was published. This method was developed through a study of a Swedish skeletal collection. It involved analysis of dental attrition, periodontal attachment, secondary dentine deposition within the pulp, apposition of cementum on the root surface, resorption of the root apex, and root transparency. It was critiqued for giving equal weight to each of the age indicators and many modifications of this method followed (e.g. Bang and Ramm 1970; Johanson 1971; Vlcek and Mrklas 1975; Burns and Maples 1976; Maples and Rice 1979; Monzavi et al. 2003; Maples 1978; Lamendin et al. 1992; Singhal et al. 2010), including software, called *Dental Age Estimation* (Willems 2000), which calculates many of the Gustafson-related equations when data are entered.

Through studies of dental wear in successive molars, Zuhrt (1955) was able to approximate the rate of occlusal wear in successive molars over a fixed period of time. Using these data, he created a standard for estimating age in a historic German skeletal collection and a Neolithic Nubian skeletal collection (Brothwell 1963a; Rose and Ungar 1998). Zuhrt's (1955) dental wear scoring method was published in German and received little attention despite providing the foundation for one of the most popular dental wear age estimation methods - the Miles method (1962, 1963, 1978). The latter method, originally developed for use on an Anglo-Saxon skeletal collection, is population-specific for age determination through the estimation of occlusal wear rate based on the calibration of occlusal dental wear against dental eruption timing. Although this method is recognized for its accuracy, it has not been fully embraced in practice because it requires a significant sample of subadults for reference, and it is time-intensive. Recently, however, Gilmore and Grote (2012) modified the Miles (1962) method by eliminating the need for subadult calibration. Despite the decreased limitations of the Gilmore and Grote (2012) method, in April of 2019 Google Scholar reported only 22 citations of the Gilmore and Grote method**.**

The success of the original Miles method may have been partially dampened by Brothwell's (1963a) publication of a less complex dental wear age estimation standard only a year after Miles' (1962) initial publication. This standard required only the comparison of patterns of dentine exposure to reference drawings in order to estimate age-at-death. It is now the most commonly used dental wear age estimation standard worldwide, despite the fact that it was originally created for the purpose of studying prehistoric to early medieval British human remains (Hillson 1996; Rose and Ungar 1998). Brothwell argued that there was little difference between Neolithic and Medieval periods, encouraging the use of this standard on all ancient British populations, but he cautioned against using it on non-British populations (Rose and Ungar 1998). Ubelaker, however, argues that this standard is diet- and culture-specific and therefore discourages its use on chronologically or geographically diverse populations (Rose and Ungar 1998). Not only is this indiscriminate usage questionable, given that dental wear is known to be affected by many biological and environmental factors (See Section 4.2), its use for prehistoric and early medieval British remains is arguable, given that the research on which this standard was based remains unpublished (Brothwell 1989; Hillson 1996).

More recently, Kim et al. (2000) presented a new population-specific age estimation method based on the macroscopic observation of occlusal dental wear patterns in a modern population. This method assigns scores to first and second premolars and molars according to counts of point-like wear facets, linear wear facets, surface-like wear facets, and band-like wear facets, and according to the ratio of worn to unworn occlusal surface, with further consideration of concavity of the worn surface. To calibrate a standard for age estimation, these scores must be taken on a large population sample and multiple linear regression analysis based on the method of least squares is used to evaluate the relationships between scores and known ages and an intercept. This culminates in the creation of sex-specific calculating tables for age estimation as well as calculation tables specific to certain age cohorts for cases in which the age can be estimated by other means. These calculating tables provide numerical values for each tooth wear score, which are then summed with the calculated intercept to provide an age estimate. Yun et al. (2007) later expanded this method to include scores for incisors and canines. Both the Kim et al. (2000) method and the modified method by Yun et al. (2007) have shown great accuracy and specificity, likely due to their population- and sex-specificity, as well as the multiple regression methods used in the creation of standards, however they are limited by their reliance on large samples and the need for observation of all teeth.

Some researchers remained skeptical of dental wear age estimation methods because of their vulnerability to cultural, environmental, and dietary habits as well as differences in dental morphology. As a result, a significant number of researchers opted to record dental wear for comparative studies without age estimation. For example, between the publication of the works of Zurht (1955) and Miles (1962, 1963, 1978), Murphy (1959a) published a standard for recording dental wear based on a study of Australian aboriginal dentition housed in the University of Adelaide's Department of Anatomy and the South Australian Museum. This standard utilized 8 attrition levels (labeled a to h) that grouped incremental patterns of dentine exposure without relating them to chronological age. In this same year, Murphy (1959b) published the ‘modal forms’ of dental wear observed in this same collection of human remains, which illustrated variations in patterns of dentine exposure. Molnar (1971) later modified Murphy’s (1959a) scoring method by adding separate scores for facet orientation and the form of the worn surface relative to crown height, wear shapes, and wear planes. Although these methods have been used widely for the recording of dental wear, neither of them were designed for the estimation of age. Two more methods for the macroscopic documentation of dental wear, namely the Scott (1979) and Smith (1984) methods, have since gained popularity, having been included in Buikstra and Ubelaker’s (1994) *Standards for data collection from human skeletal remains.* Both of these dental wear scoring systems were also based on written descriptions of occlusal wear patterns, as in the Murphy (1959a) standard, but the stages of wear are more specific and are supplemented with reference drawings. The Scott (1979) system requires the recording of occlusal wear for each molar quadrant, resulting in dental wear scores between 0 and 40 for each molar, with 0 being unobservable. The Smith (1984) method assigns scores from 0 to 8 for each tooth using different scoring characteristics for incisors and canines, premolars, and molars, with variations in their appearance demonstrated in the provided reference drawings. These methods of macroscopically recording occlusal dental wear have been used extensively for the comparison of dental wear between individuals and populations, often in conjunction with dietary reconstruction studies.

In addition to the aforementioned methods for documenting dental wear and estimating age through macroscopic observations of dental wear, several other more technologically advanced macroscopic methods have been presented for use. These include the measurement of cusp height (e.g. Tomenchuk and Mayhall 1979, Molnar et al. 1983a,b), crown height (e.g. Van Reenen 1982), and a combination of crown height and angle of wear (e.g. Walker et al. 1991). Unfortunately, these methods are somewhat subjective as they either assume a standard original cusp height or require the researcher to estimate the original crown or cusp height in order to contextualize their results. Another method that has received some attention is the measurement of occlusal tooth surface, wear facets or dentine exposures using a planimeter (e.g. LeBlanc and Black, 1974; Walker 1978). Richards and Brown (1981b) reported the percentage of exposed dentine in relation to the occlusal surface through the use of a digitizer that was programmed for use as a planimeter.

Digital photogrammetry was introduced to the 3 dimensional measurement of dental wear by Teaford (1983), who measured the area of exposed dentine based on the length, width and depth of the planes of dentine exposures in macaques and langurs. A variety of 2- and 3-dimensional methods for the measurement of occlusal wear facets and dentine exposures have since been investigated, (e.g. Smith 1984; Richards 1984; van der Bijl et al. 1989; Lambrechts et al. 1989; Teaford and Oyen 1989; Richards 1990; Richards and Miller 1991; Kambe et al. 1991; Krejci et al. 1994; Mayhall and Kageyama 1997; Kaifu et al. 2003; Kullmer et al. 2009; Deter 2006, 2009). One promising method is a 2D photogrammetric method developed by Phillips-Conroy et al. (2000) for baboons and later applied to humans by Deter (2006, 2009). With a nod to Richards and Brown's (1981b) calculations of relative area of exposed dentine, the method by Phillips-Conroy et al. (2000) uses the NIH-provided photogrammetry program, IMAGE v.1.5.7. to determine areas within outlines of the dentine exposures and occlusal surface, respectively, to determine the percentage of occlusal dentine exposure. Deter's (2006, 2009) method similarly uses image analysis software to determine the relative area of dentine exposure in relation to occlusal surface through the precise measurement of pixels within these respective areas through the use of SigmaScan Pro 5. These methods were based on dentine exposure because they are more visible in photographs and amenable to quantification than wear facets (Hillson 1996). Due to the precision of this method and its applicability to the photographic dental occlusal data available from prior studies of the Kellis 2 population, the Deter (2006, 2009) method was chosen for use in the current study, however, the free online FIJI(is just imageJ) software was used instead of SigmaScan Pro 5.

4.4 On the Aetiology of Dental Wear in Ancient Egypt: A History of Scholarly Viewpoints

The development of dental wear in ancient Egyptian dentition has been noted in paleopathological contexts at least since the description of a mummy from Stuttgart in Dr. Storr's (1780) tome on the classification of mammals, *Prodromvs methodi mammalivm*. Blumenbach (1794) later commented on his observation of this "extraordinary phenomenon" in several ancient Egyptian dentitions. Although Blumenbach described the dental attrition as "considerably worn away at that edge which is usually sharp" (1794:

184), Mummery (1870) claims that Blumenbach incorrectly believed the shape and size of the teeth affected by severe wear were morphological variants. Mummery posited that "[he has himself observed this condition] in many Egyptian skulls; recognizing it, however, as undoubtedly the result of severe attrition" (1870: 6) and states that he recorded the estimated age of individuals along with dental wear, perhaps according to the 4 stages of dental wear outlined by Broca (1879)[1], though he does not provide further details. In any case, it seems that Mummery was correct in pointing out the tendency to misdiagnose severe attrition in ancient Egyptians, as Dr. William Rushton demonstrated at the end of his 1910 paper on a clinical case of severe attrition. In his last paragraph, he lamented the repeated misdiagnosis of severe attrition as caries in a collection of ancient Egyptian skulls on display at the Royal College of Surgeons Museum (Rushton 1910).

In 1913, Turner and Bennett published their observations on 26th-30th dynasty ancient Egyptian remains excavated by Sir Flinders Petrie for Karl Pearson. In this study, Turner and Bennett noted that the rate of caries was three times lower than that in modern populations and attributed this paucity of decay to a higher rate of attrition from rough bread and lower consumption of refined carbohydrates (Rose and Burke, 2006).

Not long after this, following Sir Marc Armand Ruffer's death, his wife, Alice Ruffer, completed and published several of his unfinished papers, including "Study of Abnormalities and Pathology of Ancient Egyptian Teeth" (Ruffer 1920). This paper contains the first large-scale study of dental wear in ancient Egyptians. Within it, he describes the macroscopic process of dental wear in detail, referring to Broca's (1879) levels of wear, and expanding this with descriptions of the shapes and locations of exposed dentine throughout the dental wear process, noting the gradient of wear's accordance with the pattern of dental eruption. He discusses oblique and abnormal wear patterns and attributes differences in normal occlusal dental wear to 1) the nature of the food, 2) dental tissue density, and 3) the character of the bite. He refers to Smith and Wood-Jones' (1910)

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[1] Broca's (1879) stages of wear: 1) wear without dentine exposure, 2) disappearance of cusps and some dentine exposure, 3) complete dentine exposure, 4) wear extends beyond the crown to the root

hypothesis regarding the perceived change in the shape of dental wear in Nubians over time – namely, that predynastic Nubian occlusal wear appeared to be level, while more modern Nubians tended to form a deep cavity in the center of the occlusal surface that they attributed to wear. In response, Ruffer stated that "the study of several hundreds of skulls did suggest that the teeth were ground down evenly in some cases, whereas in others attrition was characterized by the formation of deep cavities, surrounded by a ring of strong dentine" (Ruffer 1920: 352). This description sounds similar to dentine cupping, discussed further in section 4.2.4.

Ruffer (1920) noted that dental wear severity differed greatly across populations with predynastic Upper Egyptians among the most severely affected and Greco-Roman Period Lower Egyptians among the least affected. Referring back to dental wear in ancient Egyptians, he states that although there is great variation among individuals, "the impression gained from the examination of hundreds of skulls is that attrition in these people proceeded very rapidly up to the age of 25 or so, and then became almost completely arrested. This is not in accord with observations on other peoples" (Ruffer 1920: 353). Ruffer attributed this rapid dental wear to a mixed diet with plenty of vegetables and coarse bread, arguing that "to explain the attrition it is not necessary to assume, as has been done, that the Egyptians ate earth or that the food was contaminated with sand" (Ruffer 1920: 356). As a result of this potential for variation in rates of dental wear, he cautioned against the early paleoanthropological attempts to estimate age based on unknown dental wear rates.

Further investigation into the aetiology of the significant dental wear seen in ancient Egyptians was conducted by Leek (1972) in his examination of "Teeth and Bread in Ancient Egypt". Leek's focus on the role of bread in dental attrition was derived from the apparent importance of bread in the ancient Egyptian diet, as indicated by ancient Egyptian writing, accounting, iconography, and funerary customs. Leek (1972) questioned the accepted notion that fibrous foods were largely responsible for severe dental wear, highlighting the fact that there is great variability in dental wear among populations eating hard and fibrous foods. In an effort to examine the composition of the bread consumed regularly by ancient Egyptians for clues to the aetiology of dental wear, Leek conducted petrographic and radiographic studies of inorganic residues from ancient Egyptian bread samples. These studies revealed a wide variety of rounded mineral and stone fragments, like those found in desert sand, as well as angular stone grains perhaps derived from food processing methods. Through archaeological research, Leek (1972) thus determined that gritty inclusions in bread may have included: 1) soil from harvesting, 2) fragments from harvesting tools, 3) wind-blown contaminants introduced during winnowing, 4) dirt or container fragments from storage, 5) fragments from grinding stones, 6) grit added to aid in the grinding of the grain, and 7) surface or near-surface inclusions obtained during baking, and 8) wind-blown sand contamination after baking or during consumption. He concluded that "it became quite evident that the abrasive particles found in these samples of bread would more than account for all the attrition to be seen on the teeth in ancient Egyptian skulls, so much so that further investigation was clearly unwarranted" (Leek 1972:132). This hypothesis on the aetiology of ancient Egyptian dental wear has been time honoured.

Puech et al. (1983) published the first attrition study using dental microscopy which supported Leek's hypothesis. In it the authors posited that the microscopic proximal wear in the observed Egyptian sample was similar to that documented in European *Homo erectus* and *Meganthropus*, thus indicating that ancient Egyptian dental wear was likely a result of strong occlusal pressures and foods resistant to mastication. Unfortunately, it is unclear what, if any, other reference samples were included for comparison with these microscopic observations of ancient Egyptian dentition – an important consideration given the early stage of dental microwear studies at the time. In any case, following from Dixon's (1972) paper on masticatories in ancient Egypt, Puech et al. (1983) pointed out that in addition to the consumption of fibrous or hard foods, habitual chewing of tough, fibrous, and phytolith-laden papyrus may have also contributed to dental wear rates. They also hypothesized that the preservation of foods in salt may potentially have contributed to some dental attrition (Puech et al. 1983).

4.4.1 But what about Dental Erosion?

Although the significant dental wear in ancient Egyptians has long been attributed to increased attrition, little attention has been paid to the possible role of dental erosion in overall dental wear of the past, despite our modern understanding of the interaction between chemical and mechanical forces in dental wear (Coupal and Soltysiak 2017). For example, recent dental tribological analysis demonstrated significantly higher wear volumes in dental tissues immersed in citric acid solution (pH = 3.2) than those immersed in artificial saliva (pH = 7) under lower normal mechanical loading levels. It was determined that the enamel's wear resistance decreased significantly in the citric acid solution and the wear mechanism changed to mainly adhesion delamination. The enamel wear in citric acid solution rose to around 2.5 times higher than that obtained in the artificial saliva under a mechanical load of 10 N, and this difference dissipated with increasing mechanical load (Zheng et al. 2011; Zhou et al. 2013). Since the normal mechanical load for human mastication ranges from 3-36 N (Dowson 1998), and there was a significant increase in dental wear in citric acid solution at 10N and 20N mechanical loads, with a non-significant increase in citric acid solution at a 40N mechanical load (Zheng et al. 2011; Zhou et al. 2013), it may be assumed that acid erosion will have a significant impact on dental wear in most human dentition. Given this, it is worth considering the impact of dental erosion on ancient Egyptian dental wear.

In archaeological remains, erosive wear has been identified as "a shiny glazed surface with rounded edges, followed by crown height loss with flattening of occlusal enamel and 'cupping' or 'scooping' of dentine, if exposed (Jager 2015)" (Coupal and Soltysiak 2017). These characteristics have been implicated in several archaeological studies worldwide (e.g. Cruwys & Duhig 1993; Kieser et al. 2001; Lanigan & Bartlett 2013; Richter and Eliasson 2016) and are commonly found within ancient Egyptian dentition. Although dentine cupping, or concavity of exposed dentine, is common in ancient Egyptians, it is rarely noted in relation to dental erosion as it has also been attributed to the effect of severe attrition on a material (dentine) that is less dense than enamel and higher in organic content (Coupal and Soltysiak 2017). The investigation of dental cupping deserves consideration as it is very prevalent in hunter-gatherers and fossil hominins who relied on abrasive food staples and had a higher ratio of vertical-force mastication (for crushing) to horizontal force mastication (for grinding) than in modern populations (Deter 2006, 2009; Jungers and Kaifu 2011; Kaifu et al. 2005; Kieser et al. 2001; Scott et al. 2012; Smith 1984; Coupal and Soltysiak 2017). Similarly, Ganss et al. (2002) found that in a comparative study of populations with abrasive, acidic, and intermediate diets, dentine cupping was most prominent in the population with the abrasive diet, followed by the population with the acidic diet, with little dentine cupping in the population with the intermediate diet. As such, Coupal and Soltysiak (2017) recommend that occlusal dentine cupping should be used as an indicator of both mechanical and erosive wear, or mechanical wear alone, but not as a sole indicator of erosive wear. Furthermore, Ganss (2008) cautions against the use of dentine cupping alone as an indicator of dental erosion, as there may be significant variation in lesion shape as a result of dental erosion. Consequently, it is recommended that dental erosion is identified through a three-step protocol, which includes SEM analysis of occluding and non-occluding surfaces of the teeth, taphonomic studies of the associated bone and soil pH, and dietary research to investigate possible causative agents for erosive dental wear (Coupal and Soltysiak 2017). As SEM analysis and taphonomic studies are not possible within the scope of this study, the following research focuses on the ancient Egyptian dietary components that may have contributed to dental erosion.

Dental enamel has a critical pH level of 4.5-5.5, depending on the slightly variable elemental composition of the hydroxyapatite, below which the enamel begins to demineralize and soften, enabling more severe attrition (West and Joiner 2014; Axelsson 2000). It is, however, important to note that the acidity in the oral cavity is only changed for a few minutes following consumption of beverages, but generally remains acidic for longer when chewing acidic foods (Zhou et al. 2013). Of course, constant sipping of acidic beverages throughout the day may still result in higher rates of dental wear than that achieved through the consumption of acidic foods**.** Although it is difficult to ascertain the amount of each food or beverage consumed by individuals in ancient Egypt, which certainly differed relative to socioeconomic status, geography, time period, agricultural yield, and personal preference, we may begin to understand the erosive potential of the ancient Egyptian diet by analyzing the acid-base values in the available foods. Through a brief study of ancient Egyptian foods in literature, iconography and archaeology, the following foods were identified as having erosive potential (i.e. their pH surpassed the critical pH for enamel**)**: wine, grapes, raisins, vinegar, pomegranates, plums, honey, onions, pickled foods, fermented milk products, and other fermented foods, such as beer. Some modern types of breads (e.g. sourdough bread) also exceed the erosive threshold for enamel (cf. Wieschebrock et al. 2011; Yağmur et al. 2016), and thus further studies are recommended to determine whether ancient Egyptian bread should also be included in this list.

Given the importance of bread and beer in ancient Egypt, it is perhaps reasonable to assume that most individuals, including older infants and children would regularly have consumed these dietary staples. Thus, it is important to understand the characteristics of these particular foods and their effects on the dentition. Although Coupal and Soltysiak (2017) state that "it is not possible to determine the concentration of erosive or non-erosive components from artefacts" (based on McGovern et al.'s [2004] inconclusive studies of pH, phosphate and calcium concentrations in wine vessels from Jiahu, China), a number of researchers have successfully extracted and/or analyzed the components of food from artefacts (e.g. Swift 1966; Copely et al. 2001; Evershed 2008). Biomolecular analyses of ancient foods may be particularly worthwhile in Egypt as ancient Egyptian funerary food offerings abound and are often exceptionally well preserved. Of course, challenges are inherent in the comparison of modern and ancient foods. For example, many ancient wines were likely more acidic than modern wines as a result of difficulties sealing ceramic vessels. Consequently, the storage of wine in an aerobic environment would enable the transformation of wine into vinegar, essentially introducing acetic acid to the wine (McGovern 2003). This revelation demonstrates that although many aspects of food production and storage may be assumed to be similar in modern and ancient foods, experimental archaeology has the potential to uncover unsuspected differences and to contextualize biomolecular findings. For example, in a study of archaeological samples, Swift (1966) determined that ancient Egyptian beer residue from the 18th dynasty tomb of Princess Meryet-Amun, daughter of Thutmoses III, had a pH of 3.4, and ancient Egyptian bread from the tomb of Mentuhotep II had a surprisingly low pH of 4.2. Of course, these pH levels are problematic as the presence of water and/or alcohol would have affected this pH level in the pre-desiccated products. Moreover, Swift (1966) determined that the aforementioned beer residue that produced a pH level of 3.4 had experienced a secondary infection and became sour before desiccation, thus producing this low pH level. Unfortunately, the pH level of the comparative beer sample from the tomb of Wah was not disclosed.

Although the pH of ancient Egyptian beer remains unknown, Swift (1966) conducted a productive experimental archaeological study in which his attempt to replicate ancient Egyptian bread revealed that ancient Egyptians had intentionally soured their bread before baking. Although Swift's bread was not considered to be a perfect replica of ancient Egyptian bread, as it did not appear quite as raised and had more residual sugar than the originals (meaning that the replicas may not have been sour – or acidic - enough prior to baking), it still had a relatively acidic pH level of 4.9. Through further investigation of the ancient bread, revealing undetectable levels of yeast in the body of the bread, it was hypothesized that the bakers used a "white sour sponge to sour and give character to the finished bread"[2] (Swift 1966: 218) as well as a small amount of wheat to maintain the sour culture. Although Swift (1966) did not report the pH levels for the two other ancient bread samples he examined, he noted that one of the 18th dynasty samples differed significantly from the others, as it was sweeter and more akin to a Danish pastry than bread. This observation is germane as pastry dough is considerably less acidic than sour bread and if the ancient Egyptians did not differentiate between sweet and sour "breads" as their staples, then the regularity of sour bread consumption, and thus the regularity of the consumption of breads that may cause dental erosion, may be further questioned.

"Shamsy" bread (translated sun bread) is the type of bread most commonly baked in Upper Egyptian homes (El-Gendy 1983). According to El-Gendy (1983), it was a simple recipe with few ingredients made similarly to common Western sourdough breads. The dough is composed of a mixture of wheat flour and water with a sprinkle of salt and the addition of the 'starter'. This 'starter' is a mixture of around 100g of dough from a finished batch of

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[2] The final version of a sourdough starter is called a "mother sponge" (Corsetti 2012), thus shedding some light on Swift's (1966) hypothesis.

bread with 2 kg of flour and 0.5L of water. It is left in a warm location overnight to ferment and sour (El-Gendy 1983). As in modern sour dough breads, the use of a naturally fermented 'starter' precludes the use of manufactured yeast, otherwise known as 'bakers' yeast' (Corsetti 2013). This is reminiscent of the yeast-free body of the ancient Egyptian bread. In the morning, the starter is hand mixed with the other bread ingredients for about 25 minutes before it is left for around 45 minutes to rest. It is then divided into loaves on boards covered with wheat bran, which is also reminiscent of the traces of wheat found in the ancient bread samples that Swift (1966) stated was necessary to maintain the sour culture. The loaves are again exposed to the sun for around an hour, depending on the strength of the sunshine, to further ferment and rise, after which they are flattened by hand to about 20 cm in diameter and placed back in the sun for around the same time. El-Gendy (1983) documented an additional phase: "After reversing the dough pieces, they are left in the shade for 30 min as a fourth fermentation period." (El-Gendy 1983: 366). However, modern sources from Luxor indicate that the latter phase is omitted and that the dough is often left in the sun until baking. The final stage of the process is the baking of the dough in a clay oven. In its' processing, shamsy bread is similar to modern sourdough bread, however it is not left to ferment as long as sourdough bread and is therefore not as sour or acidic as sourdough, which has an approximate pH level of 3.8-4.6 (Corsetti 2013). Given the low pH of the ancient Egyptian bread tested by Swift (1966), it is possible that ancient Egyptian bread more closely resembled sourdough bread. Although they would provide only a rough comparison, pH tests of popular Egyptian manufactures breads, shamsy bread, sourdough bread, and ancient Egyptian bread samples would be informative. Continued experimental research on this problem is to be encouraged as it may reveal information that could explain changes in dental wear patterns over time in Egypt. For example, in El-Gendy's report, less than 10% of the Egyptian national bread consumption in 1983 was Shamsy. Most modern Egyptian manufactured breads are sweeter and less acidic due to their inclusion of manufactured yeast, thus having an approximate pH level of 5.3-5.8 (Corsetti 2013). If we assume that ancient Egyptians predominantly consumed shamsy-like bread, or a more fermented sourdough-like bread, this decrease in the popularity of acidic breads, beer and wine over time may, at least partially, explain the differences in ancient and modern Egyptian dental wear. Of course, past populations may have also consumed more bread than today's population due to increased acculturation of food types internationally**.**

Although studies of pH levels can provide a general idea of the erosive potential of foods, other elements present in foods and beverages, such as calcium (Ca), potassium (K), and fluorine (F), also affect the solubility of enamel's hydroxyapatite (Larsen and Nyvad 1999). For example, in a study of the erosive potential of yogurt, Lussi et al. (2012) demonstrated that if the Ca, K and F concentration of a product is higher than that in plaque fluids, dental erosion is prevented. As such, future research on the composition of ancient Egyptian breads and beer residues are required to ascertain their true erosive potentials. Regardless, caution should be exercised in attributing any single mechanism of dental wear given the complexity of the interacting intrinsic and extrinsic variables previously discussed (see Johansson et al. 1993). Determination of causality of dental wear can be further complicated in older individuals as a result of antemortem tooth loss and severe dental wear.

4.5 Materials and Methods

4.5.1 Materials: Digital Dental Photos from Kellis 2 cemetery

Dakhleh is one of five Oases or huge depressions, located in Egypt's Western Desert (Tocheri et al. 2005). Studying the past human-environmental interactions in Dakhleh has been the research theme of the Dakhleh Oasis Project (D.O.P.) since its inception in the late 1970s (Molto 2001). Analysis of human skeletal remains is the domain of the Bioarchaeology Team which since the early 1990s has focused on cemeteries associated with the ancient centre of Kellis which is located in the central part of the Dakhleh Oasis. Kellis, was a small but important urban centre (Bagnall 1993), which was occupied from Ptolemaic to late Roman times. It was abandoned circa 450 A.D. (Hope 2001; Bowen 2003). At its zenith in the late 3rd century A.D. Kellis may have housed over 2000 people (Molto 2001). In the 1st century A.D. the people of Kellis gradually endorsed Christianity and shifted their burial program from family crypts carved into the hills northwest of Kellis called Kellis 1, to a large cemetery slightly northeast of Kellis proper called Kellis 2 (Molto

2001). The K2 burials were single, extended interments, oriented east-west, with heads to the west; the Christian position. The exact orientation of the burials however varies slightly according to the seasonal solar alignment (Williams 2008). AMS radiocarbon dates (N =42) show that K2 was in use from AD 50-450 (Stewart et al. 2003), and was organized by family groups around superstructures (Molto 2001, Keron 2015). Since the beginnings of excavations in the early 1990s, over 700 usually well preserved skeletons have been excavated and analyzed, of the 3000-4000 burials estimated to be interred in K2 (Molto 2001). The excellent preservation and representations of the skeletons is due to the hyperaridity of the climate, low soil acidity and the burial mode (Bleuze et al. 2014). Though generally the Kellis 2 burials had limited grave offerings many had been looted often involving the head-neck region (Wheeler 2009; Williams 2008). Approximately 35% of these individuals are adults, with the remaining 65% identified as juveniles (Wheeler 2009). Given the extraordinary preservation at this site, due to the low acidity of the soil and the arid climate (Bleuze et al. 2014), and the observed paleodemographic pattern, it has been suggested that the mortality profile at Kellis 2 is similar to that expected in a natural mortality distribution in pre-industrial populations (Tocheri et al. 2005). The current climate in the Dakhleh Oasis has little rainfall (0.3mm/year) and moderately low humidity, and is believed to be similar to the climate endured by the Roman Period inhabitants of the Kellis townsite, though there was likely more precipitation at that time (Bleuze et al. 2014; Dupras and Schwarcz 2001; Sutton 1947; Giddy 1987; Stewart et al. 2003). Isotopic and documentary studies indicate that the population interred at Kellis 2 consumed an omnivorous diet that included animal proteins, C3 and C4 crops, garden vegetables, fruit, nuts, cow/goat dairy, honey, and various herbs/seasonings (See Table 4.1; Dupras 1999). Dietary differences were observed between males and females, with males consuming more millet or ^{13}C enriched meat (specifically goat or cow) and females consuming relatively more C3 grains (such as wheat and barley) (Dupras 1999). Apart from dietary changes related to infant weaning, which occurred between the ages of approximately 6 months and 3 years (Dupras and Tocheri 2007), isotopic studies did not indicate any change in diet between age cohorts in the Kellis 2 population (Dupras 1999). As such, it may be assumed that diet remained relatively stable throughout the lives of Kellis inhabitants.

Table 4.1. Dietary Components at Roman Period Kellis According to Documentary and Isotopic Evidence. [Adapted from Dupras 1999: 104]

Animal Protein	Field Crops	Garden Vegetables	Fruits	Nuts and Seeds	Other
Cows (milk and meat)	Wheat (C3)	Turnips	Dates	Almonds	Honey
Goats (milk and meat)	Barley (C3)	Garlic	Doum Palm Nuts	Walnuts	Coriander
Pigs	Millet (C4)	Legumes	Figs	Pistachios	Cumin
Donkeys	Sesame (C3)	Onions	Olives	Hazelnuts	Dill
Camels		Cucumber	Pomegranates	Pine nuts	Fennel
Pigeons		Gourds	Jujubes		Marjoram
Geese		Artichokes	Carob		Mint
Ducks			Apricots		Rosemary
Eggs			Peaches		Safflower
Fish			Pears		Thyme
Gazelle			Cherry		Mustard
Oryx			Citron		Ami
Hartebeest			Apples		Anise
Hare					Caper
Chickens					Laurel
					Pepper

The large number of well-preserved burials from various age groups excavated from the Kellis 2 cemetery makes this site's adult population an ideal sample for the examination of adult dental wear standards. Digital images of adult occlusal dentition were used for dental wear analysis. Many of the images analyzed in this project were provided via the internet by Dr. Scott Haddow and were taken at the Kellis 2 site in Dakhleh Oasis, Egypt under the supervision of Dr. El Molto. Additional photographs taken by Dr. El Molto and Dr. Peter Sheldrick were digitized from slide format for this project by slidestodigital.com. Skeletal sex and age estimates were determined through the independent and blind analysis of separate skeletal indicators by members of the Dakhleh Oasis Project's Kellis 2 bioarchaeological team. For adult skeletons, factors considered in the estimation of age-at-death included: iliac crest and medial clavicular epiphyseal fusion (Webb and Suchey 1985), S1-S2 vertebral epiphyseal fusion (McKern and Stewart 1957), symphysis pubis morphology (Brooks and Suchey 1990; Suchey and Katz 1986), auricular surface (Lovejoy et al. 1985; Meindl and Lovejoy 1989), and rib morphology (İşcan and Loth 1986), cranial suture fusion (Buikstra and Ubelaker 1994), dental wear (independently calibrated against other skeletal age indicators), antemortem tooth loss, bone mineralization and degenerative joint disease. Factors contributing to the estimation of sex included: os coxae ventral arc, subpubic concavity, and ischiopubic ramus ridge morphology (Phenice 1969), as well as greater sciatic notch, preauricular sulcus morphology (Buikstra and Ubelaker 1994), and nuchal crest, mastoid process, supraorbital margin, glabella, and mental eminence morphology (Acsádi and Nemeskéri 1970; Buikstra and Ubelaker 1994).

4.5.2 Sample Selection

The original research design was planned for analysis of the Dakhleh dentitions housed in a regional storage magazine in Mut, Dakhleh Oasis, Egypt. This would have facilitated the analysis of the pattern of dental pathology and normal variation in tooth alignment that influence the pattern of attrition as discussed previously. However, following major political upheavals in Libya and Egypt, permissions for work in the Egyptian Western Desert were stopped, precluding this important research. Consequently, the population sample used in this study was limited to individuals whose occlusal dentition had been previously photographed and whose infra-cranial remains allowed for multifactorial age and sex estimation. Accordingly, individuals included in this study were limited to those with skeletal age estimates above 17 years in order to ensure the possibility for skeletal sex estimation. Pathology was observed and documented from the available photographs, with caries noted as the number of teeth affected in an individual. Individuals with visible evidence of leprosy were excluded from this study as this disease is known to affect the dentition. Lastly, the sample used in this study was limited to photographs taken approximately perpendicular to the occlusal surface of the examined teeth with sufficient clarity of enamel-dentine borders.

As a result, the sex and age distribution of the sample was 45 males and 64 females, with a total of 109 individuals (see Table 4.2). In addition to these limitations, this study focused only on first and second molars, however, some of these molars were excluded due to malocclusion, dental caries, post-mortem breakage, or abnormal wear. Some of the molars of interest were also unobservable due to ante-mortem or post-mortem tooth loss. First and second molars were chosen for analysis due to their resistance to post-mortem tooth loss and breakage, their relatively consistent rate of wear, and their long period of wear throughout adulthood.

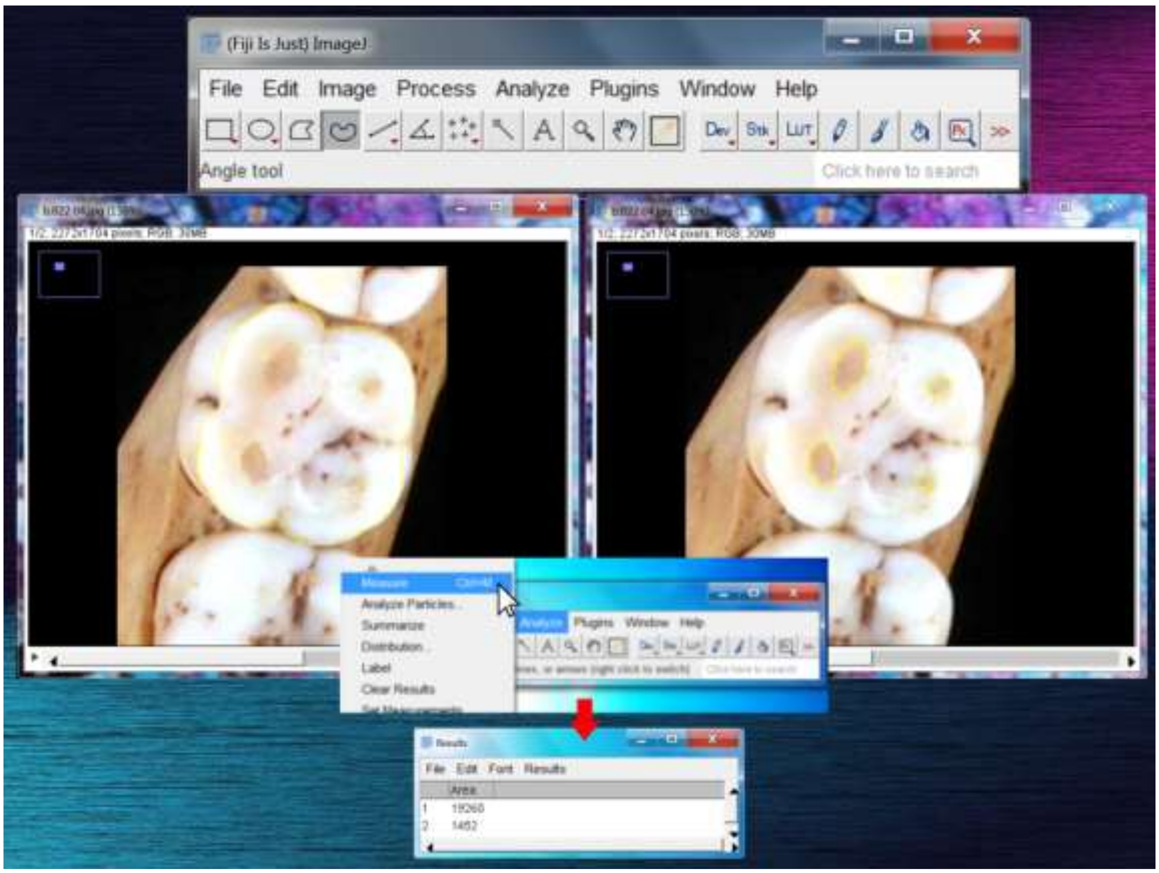
Table 4.2. Age and Sex Distribution of the Population Sample

	17-25	26-35	36-45	>45	Total
Female	16	15	9	24	64
Male	12	12	10	11	45
Total	28	27	19	35	109

4.5.3 Data Collection Methods

A new photogrammetric method for the quantification of exposed dentine, first introduced by Phillips-Conroy et al. (2000) in a study of Ethiopian and Tanzanian baboons and modified for use in humans by Deter (2006, 2009), was investigated in this study. Data were collected through the use of the digital image analysis program *FIJI (is just ImageJ)*, which is free online. Within this program, there is a freehand selection tool that was used to outline the area of the occlusal surface. This area was then measured in pixels using the tool Analyze > Measure (See Figure 4.3: Screen shot illustrating the method for quantification of the occlusal and exposed dentine surfaces through (Fiji Is Just) ImageJ). Although it is possible to calibrate measurements to real scales included in the original images, this was not necessary in this case as the data sought were percentages, and so measurements of pixels were sufficient. This method also reduced any challenges that might be associated with scales missing from photographs, illegible from glare, or positioned in a manner that might make calibration difficult.

Figure 4.3: Screen shot illustrating the method for quantification of the occlusal and exposed dentine surfaces through (Fiji Is Just) ImageJ. Quantified areas are isolated with yellow thresholds.



Following the measurement of the occlusal surface, this selection was deleted and areas of exposed dentine were similarly outlined and measured. Pressing the SHIFT key when making selections allowed for the selection of multiple areas at one time. Using the measurements produced through this method, the area of the occlusal surface and the summed areas of the exposed dentine were cut and pasted into adjacent columns in an

Excel sheet. In a third column, a formula was used to automatically calculate the percentage of the occlusal surface that is occupied by exposed dentine.

For the purpose of this study, the lower left first molars (LLM1) were chosen as the preferred "primary M1" data since it is usually recommended that dental scores are derived from these molars, if possible. Data from the rest of the dentition (adjacent M2, isomere, antimere) were collected in direct relation to the selected "primary M1". As such, unless otherwise indicated by tooth code and colour coded data cells in the Excel sheet, other data for the same individual was derived from the adjacent M2, the tooth in direct occlusion with the primary M1, and the tooth directly opposite the primary M1. In some cases data could not be collected from the lower left first molar due to caries, breakage, malocclusion, antemortem tooth loss, or post-mortem tooth loss. Following the recommended dental scoring methods, the "primary M1" data were collected from the LRM1, ULM1, or URM1 (in order of preference) and this variation from the protocol was indicated in the Excel sheet with the code of the examined tooth (e.g. LRM1) in a colour coded data cell. The adjacent M2, isomere, and antimere were then selected according to their direct relationship with the "primary M1" variant (e.g. if the primary M1 variant = LRM1, then M2 = LRM2 and isomere = URM1). Variation from this pattern was also indicated by the inclusion of the examined tooth code and a colour coded data cell in the Excel sheet. Colour coding within Excel was done to facilitate identification of variations in data collection protocol and removal of selected data for specific calculations. The colours included in the Excel sheet include:

Yellow – Lower left M1 not recordable, "primary M1" data collected from antimere (LRM1).

Orange – "primary M1" data collected from maxilla (ULM1 or URM1). Unless otherwise indicated by red data cell, all secondary data (antimere, M2) are in direct relation to the new "primary M1".

Red – Relationship of tooth not ideal with "primary M1" data (e.g. isomere data collected from tooth not in direct occlusion with "primary M1", or M2 data collected from opposite side of "primary M1").

In addition to these data, the percentage of exposed dentine data were collected a second time from a randomly selected sample of lower left first molars to assess intra-observer error. Another sample of the population was examined by a volunteer, Diane Kirkpatrick, who was trained in the collection of the percentage of exposed dentine data to assess inter-observer error. Given the perceived relationship between antemortem tooth loss and chronological age, wherever possible the number of teeth lost antemortem in the maxilla and mandible were also separately recorded. Descriptions of pathology were included in the Excel sheet and scores for the LLM1 and LLM2 were determined through the use of Brothwell (1963a) and Scott (1979) standards. Although the Scott (1979) standard for recording dental wear was not designed for age estimation, it is commonly used in the study of human dental remains due to its low intra- and inter-observer variability and relative precision (Cross et al. 1986). As such, Scott (1979) scores were documented in case of future comparative study. Following data collection, independently calculated multifactorial skeletal age and sex estimates for each individual were added to this Excel sheet to facilitate comparative statistical analysis and data were divided into two Excel sheets according to skeletal sex estimates (See Appendix 6). As previously mentioned, these skeletal sex and age estimates were determined through the independent and blind analysis of separate skeletal indicators by members of the Dakhleh Oasis Project's Kellis 2 bioarchaeological team. Factors considered in the estimation of age-at-death included: iliac crest and medial clavicular epiphyseal fusion (Webb and Suchey 1985), S1-S2 vertebral epiphyseal fusion (McKern and Stewart 1957), symphysis pubis morphology (Brooks and Suchey 1990; Suchey and Katz 1986), auricular surface (Lovejoy et al. 1985; Meindl and Lovejoy 1989), and rib morphology (İşcan and Loth 1986), dental attrition (Brothwell 1963a), cranial suture fusion (Buikstra and Ubelaker 1994), antemortem tooth loss, bone mineralization and degenerative joint disease. Factors contributing to the estimation of sex included: os coxae ventral arc, subpubic concavity, and ischiopubic ramus ridge morphology (Phenice 1969), as well as greater sciatic notch, preauricular sulcus morphology (Buikstra and Ubelaker 1994), and nuchal crest, mastoid process, supraorbital margin, glabella, and mental eminence morphology (Acsádi and Nemeskéri 1970; Buikstra and Ubelaker 1994).

4.5.4 Data Analysis

Following data collection, using the R statistical program, intra-class correlation tests were conducted to assess intra- and inter-observer error for the data collection relating to the percentage of exposed dentine in first molars. Paired t-tests were then used to examine the differences between first molar antimeres and isomeres. In the case of the isomeres, whose t-test results showed statistically significant differences at a 90% confidence interval (as per the statistical reporting convention recommended by Dahiru 2008), a simple plot of differences was graphed to visualize the differences between isomeres. In the case of antimeres, a Tukey's boxplot was created to identify outliers in the difference between antimeres. These outliers were then compared to their associated descriptions of pathology to determine if there was a clear pattern that might explain asymmetrical dental wear as a consequence of oral pathology. Oral pathology was further investigated through plots of: teeth with carious lesions vs. skeletal age and sex, percentage of observed teeth with carious lesions vs. skeletal age and sex, and the sum of teeth with carious lesions or antemortem tooth loss vs. skeletal age and sex. Pearson's r correlation scores were calculated for each of these plots, demonstrating a linear correlation only between the sum of antemortem tooth loss vs. skeletal age and sex. In order to determine whether skeletal age was better correlated with antemortem tooth loss (AMTL) alone, another plot and Pearson's r score was calculated for antemortem tooth loss vs. skeletal age and sex. Linear regression models were created for the two latter plots and regression diagnostics (i.e. Residual standard deviation [RSD], R-squared, F-statistic, Residuals vs. Fitted Values, Normal Q-Q, Scale-Location, and Residuals vs. Leverage graphs, Histogram of Residuals, and Akaike Information Criterion [AIC]) were conducted and compared. Since the AMTL linear regression diagnostics revealed skewed histograms of residuals, polynomial regression models were also graphed and they were determined to have a better goodness of fit than the linear models through a comparison of regression diagnostics. Pearson's r, plots against skeletal age and sex, linear regression models, and regression diagnostics were similarly completed for the percentage of the occlusal surface occupied by exposed dentine in first and second molars. Regression diagnostics were used to identify outliers that were subsequently removed before creating new versions of the model which were compared to determine the most reasonable model. These diagnostics were also used to note any deviations from normality in residual analysis, as they may provide biased regression coefficient estimates. ANCOVA tests were applied to the final AMTL, M1 wear, and M2 wear linear regression models, as well as the AMTL polynomial regression model, to determine the effects of age and sex on occlusal dentine exposure. For each regression model, algebraic equations were derived from the estimated coefficients provided through the "summary" output in RStudio. Confidence intervals of 95% were also calculated for these models by rounding the residual standard deviation to the nearest 0.5 and doubling it. In accordance with Raschka (2018), Prabhakaran (2017), and Gagneja (2018), and due to the small sample size, an 80:20 bootstrapping technique without replacement was used to test these models by splitting the data (80:20) to create a new model and a test sample, respectively. The difference between the resulting skeletal age predictions and the actual skeletal ages was assessed using Pearson's r and paired t-tests. These tests provided a general idea of the accuracy of the new models. In future, upon expansion of the reference data, a repetitive bootstrapping without replacement test is recommended.

The percentages of the occlusal surface occupied by exposed dentine were photogrammetrically quantified from the Brothwell (1963a) dental wear atlas, in the same manner as first and second molar wear in the Kellis 2 population. These data were then graphed along with the M1 and M2 wear linear regression models for comparison. As the regression models were found to be more specific and perhaps more accurate than the Brothwell method of dental age estimation, multiple regression models for different combinations of AMTL, M1 wear, and M2 wear were created for males, females, and individuals of unknown sex. Once again, regression diagnostics were consulted and an 80:20 bootstrapping method without replacement was applied, from which the predicted skeletal age estimates were compared to actual skeletal age through a paired t-test.

4.6 Results

4.6.1 Intra-observer error study of dentine quantification methods

Google's random number generator was used to select 30 individuals with quantified normal M1 wear from the available dataset for inclusion in a test for intra-observer error.

For this purpose, the percentage of exposed dentine was calculated a second time (n = 30) and recorded in an "intra-observer error" column for comparison with the original primary M1 data in the "% exposed dentine" column (See Appendix 6). Intra-class correlation (ICC) was then calculated in RStudio to assess the strength of the correlation between the original and secondary (intra-observer) data (See Appendix 5 for all R codes used in this dissertation). This test indicated that there is a very strong correlation (99%) between the original and intra-observer data at a statistically significant level of $p<0.0001$.

4.6.2 Inter-observer error study of dentine quantification methods

Similar to the above intra-observer test, Google's random number generator was used to select 30 individuals with quantified normal M1 wear from the available data set for inclusion in a test for inter-observer error. For this purpose, a volunteer, Diane Kirkpatrick, was trained in the quantification method and the identification of exposed dentine. Following this training, the volunteer independently quantified and calculated the percentage of exposed dentine for the primary first molars from the selected sample of M1 occlusal photographs (n = 30). These data were recorded in the "inter-observer error" column and were then compared to the primary M1 wear data in the "% exposed dentine" column (See Appendix 6). Intra-class correlation (ICC) was then calculated in RStudio to assess the correlation between the original and intra-observer data (See Appendix 5 for all R codes used in this dissertation). These test results indicate that there is a very strong correlation (98%) between the original and intra-observer data at a statistically significant level of $p<0.0001$.

4.6.3 Wear in Upper vs. Lower Left First Molars

Paired t-tests were conducted on male, female, and combined isomere data (n = 14) in RStudio to determine if there was a significant difference in dental wear between opposing maxillary and mandibular first molars (See Appendix 5 for all R codes).

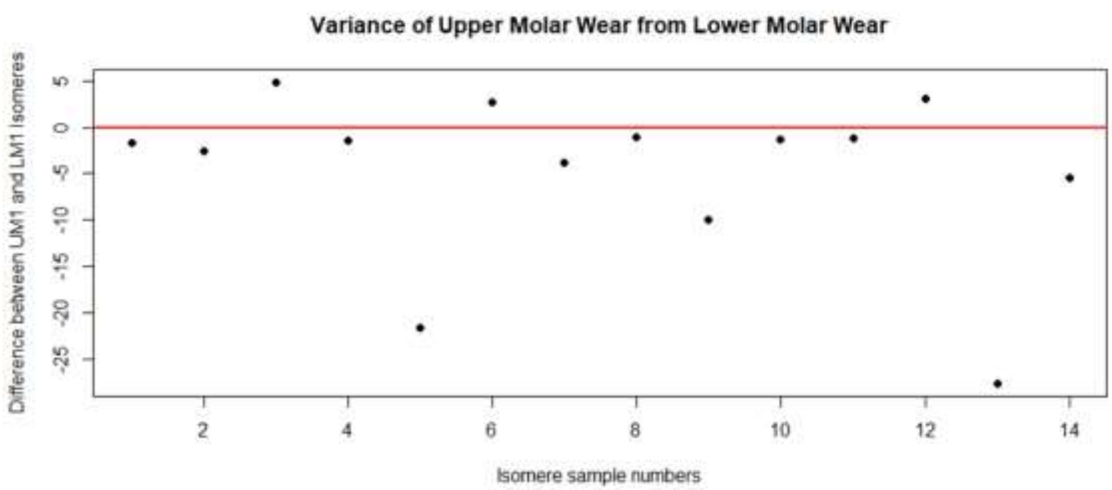
t-test for Male Isomeres
Mean of the differences: 6.21, $t(6) = 1.59$, p-value = 0.1626

t-test for Female Isomeres
Mean of the differences: 3.34, $t(6) = 1.02$, p-value = 0.345

t-test for Combined Male and Female Isomeres
Mean of the differences: 4.78, t(13) =1.93, p-value = 0.07576

These paired t-tests showed a statistically significant difference between isomeres in the male or female data at a 90% confidence interval (as per the statistical reporting convention recommended by Stern and Smith 2001 and Dahiru 2008). This difference was weighted toward heavier wear in the lower molars, with only three cases in which the upper molar had a higher percentage of exposed dentine (see Figure 4.4).

Figure 4.4. Simple Plot of Differences between First Molar Isomeres (n = 14)



4.6.4 Wear in Lower Left vs. Lower Right First Molars

Paired t-tests were conducted on male, female, and combined antimere data (n = 31) to determine if there was a significant difference in dental wear between the left and right sides of the dentition (See Appendix 5 for all R codes).

Male Antimeres Dental Wear
Mean of the differences: 4.85, t(11) = 1.12, p-value = 0.2872

Female Antimere Dental Wear
Mean of the differences: 2.25, t(18) = 1.02, p-value = 0.3223

Combined Male and Female Antimere Dental Wear
Mean of the differences: 3.26, t(30) = 1.53, p-value = 0.136

Results of the above paired t-tests indicate that there is no evidence of a statistically significant directional difference between dental wear in lower left and right first molars in males, females, and combined samples at a 95% confidence interval. Following this test, the absolute difference between lower first molar antimeres was also subjected to a paired t-test.

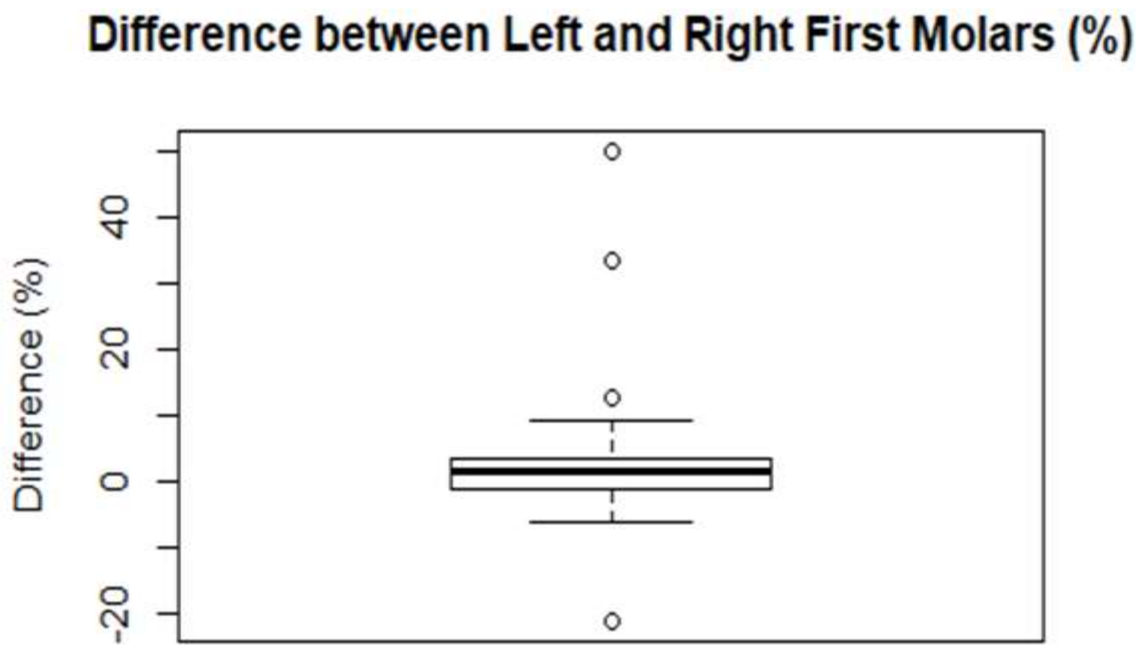
Absolute Differences between Combined Male and Female Antimere Dental Wear
Mean of the differences: 6.16, t(30) = 3.24, p-value = 0.002919

The results of this t-test of the absolute differences between antimeres indicate a statistically significant difference between left and right dental wear, independent of direction, at a 95% confidence interval.

4.6.5 Asymmetrical dental wear and oral pathology

Given that the previous results indicate significant differences in dental wear between antimeres, independent of direction, extreme examples of dental wear asymmetry were investigated in relation to oral pathology. Outliers of the differences between "Combined Male and Female Antimeres" were identified through Tukey's (1977) rule, which defines outliers as data points below Q1-1.5(IQR) and above Q3+1.5(IQR), where Q1 is the data value greater than or equal to ¼ of the data points, Q3 is the value greater than or equal to ¾ of the data points, and IQR is the difference between Q3 and Q1. These outliers are represented by singular data points in Tukey's boxplot (See Figure 4.5). In this case, a boxplot of differences between dental wear of antimeres was plotted and the outliers of interest represented the largest differences between left and right lower first molars in a sample of both males and females.

Figure 4.5. Tukey's Boxplot of Differences in Dental Wear between First Molar Antimeres (n = 31)



Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
-21.210	-1.190	1.670	3.257	3.490	49.950

The identified outliers were investigated in relation to the associated descriptions of pathology (See Table 4.3) to determine whether these differences in wear were related to preferred-side mastication resulting from pathology-related pain. This study revealed that the outliers were made up of two females (ages 30+/-5 and 30+/-3) and two males (ages 23+/-3 and 55+/-5).

Table 4.3. Dental Pathology and Tooth Loss in Individuals with the Greatest Dental Wear Asymmetry

Burial	Sex	Skeletal Age	Side with Greater Dental Wear	Description of Dental Pathology
52	F	30+/-3	Right	caries LLM1, LRM1, URM1,URM3; abscess LRM1; agenesis URM3?; AMTL LM2-3 (remodeling more complete in right side); **CARIES ON OCCLUSAL SURFACES OF LLM1 and LRM1**
265	M	55+/-5	Left	caries ULM1; AMTL LRM2, URM1; **ABNORMAL WEAR LLM1, ULP2, ULM1**
268	M	23+/-3	Left	caries LRM2; AMTL URM1, ULM2; abscess ULM2
409	F	30+/-5	Left	gross caries LLM2; **NO MAXILLA**

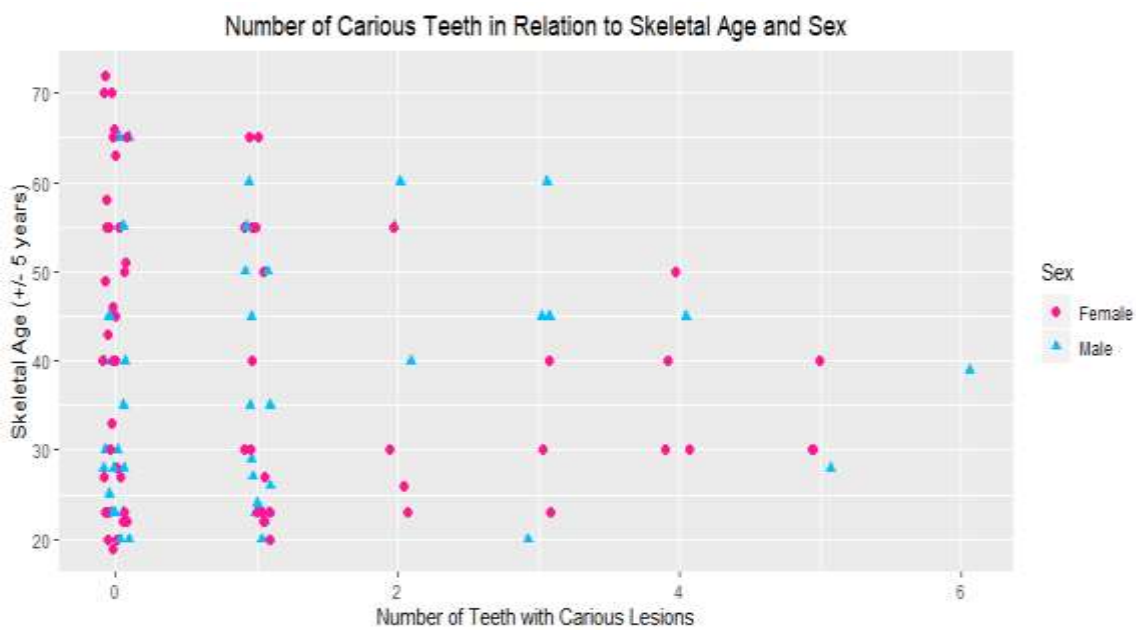
Only the 30 +/- 3 year old female had more wear in the LRM1 than the LLM1; all others had more significant wear in the LLM1. It must be noted though that the occlusal surface of the LRM1 was affected by caries; this required the estimation of boundaries for dentine exposure during photogrammetry. Consequently, this data point must be discarded. In the remaining individuals, the 55+/-5 year old male had abnormally severe wear on his LLM1, ULM1, and ULP2, compared to the surrounding teeth. This asymmetrical wear may have been the result of the use of teeth as tools in some capacity and cannot, therefore, be a consequence of the loss of dental surfaces or dental pain and discomfort. Additionally, the 30 +/-5 year old female presented with gross caries on the same side of the mandible that had more severe dental wear, however, the maxilla was not available for analysis so it is unknown if there was also pathology on the opposite side of the dentition. Finally, the 23+/-3 year old male had an abscess on the side of the mouth with more severe dental wear and a carious lesion on the opposite side. Both sides of the upper dentition were also affected by the antemortem loss of a tooth. Given that both sides of the dentition were equally affected by antemortem tooth loss and dental pathologies with the potential for pain or discomfort, a relationship between dental wear asymmetry and sided dental pathology or antemortem tooth loss was not observed. Nevertheless, any observed correlation between dental pathology and dental wear asymmetry in this individual would be statistically meaningless. As such, the results of this investigation into the relationship between dental wear asymmetry and sided dental pathology are inconclusive.

4.6.6 Dental Pathology and Wear as Indicators of Age

4.6.6.1 Caries and Antemortem Tooth Loss

Some dental pathologies are known to increase in prevalence in association with chronological age. Two of the more common, observable and discretely quantifiable dental pathologies in bioarchaeological contexts are dental caries and antemortem tooth loss (AMTL). Given the progressive unidirectional nature of both conditions (i.e. the number of teeth affected can only increase over time), it was decided that evidence of these conditions would be investigated to test their potential for use in the estimation of age-at-death. First, the number of teeth affected by carious lesions were plotted in relation to skeletal age and sex for each individual (n = 109). The jitter function in RStudio was used to introduce some random variation to the x-coordinates in the dataset in order to make overlapping data points visible; however, it is important to understand that all original data points actually rest on the nearest whole number of teeth with carious lesions.

Figure 4.6. Number of Carious Teeth in Relation to Skeletal Age and Sex (n = 109)



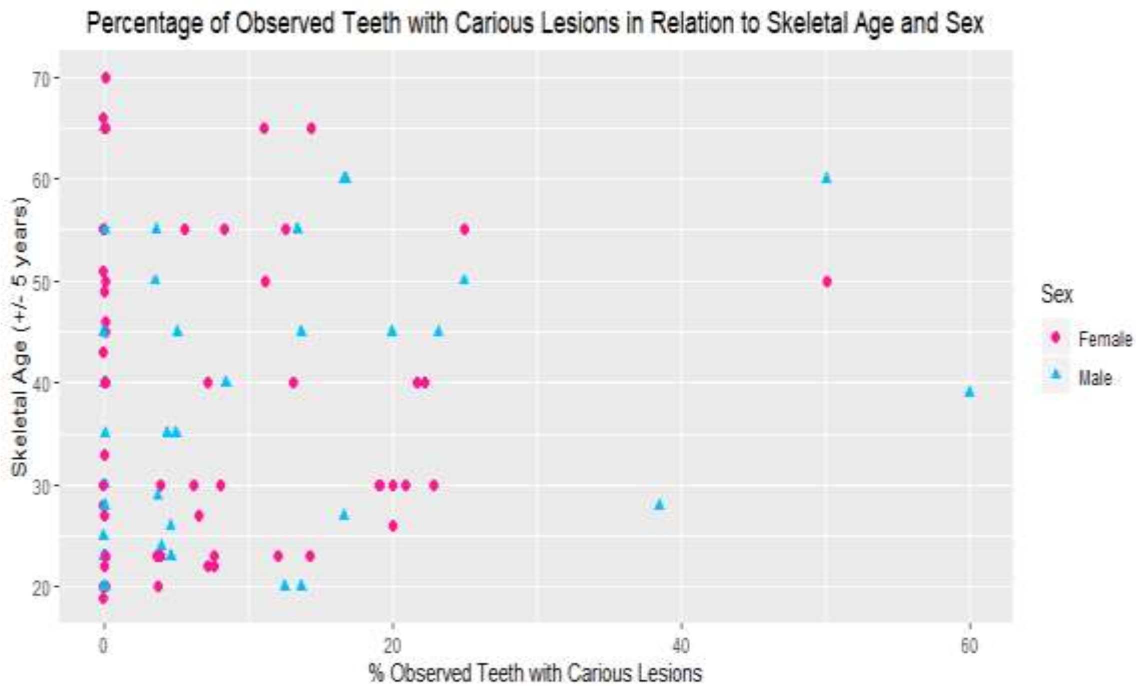
From this graph, it is clear that there is no linear relationship between the number of carious teeth and age or sex. This lack of correlation is further confirmed by the calculated Pearson's r scores as they are closer to 0 than to +1 or -1.

Correlation of Caries and Skeletal Age in Females
Pearson's r: -0.21, t(62) = -1.67, p-value = 0.1001

Correlation of Caries and Skeletal Age in Males
Pearson's r: 0.10, t(43) = 0.67, p-value = 0.506

Given that caries are known to increase in prevalence in relation to chronological age in living individuals, the observed lack of correlation is likely a result of the cross-sectional nature of this study and the deleterious effects of antemortem tooth loss. When left untreated, carious lesions often expand into the dental pulp and cause an infection, which sometimes results in the development of a periapical abscess and eventual antemortem tooth loss. Unfortunately, in bioarchaeological studies, the loss of the carious tooth results in a loss of information regarding the aetiology of this tooth loss and affects the perceived rate of dental caries. This loss of information can be further exacerbated by post-mortem tooth loss and/or missing maxillae or mandibulae. One method that has historically been used to mitigate these effects in bioarchaeology is to calculate the percentage of teeth with carious lesions from all observable teeth (Mummery 1870). For the purpose of this study, teeth were counted as "observable" if they had at least half of the crown and occlusal surface intact. Six individuals had no observable teeth and were thus excluded from these data. The following is a graph of the percentage of all observable teeth with evidence of caries in each individual with regard to skeletal age and sex (n = 103). Once again, the jitter function in RStudio was used to introduce random variation to x coordinates in the dataset in order to better visualize overlapping data points.

Figure 4.7. Percentage of Observed Teeth with Carious Lesions in Relation to Skeletal Age and Sex (n = 103)



Despite the attempt to mitigate the effects of lost teeth on caries rates, this graph does not demonstrate a linear relationship between the percentage of observed teeth with carious lesions and age or sex. Again, this lack of correlation is reflected in Pearson's r scores for the data as they are closer to 0 than to +1 or -1.

Correlation of the Percentage of Observed Teeth with Carious Lesions and Skeletal Age in Females
Pearson's r: 0.01, t(57) = 0.09, p-value = 0.9285

Correlation of the Percentage of Observed Teeth with Carious Lesions and Skeletal Age in Males
Pearson's r: 0.24, t(42) = 1.62, p-value = 0.1135

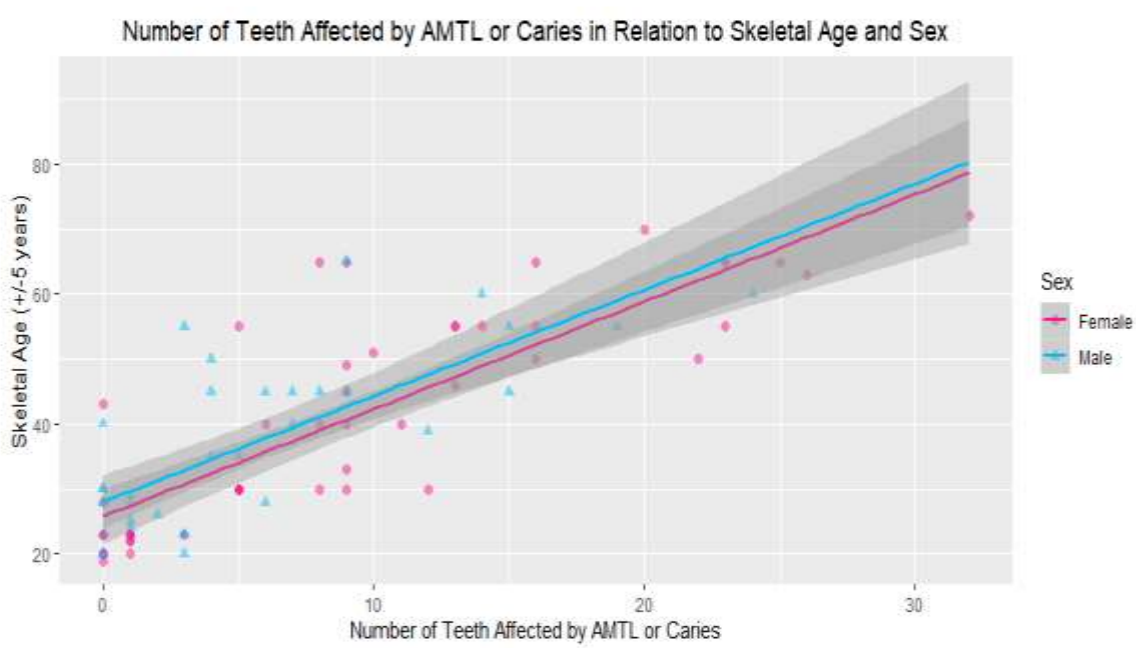
Given the apparent lack of correlation between caries and skeletal age in the examined sample, it may be concluded that caries are not a useful indicator for the estimation of skeletal age at death. However, given that antemortem tooth loss (AMTL) is a known result of gross carious lesions, and can significantly affect the observable cases of carious teeth, it may be reasonable to investigate whether there is a relationship between skeletal age and the sum of teeth affected by AMTL or caries (AMTL+Caries). In fact, this combination of carious teeth and antemortem tooth loss has been proposed as a post-mortem caries index (cf. Kelley 1991). For the current study, data were restricted to individuals with both upper and lower dental arches to reduce bias (n = 81). The data were tested using Pearson's r, which revealed a strong linear correlation between AMTL+Caries and skeletal age for males and females.

Correlation of Age and Teeth Affected by AMTL or Caries in Females
Pearson's r: 0.83, t(44) = 9.72, p-value = 1.599e-12

Correlation of Age and Teeth Affected by AMTL or Caries in Males
Pearson's r: 0.78, t(33) = 7.12, p-value = 3.701e-08

Given this correlation, linear regression models were graphed for males and females (Figure 4.8). Regression diagnostics (i.e. RSD, MAE, estimated coefficients, R-squared, F-statistic, AIC, and Residuals vs. Fitted Values, Normal Q-Q, Scale-Location, and Residuals vs. Leverage graphs) were then performed to ensure the goodness of fit of the models as well as to assess the predictive strength of the models (See Figure 4.9 andFigure 4.10). It should be noted that linear regression models assume multivariate normality, a linear relationship, little to no multicollinearity, no auto-correlation, and homoscedasticity.

Figure 4.8. Teeth Affected by AMTL or Caries in Relation to Skeletal Age and Sex (n = 81) – Colour intensity of data points reflects number of overlapping points



Residual standard deviation: *9.201 on 77 degrees of freedom*
Mean Absolute Error: *7.02 years*
Multiple R-squared: *0.659*
Adjusted R-squared: *0.6457*
F-statistic: *49.6 on 3 and 77 DF, p-value: < 2.2e-16*
AIC: *479.9779*

Figure 4.9. Diagnostics for AMTL+Caries Linear Regression Model

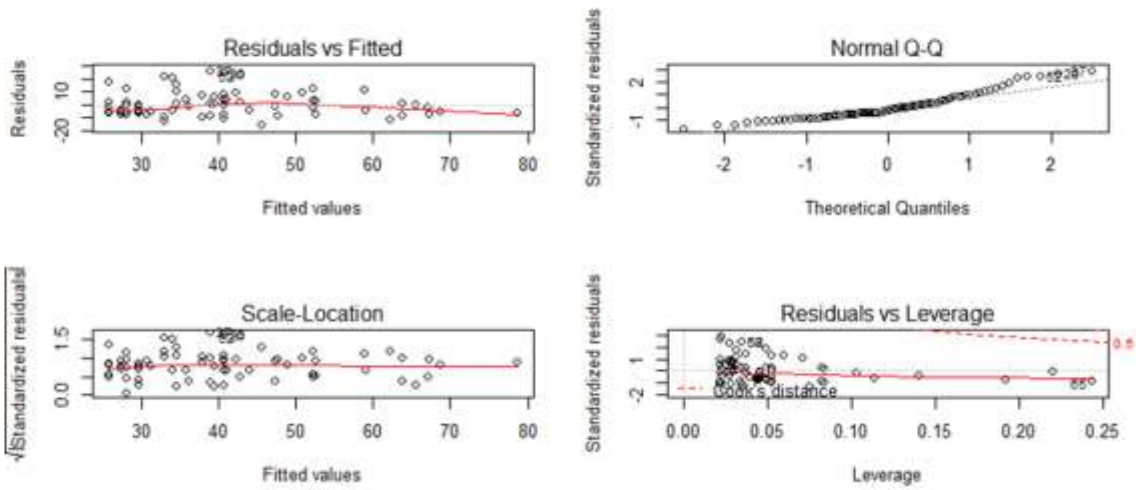
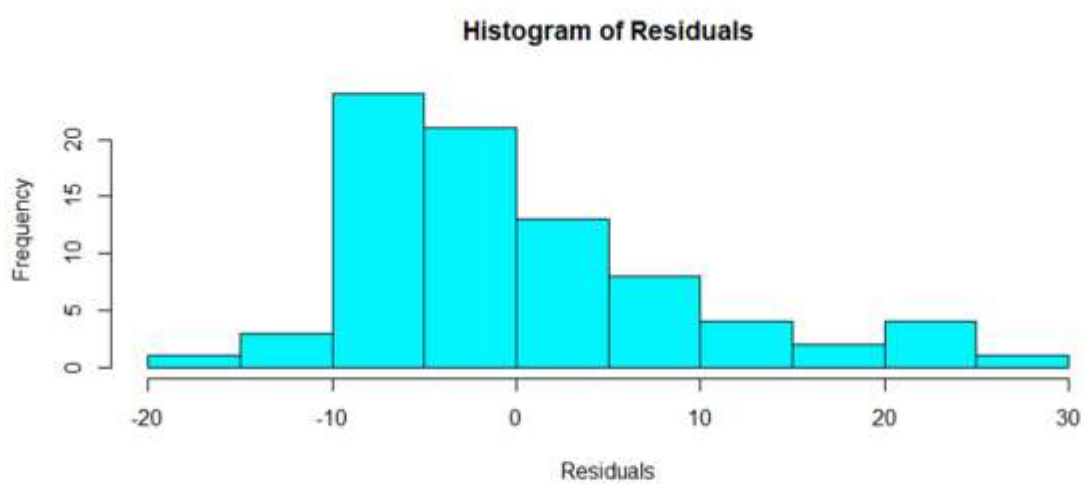


Figure 4.10. Histogram of Residuals for AMTL+Caries Linear Regression Model



These data indicate that the linear regression model for teeth affected by AMTL or caries according to age and sex has a good fit with the data, with a slightly skewed distribution of the residuals. This deviation from normality in residual analysis is likely a result of the small sample size; however, it is important to note as it may contribute to biased regression coefficient estimates. Of course, this model must be compared to a model based on antemortem tooth loss alone to determine if the inclusion of caries data improve model accuracy. Furthermore, as previously mentioned, antemortem tooth loss progresses over time and thus may be an indicator of chronological age on its own.

Pearson's r scores for the male and female models confirm that the number of teeth lost antemortem is positively correlated with skeletal age and may be a reasonable consideration when estimating age-at-death. In a comparison between the Pearson's r scores for AMTL and AMTL+Caries datasets, slightly better correlation is observed between AMTL and skeletal age than between AMTL+Caries and skeletal age. As such, these data indicate that the inclusion of caries data, even when combined with antemortem tooth loss, would not positively contribute to estimations of skeletal age at death. This may be a result of the multifactorial aetiology of antemortem tooth loss or perhaps variations in dental caries rates within the population and throughout life.

Correlation of Age and AMTL in Females
Pearson's r: 0.85, t(44) =10.89, p-value = 4.507e-14

Correlation of Age and AMTL in Males
Pearson's r: 0.80, t(33) = 7.65, p-value = 8.408e-09

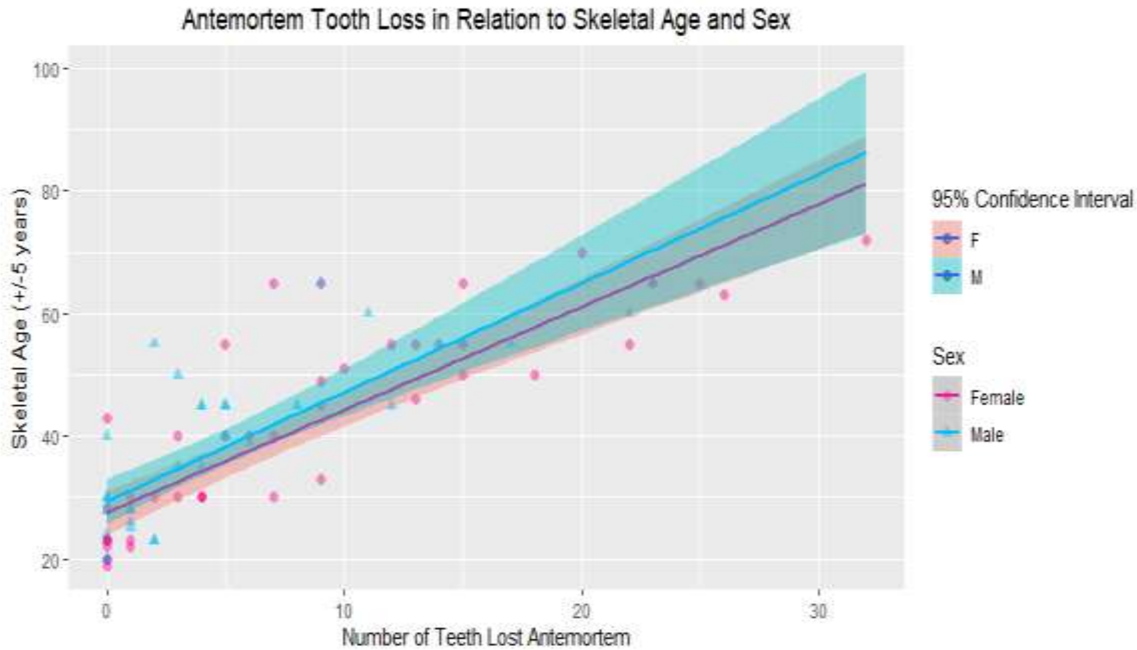
Comparison of Pearson's r Scores for Correlation of AMTL/Age and AMTL+Caries/Age Data
Females: 0.85 (AMTL) > 0.83 (AMTL+Caries)
Males: 0.80 (AMTL) > 0.78 (AMTL+Caries)

Consequently, antemortem tooth loss was plotted in relation to skeletal age and sex, with a separate model created for unknown sex based on all male and female data combined. The unknown sex model was calculated based on the combined male and female sample because skeletal sex estimation methods are not always conclusive. In all cases, RStudio fitted the regression models with 95% confidence intervals for individual points within the regression line, resulting in differing confidence intervals along the length of the graph.

This was done to provide an alternative to the static confidence interval calculated for inclusion with the regression equations. Following creation of the models, regression diagnostics (i.e. RSD, MAE, R-squared, F-statistic, AIC, Residuals vs. Fitted Values, Normal Q-Q, Scale-Location, Residuals vs. Leverage graphs, and a Histogram of Residuals) were examined to ensure goodness of fit and prediction strength. Individuals with missing mandibulae or maxillae were excluded from this dataset to avoid bias (n = 81).

Linear regression equations were derived from the estimated coefficient output in RStudio (See Appendix 5 for R code). The confidence interval included with these equations was estimated by rounding the residual standard deviation to the nearest 0.5 and doubling it to two standard deviations (CI=95%). Although this confidence interval is uniform across both models, the linear regression model shows that the variation in antemortem tooth loss number incrementally increases as skeletal age increases; meaning that individuals with skeletal ages of 65(+/-5 years) and over show a greater range in the number of antemortem teeth lost than younger individuals. As in the previous model, further diagnostic tests were performed to determine the strength of correlation between the linear regression model and the dataset (Figure 4.12 andFigure 4.13).

Figure 4.11. Antemortem Tooth Loss in Relation to Skeletal Age and Sex (n = 81) – Colour intensity of data points reflects number of overlapping points



Linear Regression Equations: ***Female*** *-> y = 1.68x + 27.55, CI = +/-18 years*
Male *-> y = 1.78x + 29.39, CI = +/-18 years*

Residual standard deviation: 8.618 *on 77 degrees of freedom*
Mean Absolute Error: *6.61 years*
Multiple R-squared: 0.7009
Adjusted R-squared: 0.6892
F-statistic: *60.14 on 3 and 77 DF, p-value: < 2.2e-16*
AIC: *584.685*

Figure 4.12. Diagnostics for Sex-specific AMTL Linear Regression Model

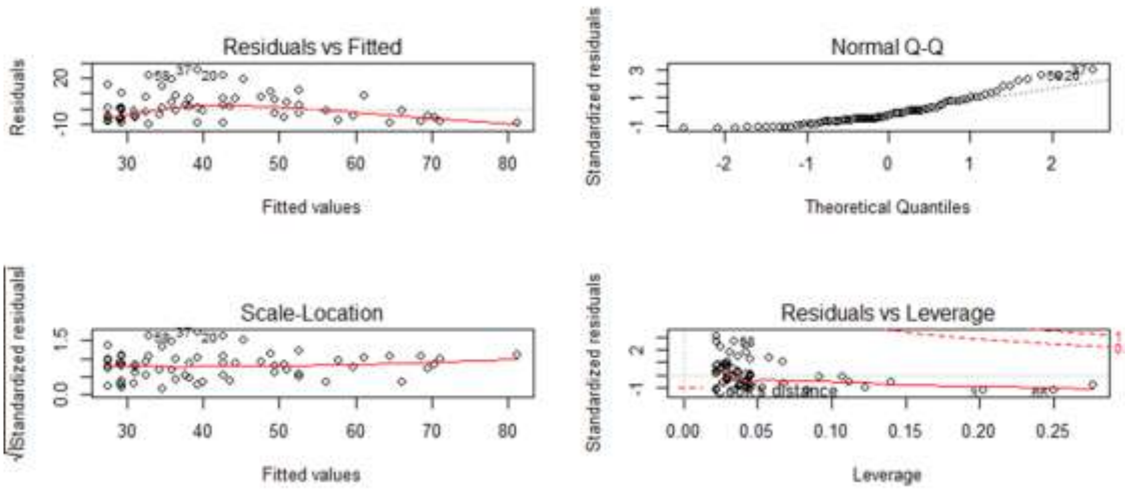


Figure 4.13. Histogram of Residuals for Sex-specific AMTL Linear Regression Model

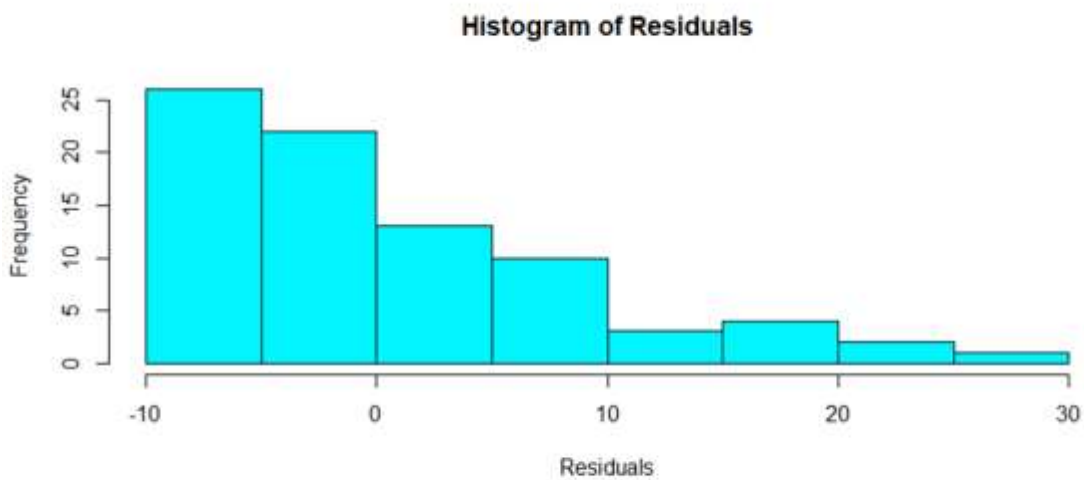
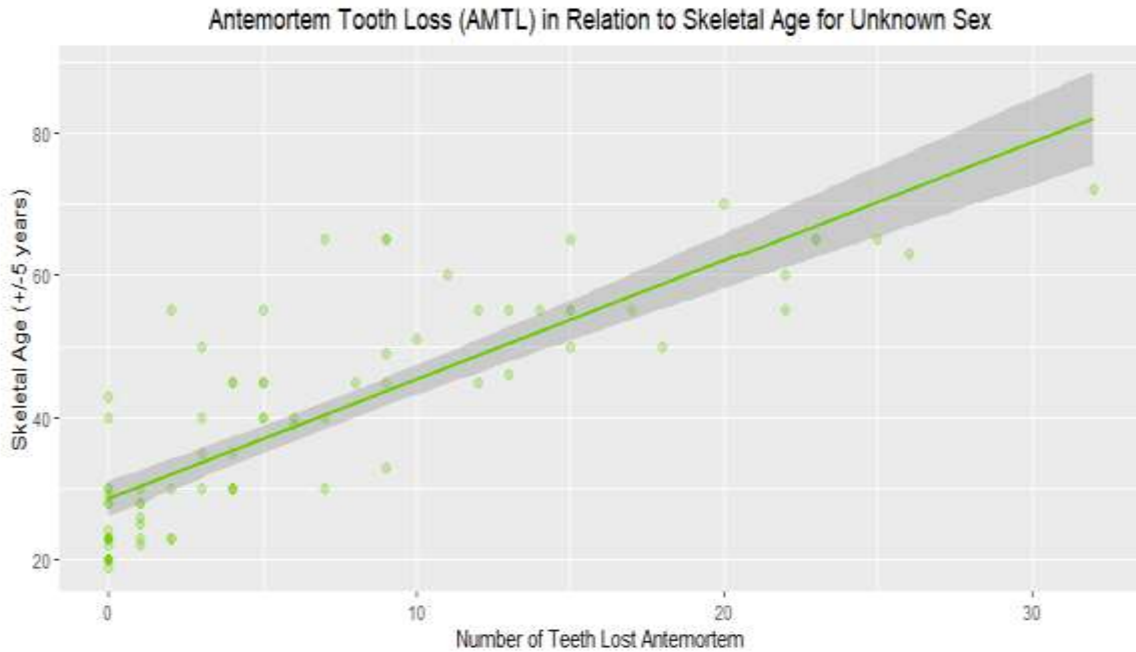


Figure 4.14. Linear Regression Model for AMTL vs. Skeletal Age for Unknown Sex (n = 81) - Colour intensity of data points reflects number of overlapping points



Linear Regression Equation for Unknown Sex ->

y = 1.67x + 28.58, CI = +/-18 years

Residual standard deviation: *8.598 on 79 degrees of freedom*
Mean Absolute Error: *6.66*
Multiple R-squared: *0.6945*
Adjusted R-squared: *0.6906*
F-statistic: *179.6 on 1 and 79 DF, p-value: < 2.2e-16*
AIC: *582.3884*

Figure 4.15. Diagnostics for AMTL Linear Regression Model for Unknown Sex

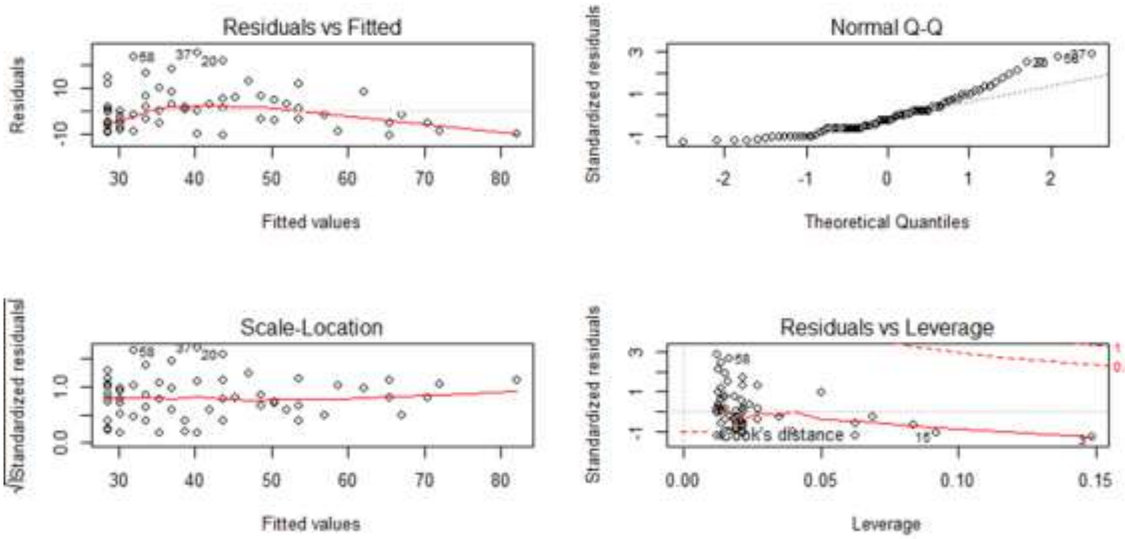
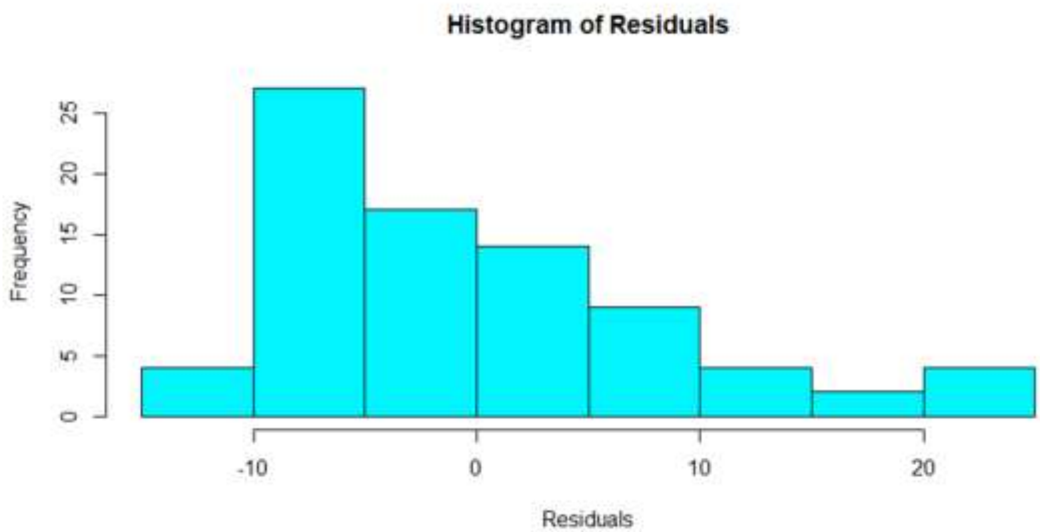
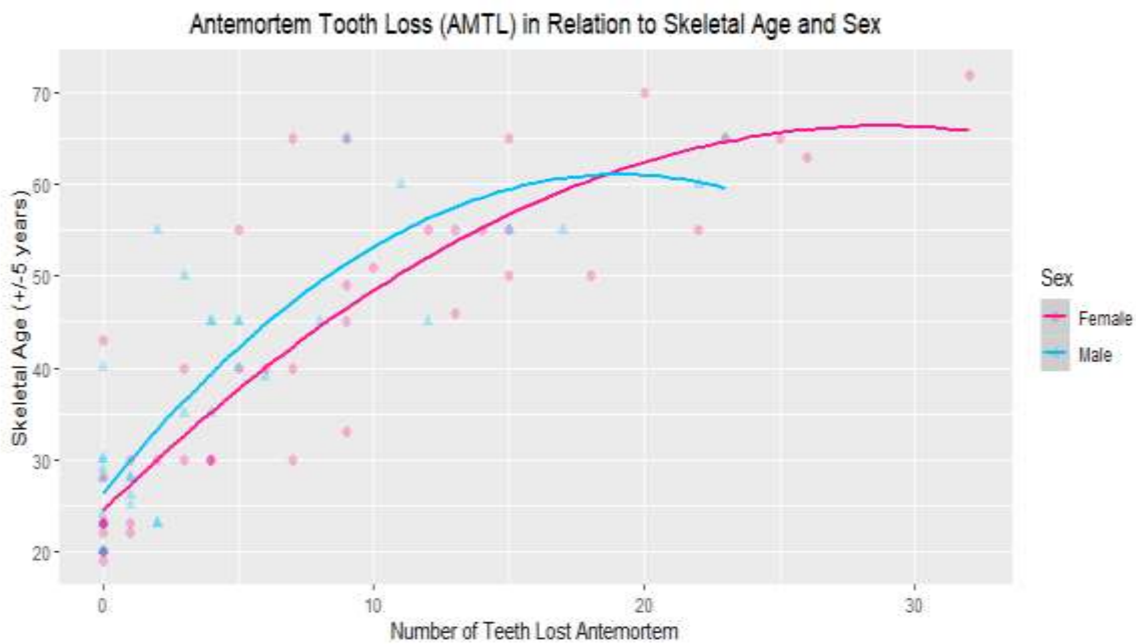


Figure 4.16. Histogram of Residuals for AMTL Linear Regression Model for Unknown Sex



Results from these tests indicate the models are well fitted to the data, with a skewed distribution of residuals in both the sex-specific and unknown sex AMTL models (Figure 4.13 andFigure 4.16). These imbalances of residual distribution indicate that non-linear regression models may be more appropriate for these data. Consequently, polynomial regression models were graphed (Figure 4.17 andFigure 4.20) and converted to algebraic equations, residual standard deviations were identified and converted to confidence intervals, and regression model diagnostics were calculated for comparison with those of the linear regression models (Figures Figure 4.18, Figure 4.19, Figure 4.21, and Figure 4.22). It should be noted that polynomial regression models assume multivariate normality, a linear or curvilinear relationship, and independence of variables.

Figure 4.17. Polynomial Regression Model for AMTL in Relation to Skeletal Age and Sex (n = 81) - Colour intensity of data points reflects number of overlapping points



Polynomial Regression Equations:

Female *->* $y = 24.38 + 2.91x - 0.05x^2$, *CI = +/- 16 years*

Male *->* $y = 26.38 + 3.64x - 0.10x^2$, *CI = +/- 16 years*

Residual standard deviation: *7.762 on 75 degrees of freedom*
Mean Absolute Error: *5.58 years*
Multiple R-squared: *0.7636*
Adjusted R-squared: *0.7479*
F-statistic: *48.46 on 5 and 75 DF, p-value: < 2.2e-16*
AIC: *569.6164*

Figure 4.18. Diagnostics for Sex-specific AMTL Polynomial Regression Model

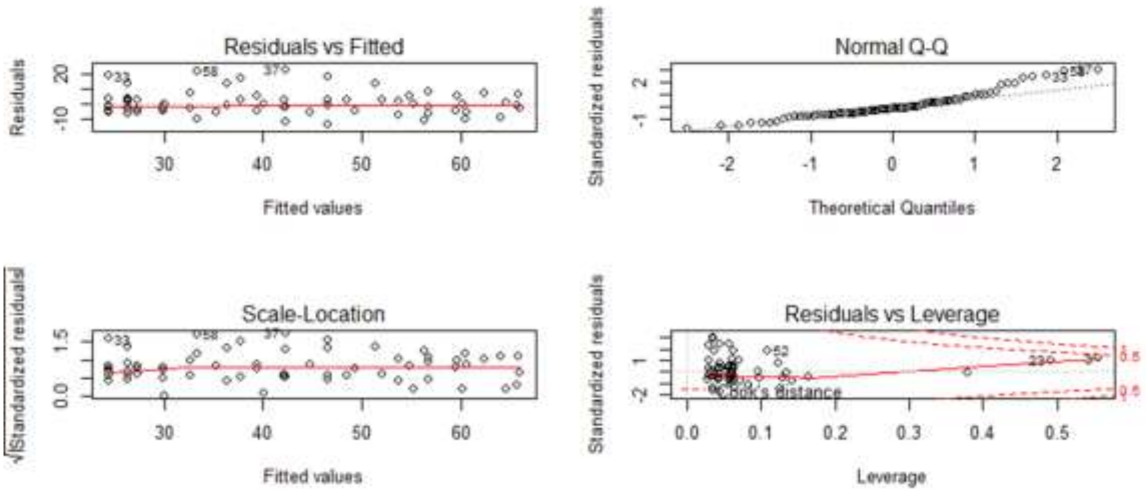


Figure 4.19. Histogram of Residuals for Sex-specific AMTL Polynomial Regression Model

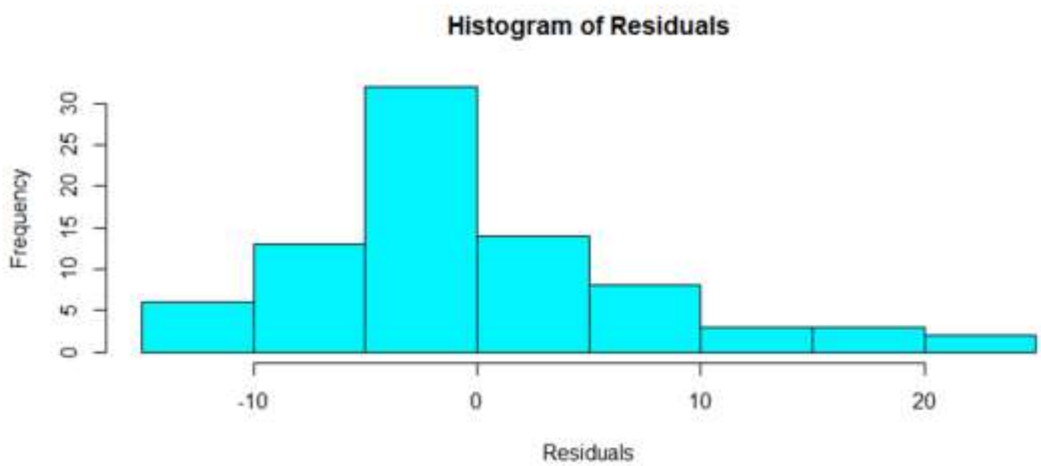
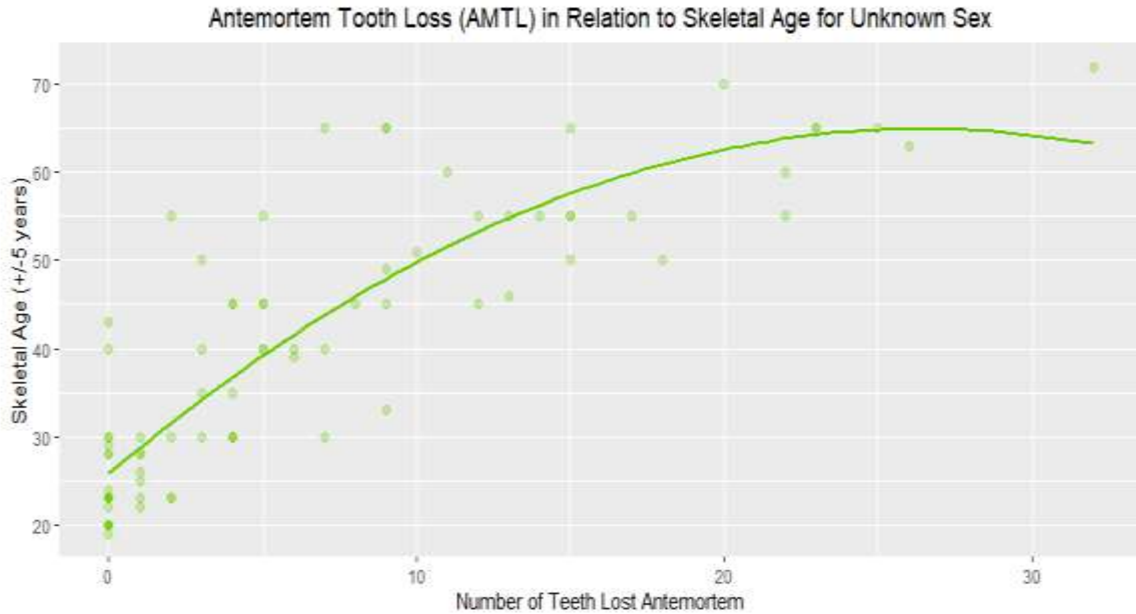


Figure 4.20. Polynomial Regression Model for AMTL in Relation to Skeletal Age for Unknown Sex (n = 81) - Colour intensity of data points reflects number of overlapping points



***Polynomial Regression Equation for Unknown Sex*:**

$$y = 25.81 + 2.95x - 0.06x^2,\ CI = +/- 16\ years$$

Residual standard deviation: *7.823 on 78 degrees of freedom*
Mean Absolute Error: *5.77 years*
Multiple R-squared: *0.7503*
Adjusted R-squared: *0.7439*
F-statistic: *117.2 on 2 and 78 DF, p-value: < 2.2e-16*
AIC: *568.0458*

Figure 4.21. Diagnostics for AMTL Polynomial Regression Model for Unknown Sex

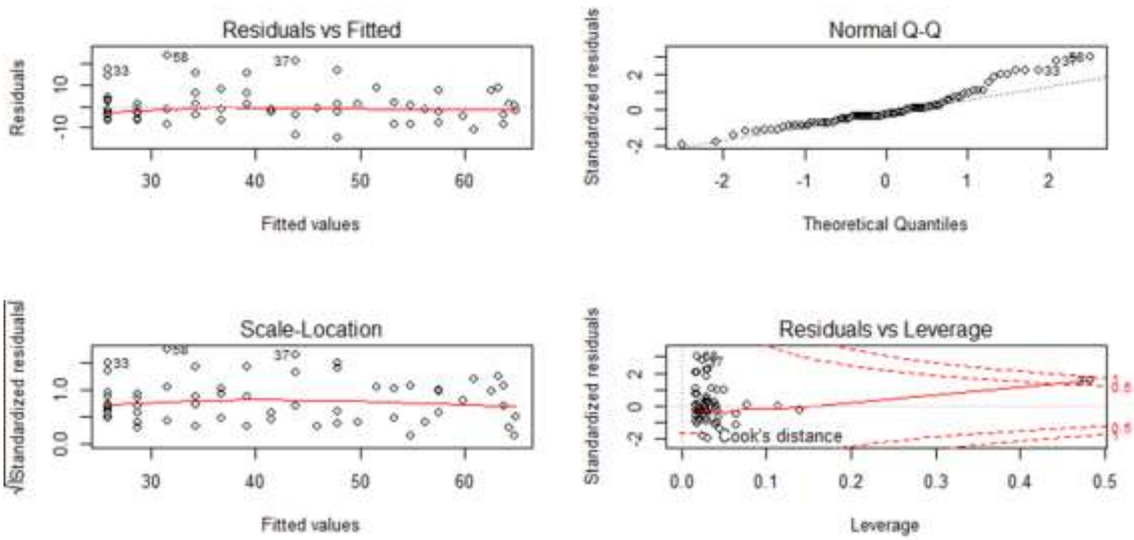
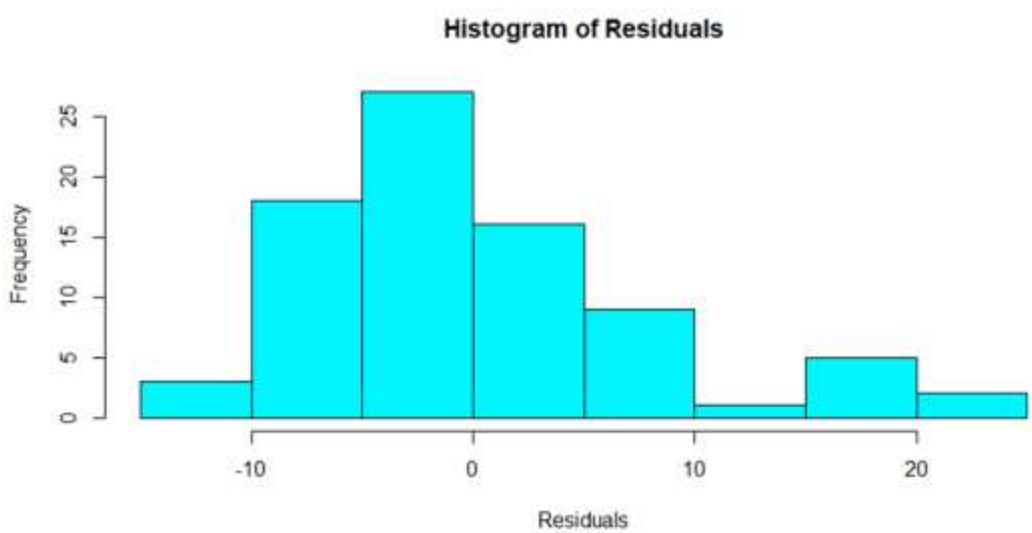


Figure 4.22. Histogram of Residuals for AMTL Polynomial Regression Model for Unknown Sex



Having ensured the goodness of fit for the polynomial regression models, the diagnostic data for the polynomial and linear regression models for AMTL were compared to determine the most suitable dental age predictor.

<u>Sex-specific Model Comparison:</u>

Residual standard deviation: *7.76 (AMTL_polynomial) < 8.62 (AMTL_linear)*
Mean Absolute Error: *5.58 years (AMTL_polynomial) < 6.61 years (AMTL_linear)*
Multiple R-squared: *0.76 (AMTL_polynomial) > 0.70 (AMTL_linear)*
Adjusted R-squared: *0.75 (AMTL_polynomial)> 0.69 (AMTL_linear)*
AIC: *569.62 (AMTL_polynomial) < 584.69 (AMTL_linear)*

Unknown Sex Model Comparison:
Residual standard deviation: *7.82 (AMTL_polynomial) < 8.60 (AMTL_linear)*
Mean Absolute Error: *5.77 (AMTL_polynomial) < 6.66 (AMTL_linear)*
Multiple R-squared: *0.75 (AMTL_polynomial) > 0.69(AMTL_linear)*
Adjusted R-squared: *0.74 (AMTL_polynomial) > 0.69(AMTL_linear)*
AIC: *568.05 (AMTL_polynomial) < 582.39(AMTL_linear)*

In both the sex-specific and unknown sex cases, the polynomial regression models outperformed the linear regression models in terms of the residual standard deviation, mean absolute error, multiple R-squared, adjusted R-squared, and the Akaike Information Criterion (AIC). All of these indicators show that the polynomial regression models have a stronger predictive strength than the linear regression models for AMTL and have a better fit among the data. As such, the polynomial regression models for AMTL may be the preferred sex-specific age estimation model based on an oral pathological indicator. Having said this, caution must be taken in using this model as there is a possibility that these models are slightly skewed by a paucity of AMTL data from older adults, as indicated by the slight skewing of the histogram of residuals. This paucity of data in the older cohorts may be particularly influential on the polynomial regression models as they run the risk of overfitting to the data. However, it should be noted that Rosing and Kvaal (1998) observed a similar polynomial relationship between the number of teeth and age in 1120 dentitions from a dental practice in Ulm, Germany. Nevertheless, all of these models should be revisited in the future when more data become available. Until then, it may be reasonable to use the polynomial regression models for individuals with only AMTL data, however, the linear regression models should not be discarded as they can be integrated into multiple regression methods with positive effects, as will be demonstrated later in this paper. In the meantime, it is worth noting that ANCOVA tests on the sex-specific models reveal that the linear regression model has a slightly stronger correlation between AMTL and skeletal age than the polynomial model, as the polynomial model is more closely associated to sex than the linear model. Nevertheless, in both linear and polynomial models, the correlation between AMTL and skeletal age far outweighs the correlation between AMTL and sex (Table 4.4).

Table 4.4. Analysis of Covariance Tables for AMTL linear and polynomial regression models (Skeletal Age vs. Sex and AMTL)

	Df	F value	Pr(>F)		Df	F value	Pr(>F)
Sex_linear	1	2.6919	0.1049	Sex_polynomial	1	3.318	0.07251
AMTL_linear	1	177.587	< 2e-16 ***	AMTL_polynomial	2	118.418	< 2e-16 ***
Sex:AMTL_linear	1	0.1338	0.7156	Sex:AMTL_polynomial	2	1.063	0.35056
Residuals_linear	77			Residuals_polynomial	75		

*** = significant to <0.0001

Following the above analyses, bootstrapping without replacement tests of the linear and polynomial AMTL models were conducted in RStudio using an 80:20 random data split for model creation and test data. Through this method, linear regression testing models were created from a random selection of 80% of the dataset. The remaining 20% of each dataset was input into respective modified regression testing models to predict skeletal ages, resulting in the following table of actual and predicted skeletal ages. This ratio was chosen because of its popularity in the literature (e.g. Raschka 2018; Prabhakaran 2017; Gagneja 2018), as well as the need for a relatively large training sample due to the small reference sample size. The dataset was split through the use of a random number generator set to select line numbers within the Excel datasheets containing the actual skeletal ages for sex-specific or combined sex datasets.

Table 4.5. Actual vs. Predicted Skeletal Age (Sex-specific AMTL Linear Regression)

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	27.29841
2	55	60.45743
3	72	82.77873
12	28	27.29841
17	40	35.96721
22	45	37.99413
26	30	28.63443
27	40	28.63443
34	20	28.63443
36	55	51.57105
51	45	43.60996
57	23	28.63443
66	30	29.03217
67	55	48.10353
73	45	51.09772
76	30	39.43473
80	50	34.25025

Mean of the differences: 0.09, t(16) = 0.05, p-value = 0.96

Table 4.6. Actual vs. Predicted Skeletal Age (AMTL Linear Regression for Unknown Sex)

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	28.01836
2	55	57.71054
3	72	83.90953
12	28	28.01836
17	40	36.75135
22	45	36.75135
26	30	28.01836
27	40	28.01836
34	20	28.01836
36	55	52.47075
51	45	41.99115
57	23	28.01836
66	30	29.76496
67	55	48.97755
73	45	48.97755
76	30	40.24455
80	50	33.25816

Mean of the differences: 0.36, t(16) = 0.19, p-value = 0.85

Table 4.7. Actual vs. Predicted Skeletal Age (AMTL Female Polynomial Regressions)

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	24.45568
2	72	56.26816
6	19	24.45568
28	23	24.45568
30	70	61.46968
35	30	27.6626
39	50	60.33796
41	23	24.45568
43	30	43.90616
45	40	41.5558

Mean of the differences: -1.00, t(9) = -0.37, p-value = 0.72

Table 4.8. Actual vs. Predicted Skeletal Age (AMTL Male Polynomial Regressions)

Line	Actual Skeletal Age	Predicted Skeletal Age
1	55	61.81492
3	29	24.86593
6	45	41.24788
20	45	41.24788
25	45	38.33913
28	55	31.97017
33	55	60.22528

Mean of the differences: 4.18, t(6) = 1.13, p-value = 0.30

Table 4.9. Actual vs. Predicted Skeletal Age (AMTL Polynomial Regression for Unknown Sex)

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	24.38269
2	55	60.73889
3	72	52.67549
12	28	24.38269
17	40	40.09349
22	45	40.09349
26	30	24.38269
27	40	24.38269
34	20	24.38269
36	55	57.83555
51	45	47.51285
57	23	24.38269
66	30	27.85937
67	55	55.06369
73	45	55.06369
76	30	45.20699
80	50	34.31095

Mean of the differences: 1.31, t(16) = 0.60, p-value = 0.56

Paired t-tests of the actual and predicted skeletal ages estimated through the 80:20 bootstrapping without replacement technique revealed relatively low means of differences in all of the linear and polynomial regression models. Unfortunately, due to the small sample sizes, all t-values were statistically insignificant.

4.6.6.2 Dental Wear

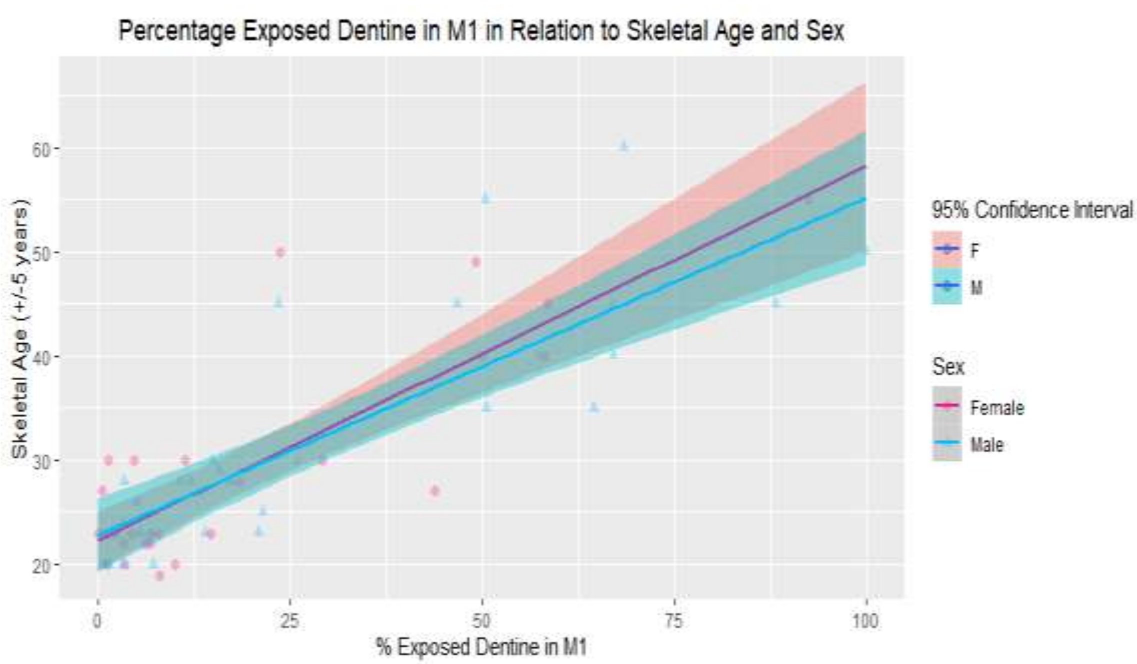
Following the above study of discrete oral pathologies in relation to skeletal sex and age, continuous data from dental wear was investigated in relation to skeletal age and sex through a study of the percentage of occlusal dentine exposure in first and second molars. The identified outliers from the study of asymmetrical dental wear among antimeres (Section 4.6.5) were excluded from the data used for these models. Pearson's r scores indicate that there is a strong positive correlation between first and second molar wear and skeletal age.

Correlation of M1 Wear and Skeletal Age
Pearson's r: 0.84, t(55) = 11.33, p-value = 5.321e-16

Correlation of M2 Wear and Skeletal Age
Pearson's r: 0.82, t(48) = 9.95, p-value = 2.967e-13

Beginning with M1 wear, regression diagnostic tests (i.e. Residuals vs. Fitted Values, Normal Q-Q, Scale-Location, and Residuals vs. Leverage graphs, see Figure 4.24 and Figure 4.25) were used to identify three individuals with abnormally low dental wear for their skeletal age and one individual with abnormally high dental wear for her skeletal age. Linear regression models of M1 wear, with and without these outliers, were compared for goodness-of-fit resulting in final models (n = 57) based on linear regression that excluded the aforementioned outliers (Figure 4.23 and Figure 4.26). Linear regression equations and 95% confidence intervals were calculated in the same manner as was described in the creation of the linear regression models for antemortem tooth loss. As previously mentioned, it should be noted that linear regression models assume multivariate normality, a linear relationship, little to no multicollinearity, no auto-correlation, and homoscedasticity.

Figure 4.23. Percentage of Exposed Dentine in M1 in Relation to Skeletal Age and Sex (n = 57) - Colour intensity of data points reflects number of overlapping points



Linear Regression Equations: Female *-> y = 0.36x + 22.29, CI = +/-13 years*
Male *-> y = 0.32x + 22.84, CI = +/-13 years*

Residual standard deviation: *6.079 on 53 degrees of freedom*
Mean Absolute Error: *4.14 years*
Multiple R-squared: *0.7019*
Adjusted R-squared: *0.685*
F-statistic: *41.59 on 3 and 53 DF, p-value: 5.859e-14*
AIC: *373.3698*

Figure 4.24. Diagnostics for Sex-specific M1 Linear Regression Model

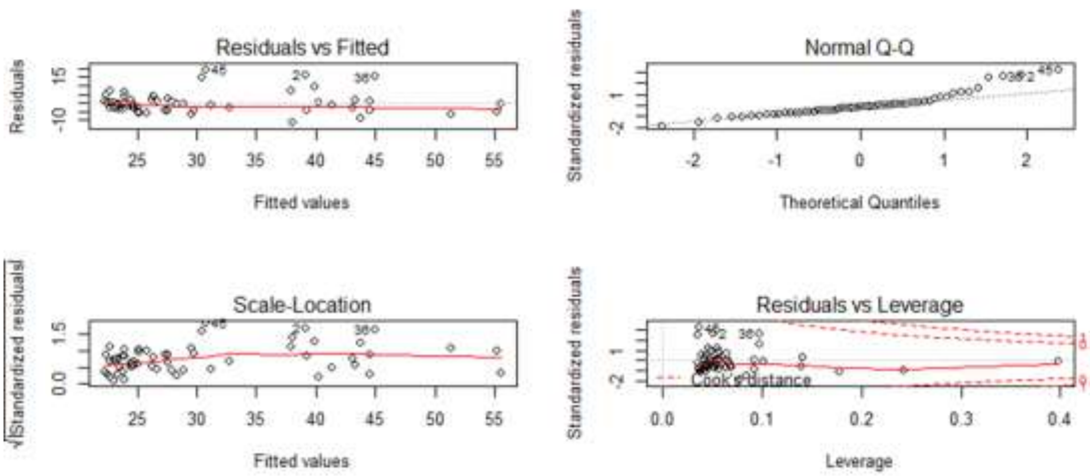


Figure 4.25. Histogram of Residuals for Sex-specific M1 Linear Regression Model

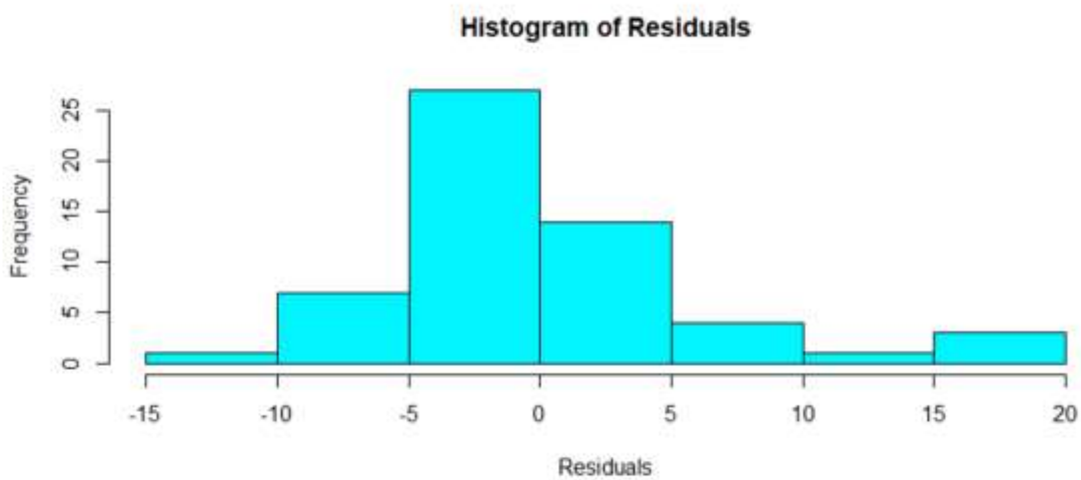
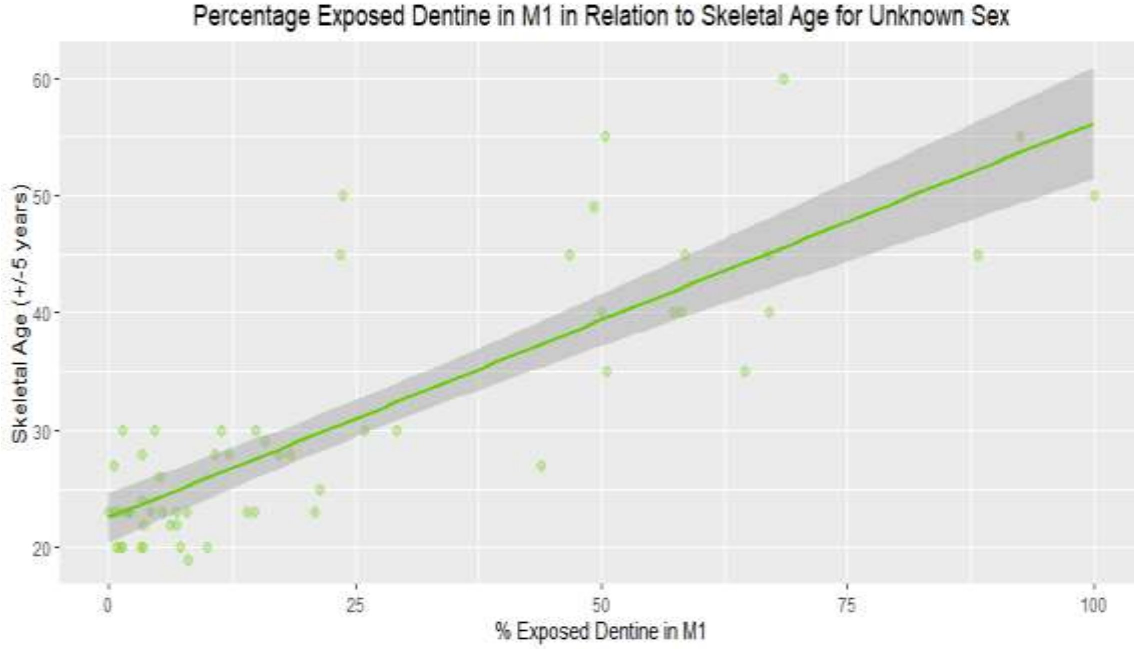


Figure 4.26. Percentage of Exposed Dentine in M1 in Relation to Skeletal Age for Unknown Sex (n = 57) - Colour intensity of data points reflects number of overlapping points



Linear Regression Equation for Unknown Sex:

y = 0.34x + 22.58, CI = +/-12 years

Residual standard deviation: *5.987 on 55 degrees of freedom*
Mean Absolute Error: *4.19 years*
Multiple R-squared: *0.7*
Adjusted R-squared: *0.6945*
F-statistic: *128.3 on 1 and 55 DF, p-value: 5.321e-16*
AIC: *369.7343*

Figure 4.27. Diagnostics for M1 Linear Regression Model for Unknown Sex

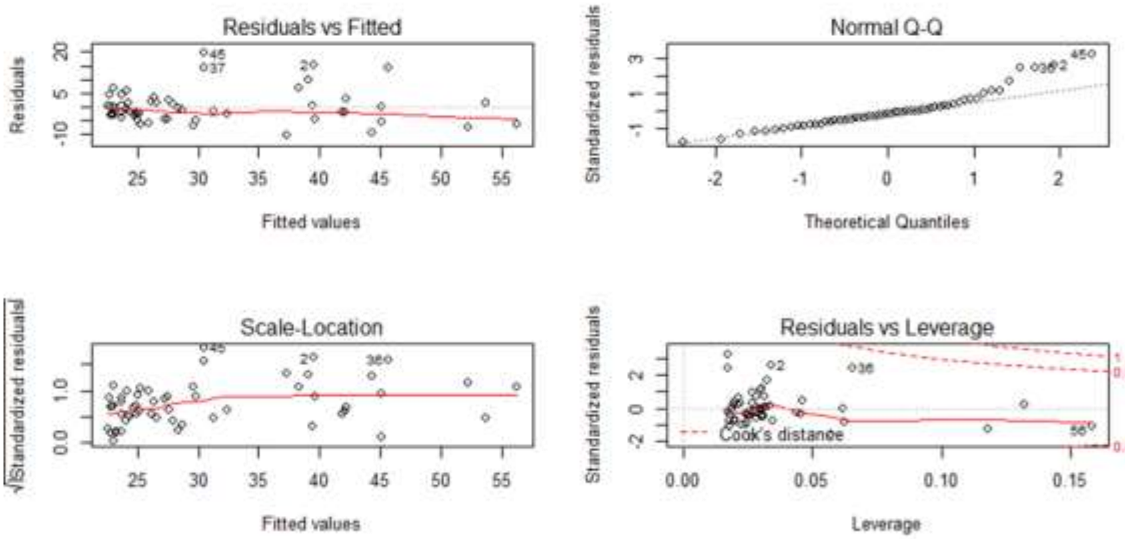
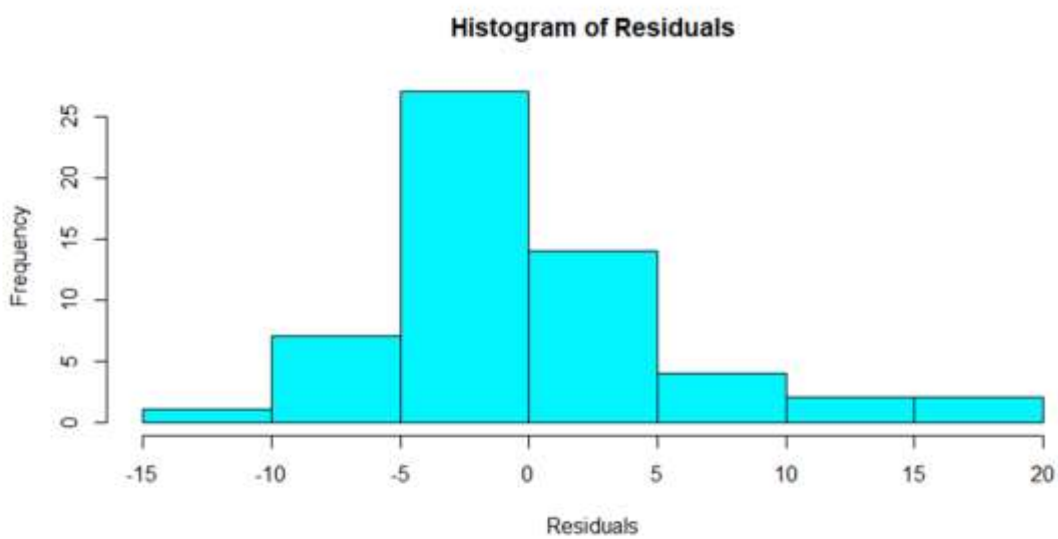


Figure 4.28. Histogram of Residuals for M1 Linear Regression Model for Unknown Sex



These final models (Figure 4.23 and Figure 4.26) were found to be well fitted with the data and approaching normal distributions of residuals (Figure 4.25 and Figure 4.28). The slight skew in the residual analyses would likely be resolved through expansion of the reference dataset, however it may result in biased, and therefore problematic, regression coefficient estimates. Similar to the AMTL models, it should be noted that variation in the percentage of exposed occlusal dentine in first molars increases with skeletal age. An Analysis of Covariance (ANCOVA) test was conducted on the sex-specific linear model of M1 wear to quantify the effects of the dependent variable (i.e. skeletal age) on the numeric independent variable (i.e. percentage dentine exposure) and the categorical independent variable (i.e. sex).

Table 4.10. Analysis of Covariance Table (Skeletal Age vs. Sex and M1 Dental Wear)

	df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Sex	1	183.5	183.5	4.9640	0.03015	p<0.05
M1	1	4416.7	4416.7	119.5056	3.404e-15	p<0.0001
Sex:M1	1	11.6	11.6	0.3132	0.57806	p<1
Residuals	53	1958.8	37.0			

The results indicate that M1 dental wear is significantly affected by skeletal age (ANCOVA: $F=119.5056$, $df=1, 53$, $p<0.0001$). The effect of sex and skeletal age was also statistically significant at a 95% confidence interval (ANCOVA: $F=4.9640$, $df=1, 53$, $p<0.05$), confirming the need for sex-specific age estimation models. However, the interaction between sex and M1 dental wear did not show statistically significant effect on skeletal age (ANCOVA: $F=0.57806$, $df=1, 53$, $p<1$).

As previously applied to the AMTL regression models, a bootstrapping without replacement technique was used to test the linear M1 wear models. Using RStudio, the dataset was randomly split 80:20 for model creation and test data, respectively (Raschka 2018; Prabhakaran 2017; Gagneja 2018; See Appendix 5 for all R codes). Through this method, a sex-specific and unknown sex linear regression testing models were created from a random selection of 80% of the dataset. The remaining 20% of the dataset were input into the linear regression testing models, resulting in the following tables of actual skeletal age and skeletal age as predicted by the sex-specific and unknown sex M1 wear linear regression testing models.

Table 4.11. Actual vs. Predicted Skeletal Age According to the Sex-specific M1 Wear Model

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	24.99171
2	55	37.71501
5	45	42.91524
7	20	22.98088
10	45	36.64586
11	40	39.98666
17	40	39.74647
21	20	25.02940
22	28	27.97184
34	45	42.58197
44	30	26.54788
55	23	25.31230

Mean of the differences: 1.71, t(11) = 0.98, p-value = 0.35

Table 4.12. Actual vs. Predicted Skeletal Age according to the M1 Wear Model for Unknown Sex

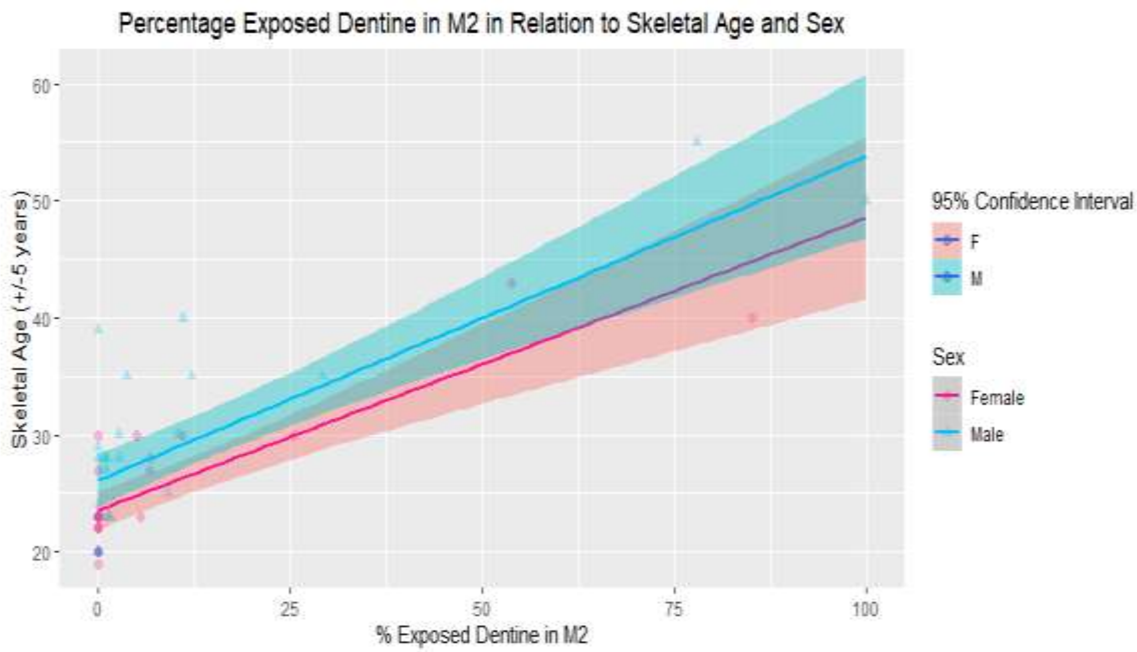
Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	24.99227
4	29	28.14803
8	20	26.07502
20	23	23.01548
26	20	23.77414
31	23	29.93029
37	45	30.84617
39	55	55.22186
40	27	22.71237
42	20	23.69070
51	27	38.02530
52	30	23.04306
53	22	23.75838
54	22	24.75029
55	23	25.31762
56	50	57.84854
57	28	26.83005

Mean of the differences: -1.29, t(16) = -0.90, p-value = 0.3816

A paired t-test comparing the actual and predicted skeletal ages according to the sex-specific M1 wear model revealed a mean of the differences of 1.71, with a t-value of 0.98 at 11DF and a p-value of 0.3481. The paired t-test comparing the actual and predicted skeletal ages according to the M1 wear model for unknown sex revealed a mean of the differences of -1.29, with a t-value of -0.90 at 16DF and a p-value of 0.3816. The high p-values indicate that the t-value is not statistically significant, likely as a result of the small sample size.

All of the processes applied to first molar dental wear were similarly applied to second molar dental wear. Through regression diagnostics, five outlying data points were identified and removed from the dataset to reduce bias (See Figure 4.30, Figure 4.31, Figure 4.33, and Figure 4.34 for diagnostics of final M2 models). All of these data points represented individuals with abnormally low dental wear on the M2 in relation to skeletal age. Linear regression models, with and without these outliers, were compared for goodness-of-fit resulting in final models (n = 50) that excluded the aforementioned outliers (Figure 4.29 and Figure 4.32). As in the other models, linear regression equations were determined through the estimated coefficient output in RStudio. Also the confidence interval included with these equations was estimated by rounding the residual standard deviation to the nearest whole number and doubling it to two standard deviations (CI=95%).

Figure 4.29. Percentage of Exposed Dentine in M2 in Relation to Skeletal Age and Sex (n = 50) - Colour intensity of data points reflects number of overlapping points



Linear Regression Equations:

Female -> y= 2.57x + 23.55, CI = +/-10 years
Male -> y=0.28x + 26.12, CI = +/- 10 years

Residual standard deviation: *4.579 on 46 degrees of freedom*
Mean Absolute Error: *3.53 years*
Multiple R-squared: *0.7053*
Adjusted R-squared: *0.6861*
F-statistic: *36.7 on 3 and 46 DF, p-value: 2.903e-12*
AIC: *299.8677*

Figure 4.30. Diagnostics for Sex-specific M2 Linear Regression Model

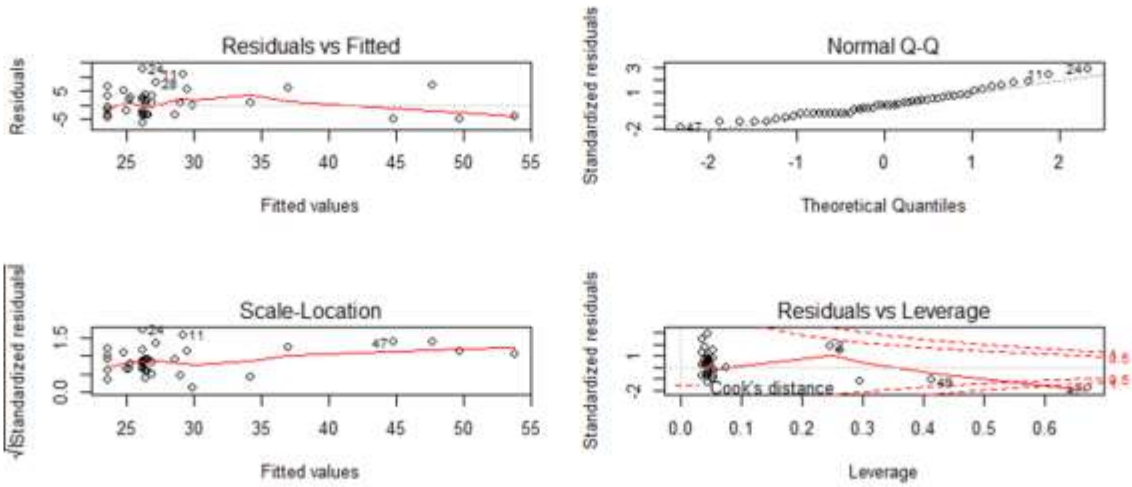


Figure 4.31. Histogram of Residuals for Sex-specific M2 Linear Regression Model

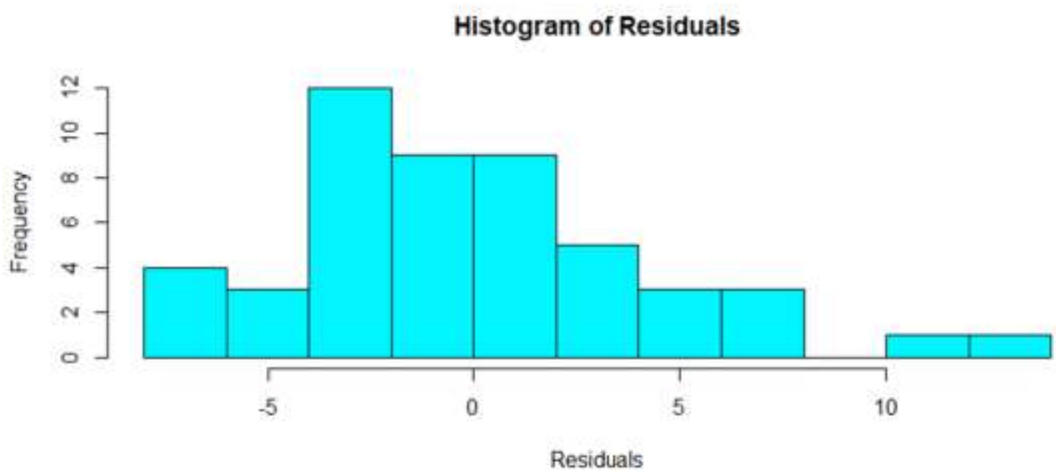
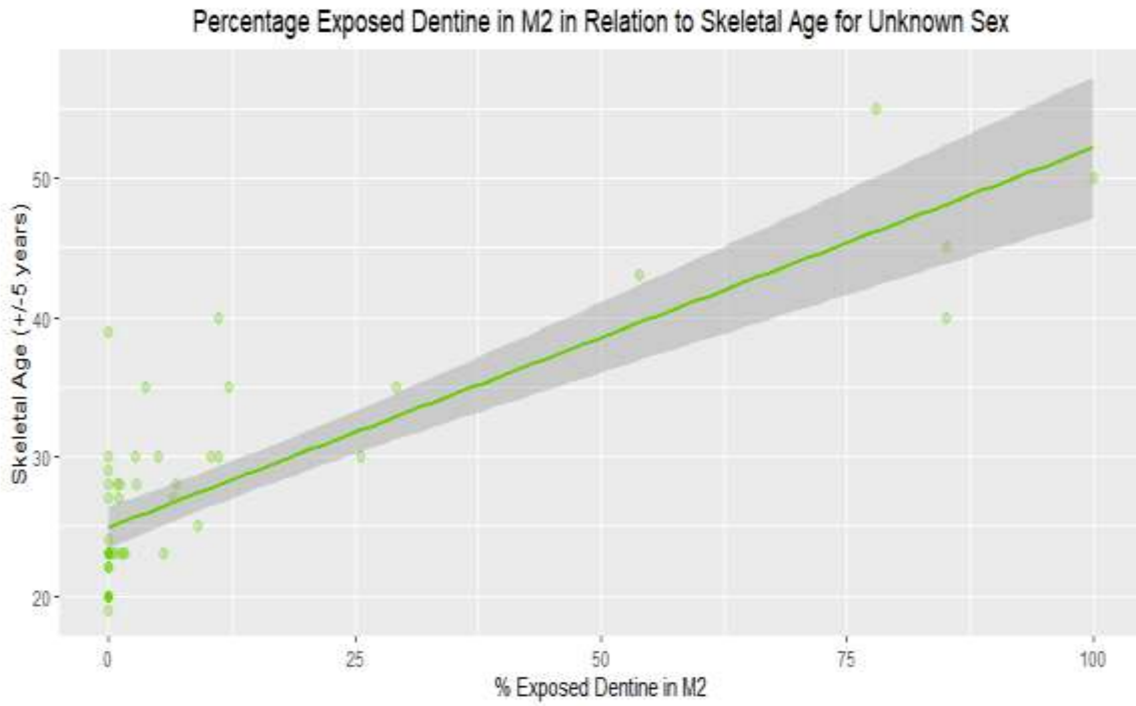


Figure 4.32. Percentage of Exposed Dentine in M2 in Relation to Skeletal Age for Unknown Sex (n = 50) - Colour intensity of data points reflects number of overlapping points



Regression Equation for Unknown Sex: *y = 0.27x + 24.93, CI = +/- 10 years*

Residual standard deviation: *4.718 on 48 degrees of freedom*
Mean Absolute Error: *3.76 years*
Multiple R-squared: *0.6735*
Adjusted R-squared: *0.6667*
F-statistic: *99.04 on 1 and 48 DF, p-value: 2.967e-13*
AIC: *300.9893*

Figure 4.33. Diagnostics for M2 Linear Regression Model for Unknown Sex

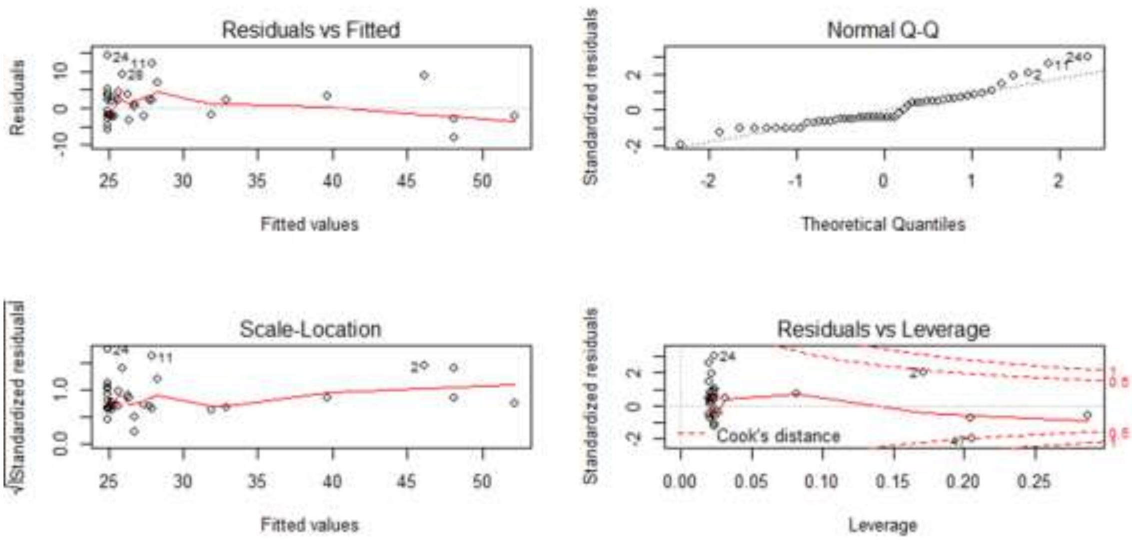
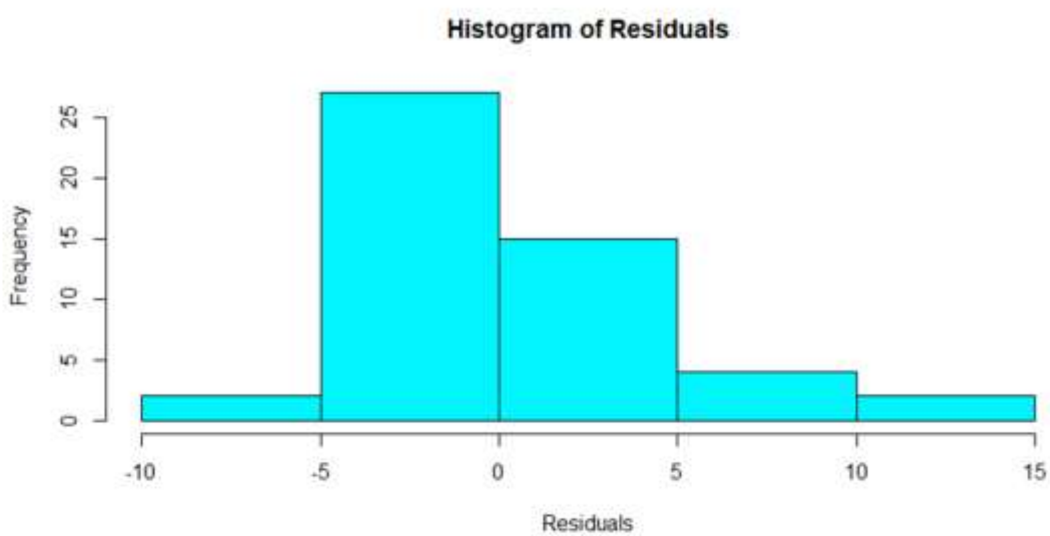


Figure 4.34. Histogram of Residuals for M2 Linear Regression Model for Unknown Sex



Both models were found to be well fitted with a slightly skewed distribution of residuals (Figure 4.31 and Figure 4.34). This deviation from normality in residual analysis is likely a result of the small sample size; however, it is important to note as it may contribute to biased regression coefficient estimates. An Analysis of Covariance (ANCOVA) test was conducted on the sex-specific model to quantify the effects of the dependent variable (i.e. skeletal age) on the numeric independent variable (i.e. percentage dentine exposure) and the categorical independent variable (i.e. sex).

Table 4.13. Analysis of Covariance Table (Skeletal Age vs. Sex and M2 Dental Wear)

	df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Sex	1	176.87	176.87	8.4363	0.005635	p<0.01
M2	1	2126.89	2126.89	101.4494	3.236e-13	p<0.0001
Sex:M2	1	4.66	4.66	0.2224	0.639428	p<1
Residuals	46	964.39	20.97			

These results indicate that sex (ANCOVA: F=8.4363, df=1, 46, p<0.01) and M2 wear (ANCOVA: F=101.4494, df=1, 46, p<0.0001) had statistically significant correlations with skeletal age. However, the interaction between sex and M2 wear was not found to be significantly correlated with skeletal age (ANCOVA: F=0.2224, df=1, 46, p<1).

Following the ANCOVA test, bootstrapping without replacement was conducted on the linear M2 models in RStudio using the an 80:20 random data split for model creation and test data (Raschka 2018; Prabhakaran 2017; Gagneja 2018). Through this method, a linear regression testing model was created from a random selection of 80% of the dataset. The remaining 20% of the dataset were input into the linear regression testing models, resulting in the following tables of actual skeletal age and skeletal age as predicted by the sex-specific and unknown sex M2 linear regression testing models.

Table 4.14. Actual vs. Predicted Skeletal Age as per the Sex-Specific M2 Wear Model

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	23.38012
4	29	26.77608
8	28	25.06274
20	20	26.77608
26	23	23.38012
31	25	29.20359
37	20	26.77608
39	30	26.12057
40	23	23.38012
42	23	23.38012

Mean of the differences: -1.12, t(9) = -0.94, p-value = 0.3704

Table 4.15. Actual vs. Predicted Skeletal Age as per the M2 Wear Model for Unknown Sex

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	25.35979
4	29	25.35979
8	28	27.16989
20	20	25.35979
26	23	25.35979
31	25	27.75874
37	20	25.35979
39	30	28.30785
40	23	25.35979
42	23	25.35979

Mean of the differences: -1.78, t(9) = -1.90, p-value = 0.08945

A paired t-test comparing the actual and predicted skeletal ages according to the sex-specific model revealed a mean of the differences of -1.12, with a t-value of -0.94 at 9DF and a p-value of 0.3704. Meanwhile, the paired t-test comparing the actual and predicted skeletal ages according to the unknown sex model revealed a mean of the differences of -1.78, with a t-value of -1.90 at 9DF and a p-value of 0.08945. The high p-values indicate that the t-value is not statistically significant, likely as a result of the small sample size.

4.6.6.3 Comparing observed dental wear at Kellis 2 to the existing dental wear standards

The quantified M1 and M2 wear models were compared to quantifications of the dental wear visualized in Brothwell's (1963a) standard for adult dental age estimation. Since Brothwell's standard provided multiple dental wear examples for each age cohort, the minimums and maximums of the age cohort and the quantified dental wear were used to graph the photogrammetrically quantified Brothwell standard (See green boxes in Figure 4.35. A Photogrammetric Comparison of the Brothwell (1963a) Dental Age Estimation Standard and the New M1 Wear Linear Regression Modelsand Figure 4.36. A Photogrammetric Comparison of the Brothwell (1963a) Dental Age Estimation Standard and the New M2 Wear Linear Regression Models. The linear regression models for M1 and M2 wear were graphed for a visual comparison (Figure 4.35 and Figure 4.36).

Figure 4.35. A Photogrammetric Comparison of the Brothwell (1963a) Dental Age Estimation Standard and the New M1 Wear Linear Regression Models (n = 57)

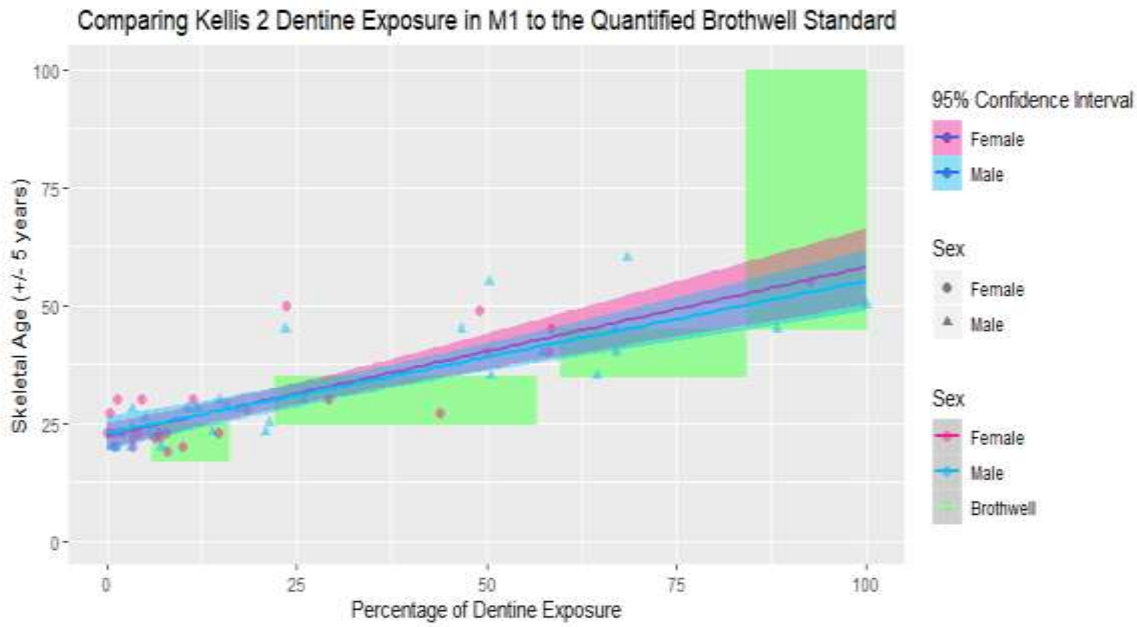
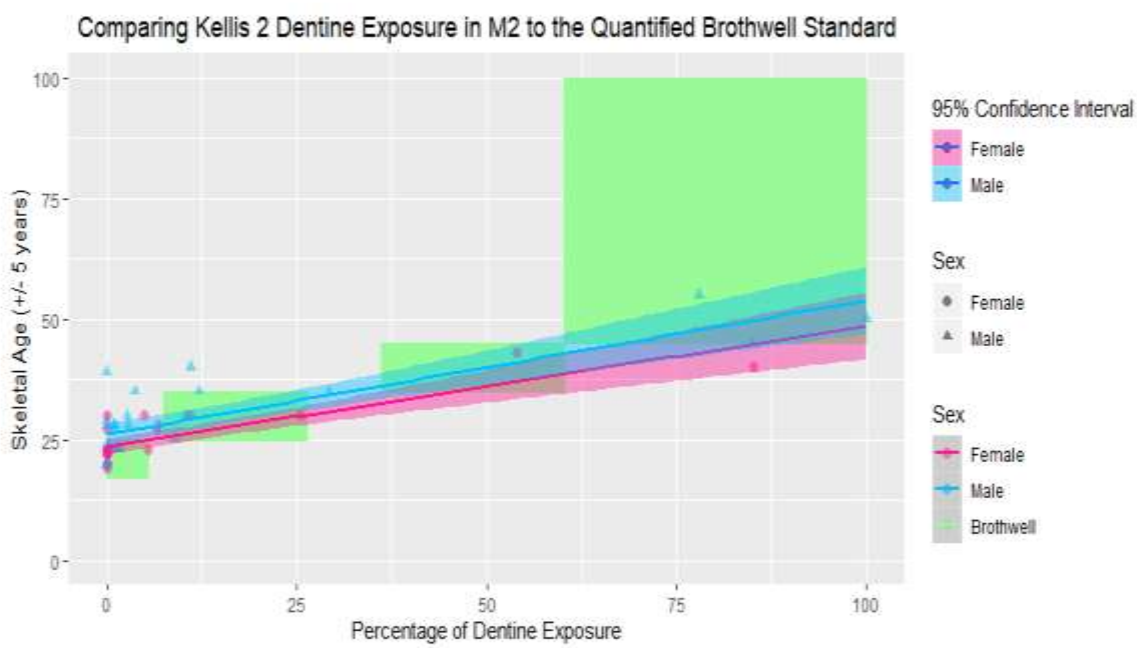


Figure 4.36. A Photogrammetric Comparison of the Brothwell (1963a) Dental Age Estimation Standard and the New M2 Wear Linear Regression Models (n = 50)



Through visualization of these data, it is apparent that there are gaps in Brothwell's dental wear representation with regard to the percentage of occlusal dentine exposed. It is also clear that the linear regression models are far more precise than the Brothwell standard and may be helpful in producing more sensitive estimations of age, particularly for older individuals. In the M1 graph, it is apparent that the same percentage of dentine exposure relates to slightly lower skeletal age estimates, on average, in the first three Brothwell age estimation ranges when compared to the linear regression models. In the M2 graph, the same percentage of dentine exposure relates to slightly lower skeletal age estimates in the youngest Brothwell cohort that transition to slightly higher skeletal age estimates in the oldest Brothwell age cohort in comparison with the associated linear regression models. Looking at the specific data points, this has translated to many underestimates of skeletal age from Brothwell's M1 wear standard and a transition from underestimation to overestimation of skeletal age from Brothwell's M2 wear standard. Given that the linear models were based on the data at hand, they are designed for goodness of fit with the data and therefore do not have such dramatic biases. Of course, it is possible that the linear regression models are slightly biased in relation to chronological age due to their reliance on skeletal age estimates. This complicates comparison with the Brothwell standard, which is presented as an estimator of chronological age. Unfortunately, modern Egyptian populations have dissimilar rates of dental wear from ancient Egyptian populations due to differences in diet and food preparation. As such, until age estimation methods for human remains significantly improve, particularly in the older cohorts, the accuracy of age estimation regression models for ancient Egyptians will continue to be based on skeletal age and therefore be at risk for systematic bias. Fortunately, skeletal age estimates based on methods not developed through the principles of the Rostock Manifesto (i.e. based on known age reference population and calculated using Bayesian statistics) are known to underestimate age in older cohorts as estimates tend toward the mean (Hoppa and Vaupel 2002). Consequently, it may be inferred that the use of skeletal age estimates in the creation of the linear regression models may result in an underestimation of skeletal ages when using these regression models. If this is true, and the regression models for the chronological age of the Kellis 2 population can be assumed to be higher on the skeletal age scale than those depicted above, then the Brothwell method may actually underestimate chronological age in the 17-45 year cohorts for M1 wear in the Kellis 2 population to a greater degree than is depicted in Figure 4.35. Similarly, the Brothwell method may also underestimate chronological age in the 17-25 year cohort for M2 wear in the Kellis 2 population more significantly than is observable in Figure 4.36.

4.6.6.4 Multiple Regression Models for the Estimation of Age

In an attempt to refine the age estimation methods, multiple regression models were created for all combinations of first molar wear (M1), second molar wear (M2), and antemortem tooth loss (AMTL) for males, females, and individuals of unknown sex. Regression diagnostics (i.e. RSE, Mean Absolute Error, R-squared, F-statistic, AIC, histogram of residuals, Residuals vs. Fitted, Normal Q-Q, Scale-Location, and Residuals vs. Leverage) were performed to ensure goodness of fit and predictive value of each model. The results of the RSE, Mean Absolute Error, R-squared, F-statistic, and AIC are presented along with their associated multiple regression equations and calculated confidence intervals below. It should be noted that multiple regression models assume multivariate normality, a linear relationship, no multicollinearity, and homoscedasticity. As in the previous regression models, an 80:20 bootstrapping technique without replacement was then applied to each model (Raschka 2018; Prabhakaran 2017; Gagneja 2018). The predicted skeletal ages derived from the bootstrapped model were then compared to the actual skeletal age values through a paired t-test to demonstrate the predictive value of each model.

The following multiple regression equation was developed for use with first and second molar dental wear data as well as antemortem tooth loss in males. A confidence interval of +/-9 years was calculated and a paired t-test of bootstrapped data gave results of $t(6) = 1.34$, $p = 0.2296$. These results are statistically insignificant; however, the mean of differences between actual and predicted skeletal age is 7.27 years, which is within the calculated confidence interval.

M1+M2+AMTL (Males) ->

y = 0.23(M1) + 0.03(M2) + 1.03(AMTL) + 21.70, CI = +/- 9 years

Residual standard deviation: *4.054 on 13 degrees of freedom*
Mean Absolute Error: *3.25 years*
Multiple R-squared: *0.8307*

Adjusted R-squared: *0.7916*
F-statistic: *21.26 on 3 and 13 DF, p-value: 2.72e-05*
AIC: *54.25035*

Table 4.16. Actual vs. Predicted Skeletal Age as per the M1+M2+AMTL Multiple Regression Model for Males

Line	Actual Skeletal Age	Predicted Skeletal Age
1	55	16.86195
2	28	19.35500
3	29	25.26267
13	24	21.05431
14	30	29.89427
17	20	26.56549
43	28	24.13897

Mean of the differences: 7.27, t(6) = 1.34, p-value = 0.2296

This next multiple regression equation was developed for use with first and second molar dental wear data as well as antemortem tooth loss in females. A confidence interval of +/-4 years was calculated and a paired t-test of bootstrapped data gave results of t(6) = 0.51, p = 0.6294. These results do not show strong confidence in the t value due to the small sample size; however, the mean of differences between actual and predicted skeletal ages is 0.66 years. This is well within the calculated confidence interval.

M1+M2+AMTL (Females) ->
y = -0.16(M1) + 1.01(M2) – 4.44(AMTL) + 22.11, CI = +/-4 years

Residual standard deviation: *1.875 on 8 degrees of freedom*
Mean Absolute Error: *1.38 years*
Multiple R-squared: *0.7611*
Adjusted R-squared: *0.6715*
F-statistic: *8.494 on 3 and 8 DF, p-value: 0.00721*
AIC: *26.41295*

Table 4.17. Actual vs. Predicted Skeletal Age as per the M1+M2+AMTL Multiple Regression Model for Females

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	20.36403
6	19	20.10966
9	28	23.23229
26	23	21.46454
30	30	35.91823
46	22	21.17345
50	23	20.15060

Mean of the differences: 0.66, t(6) = 0.51, p-value = 0.6294

The next multiple regression equation was developed for use with first and second molar dental wear data, as well as antemortem tooth loss data, in individuals of unknown sex. A confidence interval of +/-7 years was calculated and a paired t-test of bootstrapped data gave results of t(5) = 1.45, p = 0.2066. These results are statistically insignificant; however, the mean of differences between actual and predicted skeletal age is 6.45, which falls within the calculated confidence interval.

M1+M2+AMTL (Unknown Sex) ->
y = 0.24(M1) + 0.03(M2) + 1.02(AMTL) + 21.07, CI = +/- 7 years

Residual standard deviation: *3.294 on 25 degrees of freedom*
Mean Absolute Error: *2.68 years*
Multiple R-squared: *0.8278*
Adjusted R-squared: *0.8072*
F-statistic: *40.06 on 3 and 25 DF, p-value: 1.061e-09*
AIC: *114.1931*

Table 4.18. Actual vs. Predicted Skeletal Age as per the M1+M2+AMTL Multiple Regression Model for Unknown Sex

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	22.70786
2	55	27.10125
4	28	21.21721
26	30	24.98119
27	40	37.07117
49	23	25.69477

Mean of the differences: 6.54, t(5) = 1.45, p-value = 0.2066

The following multiple regression equation was developed for use with first molar wear and antemortem tooth loss data in males. A confidence interval of +/-11 years was calculated and a paired t-test of bootstrapped data gave results of t(6) = 0.65, p = 0.5421. These results do not show confidence in the t value due to the small sample size; however, the mean of differences between actual and predicted skeletal ages is 2.19 years. This is well within the calculated confidence interval.

M1+AMTL (Males) -> y = 23.24 + 0.21(M1) + 1.04(AMTL), CI = +/- 11 years

Residual standard deviation: *5.176 on 21 degrees of freedom*
Mean Absolute Error: *3.92 years*
Multiple R-squared: *0.785*
Adjusted R-squared: *0.7645*
F-statistic: *38.33 on 2 and 21 DF, p-value: 9.802e-08*

AIC: *151.819*

Table 4.19. Actual vs. Predicted Skeletal Age as per the M1+AMTL Multiple Regression Model for Males

Line	Actual Skeletal Age	Predicted Skeletal Age
1	55	42.11582
2	28	22.73382
5	40	43.75519
27	25	28.35780
32	45	29.88427
40	45	52.42030
42	50	53.42676

Mean of the differences: 2.19, t(6) = 0.65, p-value = 0.5421

The next multiple regression equation was developed for use with first molar dental wear and antemortem tooth loss data from females. A confidence interval of +/-6 years was calculated and a paired t-test of bootstrapped data gave results of t(5) = -0.82, p = 0.4497. These results are statistically insignificant; however, the mean of differences between actual and predicted skeletal age is -0,80, which falls within the calculated confidence interval.

M1+AMTL (Females) ->

y = 20.77 + 0.27(M1) + 1.30(AMTL), CI = +/- 6 years

Residual standard deviation: *2.628 on 18 degrees of freedom*
Mean Absolute Error: *2.09 years*
Multiple R-squared: *0.952*
Adjusted R-squared: *0.9467*
F-statistic: *178.6 on 2 and 18 DF, p-value: 1.346e-12*
AIC: *104.9468*

Table 4.20. Actual vs. Predicted Skeletal Age as per the M1+AMTL Multiple Regression Model for Females

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	22.91336
6	19	23.21272
9	28	26.04078
26	23	21.61819
30	30	32.85877
50	23	23.16454

Mean of the differences: -0.80, t(5) = -0.82, p-value = 0.4497

The following multiple regression equation was developed for use with first molar wear and antemortem tooth loss data in individuals of unknown sex. A confidence interval of +/-9 years was calculated and a paired t-test of bootstrapped data gave results of t(8) = 0.38, p = 0.7109. These results do not show confidence in the t value due to the small sample size; however, the mean of differences between actual and predicted skeletal ages is 0.48 years. This is well within the calculated confidence interval.

M1+AMTL (Unknown Sex) -> y = 22.10 + 0.23(M1) + 1.18(AMTL), CI = +/- 9 years

Residual standard deviation: *4.238 on 42 degrees of freedom*
Mean Absolute Error: *3.15 years*
Multiple R-squared: *0.8564*
Adjusted R-squared: *0.8495*
F-statistic: *125.2 on 2 and 42 DF, p-value: < 2.2e-16*
AIC: *262.5721*

Table 4.21. Actual vs. Predicted Skeletal Age as per the M1+AMTL Multiple Regression Model for Unknown Sex

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	23.56684
2	55	54.24015
4	28	23.97497
16	40	39.57663
18	40	43.50033
26	30	25.39849
27	40	35.17079
45	40	39.00589
49	23	29.22075

Mean of the differences: 0.48, t(8) = 0.38, p-value = 0.7109

The next multiple regression equation was developed for use with second molar dental wear and antemortem tooth loss data from males. A confidence interval of +/-11 years was calculated and a paired t-test of bootstrapped data gave results of t(4) = 2.21, p = 0.09186. These results are statistically insignificant; however, the mean of differences between actual and predicted skeletal age is 4.23 years, which falls within the calculated confidence interval.

M2+AMTL (Males) -> y = 25.37 - 0.03(M2) + 1.90(AMTL), CI = +/-11 years

Residual standard deviation: *5.338 on 18 degrees of freedom*
Mean Absolute Error: *3.91 years*
Multiple R-squared: *0.6529*
Adjusted R-squared: *0.6143*
F-statistic: *16.93 on 2 and 18 DF, p-value: 7.32e-05*
AIC: *134.6982*

Table 4.22. Actual vs. Predicted Skeletal Age as per the M2+AMTL Multiple Regression Model for Males

Line	Actual Skeletal Sex	Predicted Skeletal Sex
1	55	45.66686
2	28	26.66192
22	39	33.05085
27	25	26.49539
28	35	28.99278

Mean of the differences: 4.23, t(4) = 2.21, p-value = 0.09186

The following multiple regression equation was developed for use with second molar wear and antemortem tooth loss data from females. A confidence interval of +/-5 years was calculated and a paired t-test of bootstrapped data gave results of t(6) = 1.07, p = 0.3241. These results do not show confidence in the t value due to the small sample size; however, the mean of differences between actual and predicted skeletal ages is 0.97 years. This is well within the calculated confidence interval.

M2+AMTL (Females) -> y = 21.78 + 0.28(M2) + 0.91(AMTL), CI = +/-5 years

Residual standard deviation: *2.074 on 11 degrees of freedom*
Mean Absolute Error: *1.55 years*
Multiple R-squared: *0.7164*
Adjusted R-squared: *0.6648*

F-statistic: *13.89 on 2 and 11 DF, p-value: 0.0009774*
AIC: *64.78183*

Table 4.23. Actual vs. Predicted Skeletal Age as per the M2+AMTL Multiple Regression Model for Females

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	21.20000
6	19	21.20000
9	28	23.43276
26	23	21.20000
30	30	31.96606
36	30	28.03644
50	23	21.20000

Mean of the differences: 0.97, t(6) = 1.07, p-value = 0.3241

The next multiple regression equation was developed for use with second molar wear and antemortem tooth loss data from individuals of unknown sex. A confidence interval of +/- 10 years was calculated and a paired t-test of bootstrapped data gave results of t(6) = 0.10, p = 0.9217. These results are statistically insignificant; however, the mean of differences between actual and predicted skeletal age is 0.39 years, which falls well within the calculated confidence interval.

M2+AMTL (Unknown Sex) ->
y = 23.93 + 0.002(M2) + 1.91(AMTL), CI = +/- 10 years

Residual standard deviation: *4.544 on 32 degrees of freedom*
Mean Absolute Error: *3.34 years*
Multiple R-squared: *0.6468,*
Adjusted R-squared: *0.6247*
F-statistic: *29.3 on 2 and 32 DF, p-value: 5.876e-08*
AIC: *210.1538*

Table 4.24. Actual vs. Predicted Skeletal Age as per the M2+AMTL Multiple Regression Model for Unknown Sex

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	22.81015
2	55	69.27939
4	28	25.55462
26	30	22.80369
27	40	22.78368
49	23	28.29544
74	30	33.76148

Mean of the differences: 0.39, t(6) = 0.10, p-value = 0.9217

The following multiple regression equation was developed for use with first and second molar wear in males. A confidence interval of +/-9 years was calculated and a paired t-test of bootstrapped data gave results of t(2) = 1.15, p = 0.3704. These results do not show confidence in the t value due to the small sample size; however, the mean of differences between actual and predicted skeletal ages is 8.01 years. This is within the calculated confidence interval.

M1+M2 (Males) -> y = 21.21 + 0.28(M1) + 0.15(M2), CI = +/- 9 years

Residual standard deviation: *4.401 on 18 degrees of freedom*
Mean Absolute Error: *3.35 years*
Multiple R-squared: *0.7438*
Adjusted R-squared: *0.7153*
F-statistic: *26.13 on 2 and 18 DF, p-value: 4.753e-06*
AIC: *126.5963*

Table 4.25. Actual vs. Predicted Skeletal Age as per the M1+M2 Multiple Regression Model for Males

Line	Actual Skeletal Age	Predicted Skeletal Age
1	55	33.71688
2	28	22.80879
27	25	27.43647

Mean of the differences: 8.01, t(2) = 1.15, p-value = 0.3704

The next multiple regression equation was developed for use with first and second molar dental wear in females. A confidence interval of +/-6 years was calculated and a paired t-test of bootstrapped data gave results of t(6) = 0.49, p = 0.6397. These results are statistically insignificant; however, the mean of differences between actual and predicted skeletal age is 0.65 years, which falls well within the calculated confidence interval.

M1+M2 (Females) -> y = 22.18 + 0.06(M1) + 0.26(M2), CI = +/- 6 years

Residual standard deviation: *2.816 on 13 degrees of freedom*
Mean Absolute Error: *1.78 years*
Multiple R-squared: *0.4274*
Adjusted R-squared: *0.3393*
F-statistic: *4.851 on 2 and 13 DF, p-value: 0.02668*
AIC: *83.21183*

Table 4.26. Actual vs. Predicted Skeletal Age as per the M1+M2 Multiple Regression Model for Females

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	23.10867
6	19	23.22417
9	28	23.84644
26	23	22.60897
30	30	23.69044
46	22	22.74114
50	23	23.20558

Mean of the differences: 0.65, t(6) = 0.49, p-value = 0.6397

Lastly, the following multiple regression equation was developed for use with first and second molar wear in individuals of unknown sex. A confidence interval of +/-8 years was calculated and a paired t-test of bootstrapped data gave results of t(6) = 1.57, p = 0.167. These results do not show confidence in the t value due to the small sample size; however, the mean of differences between actual and predicted skeletal ages is 4.95 years. This is within the calculated confidence interval.

M1+M2 (Unknown Sex) -> y = 21.28 + 0.24(M1) + 0.16(M2), CI = +/-8 years

Residual standard deviation: *3.898 on 34 degrees of freedom*
Mean Absolute Error: *2.97 years*
Multiple R-squared: *0.6999*
Adjusted R-squared: *0.6822*

F-statistic: *39.64 on 2 and 34 DF, p-value: 1.302e-09*
AIC: *210.5422*

Table 4.27. Actual vs. Predicted Skeletal Age as per the M1+M2 Multiple Regression Model for Unknown Sex

Line	Actual Skeletal Age	Predicted Skeletal Age
1	22	23.26993
2	55	32.91064
4	28	22.48656
26	30	25.04476
27	40	34.51969
49	23	26.39612
81	23	21.73151

Mean of the differences: 4.95, t(6) = 1.57, p-value = 0.167

4.7 Discussion and Conclusion

This study of dental age estimation in adults from Kellis 2, Dakhleh Oasis, Egypt demonstrated the usefulness of dental indicators in the estimation of age and, more importantly, the potential for improved adult dental age estimation methods in Egypt. To this end, a photogrammetric method for the quantification of occlusal dentine exposure in relation to the occlusal surface was identified and tested. This method, originally proposed by Phillips-Conroy et al. (2000) and modified by Deter (2006, 2009), was applied using the free online software FIJI (Is Just ImageJ). All subsequent calculations, plots, graphs and models were calculated in RStudio and the related R codes are accessible in Appendix 5. For this study, FIJI (Is Just ImageJ) was used to quantify the percentage of exposed occlusal dentine in relation to the occlusal surface. These data were collected for lower left first molars from a photographic sample of human occlusal dentition from Kellis 2 cemetery. When the lower left molar was missing, the lower right molar or the upper left molar was used as a substitute, in this order. This category of primary M1 data reflects the inclusion of these substitutions. In an effort to test the accuracy and repeatability of this method, primary M1s were quantified a second time by the author and a third time by a volunteer, who was briefly trained in this method. An intra-class comparison (ICC) of intra-and inter-observer data to the original data demonstrated reasonably low intra- and inter-observer error rates (intra: 99% correlation; inter: 98% correlation). These rates compare favourably to intra- and inter-observer error rates seen through the use of the Brothwell (1963a) atlas-style dental age estimation method (intra: 80% correlation; inter: 80% correlation) (Alayan 2018).

Following these tests, the quantification method was used to collect data for second molars adjacent to the primary M1, primary M1 antimeres (i.e. lower right first molar), and primary M1 isomeres (i.e. upper left first molar). These data were used to compare the percentages of exposed occlusal dentine between primary M1 antimeres (opposing left and right pairs within individuals) and isomeres (occluding upper and lower teeth). The paired t-tests of these datasets revealed that there was a statistically significant difference between isomeres in the male or female data at a 90% confidence interval. A simple plot of differences demonstrated that most individuals had a higher percentage of exposed dentine in the lower molar than in the occluding upper molar. This tendency may be explained by greater bucco-lingual dimensions and enamel thickness in the upper molars compared to lower molars (Grine 2005; Smith et al. 2006). These results are congruent with findings from the dentition of prehistoric and contemporary Australian Aboriginals (Molnar et al. 1983a,b), but are in contrast with a number of other populations which show a tendency toward higher dental wear in the maxillary teeth than the mandibular teeth (Molnar 1971). McKee (1986) hypothesized that differences in face form and premolar eruption pattern may account for these population-level differences in maxillary and mandibular wear, though the aetiology remains unclear (Molnar and Molnar 1990). In any case, given that most common age estimation standards based on dental wear rely on the observation of dentine exposure patterns, these data show that upper and lower molar wear patterns are not interchangeable. These data support the observations of Murphy (1959b) and Pal (1971) and are an important consideration when scoring dental wear.

The comparison of percentages of occlusal dentine exposure in antimeres uncovered that although there was no directional difference in the severity of dental wear according to the side of the mouth, on average, there was a statistically significant absolute difference in the severity of dental wear between sides of the mouth. These findings stand in stark contrast to the longstanding belief that there is little difference in wear between left and right sides of the dentition (Campbell 1925; Hillson 1996). Significant asymmetry in dental wear among antimeres may be a consequence of a number of factors, including differences in occlusal relationship, arbitrary preference for a chewing side, a tendency to sided chewing as a result of dental pain or discomfort, or a tendency to one-sided chewing to compensate for lost chewing surfaces through dental disease or antemortem tooth loss. The aforementioned pathological hypotheses were investigated on 4 individuals who demonstrated the largest differences in dentine exposure between antimeres. These individuals were identified as outliers via Tukey's (1977) rule, which defines outliers as data points outside of the fences (or lines) on the whiskers of a Tukey's boxplot (see Section 4.6.5 for statistical details). The outliers included two females (ages 30+/-5 and 30+/-3) and two males (ages 23+/-3 and 55+/-5), with age and sex estimated by Dr. El Molto. Only the 30+/-3 year old female had more wear in the LRM1 than the LLM1; all others had more significant wear in the LLM1. It must be noted though that the occlusal surface of the LRM1 was affected by caries; this required the estimation of boundaries for dentine exposure during photogrammetry. Consequently, this data point was discarded. In the remaining individuals, the 55+/-5 year old male had abnormally severe wear on his LLM1, ULM1, and ULP2, compared to the surrounding teeth. As such, the observed asymmetry in left and right first molar wear may have been the result of the use of teeth as tools in some capacity and cannot, therefore, be a consequence of the loss of dental surfaces or dental pain and discomfort. Additionally, the 30 +/-5 year old female presented with gross caries on the same side of the mandible that had more severe dental wear; however, the maxilla was not available for analysis so it is unknown if there was also pathology on the opposite side of the dentition. Finally, the 23+/-3 year old male had an abscess on the side of the mouth with more severe dental wear and a carious lesion on the opposite side. Both sides of the upper dentition were also affected by the antemortem loss of a tooth. Given the small sample size (1 individual), and the fact that both sides of the dentition were equally affected by antemortem tooth loss and dental pathologies with the potential for pain or discomfort, a relationship between dental wear asymmetry and dental pathology or antemortem tooth loss was not observed. As such, a much larger study of dental pathology, AMTL, and asymmetrical dental wear is recommended to determine the relationship, if any, between dental wear and dental pathology. Furthermore, since the caries data collected in this study were gleaned from available photographs, these data are considered to be preliminary in nature and insufficient for a dental pathological study. However, this avenue will be pursued in the future.

Two of the more common, observable and discretely quantifiable dental pathologies in bioarchaeological contexts are dental caries and antemortem tooth loss (AMTL). Given the progressive unidirectional nature of both conditions (i.e. the number of teeth affected can only increase over time), it was decided that evidence of these conditions would be investigated to test their potential for use in the estimation of age-at-death. The relationship between dental caries and skeletal age was plotted and calculated using Pearson's r. Neither Pearson's r nor the visualization of the data revealed a linear relationship between the two variables. Since the number of teeth affected by dental caries is known to increase with chronological age in living populations (i.e. their number can only go up with time), these results were attributed to a loss of caries evidence through antemortem tooth loss. As a result, two variations on the reporting of dental caries were investigated in an attempt to mitigate theses effects. The first variation was the translation of the number of dental caries to the percentage of observable teeth with dental caries. For the purpose of this study, teeth were counted as "observable" if they had at least half of the crown and occlusal surface intact. Six individuals had no observable teeth and were thus excluded from these data. The resulting percentages were similarly compared with the related skeletal age data and, again, both Pearson's r and the visual representation of data failed to identify a linear correlation.

The second variation was inspired by the longstanding historical caries index for bioarchaeological material in which the sum of observed dental decay and teeth lost antemortem represents the adult prevalence of carious lesions (Mummery 1870). In the creation of the AMTL+Caries dataset, individuals who were missing their mandible or maxilla were excluded to reduce bias. The resulting data showed a clear positive linear relationship with skeletal age when plotted, and in accordance with Pearson's r. Of course, since antemortem tooth loss data were combined with caries data in this model, it was necessary to compare the correlative strength of the antemortem tooth loss data with and without caries data. Comparisons of Pearson's r scores indicated that the sum of antemortem tooth loss and caries data were less strongly correlated to skeletal age than the antemortem tooth loss data alone. Although analysis of the caries data collected during this study may be limited by their observation through photographs, the age related patterns seen in caries and antemortem tooth loss support Meiklejohn et al.'s (1992) assertion that dental wear and caries are independent factors and, as can be seen in the population studied here, are not mutually exclusive.

Since antemortem tooth loss proved to be strongly correlated with skeletal age, this relationship was plotted and linear regression models were calculated and plotted for males, females, and individuals with unknown sex. The inclusion of a regression model for individuals of unknown sex was deemed necessary as it is sometimes difficult or impossible to ascertain the sex of human remains. As a result, this model was derived from the combined male and female data. For each model, the estimated coefficients derived from the "summary" output from R were used to create an algebraic expression of each model and the 95% confidence interval was calculated by rounding the residual standard deviation to the nearest whole number and doubling it. Although the confidence intervals included with the regression equations are uniform across their respective models, graphs for each model showed that variation in the quantified dental indicator incrementally increases as skeletal age increases; that is individuals with skeletal ages of 65(+/-5 years) and over show a greater range in the number of antemortem teeth lost than younger individuals.

Each antemortem tooth loss vs. skeletal age and sex models were examined diagnostically through RSD, MAE, R-squared, F-statistic, and AIC calculations. Graphs for Residuals vs. Fitted Values, Normal Q-Q, Scale-Location, and Residuals vs. Leverage. These linear models showed skewed histograms of residuals. Consequently, polynomial regression models were created and similarly examined for goodness of fit. Equations for these models are as follows:

AMTL_linear (Female) *->* $y = 1.68x + 27.55$, *CI = +/-18 years*
AMTL_linear (Male) *->* $y = 1.78x + 29.39$, *CI = +/-18 years*
AMTL_linear (Unknown Sex) *->* $y = 1.67x + 28.58$, *CI = +/-18 years*

AMTL_polynomial (Female) *->* $y = 24.38 + 2.91x - 0.05x^2$, *CI = +/- 16 years*

AMTL_polynomial (Male) *->* $y = 26.38 + 3.64x - 0.10x^2$, *CI = +/- 16 years*
AMTL_polynomial (Unknown Sex) *->* $y = 25.81 + 2.95x - 0.06x^2$, *CI = +/- 16 years*

Regression diagnostics for the polynomial and linear regression models were then compared to determine the ideal model for skeletal age estimation. In both the sex-specific and unknown sex cases, the polynomial regression models outperformed the linear regression models in terms of the residual standard deviation, mean absolute error, multiple R-squared, adjusted R-squared, and the Akaike Information Criterion (AIC). All of these indicators show that the polynomial regression models have a stronger predictive strength than the linear regression models for AMTL and have a better fit among the data. As such, the polynomial regression models for AMTL may be the preferred age estimation models based on an oral pathological indicator. However, it is worth noting that ANCOVA tests on the sex-specific models reveal that the linear regression model has a slightly stronger correlation between AMTL and skeletal age than the polynomial model, as the polynomial model is more closely associated to sex than the linear model. Nevertheless, in both linear and polynomial models, the correlation between AMTL and skeletal age far outweighs the correlation between sex and skeletal age. In an attempt to verify the predictive strength of the AMTL models, their data were subjected to an 80:20 bootstrapping without replacement technique in which 20 percent of the data were used to test a model based on 80 percent of the data. This 80:20 split was chosen in accordance with Raschka (2018), Prabhakaran (2017), and Gagneja (2018), and as a result of the small sample size. The difference between the resulting skeletal age predictions and the actual skeletal ages was assessed using paired t-tests. Although the calculated t-values were not statistically significant as a result of the small sample size, the mean of differences was found to be consistently low, with all means of differences falling below the mean absolute errors and the residual standard deviations for the original models, and under 2 years on average between the actual and predicted skeletal age. These tests provided a general idea of the accuracy of the new models. In future, upon expansion of the reference data, a repetitive bootstrapping without replacement test is recommended.

Following this, attention was focused on the relationship between the percentages of exposed dentine in the occlusal surfaces of first and second molars. To accomplish this, the data that were collected at the beginning of this study through photogrammetric methods were compared to skeletal ages for those respective individuals. Pearson's r scores revealed strong positive correlations between skeletal age and both first and second molar dentine exposure.

Using the same methods for the regression model, sex-specific models and models for individuals of unknown sex were created for the prediction of skeletal age based on first molar or second molar dentine exposure. Regression diagnostic tests (i.e. Residuals vs. Fitted Values, Normal Q-Q, Scale-Location, and Residuals vs. Leverage graphs) were used to identify any possibility for bias in the regression models. They were also used to identify three individuals with abnormally low dental wear for their skeletal age and one individual with abnormally high dental wear for her skeletal age in the first molar wear dataset. Likewise, five outlying data points were identified and removed from the M2 wear dataset to reduce bias. All of these data points represented individuals with abnormally low dental wear on the M2 in relation to skeletal age. Linear regression models, with and without these respective outliers, were compared for goodness-of-fit, resulting in final models that excluded the aforementioned outliers. These final models were found to be well fitted with the data, with slightly skewed distributions of residuals. This deviation from normality in residual analysis may contribute to biased regression coefficient estimates, but this issue can likely be resolved by expansion of the reference data. Similar to the AMTL models, it should be noted that variation in the percentage of exposed occlusal dentine in both first and second molars increases with skeletal age. Additionally, first molar regression models show that for the same skeletal age, females have a slightly higher percentage of occlusal dentine exposure. In contrast, second molar regression models show a significantly lower percentage of occlusal dentine exposure in females when compared to males.

In studies of Inuits (Pedersen 1938), Australians (Campbell 1938; Molnar et al. 1983a,b), and American Indians (Molnar 1971), it has been found that females generally presented with more rapid and severe dental wear than males starting in adolescence (Molnar and Molnar 1990). However, the opposite has been observed in medieval human remains from Denmark (Lunt 1978), while other populations have not shown statistical differences in dental wear between the sexes (Molnar and Molnar 1990). Given this inconsistency, it has been proposed that significantly higher rates of dental wear in females may be a result of dietary differences (Heithersay 1959) and/or the use of teeth in work dominated by females, such as the chewing of animal skins and plant fibers (Molnar 1972). This hypothesis may be particularly apt as females are known to have significantly thicker enamel than males on average (Smith et al. 2006). As such, they may be expected to generally have slightly lower rates of dental wear than males if a difference can be observed. The quantified pattern of wear observed in this study, however, requires further study and consideration with regard to sex-specific differences in diet as well as sex-specific differences in dental developmental timing and enamel thickness.

At the same time, algebraic equations for each molar wear regression model were derived from the estimated coefficients provided through the "summary" output in RStudio. Confidence intervals were again calculated by rounding the residual standard deviation to the nearest 0.5 and doubling it. Equations for all of these models are as follows:

M1 (Female) *-> y = 0.36x + 22.29, CI = +/-12 years*
M1 (Male) *-> y = 0.32x + 22.84, CI = +/-12 years*
M1 (Unknown Sex): *y = 0.34 x + 22.58, CI = +/-12 years*

M2 (Female) *-> y= 2.57x + 23.55, CI = +/-10 years*
M2 (Male) *-> y=0.28x + 26.12, CI = +/- 10 years*
M2 (Unknown Sex) *-> y = 0.27x + 24.93, CI = +/- 10 years*

Analysis of Covariance (ANCOVA) tests were conducted on the sex-specific linear models to quantify the effects of the dependent variable (i.e. skeletal age) on the numeric independent variable (i.e. percentage dentine exposure) and the categorical independent variable (i.e. sex). As expected, results for all models indicated that dental wear is significantly affected by skeletal age with varying lower effects from sex and negligible effects of the interaction between age and sex.

As previously applied to the AMTL regression models, a bootstrapping without replacement technique was used to test the molar wear regression models. Again, using RStudio, the datasets were randomly split 80:20 for model creation and test data (cf. Raschka 2018; Prabhakaran 2017; Gagneja 2018). Linear regression testing models were created from a random selection of 80% of the dataset. The remaining

20% of the dataset were input into the linear regression testing models, resulting in predictions of skeletal age for those individuals. These predictions were compared to the actual skeletal age by way of a t-test. Although all of the resulting t-values were statistically insignificant due to small sample sizes, the means of the differences between predictions from the bootstrapped regression models and the actual skeletal ages of the individuals included in the test samples were between +/-2 years for all dental wear regression models. These tests demonstrate that these models generally tend to produce age estimates within the given confidence intervals when compared to skeletal age. Antemortem tooth loss models showed similar results with only one model producing a mean of differences larger than +/=2 years (See

Table 4.8. Actual vs. Predicted Skeletal Age (AMTL Male Polynomial Regressions))). Paired t-tests of bootstrapped data for the multiple regression models showed larger means of differences, with four models including male data (See Table 4.16. Actual vs. Predicted Skeletal Age as per the M1+M2+AMTL Multiple Regression Model for Males,Table 4.19,Table 4.22, Table 4.25) and two unknown sex models (See Table 4.18 andTable 4.27) producing means of differences between +/-2 and +/-10 years. Nevertheless, in all cases, the mean of differences falls below the calculated uniform confidence interval. In the future, if the reference data are expanded, more meaningful tests of these models could be completed through repetitive bootstrapping without replacement methods, and it is hoped that this expansion will result in lower means of differences.

Following the creation and testing of age estimation models based on quantified dental wear in first and second molars, these models were compared to the Brothwell (1963a) standard for age estimation based on dental wear. The Brothwell (1963a) standard is the most commonly used dental wear age estimation method in Egypt although it was designed for use in prehistoric to early medieval British populations (Hillson 1996). In order to compare the Brothwell standard to the new regression models, it was necessary to quantify the percentage of exposed dentine in the reference image provided by Brothwell (1963a).

Since Brothwell's standard provides multiple dental wear examples for each age cohort, the minimums and maximums of the age cohort and the quantified dental wear were used to graph the photogrammetrically quantified Brothwell standard. The linear regression models for M1 and M2 wear were graphed for a visual comparison.

Through this data visualization, it is apparent that there are gaps in Brothwell's dental wear representation with regard to the percentage of occlusal dentine exposed. This is not surprising as the reference images were selected according to their general shape and pattern of dentine exposure, rather than its relative area. However, this lack of precision and the possibility for variation in these shapes and patterns throughout the dental wear process have been known to result in challenges in the assignment of a dental wear pattern to a Brothwell age category.

In the comparative graph, it is also clear that the linear regression models are far more precise than the Brothwell (1963a) standard and may be helpful in producing more sensitive estimations of age, particularly for older individuals. In the M1 graph, it is apparent that the same percentage of dentine exposure relates to slightly lower skeletal age estimates on average in the first three Brothwell age estimation ranges when compared to the linear regression models. In the M2 graph, the same percentage of dentine exposure relates to slightly lower skeletal age estimates in the youngest Brothwell cohort and slightly higher skeletal age estimates in the oldest Brothwell age cohort in comparison with the associated linear regression models. Looking at the specific data points, this has translated into many underestimates of skeletal age from Brothwell's M1 wear standard and a transition from underestimation to overestimation of skeletal age from Brothwell's M2 wear standard. Given that the linear models were based on the data at hand, they are designed for goodness of fit with the data and therefore do not have such dramatic biases. Of course, it is possible that the linear regression models are slightly biased in relation to chronological age due to their reliance on skeletal age estimates. This complicates comparison with the Brothwell (1963a) standard, which is presented as an estimator of chronological age. Unfortunately, modern Egyptian populations have dissimilar rates of dental wear from ancient Egyptian populations due to differences in diet and food preparation. As such, until age estimation methods for human remains significantly improve, particularly in the older cohorts, the accuracy of age estimation regression models for ancient Egyptians will continue to be based on skeletal age and therefore be at risk for systematic bias. Fortunately, skeletal age estimates based on methods not developed through the principles of the Rostock Manifesto (i.e. based on known age reference population and calculated using Bayesian statistics), are known to underestimate age in older cohorts as estimates tend toward the mean (Hoppa and Vaupel 2002). Consequently, it may be inferred that the use of skeletal age estimates in the creation of the linear regression models may result in an underestimation of skeletal ages when using these regression models. If this is true, and the regression models for the chronological age of the Kellis 2 population can be assumed to be higher on the skeletal age scale than those depicted above, then the Brothwell (1963a) method may actually underestimate chronological age in the 17-45 year cohorts for M1 wear in the Kellis 2 population to a greater degree than is depicted in the comparative graph for M1 wear. Similarly, the Brothwell method may also underestimate chronological age in the 17-25 year cohort for M2 wear in the Kellis 2 population more significantly than is observable in the comparative graph for M2 wear.

Since the molar wear regression models were shown to be less ambiguous in their interpretation than Brothwell's (1963a) standards as well as more specific in their age estimates, multiple regression models were investigated for their ability to cross-reference linear regression models to achieve more accurate and specific predictions. To this end, algebraic multiple regression equations for all combinations of M1 wear, M2 wear and AMTL were derived from the estimated coefficients presented through the "summary" output for the relevant R codes in RStudio. As was done in all previous regression models, regression diagnostics were examined for goodness of fit and predictive value and confidence intervals were calculated by rounding the doubled residual standard deviation. The resulting multiple regression equations are as follows:

M1+M2+AMTL

Males -> *y = 0.23(M1) + 0.03(M2) + 1.03(AMTL) + 21.70, CI = +/- 9 years*
Females -> *y = -0.16(M1) + 1.01(M2) – 4.44(AMTL) + 22.11, CI = +/-4 years*
Unknown Sex -> *y = 0.24(M1) + 0.03(M2) + 1.02(AMTL) + 21.07, CI = +/- 7 years*

<u>M1+AMTL</u>
Males -> *y = 23.24 + 0.21(M1) + 1.04(AMTL), CI = +/- 11 years*
Females -> *y = 20.77 + 0.27(M1) + 1.30(AMTL), CI = +/- 6 years*
Unknown Sex -> *y = 22.10 + 0.23(M1) + 1.18(AMTL), CI = +/- 9 years*

<u>M2+AMTL</u>
Males -> *y = 25.37 - 0.03(M2) + 1.90(AMTL), CI = +/-11 years*
Females -> *y = 21.78 + 0.28(M2) + 0.91(AMTL), CI = +/-5 years*
Unknown Sex -> *y = 23.93 + 0.002(M2) + 1.91(AMTL), CI = +/- 10 years*

<u>M1+M2</u>
Males -> *y = 21.21 + 0.28(M1) + 0.15(M2), CI = +/- 9 years*
Females -> *y = 22.18 + 0.06(M1) + 0.26(M2), CI = +/- 6 years*
Unknown Sex -> *y = 21.28 + 0.24 + 0.16, CI = +/-8 years*

To test the predictive value of these models, the 80:20 bootstrapping without replacement method was used to predict skeletal age estimates, which were then compared with the actual skeletal age through a t-test (cf. Raschka 2018; Prabhakaran 2017; Gagneja 2018). Although most models showed means of differences between actual and predicted skeletal ages under half of the calculated mean absolute error, 3/4 regression models for males (all of the male equations that included M2 wear data) and 2/4 regression models for unknown sex (which also included M2 wear data) showed relatively higher means of differences. This pattern is reflected in the fact that all M1+AMTL models had relatively high R-squared values (which is preferred) when compared to M2+AMTL, M1+M2, and M1+M2+AMTL models. Moreover, within each of these model types, female models had AICs that were significantly lower (and thus had greater predictive strength) than the AICs for comparative male or unknown sex models. As such, the multiple regression models that are partly based on M2 wear in males should be used with caution.

It is hoped that revisiting these methods for model creation with an expanded dataset in the future will resolve the identified challenges. Furthermore, the resulting standards will subsequently be tested on geographically diverse Roman Period populations to determine the regional applicability of the standards and calculate correction factors as needed. Similarly, the standards will then be tested on populations from differing time periods within Egypt to determine its chronological applicability and any relevant correction factors for use of the standard in these populations. To this end, a call to action was included in a presentation of the preliminary findings of this study at the 2019 Bioarchaeology in

Ancient Egypt conference in Cairo. This call to action was well-received and several researchers expressed their interest in contributing data for this purpose. Plans for the expansion and systematic testing of the new standards for dental age estimation in adults are further discussed in the Future Research section (Section 4.10.4).

Overall, this study demonstrates that significant improvements can be made in the estimation of age through new methods and standards for analysis of adult dental age indicators. Specifically, a photogrammetric method of quantifying dental wear was shown to have little intra- and inter-observer error and removed the ambiguity inherent in the use of the Brothwell (1963a) atlas of dental wear in relation to age. The strong correlations between the quantified dental wear and skeletal age resulted in the creation of regression models that are far more specific than the Brothwell (1963a) standards in the estimation of skeletal age. Through the use of the uniform confidence interval included with these regression models, the accuracy of these models is relatively high as well. The number of teeth lost antemortem was also proven to be strongly correlated in relation to skeletal age and was integrated into multiple regression models with quantified dental wear data for first and second molars in the Kellis 2 population. These regression models show great potential although the models that include male second molar data may require revision with an expanded dataset. Nevertheless, the female regression models represent a significant improvement in macroscopic adult dental age estimation. As such, the null hypothesis for this part of the dissertation, (H_o): “Current dental age estimation standards based on dental wear cannot be improved”, can be rejected. Furthermore, this new method for adult dental age estimation would be beneficial to the fields of forensics, immigration, and bioarchaeology, among others.

4.8 Research Ethics

The University of Western Ontario does not require Research Ethics Board approval for the study of unidentified human remains. A letter from the Research Ethics department has been written to confirm these regulations and to demonstrate the university’s approval of the use of human remains in the proposed study and is available upon request. With regards to the treatment of these remains, all specimens will be handled with respect and care and the study will be non-destructive in nature. While handling the human remains, I will abide by the Vermillion Accord on Human Remains, which has been adopted for use by the UWO Department of Anthropology.

4.9 Limitations

Although the image analysis method used in this study is an excellent way to quantify dental wear and has little inter- and intra-observer error, it does have some limitations. For example, it can be difficult to distinguish the occlusal surface from the other surfaces depending on the lighting and the photographic angle. Given this, the photographic angle of the occlusal surface can only significantly affect data collection when the angle is quite obviously off (Hillson, personal communication). Another limitation of the current method is the time requirement for manual selection of the elements to be measured within the image analysis software. This limitation may be eliminated in the near future as a trainable weka segmentation program (artificial intelligence software) is being investigated by the current author as an alternative to the manual image analysis method. If this artificial intelligence program is found to be accurate, it may be applied to batches of images, further decreasing the time requirement for this type of dental wear analysis.

This study was limited by the size of the population sample available for study due to its reliance on pre-existing photographs of occlusal dentition. Statistical significance would have be stronger with a larger number of individuals in each age cohort. Thankfully, this reference sample can be expanded when work permissions resume as more than 700 individuals have been excavated at this site to date.

Inherent in the study of dental wear in ancient Egyptians is the problem of accuracy and specificity in the skeletal age estimates which were necessarily taken as the “known” age of individuals. Since the rates of dental wear in ancient Egyptians are not comparable to modern populations, it was necessary to conduct a study of the ancient population directly. With the limitations and biases of current macroscopic skeletal age estimation methods,

The paleodemographic structure of the sample reference population will produce a bias in any age estimation standards created in their image (Hoppa and Vaupel 2002). Bayesian statistical methods have been successfully used to normalize the tendency for age estimates to regress toward the mean (Hoppa and Vaupel 2002). Unfortunately, since this study required the use of skeletal age estimates based on non-Bayesian methods as the "known" age of individuals, application of Bayesian statistical methods to the new standard would result in age estimates that are still biased. In the future, if less time-intensive methods for ultra-specific and -accurate age estimation are accessible and permissible in Egypt, the creation of a standard using Bayesian statistical analysis of quantified exposed dentine in a population whose age can be considered "known" is recommended.

4.10 Future Research

4.10.1 Examining the Effects of Dental Erosion in Ancient Egyptians

A study of the remains of ancient Egyptian bread and beer in collaboration with UCLA PhD candidate, Amr Shahat, is currently in progress. This study involves the testing of pH as well as the quantity of residual sugars and concentrations of calcium, fluoride, and potassium (cf. Swift 1966; Larsen and Nyvad 1999). The quantification of residual sugars will be conducted to verify results of the pH analysis, according to the methods of Swift (1966). Together, the pH and concentrations of calcium, fluoride and potassium will be used in the first study to estimate the erosive potential of ancient Egyptian food staples, bread and beer.

4.10.2 An atlas-type standard?

Although atlases based on dental development are necessarily subjective, their convenience is unequivocal. During a recent presentation of this dental wear research at the Bioarchaeology in Egypt 2019 conference, I polled the audience to determine the level of interest in an atlas-type age estimation tool for dental wear. This poll revealed some interest in a sex-, region-, and time-specific dental wear atlas, though this level was far lower than the interest expressed in the possibility of dental quantification and age estimation software. As such, an atlas-type standard was not prioritized for this dissertation, but may be considered in the future.

4.10.3 Computer assisted age estimation from dental wear?

Since the quantification of dentine may be more closely related to age than the categorical images of dental wear presented in atlas-type standards, an automated system for the quantification of exposed dentine is being investigated. Within the FIJI (is just ImageJ) program, there is an application in Plugins > Segmentation > Trainable Weka Segmentation. This artificial intelligence application can be trained to recognize specific colours and borders separating these colours. This colour thresholding method is more specific than the freehand selection method used to quantify dentine to date, as individual pixels are classified based on shared visual characteristics, such as colour. Since the occlusal surface can be isolated through the use of a blue rubber dental dam surrounding the tooth during photography, it may be possible to train this classifying application to recognize the differences in colour between enamel and exposed dentine without confusion from similar colouring in the background. This dental dam would also serve to delimit the occlusal surface from the adjacent surfaces, solving the problems associated with the identification of occlusal edges in photographs. If possible, then this trained classifier code may be shared online with other Fiji users, who could then apply this classifier to quickly measure areas of exposed dentine and the occlusal surface in a number of occlusal images at once. Ideally, the trained application would be able to identify the differences between stained enamel, caries, and exposed dentine. However, it may be necessary to develop code for a new function within this application to deselect areas in cases where the classifier makes incorrect selections. Lastly, a macro program may be created to automate all of these functions in *FIJI (is just ImageJ)* and input the generated data into a simple calculator to determine age at death. The feasibility of the proposed automated system will be investigated in future studies.

4.10.4 Expansion of the New Standards and Testing on Different Populations

Although the age estimation equations provided above are currently only recommended for use on Roman Period populations from the Dakhleh Oasis, this standard will be revised in the near future. As per the suggestion of Dr. Brenda Baker and Dr. Jerome Rose, in the future, the new standard may be compared to standards resulting from the Miles' (1962) protocol to ensure the accuracy of the new method. Additionally, approximately 500 skulls from the Fag El-Gamous cemetery in Seila, Fayoum, Egypt will be analyzed as above and the rates of wear will be compared with the data from Kellis 2. This study will determine if there were significant differences in dental wear over geological space within Upper and Lower Egypt during the Roman Period. The Fag El-Gamous data will then be integrated into the age estimation equations to broaden the scope of the standard so that it may be applied to all populations within Roman Egypt. At this time, consideration may also be given to the expansion of the standard to include dental wear scoring for anterior teeth.

Following this geographical expansion, further studies are planned to examine dental wear in Upper and Lower Egypt through different time periods. These studies would enable the systematic expansion of the sex-, region-, and time-specific dental wear standard while allowing for the identification of significant changes in food production/processing methods or differences in food preferences throughout Egyptian history. Some Egyptian bioarchaeologists have already offered to provide quantified occlusal wear data from earlier time periods to enable this extended study.

Subsequent to the expansion of the reference data, the regression models should be re-evaluated and Poisson regression can be investigated for goodness-of-fit. Data may also be divided according to age to investigate whether a combination of different regression models may better suit the different age cohorts within the reference data.

Chapter 5

5 Dissertation Summary and Conclusion

This dissertation has demonstrated the value of dental anthropology through its significant contributions to scientific and anthropological knowledge, and proven the particular value of macroscopic dental age estimation methods. Despite the relative accuracy and specificity of the existing macroscopic dental age estimation standards (as demonstrated in an abbreviated preliminary meta-analysis), this dissertation also revealed that there is room for improvement in dental age estimation, particularly through the development of (time-,) sex- and region-specific standards based on innovative methods for data collection and statistical analysis.

In the case of subadult dental age estimation standards, a proposed method for the creation of a sex- and region-specific standard was designed and determined to be feasible. This method was subsequently compared to the methods used for the creation of pre-existing age estimation standards based on dental development. This meta-analysis demonstrated that all of the identified methodological errors present in the pre-existing standards can be eliminated through the use of the proposed method. This method was designed in accordance with the Rostock Manifesto (Hoppa and Vaupel 2002), which requires the collection of data from a large population sample with known ages and the use of Bayesian statistics for predictive modelling. Bayesian statistical methods have been shown to improve age estimates in human remains by reducing biases related to the composition of the reference population, as other statistical methods produce estimates that tend toward the mean (Hoppa and Vaupel 2002). For the purpose of the proposed study, the chosen reference population was specific to the region, could be aged to within a month of their birthdate, and could be mined for data relating to factors affecting dental development. Since radiographic study is the most effective macroscopic method for the observation of dental development, it was necessary to integrate radiographic data collection into a larger public dental health program. This public health initiative would also enable the collection of gingival emergence data for comparison with alveolar eruption timing. This research model was planned out in detail to ensure that the proposed study was ethical and in accordance with the risk-benefit philosophy (Davidson and O'Brien 2009; Iannucci and Howerton 2012), which requires that the benefit to a patient outweighs any risks involved in medical radiation exposure. To this end, digital panoramic radiographic data collection was recommended for use in the proposed method as it is known to have a significantly lower effective dose of radiation than other dental radiographic methods. All details of the proposed methods for standard creation were made available to readers to ensure transparency in the methods of creation, their replication, and their potential for application. This transparency represents yet another improvement on several existing subadult dental age estimation standards.

Errors in methodology from the existing subadult dental age estimation standards were compared to the proposed methodological model. This meta-analysis further demonstrates the many methodological improvements that are addressed in the proposed model and have the potential to contribute to more accuracy and unbiased subadult age estimates. This study of subadult dental age estimation methodology thus rejects the null hypothesis "Current macroscopic subadult dental age estimation standards cannot be improved".

This null hypothesis was subsequently tested with a focus on macroscopic adult dental age estimation standards, which is traditionally based on dental wear. During a review of the literature relevant to age estimation based on dental wear, it was discovered that the role of dental erosion in the relative severity of ancient Egyptian dental wear may be greatly underestimated. Through an examination of ancient Egyptian iconographic, textual and archaeological evidence, I identified several foods and drinks with the potential to erode dental enamel. These findings indicated that the staple foods in ancient Egypt, namely bread and wine, likely had high erosive potential. Although further investigation of this finding was outside the scope of this dissertation, a collaborative study has been commenced to specify the erosive potential through chemical tests of ancient bread and beer residues.

Following this review of the literature, a new method for quantifying the percentage of occlusal dentine exposure was applied to a photographic sample of occlusal dentition from the Kellis 2 cemetery population in Egypt. This method, introduced by Phillips-Conroy et al. (2000) and modified by Deter (2006, 2009), uses image analysis software to count the number of pixels within an outlined area (i.e. the exposed dentine patches and the occlusal surface) which are then used to calculate the percentage of the occlusal surface occupied by exposed dentine. This is the first time this method of dental wear quantification has been applied to age estimation. Intra- and inter-observer tests were conducted through the use of intra-class correlation on first molar data to ensure the accuracy and reliability of this method. Results showed a 99% correlation between intra-observer data and a 98% correlation between inter-observer data. These data represent an improvement on the intra- and inter observer rates seen through the use of the Brothwell (1963a) method, in which intra- and inter-observer errors were both 80%) (Alayan 2018).

Following these tests of intra- and inter-observer error, examinations of variation within individual dentition were conducted to determine whether quantities of dental wear on different 1st molars show significant differences. T-tests of isomere data revealed a statistically significant difference between wear in upper and lower first molars at a 90% confidence interval. A simple plot of differences showed that lower first molars tended to have more severe wear than upper first molars. It was concluded that this was likely a result of larger tooth diameter and thicker dental enamel in the upper molars. This finding was significant as it confirms that dental wear scores (or in this case percentages) in upper and lower first molars are not interchangeable. These data support the observations of Murphy (1959b) and Pal (1971) and are an importance consideration when scoring dental wear

T-tests of antimere data revealed a statistically significant difference between dental wear in lower left and right molars, independent of direction. These results stand in contrast to the commonly held belief that there is little difference in dental wear between left and right sides of the dentition (Hillson 1996). Similarly to the observed difference between isomeres, this asymmetry of dental wear in antimeres should be taken into consideration when estimating age based on dental wear. In the meantime, this asymmetry in dental wear among antimeres was investigated in relation to dental pathology to determine if pain, discomfort, or a loss of tooth surface could be the reason for more wear on one side of the mouth. To this end, individuals with the greatest dental wear asymmetry were identified through the use of a Tukey's boxplot and Tukey's (1977) rule for identifying outliers.

Unfortunately, results of a detailed comparison of dental pathology to dental wear asymmetry were inconclusive as three of the four individuals were excluded due to use of the teeth as tools, gross caries on the occlusal surface, and a missing maxilla. The remaining individual presented with pathology and tooth loss on both sides of the mouth, giving little indication of the reason for the asymmetrical dental wear pattern. Although the results of this study were inconclusive, it demonstrates some of the factors that need to be considered when estimating age based on the quantification of dental wear. It is hoped that future research may shed more light on the relationship, if any, between dental wear asymmetry and dental pathology.

Following this investigation of dental pathology, the two most common and quantifiable discrete pathologies, dental caries and antemortem tooth loss, were examined in relation to skeletal age. Pearson's r scores and data plots indicated no linear relationship with skeletal age for the number of caries per individual or the percent of observed teeth with carious lesions. However, the sum of caries with teeth lost antemortem, as well as the number of teeth lost antemortem, were shown to have strong linear correlations with skeletal age. Through a comparison of Pearson's r scores for each of these datasets, it became clear that the inclusion of dental caries with antemortem tooth loss did not improve the correlation. Thus, antemortem tooth loss alone was identified as a strong indicator of skeletal age with statistically significant Pearson's r scores of 0.85 and 0.80 for female and male datasets, respectively. Although analysis of the caries data collected during this study may be limited by their observation through photographs, the age related patterns seen in caries and antemortem tooth loss, and their coexistence within individuals, support Meiklejohn et al.'s (1992) assertion that dental wear and caries are independent factors and are not mutually exclusive.

For the purpose of age estimation, linear and polynomial regression models were designed and tested for goodness of fit. Through a comparison of these models, the polynomial models were found to be better fitted to the data and had a stronger predictive value. Having said this, caution must be taken in using these models as there is a possibility that they are skewed by the limited number of older adults included in the dataset. This paucity of data in the older cohorts may be particularly influential on the polynomial regression models as they run the risk of overfitting to the data. However, it should be noted that Rosing and Kvaal (1998) observed a similar polynomial relationship between antemortem tooth loss and age. Nevertheless, all of these models should be revisited in the future when more data become available. In the meantime, it is worth noting that ANCOVA tests on the sex-specific models reveal that the linear regression model has a slightly stronger correlation between AMTL and skeletal age than the polynomial model, as the polynomial model is more closely associated to sex than the linear model.

Consequently, it was decided that the polynomial equation should be used as a predictor of skeletal age based on antemortem tooth loss until additional data can be integrated and this hypothesis tested. In the meantime, the linear regression model would be integrated into subsequent multiple regression models for skeletal age estimation based on dental indicators. Both linear and polynomial models were further tested through an 80:20 bootstrapping without replacement technique in which 80% of the dataset were used to create a new regression model and the remaining 20% were input into this model to predict skeletal ages (cf. Raschka 2018; Prabhakaran 2017; Gagneja 2018). The means of the differences between the actual skeletal age and the predicted skeletal age for the test data were found to be smaller than +/-1.5 years in all but the male polynomial model for AMTL. The mean of the differences for the bootstrapped male polynomial model was 4.18 years, which is still well below the mean absolute error (5.58 years) and the residual standard deviation (7.76 years).

Following this investigation into discrete pathological indicators for age, the quantified dental wear data were tested for correlation with skeletal age. Pearson’s r scores indicated strong linear correlations between skeletal age and both first and second molar wear for male and female datasets. Consequently, linear regression models were similarly designed and tested. These models showed relatively high predictive value and small confidence intervals. When compared to a quantified version of the Brothwell standard for dental age estimation, the linear regression models were more specific and more accurate. The quantified Brothwell standard showed gaps in dental wear representation, which is not surprising given the imprecision of the method. This precision in the use of photogrammetric quantification of dental wear helps to avoid the subjectivity inherent in atlas-type and description-type age estimation standards.

Following this proof of concept for the use of regression models based on quantified dental wear, multiple regression models were designed for all combinations of antemortem tooth loss and first and second molar wear. Regression diagnostics and the bootstrapping without replacement test indicated that small reference sample sizes may have negatively affected the accuracy and precision of the models that incorporated male first molar wear data. However, all other multiple regression models demonstrated great accuracy and precision with error rates well within the given confidence intervals. This shows that there is potential for significant improvement in age estimation based on dental indicators of age. However, in future, following expansion of the reference dataset, it is recommended that these models are all retested using a repetitive bootstrapping without replacement technique to ensure that outliers do not have a significant negative impact on prediction accuracy.

In conclusion, the null hypothesis '(H_o): "Current dental age estimation standards based on dental wear cannot be improved", can be rejected, given the proven potential for improvement in subadult standards and the presentation of new and improved adult standards. This dissertation also demonstrates the utility of photogrammetric dental wear quantification in age estimation and identifies several areas for future study. Among the listed future research topics, the development of a trainable artificial intelligence program for the quantification of exposed occlusal dentine is in progress. This would allow for large collections of photographs of occlusal dentition to be analyzed instantaneously, enabling widespread use of this system within the intended region. Given that the designed regression models are more accurate and precise than Brothwell's universally-applied standard, it is recommended that, following recalculation of these standards with a larger reference sample, they are tested on geographically and chronologically diverse populations in the regions of Egypt and the Middle East. Through this process, a sex-, region-, and time-specific standard can be developed with correction factors for deviant populations. This method would be more user-friendly and less time-consuming than the Miles method with better accuracy and precision than the Brothwell method.

This improvement in macroscopic dental age estimation could be beneficial to the fields of dentistry, pediatric medicine, orthodontics, biology, health sciences, evolutionary medicine, forensics, immigration, socio-cultural studies, and bioarchaeology. Within bioarchaeology, alone, improved age estimation would have applications in evolutionary studies, osteobiographies, paleo-pathology, paleo-epidemiology, and paleo-demography. In particular, it would contribute to significantly improved lifetable reconstructions based on past populations, as improved dental wear aging standards have the greatest potential, among macroscopic aging methods, to rectify the problem of age underestimation in older cohorts. Given their relative accuracy, specificity, and applicability to a large age range, dental age estimation methods remain the best macroscopic single indicators of age. However, this dissertation has shown that there is room for improvement in these methods and, to paraphrase Konigsberg and Frankenberg (1992: 253), we must continue to strive to make age estimation more of a science than an art.

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Appendices

Appendix 1

Western Applicant/Participant Number______

Project Title: Comparing the Accuracy of Region-specific and Universal Dental Aging Standards in Egypt
Principal Investigator: Dr. Joseph Eldon Molto, Department of Anthropology, Western University

Letter of Information

1. **Invitation to Participate**

   You are being invited to participate in this research study about dental development in Egyptian children because you are an Egyptian child between the ages of 1 and 17.

2. **Purpose of the Letter**

   The purpose of this letter is to provide you with information required for you to make an informed decision regarding participation in this research.

3. **Purpose of this Study**

   The purpose of this study is to gather further knowledge about dental growth and development in relation to age, while educating Egyptian children and their guardians about dental health and hygiene and offering free dental services (i.e. dental examination, panoramic dental x-ray and dental health report). Information gathered from this study will be used to create a region-specific dental age estimation standard, which will have applications for health assessment and treatment planning in medical, dental and orthodontic practices, and applications for the identification of human remains in forensic and archaeological contexts.

4. **Inclusion Criteria**

   Upper Egyptian individuals who fall between the ages of 1 and 17 are eligible to participate in this study if their birthdates fall within one month of available study dates and can be verified through the participant's school or official documents. 1020 participants in total, will be accepted for participation in this study (30 males and 30 females for each age from 1 to 17 years old). If demand for participation is high, eligible participants will be accepted following a lottery for each age/sex cohort. Otherwise, participants will be accepted upon receipt of their completed 'Participant Questionnaire' and 'Consent'/'Assent' form(s) until the number of participants necessary for each cohort is satisfied.

5. **Exclusion Criteria**

   Individuals whose birthdate cannot be verified to fall within one month of an available study date will not be eligible for participation in this study. Individuals who are more than one month younger than 1 year old, or one month older than 17 years old, during the study period will not be eligible for

Version 08/12/2013 Page 1 of 6 Participant/Guardian Initials ___ participation in this study. Individuals may also be excluded from participation in this study if they have had any dental interventions that have significantly affected normal dental development, or if they fall into an age category that has reached the required number of individuals. Additionally, individuals who have participated in other research studies using ionizing radiation, or who have been exposed to radiation in other ways, like through work or radiation therapy, are not eligible to participate in this study. Pregnant women are also not eligible for participation in this study.

6. Study Procedures

If you agree to participate, you and/or your legal guardian will be asked to complete a questionnaire about factors that may affect dental health and development, you will then be assigned an appointment to receive a free panoramic dental x-ray, and an appointment for a free dental examination and dental health advice from a local dentist. It is anticipated that the entire task will take less than 2 hours and a select few will be asked to return for a second clinical examination (without an x-ray) to assess the accuracy and consistency of the examining dentists. The task(s) will be conducted by a local dentist and radiologist in the Radwania Radiological Laboratory on Sharia Television in Luxor. There will be a total of 1020 local participants in this study.

7. Possible Risks, Harms, or Inconveniences

Participants will be exposed to a small amount of radiation called 'ionizing radiation', which in large amounts has been known to contribute to the development of cancer. The amount of radiation received from a panoramic dental x-ray is extremely small compared to other naturally occurring sources of radiation such as minerals in the soil, radon, and cosmic radiation from outer space. Localized x-rays may also be taken if it is considered necessary for diagnosis during the dental examination and it is believed that the benefits to the participant will outweigh the risks. Precautions will be taken to protect participants from receiving unnecessary radiation and the panoramic x-ray machine will be set to minimize radiation exposure without sacrificing the quality of the radiograph. With guardian and school permission, participants may be required to attend a dental appointment during normal school or working hours. This is due to the fact that dental offices in Egypt are usually open after regular working hours and it will be possible to conduct this study during the daytime without negatively affecting access to dental health care for the general population.

8. Possible Benefits

The possible benefits to participants include receiving a free dental examination from a local dentist, with child-friendly introductions to the dentist for younger participants. Participants will receive free education regarding dental hygiene techniques, and may receive tools or samples to encourage preventative health care practices at home. Participants will receive a free panoramic dental x-ray, which will be assessed, and may receive a copy of the x-ray to take home.

The possible benefits to society may be the improvement of knowledge about dental development in children, which will have applications to dentistry, orthodontics, forensics, archaeology, sociocultural studies, law and immigration. This project will also result in the creation of a region-specific dental aging standard, which will be useful in the diagnosis of growth disruption disorders in children and the identification of young human remains in forensic and archaeological contexts. This new model for the creation of age estimation standards will also be useful for the creation of other region-specific aging standards. It is also hoped that the education of children, and their guardians, about dental health and hygiene will raise public awareness about preventative oral health care and lower rates of dental disease in Upper Egypt. Results from this study will also be used in the development of a public dental health report following completion of the current study. This report will attempt to identify areas for improvement of access to dental services and education about oral health and hygiene in Upper Egypt.

9. Compensation

You will not be compensated for your participation in this research.

10. Voluntary Participation

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your future care or academic status.

11. Confidentiality

Participants will be assigned numbers which will be used on all data collection forms and x-rays. Identifying information will be deleted from all documents before the documents are uploaded or scanned, and encrypted and saved on a laptop and an external hard drive. Original forms will be shredded and destroyed before disposal, apart from original x-rays which will be given to participants. An encrypted master list, available only to the study investigator, Dr. Molto, and his student, Casey Kirkpatrick, will be created to store identifying information in relation to the assigned number. This master list will be deleted after 15 years, and it will be kept confidential until its deletion.

Upon publication of this study, your name will not be used; only the assigned participant number and sex and age of the participant will be made available to the public due to their requirement for contextualization of the collected information. Following this study, the unidentifiable survey data will be stored indefinitely in a digital archive managed by Casey Kirkpatrick which will be made available to scholars upon Casey Kirkpatrick's approval of a study proposal. A suitable academic or institution will be assigned to keep the archives upon Casey Kirkpatrick's retirement from academia. Unidentifiable data may also be submitted to the World Health Organization's database. And a report on the general anonymous findings of this study will be provided to participating schools in an effort to encourage school-based dental health and hygiene educational programs. If you choose to withdraw from this study, your data will be removed and destroyed from these archives. While we will do our best to protect your information there is no guarantee that we will be able to do so. The inclusion of your sex, your date of birth and the name of your school may allow someone to link the data and identify you. Representatives of The University of Western Ontario Health Sciences Research Ethics Board may also contact you or require access to your study-related records to monitor the conduct of the research.

12. Contacts for Further Information

If you require any further information regarding this research project or your participation in the study you may contact Dr. Joseph Eldon Molto at [redacted] or [redacted]. Locally, you may contact Dr. Molto's student, Casey Kirkpatrick at [redacted] or [redacted]. If you have any questions about your rights as a research participant or the conduct of this study, you may contact The Office of Research Ethics [redacted], email: [redacted].

13. Publication

Results from this study will be published in an online dissertation and academic journal articles. Software will also be created using the collected radiographic data. Results from this study may also be used in the development of a public dental health report following completion of the current study, if desired by the Egyptian Ministry of Health. If the results of this study, or any future study using the collected data, are published, your name will not be used. If you would like to receive a copy of any potential study results, please contact Casey Kirkpatrick at [redacted].

14. Consent

Completion of the questionnaire and the consent/assent forms, as required, are indications of your consent to participate in this study.

This letter is yours to keep for future reference.

Please return the following completed forms if you would like to participate in this study.

Western

Applicant/Participant Number______

Project Title: Comparing the Accuracy of Region-specific and Universal Dental Aging Standards in Egypt

Study Investigator's Name: Dr. Joseph Eldon Molto

Consent Form

I have read the Letter of Information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Participating Child's Name: ______________________________

Participating Child's Sex/Birthdate: ______________________________

Participant's Signature (age 13-18): ______________________________

Date: ______________________________

Parent/Legal Guardian/Legally Authorized Representative Print: ____________________

Parent/Legal Guardian/Legally Authorized Representative Sign: ____________________

Parent/Legal Guardian/Legally Authorized Representative Date: ____________________

☐ I agree to being contacted in the future by the current researchers, in the event of a follow-up study or if another opportunity for discounted dental services arises.

Address:____

Phone Number: ____________________

Person Obtaining Informed Consent (please print): ____________________

Signature: ____________________

Date: ____________________

Birthdate verified by:________________________

Document observed:________________________

Date: ________________________

Version 08/12/2013 Page 5 of 6 Participant/Guardian Initials ___

Western Applicant/Participant Number______

Project Title: Comparing the Accuracy of Region-Specific and Universal Dental Aging Standards in Egypt
Principal Investigator: Dr. Joseph Eldon Molto, *Department of Anthropology, Western University*

Assent Letter (for children aged 7-12)

1. **Why you are here.**
   Dr. Molto wants to tell you about a study that will look at why children's teeth grow. He wants to see if you would like to be in this study. There will also be other researchers working with Dr. Molto on this study, like Ms. Kirkpatrick.

2. **Why are they doing this study?**
   Dr. Molto and his researchers want to see if teeth grow differently in children in different parts of Egypt and in children outside of Egypt.

3. **What will happen to you?**
   If you want to be in the study three things will happen:
   1. Your mother, father or guardian will answer questions about your health and your family.
   2. You will have a picture taken of your teeth for free with an x-ray machine and you will get a copy of the picture to take home with you.
   3. You will get a free visit to a dentist's office, who will count your teeth, tell you about your teeth, and teach you how to keep your teeth clean, happy and healthy.

4. **Will there be any tests?**
   No, there will not be any tests or marks on your school's report card from this study.

5. **Will the study help you?**
   This study will help you in some ways, as you get an x-ray picture of your teeth for free and you will get a free visit with a dentist, who will tell you about your teeth and how to keep your teeth clean and healthy. If there is a problem with your teeth you might have a chance to have them fixed for free. The results of this study will not help you directly, but they might help other children by giving dentists, doctors and archeologists more information about how children's teeth grow and information about how to keep Egyptian children's teeth clean and healthy.

6. **What if you have any questions?**
   You can ask questions at any time, now or later. You can talk to the teachers, your family or someone else.

7. **Do you have to be in the study?**
   You do not have to be in the study. No one will be mad at you if you do not want to do this. If you do not want to be in the study, just say so. Even if you say yes, you can change your mind later. It is up to you.

I want to participate in this study.

Print Name of Child ____________________ **Sex/Birthdate**____________________

Signature of Child ____________________ **Date**____________________

Signature of Person Obtaining Consent ______________________________

Appendix 2

Western

Applicant/Participant Number: ________ Birthdate: ___/___/___ Sex (M/F):___

CONFIDENTIAL

First, we would like you to answer some questions concerning yourself and your teeth. Please tick the box next to your answer or write your answer on the line provided.

Medical History

1. Were you born prematurely and/or with a low birth weight?

   ☐ Yes ☐ No ☐ Unsure

2. At what age were you weaned from breast milk?

   ☐ 0 months - under 1 month ☐ 1 month - under 3 months ☐ 3 months - under 6 months
   ☐ 6 months - under 12 months ☐ 1 year - under 2 years ☐ 2 years - under 3 years
   ☐ 3 years - under 4 years ☐ 4 years or older

3. A) Were you born with any medical conditions?

   ☐ Yes ☐ Unsure ☐ No-(GO TO QUESTION 4)

   B) Please specify: ____

   ____

4. A) Do you have any chronic medical conditions?

   ☐ Yes ☐ Unsure ☐ No-(GO TO QUESTION 5)

   B) Please specify:____

   ____

5. A) Have you had any long periods of illness, stress or inability to eat?

   ☐ Yes ☐ Unsure ☐ No-(GO TO QUESTION 6)

   B) If yes, please specify your age at the time: ______

6. A) Have any teeth been lost from an accident or extracted prematurely?

   ☐ Yes ☐ No-(GO TO QUESTION 7)

   B) If yes, please specify (Which tooth? Why? When?):________________________

   ____

7. Do you suck your thumb, fingers or a pacifier? ☐ Yes ☐ No

8. Have you ever had a sealant applied to your teeth to prevent cavities? ☐ Yes ☐ No ☐ Unsure

Page 1 of 6

Western Applicant/Participant Number: ________ Birthdate: ___/___/___ Sex (M/F):___

Demographic and Genetic Factors that may Affect Dental Health and Development

9. A) Do you have any known ancestry from outside of Egypt? ☐Yes ☐No

   B) If yes, what relative(s)? ____________________

   C) If yes, what nationalit(y/ies)? ____________________

10. A) Are your parents blood relatives? ☐Yes ☐No ☐Unsure

   B) If yes, what was their family relationship before marriage? ____________________

11. Has your mother or primary caregiver had active dental decay in the last 12 months?

   ☐Yes ☐No ☐Unsure

12. A) How many siblings do you have? ___ (IF NONE, GO TO QUESTION 13)

   B) Are any of your siblings participating in this study?

   ☐Yes ☐No (IF NO, GO TO QUESTION 12 D)

   C) If yes, what is/are their name(s) (or participant numbers if known)? ____________________

   ____

   D) Where do you rank in the birth order?

   ☐1st child ☐2nd child ☐3rd child ☐4th child ☐5th child

   ☐6th child ☐7th child ☐8th child ☐9th child ☐10th+ child

   E) Do you have any same-age siblings?
   - ☐ No, I was a single birth – (GO TO QUESTION 13)
   - ☐ Yes, I have ____ same age sibling(s)

   F) What sex is/are your SAME AGE sibling(s)?

   1)☐Male ☐Female 2)☐Male ☐Female 3)☐Male ☐Female 4)☐Male ☐Female 5)☐Male ☐Female 6)☐Male ☐Female

13. What is your approximate annual household income?

   ☐Under 1,000 LE ☐1,000 – 4,999 LE ☐5,000 – 9,999 LE
   ☐10,000 – 14,999 LE ☐15,000 – 29,999 LE ☐30,000 – 49,999 LE
   ☐50,000 – 99,999 LE ☐100,000 – 149,999 LE ☐150,000 –249,999 LE
   ☐250,000 LE or over

14. How many people live in your household? ___

Western Applicant/Participant Number: ________ Birthdate: ___/___/___ Sex (M/F):___

World Health Organization

Oral Health Questionnaire for Children

First, we would like you to answer some questions concerning yourself and your teeth

Identification number	Sex		Location		
	Boy	Girl	Urban	Periurban	Rural
1. ☐☐☐☐	☐	☐	☐	☐	☐
1 4	1	2	1	2	3

2. **How old are you today?** ____________________
(Years)

3. **How would you describe the health of your teeth and gums?**
(Read each item)

	Teeth	Gums
Excellent	☐ 1	☐ 1
Very good	☐ 2	☐ 2
Good	☐ 3	☐ 3
Average	☐ 4	☐ 4
Poor	☐ 5	☐ 5
Very poor	☐ 6	☐ 6
Don't know	☐ 9	☐ 9

4. **How often during the past 12 months did you have toothache or feel discomfort due to your teeth?**

Often .... ☐ 1
Occasionally .... ☐ 2
Rarely .... ☐ 3
Never .... ☐ 4
Don't know .... ☐ 9

Now please answer some questions about the care of your teeth

5. **How often did you go to the dentist during the past 12 months?**
(Put a tick/cross in one only)

Once .... ☐ 1
Twice .... ☐ 2
Three times .... ☐ 3
Four times .... ☐ 4

Western Applicant/Participant Number: ________ Birthdate: ___/___/___ Sex (M/F):___

More than four times	☐ 5
I had no visit to dentist during the past 12 months	☐ 6
I have never received dental care/visited a dentist	☐ 7
I don't know/don't remember	☐ 9

If you did not see a dentist during the last 12 months, go on to question 7

6. **What was the reason for your last visit to the dentist?**
(Put a tick/cross in one box only)

Pain or trouble with teeth, gums or mouth	☐ 1
Treatment/follow-up treatment	☐ 2
Routine check-up of teeth/treatment	☐ 3
I don't know/don't remember	☐ 9

7. **How often do you clean your teeth?**
(Put a tick/cross in one box only)

Never	☐ 1
Several times a month (2–3 times)	☐ 2
Once a week	☐ 3
Several times a week (2–6 times)	☐ 4
Once a day	☐ 5
2 or more times a day	☐ 6

8. **Do you use any of the following to clean your teeth or gums?**
(Read each item)

	Yes 1	No 2
Toothbrush	☐	☐
Wooden toothpicks	☐	☐
Plastic toothpicks	☐	☐
Thread (dental floss)	☐	☐
Charcoal	☐	☐
Chewstick/miswak	☐	☐
Other	☐	☐

Please specify______________________

9.

	Yes	No
a) **Do you use toothpaste to clean your teeth**	☐ 1	☐ 2
b) **Do you use toothpaste that contains fluoride?**	☐ 1	☐ 2
Don't know	☐ 9	

Western Applicant/Participant Number: ________ Birthdate: ___/___/___ Sex (M/F):___

10. **Because of the state of your teeth and mouth, have you experienced any of the following problems during the past year?**

	Yes 1	No 2	Don't know 0
(a) I am not satisfied with the appearance of my teeth	☐	☐	☐
(b) I often avoid smiling and laughing because of my teeth	☐	☐	☐
(c) Other children make fun of my teeth	☐	☐	☐
(d) Toothache or discomfort caused by my teeth forced me to miss classes at school or miss school for whole days................................	☐	☐	☐
(e) I have difficulty biting hard foods	☐	☐	☐
(f)I have difficulty in chewing...................	☐	☐	☐

11. **How often do you eat or drink any of the following foods, even in small quantities?**
(Read each item)

	Several times a day 6	Every day 5	Several times a week 4	Once a week 3	Several times a month 2	Never 1
Fresh fruit..................	☐	☐	☐	☐	☐	☐
Biscuits, cakes, cream cakes, sweet pies, buns etc..................	☐	☐	☐	☐	☐	☐
Lemonade, Coca Cola or other soft drinks..	☐	☐	☐	☐	☐	☐
Jam/honey....................	☐	☐	☐	☐	☐	☐
Chewing gum containing sugar	☐	☐	☐	☐	☐	☐
Sweets/candy...............	☐	☐	☐	☐	☐	☐
Milk with sugar...........	☐	☐	☐	☐	☐	☐
Tea with sugar	☐	☐	☐	☐	☐	☐
Coffee with sugar	☐	☐	☐	☐	☐	☐

(Insert country-specific items)

Western

Applicant/Participant Number: ________ Birthdate: ___/___/___ Sex (M/F):___

12. **How often do you use any of the following types of tobacco?**
(Read each item)

	Every day	Several times a week	Once a week	Several times a month	Seldom	Never
	6	5	4	3	2	1
Cigarettes, pipe or cigars ...	☐	☐	☐	☐	☐	☐
Chewing tobacco or snuff..	☐	☐	☐	☐	☐	☐

13. **What level of education has your father completed (or your stepfather, guardian or other male adult living with you)?**

No formal schooling.. ☐ 1
Less than primary school.. ☐ 2
Primary school completed .... ☐ 3
Secondary school completed.. ☐ 4
High school completed .... ☐ 5
College/university completed .... ☐ 6
No male adult in household .... ☐ 7
Don't know... ☐ 9

14. **What level of education has your mother completed?**

No formal schooling.. ☐ 1
Less than primary school.. ☐ 2
Primary school completed .... ☐ 3
Secondary school completed.. ☐ 4
High school completed .... ☐ 5
College/university completed .... ☐ 6
No female adult in household.. ☐ 7
Don't know... ☐ 9
(Insert country-specific categories)

That completes our questionnaire
Thank you very much for your cooperation!

Year	Month	Day	Interviewer	District	Country
☐☐	☐☐	☐☐	☐☐	☐☐	☐☐☐☐

Appendix 3

Script for Classroom Recruitment

Hello, my name is _____________. I am here today to talk to you about a research study about dental growth and development in modern and ancient Egyptian children which is being done under the supervision of Dr. El Molto.

I am currently recruiting participants who are between the ages of 2 and 17 years old, with a birthdate that falls within one month of available study dates, and who would like to participate in this study. Briefly, the study involves the completion of a questionnaire by you and/or your legal guardian about factors that may affect dental health and development. Eligible participants will then be assigned an appointment to receive a free panoramic dental x-ray, and an appointment for a free dental examination, oral health report and advice from a local dentist about oral health and hygiene. This study will be conducted in the Radwania Radiographic Laboratory on Sharia Television in Luxor. There will be a total of 1020 local participants in this study.

If you are interested in participating or have any questions; please contact me at the email address or phone number provided in the information form.

Thank you for considering participation in this study.

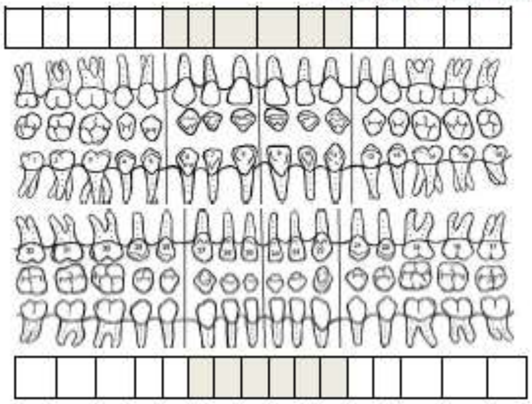
Appendix 4

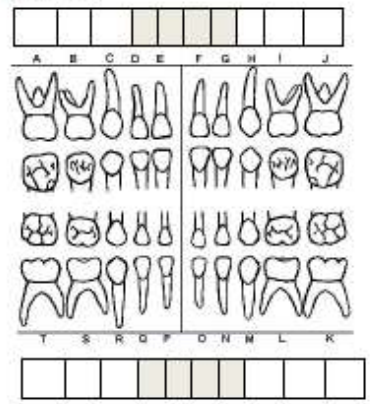
Western

The Egyptian Dental Health and Development Survey

Casey Kirkpatrick 2014

Please assign developmental scores in the boxes provided and draw caries and enamel defects on diagram in red. Briefly describe any abnormalities in number 1 below.





Developmental Scores:

Score	Definition
0	**Not erupted**: Tooth is congenitally missing or has not emerged through the gingiva
3	**Gingival emergence (Massler and Schour, Stage III)**: The emergence of any portion of the tooth crown through the gingiva. No evidence of soft tissue occlusal to the tooth crown.
4	**Attainment of the occlusal level (Massler and Schour, Stage V)**: Full occlusal contact as determined by the overall line of occlusion (Curve of Spee). If a tooth is malaligned, only a portion of the occlusal (incisal) surface must be in occlusion.
	Missing teeth:
16	-tooth has been exfoliated (score 16).
17	-tooth has been extracted (score 17)
19	-tooth was probably extracted (score 19)
99	-presence or absence of the tooth is unknown (score 99).

1. Please describe any developmental anomalies (i.e. supernumerary teeth, congenital tooth absence, hypodontia, enamel defects, deciduous tooth retentions, peg tooth, impacted tooth, etc.) or pathologies observed: ____________

2. Please describe any developmental or pathological aspects observed in the panoramic x-ray that were not visible through clinical examination (i.e. dental abscess, minor decay, etc.): ____________

THANK YOU FOR PRINTING CLEARLY! Participant Number: ________ Birthdate: ___/___/___ Sex: M / F

World Health Organization

World Health Organization
Oral Health Assessment Form for Children (by tooth surface), 2013

Leave blank	Year	Month	Day	Identification No.	Orig/Dupl	Examiner
(1) ☐☐☐☐ (4)	(5) ☐☐	☐☐	☐☐ (10)	(11) ☐☐☐☐ (14)	☐ (15)	(16) ☐☐ (17)

General information: ______________________ (Name)

Sex 1=M, 2=F ☐ (18)

Date of birth (19) ☐☐☐☐☐☐ (24)

Age in years (25) ☐☐ (26)

Ethnic group (27) ☐☐ (28) **Other group** (29) ☐☐ (30) **Years in school** (31) ☐☐ (32) **Occupation** ☐ (33)

Community (geographical location) (34) ☐☐ (35) **Location** Urban (1) Periurban (2) Rural (3) ☐ (36)

Other data ______________ (37) ☐☐ (38) **Other data** ______________ (39) ☐☐ (40)

Other data ______________ (41) ☐☐ (42) **Extra-oral examination** ________ (43) ☐☐ (44)

Dentition status by tooth surface

	17	16	55 15	54 14	53 13	52 12	51 11	61 21	62 22	63 23	64 24	65 25	26	27	
Occ					X	X	X	X	X	X					(45–52)
Mes															(53–66)
Buc															(67–80)
Dis															(81–94)
Oral															(95–108)

	47	46	85 45	84 44	83 43	82 42	81 41	71 31	72 32	73 33	74 34	75 35	36	37	
Occ					X	X	X	X	X	X					(109–116)
Mes															(117–130)
Buc															(131–144)
Dis															(145–158)
Oral															(159–172)

Primary teeth	Permanent teeth Status
A	0 = Sound
B	1 = Caries
C	2 = Filled w/caries
D	3 = Filled, no caries
E	4 = Missing due to caries
–	5 = Missing for another reason
F	6 = Fissure sealant
G	7 = Fix dental prosthesis/crown, abutment, veneer
–	8 = Unerupted
–	9 = Not recorded

THANK YOU FOR PRINTING CLEARLY! Participant Number: ________ Birthdate: ___/___/___ Sex: M / F

Periodontal status

			55	54	53	52	51	61	62	63	64	65			
	17	16	15	14	13	12	11	21	22	23	24	25	26	27	
(173)															(180)
(187)															(200)
			85	84	83	82	81	71	72	73	74	75			
	47	46	45	44	43	42	41	31	32	33	34	35	36	37	

Gingival bleeding
Score
(0) = Absence of condition (1) = Presence of condition
(9) = Tooth excluded (X) = Tooth not present

Enamel fluorosis ☐ (201)

Severity

0 = Normal
1 = Questionable
2 = Very mild
3 = Mild
4 = Moderate
5 = Severe

8 = Excluded (crown, restoration, "bracket")

9 = Not recorded (unerupted tooth)

Dental erosion

Severity

(202) ☐

0 = No sign of erosion
1 = Enamel lesion
2 = Dentinal lesion
3 = Pulp involvement

No. of teeth

(203) ☐☐ (204)

Dental trauma

Status

(205) ☐

0 = No sign of injury
1 = Treated injury
2 = Enamel fracture only
3 = Enamel and dentine fracture
4 = Pulp involvement
5 = Missing tooth due to trauma
6 = Other damage
9 = Excluded tooth

No. of teeth

(206) ☐☐ (207)

Oral mucosal lesions

Condition

(208) ☐
(209) ☐
(210) ☐

0= No abnormal condition
1= Ulceration (aphtheus herpetic, traumatic)
2= Acute necrotizing ulcerative gingivitis (ANUG)
3= Candidiasis
4= Abscess
8= Other condition
9= Not recorded

Location

(211) ☐
(212) ☐
(213) ☐

0 = Vermillion border
1 = Commissures
2 = Lips
3 = Sulci
4 = Buccal mucosa
5 = Floor of mouth
6 = Tongue
7 = Hard/soft palate
8 = Alveolar ridges/gingiva
9= Not recorded

Intervention URGENCY ☐ (214)

0 = No curative treatment needed

1 = Preventive or routine treatment needed

2 = Prompt treatment (including scaling) needed

3 = Immediate (urgent) treatment due to pain or infection of dental and/or oral origin

4 = Referred for comprehensive evaluation or medical treatment (systemic condition)

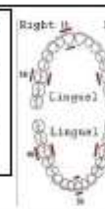
For Children Aged 15 or Over

*Periodontal Loss of Attachment**

	17/16	11	26/27	
[illegible]				[illegible]
[illegible]				[illegible]
	47/46	31	36/37	

Severity
0 = 0–3 mm
1 = 4–5 mm Cemento-enamel junction (CEJ) within black band
2 = 6–8 mm CEJ between upper limit of black band and 8.5 mm ring
3 = 9–11 mm CEJ between 8.5 mm and 11.5 mm ring
4 = 12 mm or more CEJ beyond 11.5 mm ring
X = Excluded sextant
9 = Not recorded

*Scores should be based on the teeth indicated above/below each scoring box. Boxes with two numbers should record the average score of the two teeth if possible. If the requested 1st incisor is missing, use the adjacent 1st incisor for scoring.



*Plaque (Soft debris)***

	Right Molar		Anterior		Left Molar	
	Buccal	Lingual	Labial	Lingual	Buccal	Lingual
Upper						
Lower						

****Plaque and Calculus Scoring**
0 = No plaque (soft debris) / calculus present
1 = Covers not more than 1/3 of the exposed tooth surface
2 = Covers between 1/3 and 2/3 of the exposed tooth surface
3 = Covers more than 2/3 of the exposed tooth surface

*Calculus***

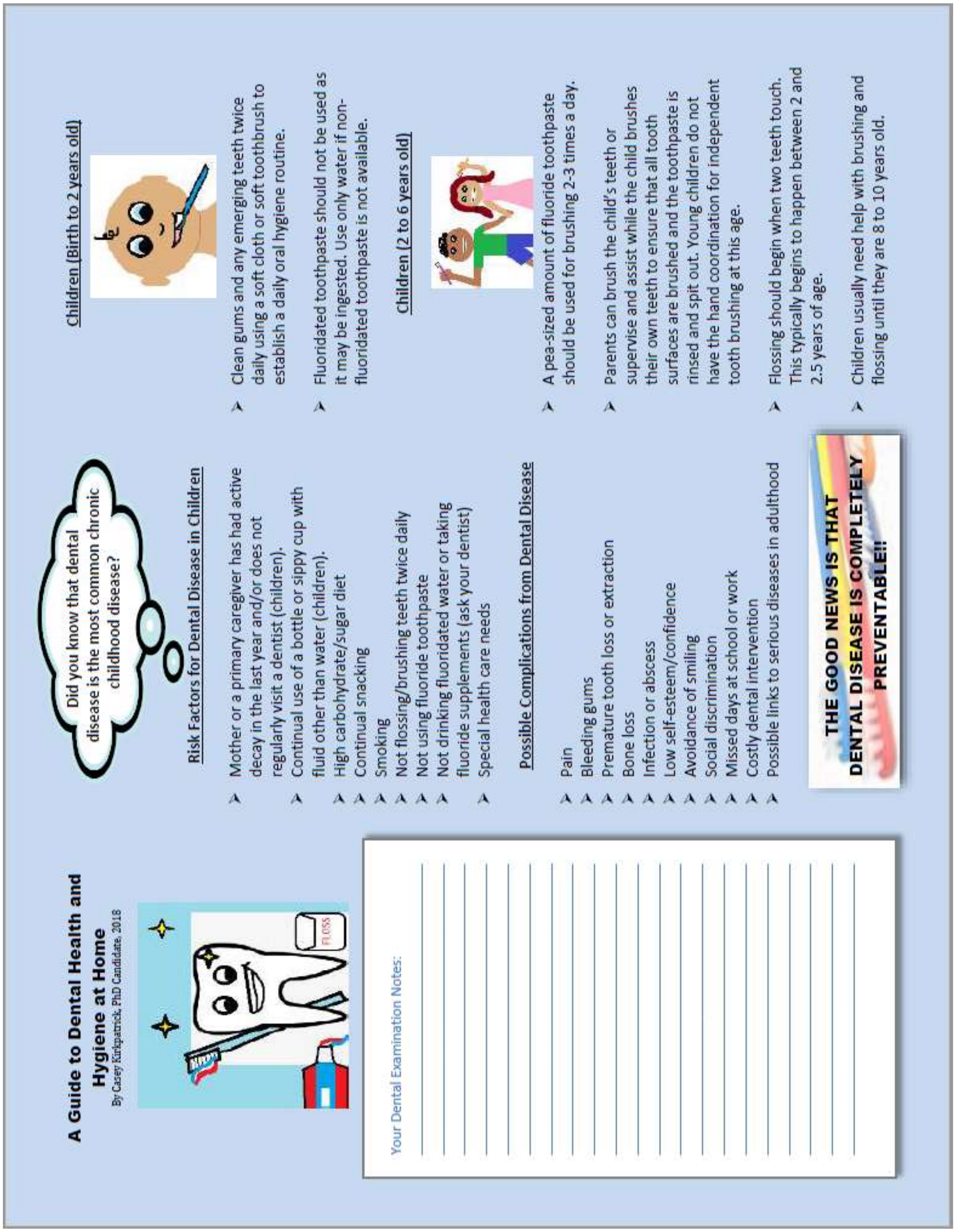
	Right Molar			Anterior			Left Molar		
	Buccal	Lingual	Intrprx	Labial	Lingual	Intrprx	Buccal	Lingual	Intrprx
Upper									
Lower									

**Plaque and Calculus scores should be based on the teeth indicated in the above diagram. The adjacent 2nd molar may be used in cases where 1st molars are missing.

THANK YOU FOR PRINTING CLEARLY! Participant Number: __________ Birthdate: ___/___/___ Sex: M / F

Appendix 5

Oral Health Education Pamphlet



Oral Hygiene Tips and Tricks for Children

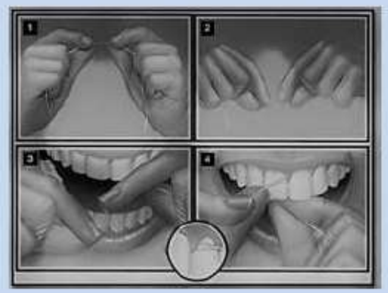
- For infants and toddlers, teeth cleaning can be incorporated into bath time.
- Although they are not necessary, specialized infant and child toothbrushes are available to make toothbrushing easier and children more interested in brushing their teeth.
- Allow children to make choices during toothbrushing time so that they feel in control. Let them pick a song to listen to while brushing, or provide more than one colourful and tasty toothpaste from which to choose!
- Toothbrushing can be made fun by playing games like pretending to chase the animals or germs out of your child's mouth with a toothbrush!
- Children may be more willing to brush their teeth if they can watch an older sibling or a parent brushing their teeth at the same time.
- Due to a lack of hand coordination, children may be better able to brush their teeth in a circular pattern, rather than the horizontal pattern recommended for adults.

- Plastic dental floss holders may help children to floss on their own. Another option is to tie a length of dental floss in a loop, making it easier to hold while flossing.

Adolescent and Adult Dental Cleaning Methods

1-2: Wrap a piece of dental floss around your fingers

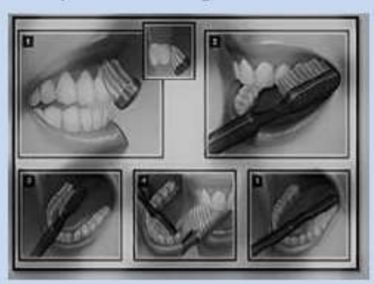
3-4: Slide the floss between upper and lower teeth, taking care to reach under the gum line by following the surfaces of the teeth.



1-3: Gently brush the inside and outside of the top and bottom dentition horizontally with the bristles at a 45 degree angle to the teeth so that the toothbrush bristles can reach under the gumline and massage the gums.

4: Don't forget to brush the inside of the top and bottom front teeth!!

5: Finally, brush the biting surfaces of all teeth.



Toothbrush Care

Your toothbrush:

- should not be shared with others
- should not be stored in a closed container
- should be rinsed after every use and air dried in a clean place
- should be replaced every 3 months, or when visibly worn, damaged or dirty

Other Oral Hygiene Tips and Tricks

- Mouth rinses can be used to loosen dental plaque and kill bacteria that contributes to dental decay and bad breath
- Brushing the tongue with a toothbruth or special tongue brush may also help prevent bad breath and dental decay
- Special brushes can be used for cleaning between teeth and around dental appliances
- Ask a local dentist about your oral hygiene routine and how you can keep your smile for years to come!

Black and white instructional images were modified from images on Normanhurstdental.com.au and moonstonedental.com.

Appendix 6

Dissertation R Codes

##Dataframes, column names, and regression models included in this list of R codes were used in dissertation calculations; however these R codes were often used to calculate, plot or graph more than one dataset or model.

Intra-class Correlation (ICC)

```
>psych::ICC(intra)
```

Paired t-test

```
>t.test(Isomere$LM1, Isomere$UM1, paired=TRUE, conf.level=0.95)
```

Simple plot of differences

```
>plot(Difference, pch = 16, ylab="Difference (UM1 - LM1)", main="Variance of Upper Molar Wear from Lower Molar Wear") + abline(0,0, col="blue", lwd=2)
```

Tukey's Boxplot

```
>boxplot(antimeres$Difference, ylab = "Difference (%)", main = "Difference between Left and Right First Molars (%)")
```

Pearson's r Correlation Test

```
>cor.test( ~ Age + AMTL, data = AMTL2, subset = Sex == "M")
```

Caries vs Age and Sex

```
> ggplot2::ggplot(AMTL, aes(x=caries,y=Age, group=Sex,colour=Sex,shape=Sex)) + geom_point(position = position_jitter(w = 0.1, h = 0.0), size=2) +  ggtitle("Number of Carious Teeth in Relation to Skeletal Age and Sex") + theme(plot.title = element_text(hjust = 0.5)) + labs(x = "Number of Teeth with Carious Lesions",y = "Skeletal Age (+/- 5 years)") + scale_colour_manual(name="Sex", labels = c("Female", "Male"), values = c("deeppink", "deepskyblue1")) +scale_shape_manual(name="Sex",labels = c("Female", "Male"), values=c(16, 17)) + theme(legend.position="right") + guides(color=guide_legend("Sex"), shape=guide_legend("Sex"))
```

Observed Teeth with Carious Lesions vs Age and Sex

```
> ggplot2::ggplot(AMTL3, aes(x=obs,y=Age,group=Sex,colour=Sex,shape=Sex)) + geom_point(position = position_jitter(w = 0.1, h = 0.0),size=2) + ggtitle("Percentage of Observed Teeth with Carious Lesions in Relation to Skeletal Age and Sex") + theme(plot.title = element_text(hjust = 0.5)) + labs(x = "% Observed Teeth with Carious Lesions",y = "Skeletal Age (+/- 5 years)") + scale_colour_manual(name="Sex", labels = c("Female", "Male"), values = c("deeppink", "deepskyblue1")) +scale_shape_manual(name="Sex",labels = c("Female", "Male"), values=c(16, 17)) + theme(leg
```

```
end.position="right") + guides(color=guide_legend("Sex"), shape=guide_l
egend("Sex"))
```

Number of Teeth Affected by AMTL or Caries vs. Age and Sex

```
> ggplot2::ggplot(AMTL2, aes(x=AMTL.Caries,y=Age,group=Sex,colour=Sex,s
hape=Sex)) + geom_point(alpha=0.3,size=2) + ggtitle("Number of Teeth Af
fected by AMTL or Caries in Relation to Skeletal Age and Sex") + theme
(plot.title = element_text(hjust = 0.5)) + labs(x = "Number of Teeth Af
fected by AMTL or Caries", y = "Skeletal Age (+/-5 years)") + scale_col
our_manual(name="Sex", labels = c("Female", "Male"), values = c("deeppi
nk", "deepskyblue1")) +scale_shape_manual(name="Sex",labels = c("Female
", "Male"), values=c(16, 17)) + theme(legend.position="right") + guides
(color=guide_legend("Sex"), shape=guide_legend("Sex"), fill=guide_legen
d("95% Confidence Interval")) + stat_smooth(method = "lm", fullrange =
TRUE)

>gd = lm(Age~Sex + AMTL.Caries + Sex:AMTL.Caries, data = AMTL2)
```

Antemortem tooth loss in relation to skeletal age and sex

```
> ggplot2::ggplot(AMTL2, aes(x=AMTL,y=Age,group=Sex,colour=Sex,shape=Se
x,fill=Sex)) + geom_point(alpha=0.5,size=2) + ggtitle("Antemortem Tooth
Loss in Relation to Skeletal Age and Sex") + theme(plot.title = element
_text(hjust = 0.5)) + labs(x = "Number of Teeth Lost Antemortem", y = "
Skeletal Age (+/-5 years)") + scale_colour_manual(name="Sex", labels =
c("Female", "Male"), values = c("deeppink", "deepskyblue1")) +scale_sha
pe_manual(name="Sex",labels = c("Female", "Male"), values=c(16, 17)) +
theme(legend.position="right") + guides(color=guide_legend("Sex"), shap
e=guide_legend("Sex"), fill=guide_legend("95% Confidence Interval")) +
stat_smooth(method = "lm", fullrange = TRUE)

> ggplot2::ggplot(AMTL2, aes(x=AMTL,y=Age)) + geom_point(alpha=0.3, size
=2, colour="chartreuse3") + ggtitle("Antemortem Tooth Loss (AMTL) in Rel
ation to Skeletal Age for Unknown Sex") + theme(plot.title = element_tex
t(hjust = 0.5)) +  labs(x = "Number of Teeth Lost Antemortem", y = "Skel
etal Age (+/-5 years)") + theme(legend.position="right") + stat_smooth(m
ethod = "lm", fullrange = TRUE, colour="chartreuse3")

>modelAMTLlm = lm(Age~Sex + AMTL + Sex:AMTL, data = AMTL2)
>AMTLU = lm(Age~AMTL, data = AMTL2)
```

AMTL polynomial regression models

```
>new_AM=cbind(AMTL2$AMTL, AMTL2$AMTL^2)
>modelAMpoly = lm(Age~Sex + new_AM + Sex:new_AM, data = AMTL2)
>summary(modelAMpoly)
>plot(modelAMpoly)
>hist(residuals(modelAMpoly), col="turquoise1", main = "Histogram of Res
iduals", xlab = "Residuals", ylab = "Frequency")

>ggplot2::ggplot(AMTL2, aes(x=AMTL,y=Age,group=Sex,colour=Sex,shape=Se
x)) + geom_point(alpha=0.3,size=2) + stat_smooth(aes(x = AMTL,y = model
AMpoly$fitted.values)) + ggtitle("Antemortem Tooth Loss (AMTL) in Relat
ion to Skeletal Age and Sex") + theme(plot.title = element_text(hjust =
0.5)) +  labs(x = "Number of Teeth Lost Antemortem", y = "Skeletal Age
(+/-5 years)") + scale_colour_manual(name="Sex", labels = c("Female", "
Male"), values = c("deeppink", "deepskyblue1")) +scale_shape_manual(nam
```

```
e="Sex",labels = c("Female", "Male"), values=c(16, 17)) + theme(legend.
position="right") + guides(color=guide_legend("Sex"), shape=guide_legen
d("Sex"))

> ggplot2::ggplot(AMTL2, aes(x=AMTL,y=Age)) + geom_point(alpha=0.3,size=
2,colour = "chartreuse3") + stat_smooth(aes(x = AMTL,y = AMTLpolyU$fitte
d.values), colour = "chartreuse3") + ggtitle("Antemortem Tooth Loss (AMT
L) in Relation to Skeletal Age for Unknown Sex") + theme(plot.title = el
ement_text(hjust = 0.5)) +  labs(x = "Number of Teeth Lost Antemortem",
y = "Skeletal Age (+/-5 years)") + theme(legend.position="right")

> set.seed(100)
> trainingRowIndex <- sample(1:nrow(AMTL2), 0.8*nrow(AMTL2))
> trainingData <- AMTL2[trainingRowIndex, ]
> testData  <- AMTL2[-trainingRowIndex, ]
> new_AM2=cbind(trainingData$AMTL, trainingData$AMTL^2)
> lmMod = lm(Age~new_AM2, data = trainingData)
> Summary(lmMod) ## use estimated coefficients to create actual algebraic
formula
> x = trainingData$AMTL
#input actual algebraic formula
> M = actual algebraic formula
> t.test(testData$Age, M, paired=TRUE, conf.level=0.95)

modelAMpoly = lm(Age~Sex + new_AM + Sex:new_AM, data = AMTL2)
AMTLpolyU = lm(Age~new_AM, data = AMTL2)
AMTLpolyF = lm(Age~new_AM, data = AMTL2F)
AMTLpolyM = lm(Age~new_AM, data = AMTL2M)
```

Percentage Exposed Dentine in M1 in Relation to Skeletal Age and Sex

```
ggplot2::ggplot(M1.5, aes(x=M1,y=Age,group=Sex,colour=Sex,shape=Sex,fil
l=Sex)) + geom_point(alpha=0.3,size=2) + ggtitle("Percentage Exposed De
ntine in M1 in Relation to Skeletal Age and Sex") + theme(plot.title =
element_text(hjust = 0.5)) + labs(x = "% Exposed Dentine in M1", y = "S
keletal Age (+/-5 years)") + scale_colour_manual(name="Sex", labels = c
("Female", "Male"), values = c("deeppink", "deepskyblue1")) +scale_shap
e_manual(name="Sex",labels = c("Female", "Male"), values=c(16, 17)) + t
heme(legend.position="right") + guides(color=guide_legend("Sex"), shape
=guide_legend("Sex"), fill=guide_legend("95% Confidence Interval")) + s
tat_smooth(method = "lm", fullrange = TRUE)

> ggplot2::ggplot(M1.5, aes(x=M1,y=Age) + geom_point(size=2, colour="cha
rtreuse3",alpha=0.3) + ggtitle("Percentage Exposed Dentine in M1 in Rela
tion to Skeletal Age for Unknown Sex") + theme(plot.title = element_text
(hjust = 0.5)) +  labs(x = "% Exposed Dentine in M1", y = "Skeletal Age
(+/-5 years)") + theme(legend.position="right") + stat_smooth(method = "
lm", fullrange = TRUE, colour="chartreuse3")

>gd = lm(Age~Sex + M1 + Sex:M1, data = M1.5)
>M1U = lm(Age~M1, data = M1.5)
```

Percentage Exposed Dentine in M2

```
>ggplot2::ggplot(M2.5, aes(x=M2,y=Age,group=Sex,colour=Sex,shape=Sex,fi
ll=Sex)) + geom_point(alpha=0.3,size=2) + ggtitle("Percentage Exposed D
entine in M2 in Relation to Skeletal Age and Sex") + theme(plot.title =
element_text(hjust = 0.5)) + labs(x = "% Exposed Dentine in M2", y = "S
keletal Age (+/-5 years)") + scale_colour_manual(name="Sex", labels = c
```

```
("Female", "Male"), values = c("deeppink", "deepskyblue1")) +scale_shap
e_manual(name="Sex",labels = c("Female", "Male"), values=c(16, 17)) + t
heme(legend.position="right") + guides(color=guide_legend("Sex"), shape
=guide_legend("Sex"), fill=guide_legend("95% Confidence Interval")) + s
tat_smooth(method = "lm", fullrange = TRUE)

> ggplot2::ggplot(M2.5, aes(x=M2,y=Age)) + geom_point(size=2, colour="ch
artreuse3",alpha=0.3) + ggtitle("Percentage Exposed Dentine in M2 in Rel
ation to Skeletal Age for Unknown Sex") + theme(plot.title = element_tex
t(hjust = 0.5)) +  labs(x = "% Exposed Dentine in M2", y = "Skeletal Age
(+/-5 years)") + theme(legend.position="right") + stat_smooth(method = "
lm", fullrange = TRUE, colour="chartreuse3")

>modelM2.5 = lm(Age~Sex + M2 + Sex:M2, data = M2.5)
>M2U = lm(Age~M2, data = M2.5)
```

Regression diagnostics

```
##Residuals, Coefficients, RSE, R-squared, F statistic,
>Summary(gd)

##Mean Absolute Error
>accuracy(list(modelAMTLlm, AMTLU, modelAMTLpoly, AMTLpolyU, gd, M1U, mo
delM2.5, M2U, ModelmregM3, modelmregF, modelmreg2, modelM1AMM, M1AMF,M1A
MU, modelmregM2AMM, M2AMF, M2AMU, M1M2M, M1M2F, M1M2U),plotit=TRUE, digi
ts=3)

##Diagnostic plots in 2x2 layout
>par(mfrow=c(2,2))
>plot(gd)

##Histogram of residuals
>hist(residuals(gd), col="turquoise1", main = "Histogram of Residuals",
xlab = "Residuals", ylab = "Frequency")
```

ANCOVA/ANOVA

```
>anova(gd)
```

M1 Wear vs. Brothwell

```
ggplot2::ggplot(M1.5, aes(x=M1,y=Age,colour=Sex,shape=Sex,fill=Sex))+ge
om_rect(aes(xmin = 6, xmax = 16.1, ymin = 17, ymax = 25,colour = "paleg
reen"), fill = "palegreen",show.legend = FALSE)+geom_rect(aes(xmin = 2
2.13, xmax = 56.55, ymin = 25, ymax = 35,colour="palegreen"), fill = "p
alegreen",show.legend = FALSE)+geom_rect(aes(xmin = 59.77, xmax = 84.1
3, ymin = 35, ymax = 45,colour="palegreen"), fill = "palegreen",show.le
gend = FALSE)+geom_rect(aes(xmin = 84.13, xmax = 100, ymin = 45, ymax =
100,colour="palegreen"), fill = "palegreen",show.legend = FALSE)+ geom_
point(alpha=0.5,size=2)+ xlim(0,100)+ylim(0,100)+ggtitle("Comparing Kel
lis 2 Dentine Exposure in M1 to the Quantified Brothwell Standard") + t
heme(plot.title = element_text(hjust = 0.5),legend.position = "right")+
labs(x = "Percentage of Dentine Exposure",y = "Skeletal Age (+/- 5 year
s)")+ scale_colour_manual(values = c("deeppink","deepskyblue1","palegre
en"),labels = c("Female", "Male","Brothwell"))+scale_shape_manual(value
s=c(16,17,NULL),labels = c("Female","Male","Brothwell"))+scale_fill_man
ual(name="95% Confidence Interval", values=c("deeppink","deepskyblue1",
"palegreen"),labels=c("Female","Male","Brothwell"))+stat_smooth(method
= "lm", se = TRUE, fullrange = TRUE)
```

M2 Wear vs. Brothwell

```
ggplot2::ggplot(M2.5, aes(x=M2,y=Age,colour=Sex,shape=Sex,fill=Sex))+ g
eom_rect(aes(xmin = 0, xmax = 5.438066, ymin = 17, ymax = 25,colour = "
palegreen"), fill = "palegreen",show.legend=FALSE)+geom_rect(aes(xmin =
7.471264, xmax = 26.36816, ymin = 25, ymax = 35,colour="palegreen"), fi
ll = "palegreen",show.legend=FALSE)+geom_rect(aes(xmin = 36.1039, xmax
= 60.15038, ymin = 35, ymax = 45,colour="palegreen"), fill = "palegreen
",show.legend=FALSE)+geom_rect(aes(xmin = 60.15038, xmax = 100, ymin =
45, ymax = 100,colour="palegreen"), fill = "palegreen",show.legend=FALS
E)+ geom_point(alpha=0.5,size=2)+ xlim(0,100)+ylim(0,100)+ggtitle("Comp
aring Kellis 2 Dentine Exposure in M2 to the Quantified Brothwell Stand
ard") + theme(plot.title = element_text(hjust = 0.5),legend.position =
"right")+ labs(x = "Percentage of Dentine Exposure",y = "Skeletal Age
(+/- 5 years)")+ scale_colour_manual(values = c("deeppink","deepskyblue
1","palegreen"),labels = c("Female", "Male","Brothwell"))+scale_shape_m
anual(values=c(16,17,NULL),labels = c("Female","Male","Brothwell"))+sca
le_fill_manual(name="95% Confidence Interval", values=c("deeppink","dee
pskyblue1","palegreen"),labels=c("Female","Male","Brothwell"))+stat_smo
oth(method = "lm", se = TRUE, fullrange = TRUE)
```

Multiple Regression

```
>modelmreg2 = lm(formula = Age ~ M1 + M2 + AMTL, data = mreg2) ##male an
d female
>modelmregM = lm(formula = Age ~ M1 + M2 + AMTL, data = mregM) ##male
>modelmregF = lm(formula = Age ~ M1 + M2 + AMTL, data = mregF) ##female
>summary(modelmreg2) ##Residuals, Coefficients, RSE, R-squared, F statis
tic

>par(mfrow=c(2,2)) ##Diagnostic plots in 2x2 layout
>plot(gd)

>hist(residuals(gd), col="turquoise1", main = "Histogram of Residuals",
xlab = "Residuals", ylab = "Frequency") ##Histogram of residuals

>AIC(lmMod)
```

Regression Model 80:20 Bootstrapping Tests Without Replacement

```
>set.seed(100)
>trainingRowIndex <- sample(1:nrow(mregF), 0.8*nrow(mregF))
>trainingData <- mregF[trainingRowIndex, ]
>testData  <- mregF[-trainingRowIndex, ]
>lmMod <- lm(Age ~ M1 + M2 + AMTL, data=trainingData)
>distPred <- predict(lmMod, testData)
>act = data.frame(cbind(actuals=testData$Age, predicteds=distPred))
>View(act)
>t.test(act$actuals, act$predicteds, paired=TRUE, conf.level=0.95)
>M = lm(Age ~ M1 + M2 + AMTL, data=mregF)
>summary()
>AIC()

AMTLU = lm(Age~AMTL, data = AMTL2)

gd = lm(Age~Sex + M1 + Sex:M1, data = M1.5)
```

Curriculum Vitae

Casey Kirkpatrick

EDUCATION

September 2019	**Western Certificate in University Teaching and Learning** The University of Western Ontario, *London, Ontario, Canada*
August 2019	**Ph.D. (Doctor of Philosophy) in Anthropology, Specialization in Bioarchaeology and Archaeology** **Dissertation Title:** "Getting Better All the Time: Re-evaluating Macroscopic Dental Age Estimation Standards in Egypt" University of Western Ontario, *London, Ontario, Canada*
October 2009	**M.A. (Masters of Arts) in Ancient Egyptian Culture** **Dissertation Title:** "Site 117: A Multidisciplinary Analysis of the Lives and Deaths of the World's First Known War Victims." (Graduated with Distinction) Swansea University, *Swansea, Wales, U.K.*
June 2007	**H.B.A. (Honors Bachelor of Arts) in Anthropology Specialization in Archaeology; Minor in Biology** University of Western Ontario, *London, Ontario, Canada* (Dean's Honor List 2006)

PUBLICATIONS

Kirkpatrick, C.L. In press. A Paleopathological Pilot Study of the Fag El Gamous Cranial Collection. In, K. Muhlestein, K. Pierce, B. Jensen (eds.), *Excavations at Fag el-Gamous and the Seila Pyramid.* Boston: Brill.

Molto, J.E., Kirkpatrick, C.L., Keron, J. 2019. A Paleoepidemiological Analysis of Sacral Spina Bifida Occulta in Population Samples from the Dakhleh Oasis, Egypt. *International Journal of Paleopathology* 26: 93-103. DOI: 10.1016/j.ijpp.2019.06.006

Kirkpatrick, C.L., Campbell, R.A., Hunt, K.J., and Willoughby, J.L. [Guest Editors] 2018. *IJPP Special Issue. Paleo-oncology: Taking Stock and Moving Forward.* Volume 21. https://www.sciencedirect.com/journal/international-journal-of-paleopathology/vol/21

Kirkpatrick, C.L., Campbell, R.A., Hunt, K.J., and Willoughby, J.L. 2018. "Preface: A Letter from the Guest Editors". *IJPP Special Issue. Paleo-oncology: Taking Stock and Moving Forward.* DOI: 10.1016/j.ijpp.2017.10.012

Kirkpatrick, C.L., Campbell, R.A., and Hunt, K.J. 2018. Introduction to Paleo-oncology: Taking Stock and Moving Forward. *IJPP Special Issue. Paleo-oncology: Taking Stock and Moving Forward. DOI:* 10.1016/j.ijpp.2018.02.001

Ragsdale, B.D., Kirkpatrick, C.L., and Campbell, R.A. 2018. Morphological Analysis of Dry Bone Specimens: General Principles and Differential Diagnosis. *IJPP Special Issue. Paleo-oncology: Taking Stock and Moving Forward.* DOI: 10.1016/j.ijpp.2017.02.002

Hunt, K.J., Roberts, C., Kirkpatrick, C.L. 2018. Taking Stock: A Meta-analysis of Published Evidence of Malignant Neoplastic Disease in Archaeological Human Remains. *IJPP Special Issue. Paleo-oncology: Taking Stock and Moving Forward.* DOI: 10.1016/j.ijpp.2018.03.002

Muhlestein, K., Harris, D., Evans, R.P., Gee, J., South, K., Kirkpatrick, C.L., Kwaspen, A., Campbell, R.A., Jensen, B. Unpublished field report. "The Fag el-Gamous 2018 Excavation Season," *Annales du Service des Antiquités de l'Egypte*.

Hunt, K., Kirkpatrick, C.L., Campbell, R, and Willoughby, J. 2017. *Cancer Research in Ancient Bodies (CRAB) Database*. https://www.cancerantiquity.org/crabdatabase.

Molto, E. and Kirkpatrick, C.L. 2018. "How Short is Short, and Why? A Possible Case of Dwarfism from Egypt's 3rd Intermediate Period from the Dakhleh Oasis, Egypt". *International Journal of Osteoarchaeology 28(1): 3-17*. DOI: 10.1002/oa.2625. [One of Wiley's top 20 downloaded papers published between July 2016 and June 2018]

Kirkpatrick, C.L., Hunt, K.J., Campbell, R., and Willoughby, J. (Content creators, editors and publishers) *Ancient Cancer Foundation* [Website]. http://www.ancientcancerfoundation.org (published online October 2015).

Kirkpatrick, C.L., Hunt, K.J., Campbell, R., and Willoughby, J. (Content creators, editors and publishers) *Paleo-oncology Research Organization* [Website]. http://www.cancerantiquity.org (published online March 2014).

Muhlestein, K., Tata, G., Harris, R., Harris, D., Evans, R.P., Blumell, L., Taylor, C., Christensen, B., Gee, J., South, K., Smith, J., Kirkpatrick, C.L., Ahmed, M.S. Unpublished field report. "The Fag el-Gamous 2014 Excavation Season," *Annales du Service des Antiquités de l'Egypte.*

Kirkpatrick, C.L. and Strong, M. (Eds.). 2010. *Coins through the Ages: Selected Collections from the Egyptian Museum in Cairo*. Catalogue for the temporary exhibit at the Egyptian Museum in Cairo. Cairo: American University in Cairo Press.

Kirkpatrick, C. 2007. "Tactical Deception and the Great Apes: Insight into the Question of Theory of Mind". *Totem: The University of Western Ontario Journal of Anthropology* 15: 31-37. https://ir.lib.uwo.ca/totem/vol15/iss1/4/.

MENTIONED IN

Killgrove, K. 2019. Archaeologists Discover A New Profession In An Ancient Egyptian Woman's Teeth. *Forbes Science*. https://www.forbes.com/sites/kristinakillgrove/2019/04/11/archaeologists-discover-a-new-profession-in-an-ancient-egyptian-womans-teeth/#3fa3f5273098

Yahia, M. 2019. That's cancer, a long long time ago. *CancerWorld* (CW+3). Official magazine for the European School of Oncology. https://cancerworld.net/cancerworld-plus/thats-cancer-a-long-long-time-ago/

Nelson, A. 2019. Mummies, memories and marginalization: the changing social roles of mummies from antiquity to today. In, M.L. Mant, A.J. Holland (eds.), *Bioarchaeology of Marginalized People*. London: Academic Press, p. 11-32.

Western Social Science. 2016. Western doctoral student makes mummified mice to better understand ancient diseases. *Western Social Science*. https://www.ssc.uwo.ca/news/2016/mummified_mice.html.

Eng, KF. 2016. Searching for the history of cancer in ancient human bones. *Ideas.TED.com*. https://ideas.ted.com/why-im-searching-for-cancer-in-ancient-human-bones/

Kamrin, J. 2015. The Egyptian Museum Database, Digitizing, and Registrar Training Projects: Update 2012. *Bulletin of the Egyptological Seminar: The Art and Culture of Ancient Egypt: Studies in Honor of Dorothea Arnold* 19:431-440.

Sourouzian H., et al. 2011. Fifth report on excavation and conservation works at Kôm el-Hettan from 9th to 12th seasons (2007-2010) by the Collossi of Memnon and Amenhotep III Temple conversion project. Part III: Work in the Peristyle Court and the Western Zone. Architectural Research and Site Protection Plan. *Annales du Service des Antiquités de l'Egypte* 85:413–552.

WORKSHOPS HOSTED

Kirkpatrick, C.L., Ragsdale, B.D. and Campbell, R. 2018. Neoplasm or Not? Morphologic Analysis of Dry Bone Specimens. *Paleopathology Association Annual Meeting*. Austin, Texas. April 9, 2018. Materials available at http://bit.ly/paleo-onc.

SYMPOSIUMS HOSTED

Co-organizer and Co-chair. Podium Symposium: Cancer and Neoplastic Disease in Bioarchaeology: Establishing a Dialogue for Future Research. *Paleopathology Association's Annual Meeting*. April 8, 2014.

CONFERENCES ORGANIZED

Conference Co-organizer. *Working Anthropology*. The University of Western Ontario Department of Anthropology. April 2013.

Program Co-editor. *Joint Conference on the Bioarchaeology of Ancient Egypt & the International Symposium on Animals in Ancient Egypt*. American University in Cairo – Tahrir Campus. January 10-13, 2019.

CONFERENCE POSTERS AND PRESENTATIONS

Kirkpatrick, C.L. 2019. "Neoplasm or Not? Considering the Accuracy of Paleo-oncological Diagnoses". [Invited Podium] *The Second International Colloquium on Palaeo-oncology: The Antiquity of Cancer - A symposium hosted by the European Society for the History of Oncology.* Athens, Greece [by video]. November 1, 2019.

Kirkpatrick, C.L. 2019. "Interactions between teeth and their environment: a study of the effect on age estimation". [Podium] *Joint Conference on the Bioarchaeology of Ancient Egypt and the International Symposium on Animals in Ancient Egypt.* Cairo, Egypt. January 11, 2019.

Kirkpatrick, C.L. 2018. "The Paleopathology of Cancer: Past, Present and Future". [Invited Podium] *The Antiquity of Cancer – A symposium hosted by the European Society for the History of Oncology*. London, England, UK. November 16, 2018.

Kirkpatrick, C.L. 2018. "Warriors or Martyrs? Untangling the trauma at Egypt's Fag El Gamous cemetery". [Podium] *Canadian Association for Physical Anthropology*. London, Ontario, Canada. November 2, 2018.

Kirkpatrick, C.L. 2017. "Bioarchaeology at Fag El-Gamous Cemetery: A New Beginning". [Invited Podium] *Studying Graeco-Roman Egypt: New Approaches in a New Generation - University of Basel Department of Classical Civilizations Research Showcase.* December 8, 2017.

Kirkpatrick, C.L. 2017. "Something To Chew On: Comparing Dentine Exposure in Ancient Egyptians and Dental Age Estimation Standards". [Poster] *American Association of Physical Anthropology Annual Meeting*. April 20, 2017.

Willoughby, J., Kirkpatrick, C.L., Koropatnik, J., and Nelson, A. 2016. “Observations on experimentally mummified tumors”. [Podium] *Canadian Association for Physical Anthropology Annual Meeting*. October 27, 2016.

Willoughby, J., Kirkpatrick, C.L., Koropatnik, J., and Nelson, A. 2016. “Experimental Mummification Project: CT Analysis of Cancer in Mummified Material”. [Podium] *9th World Congress on Mummy Studies*. August 11, 2016.

Willoughby, J., Kirkpatrick, C.L., Koropatnik, J., and Nelson, A. “Experimental Mummification Project for Radiological Detection of Cancer”. [Podium] *American Association of Physical Anthropology Annual Meeting*. April 14, 2016.

Willoughby, J., Hunt, K., Campbell, R. and C.L. Kirkpatrick, C.L. “Paleoradiology of Egyptian Mummies: A CT imaging survey of cancer in ancient remains”. [Poster] *American Research Centre in Egypt’s Annual Meeting*. April 24-25, 2015.

Kirkpatrick, C.L. “Now and Then: Linking Public Health Research to Bioarchaeological Methodology”. [Invited Poster] Invited Poster Symposium - Beyond the Bones: Engaging with Disparate Datasets. *American Association of Physical Anthropology Annual Meeting*. March 26, 2015.

Kirkpatrick, C.L. “Site 117: Reconstructing the Lives and Deaths of the Deceased at Nubia’s Earliest Known Cemetery”. [Poster] *Paleopathology Association’s Annual Meeting*. March 25, 2015.

Kirkpatrick, C.L. “Subadult Oral Health and Dental Development in Modern and Ancient Egypt” [Invited Podium] *Department of Anthropology, The University of Western Ontario*. March 13, 2015.

Kirkpatrick, C.L. and Willoughby, J. “Entanglements of Ancient and Modern: Defining Archaeological Space on the West Bank of Luxor”. [Podium] *Confronting Categories: Western Anthropology Graduate Student Conference*. March 6-8, 2015.

Kirkpatrick, C.L. and Molto, J.E. “How Short is Short? A Possible Case of Dwarfism from Egypt’s 3rd Intermediate Period from the Dakhleh Oasis, Egypt”. [Poster] *American Association of Physical Anthropology Annual Meeting.* April 10, 2014.

Kirkpatrick, C.L. “Abnormal Growths and Tumours of the Salivary Glands: A Bioarchaeological Perspective”. [Invited Podium] Symposium: Cancer and Neoplastic Disease in Bioarchaeology. *Paleopathology Association’s Annual Meeting*. April 8, 2014.

Kirkpatrick, C.L. “A New Model for the Creation of Subadult Dental Age Estimation Standards” [Podium] *Canadian Association for Physical Anthropology (CAPA) Annual Meeting*. October 19, 2013.

WORK EXPERIENCE

BYU Egypt Excavation Project – Head of Osteology
February 2018 – March 2018
Seila, Fayoum, Egypt

- Osteology team leader (ongoing seasonal position). This season was focused on organization and curation of the magazine as well as trauma analysis and documentation. Co-hosted a short osteological field school for Egyptian antiquities inspectors.

Paleo-oncology Research Organization and Ancient Cancer Foundation – Executive Director
June 2016 – Ongoing
Minneapolis, Minnesota, USA

- Executive co-founding member responsible for the development and enforcement of legal and business protocols, planning and leadership of business meetings, development of marketing strategies, website maintenance, business and academic research contributions, and team management and coordination. Guest editor, leader, and coordinator on the IJPP Paleo-oncology Special Issue project, reporting to IJPP Editor-in-Chief, Dr. Jane Buikstra, on behalf of the PRO team.

Theban Tomb 110 – Visiting Scholar
January 2017
Luxor, Egypt

- Assisted Dr. Miguel Sanchez, Dr. Jesus Herrerin, and Dr. Rosa Dinares in the radiographic analysis of pathologic bones from the collection of commingled remains excavated in TT110.

University of Memphis Mission to Theban Tomb 16 – Dental Anthropologist
December 2016 – January 2017
Dra Abu el-Naga, Luxor, Egypt

- Inventoried, documented and analyzed dentition from the collection of commingled remains excavated in TT16.

Paleo-oncology Research Organization – Director of Operations; Ancient Cancer Foundation - CFO
April 2013 – June 2016
Minneapolis, Minnesota, USA

- Executive co-founding member responsible for the development and enforcement of legal, financial and business protocols, application for incorporated non-profit business status, development of marketing strategies, social media content creator, business and academic research contributions, and team management and coordination.

The University of Western Ontario – *Teaching Assistant*
September 2014 – December 2014
London, Ontario, Canada

- Assisted lecturer in the 'Biological Anthropology' online course during semester 1. Proctored and marked exams and marked written osteological assignments.

BYU Egypt Excavation Project – *Head of Osteology*
February 2014
Seila, Fayoum, Egypt

- Responsible for the macroscopic analysis of Middle Kingdom and Early Roman Period human remains. This season focused on a preliminary bioarchaeological study of the skeletal remains in the storage magazine and resulted in a report on the potential for knowledge acquisition through further bioarchaeological study. Co-hosted a short osteological field school for Egyptian antiquities inspectors.

The University of Western Ontario – *Graduate Research Assistant*
September 2013 – April 2014
London, Ontario, Canada

- Paid position for independent research contributing to doctoral dissertation.

The University of Western Ontario – *Teaching Assistant*
September 2012 – April 2013
London, Ontario, Canada

- Assisted lecturer in the 'Individuation in Forensic Science' course during semester 1 and the 'Human Aging' course during semester 2. Held office hours, marked essays and exams, and proctored exams.

The University of Western Ontario – *Teaching Assistant*
September 2011 – April 2012
London, Ontario, Canada

- Assisted lecturers in the 'Human Aging: Bioanthropological Perspectives' course and the 'Introduction to Biological Anthropology and Archaeology' course. Gave lectures for both classes, held office hours, marked essays and exams, and proctored exams.

Lady Hudson Preventative Conservation Project – *Principle Researcher*
January 2012 – April 2012
London, Ontario, Canada

- Researched and compiled the conservation and storage history of the Lady Hudson mummy and her coffin, conducted a comparative analysis of her state of conservation since her arrival at the University of Western Ontario, monitored the environmental factors in her storage room and researched the materials making up her current display case. Reported findings along with suggestions for the installation of safer lighting, which was later implemented by the Associate Dean in the Faculty of Social Science, Dr. Andrew Nelson.

Colossi of Memnon and Amenhotep III Temple Conservation Project – ***Final Report Editor***
October 2011 – January 2012
[Via Email] Cairo, Egypt

- Edited and checked facts for the large final report covering four years of excavation, conservation and reconstruction at the Temple of Amenhotep III and the Colossi of Memnon. This report was later submitted to the Supreme Council of Antiquities in Egypt.

The University of Western Ontario – ***Volunteer GPR Survey Assistant***
October 2011
Dresden, Ontario, Canada

- Helped to complete the widely publicized ground penetrating radar (GPR) survey of Uncle Tom's Cabin Historic Site cemetery. This site is made up of the black settlement and cemetery that was established by Rev. Josiah Henson, following his escape from slavery. Henson inspired Harriet Beecher Stowe's character, Uncle Tom, in her highly influential anti-slavery novel "Uncle Tom's Cabin".

URS – ***Field Archaeologist***
May 2011 – August 2011
Southern Ontario, Canada

- Excavated, classified, mapped and documented artifacts from archaic native Canadian and 19th century archaeological sites.

The British Museum – ***Volunteer Physical Anthropologist***
September 2010 – October 2010
London, England, U.K.

- Cleaned, inventoried and analyzed human remains from the Kawa collection. Recorded measurements and non-metric skeletal traits according to the Buikstra and Ubelaker "Standards for Data Collection from Skeletal Remains". Also assisted with the collection and set-up of bone x-rays.

The Egyptian Museum in Cairo – ***Exhibit Development Consultant***
June 2010 – August 2010
Cairo, Egypt

- Assisted Dr. Sayed Hassan in the creation of the temporary exhibit "Coins through the Ages: Selected Coins from the Egyptian Museum in Cairo". Gave input on the arrangement of display cases, the objects displayed and co-wrote information panels and display case labels.

ARCE Egyptian Museum Database Project – *Collections Management System Assistant*
June 2010 – November 2010
Cairo, Egypt

- Input and cross checked object file information from register books, catalogues and reference books. Inventoried and photographed objects on display in the museum galleries. Drafted object descriptions and internet search instructions. Coordinated and managed volunteers and interns. Organized computer filing system.

Dr. Hourig Sourouzian – *Egyptological Research Assistant*
April 2010 – May 2010
Luxor and Cairo, Egypt and London, England, U.K.

- Conducted Egyptological research at Chicago House, The British Museum and various other libraries.

Colossi of Memnon and Amenhotep III Temple Conservation Project – *Documentalist Ceramicist's Assistant*
January 2010 – March 2010; January – March 2011
Luxor, Egypt

- Supervised and recorded reconstructions in the Peristyle of the Mortuary Temple of Amenhotep III. Documented new finds through photography, sketches, measurements, descriptions and the maintenance of join lists. Developed a new organizational system for pottery throughout the site. Assisted Dr. Pascale Ballet, Ceramicist, with the identification, analysis, documentation and drawing of pottery during her two week work period. Also completed a seasonal documentation report and co-wrote a seasonal restoration report.

The British Museum – *Physical Anthropology Intern*
October 2009 – November 2009
London, England, U.K.

- Cleaned, inventoried and analyzed human remains under the supervision of Dr. Daniel Antoine, Curator of Physical Anthropology. Recorded measurements and non-metric skeletal traits according to the Buikstra and Ubelaker "Standards for Data Collection from Skeletal Remains". Also assisted with unpacking newly acquired mummies and organizing them for storage.

The Egypt Centre – *Volunteer Gallery Attendant*
September 2008 – January 2010
Swansea, Wales, U.K.

- Monitored the galleries, provided information and gave demonstrations. Also cared for and researched selected artifacts under the 'Object Handling Scheme'.

Dr. Choi Dental Office – *Dental Assistant / Dental Nurse*
Aug 2007 – Sept 2008
Mississauga, Ontario, Canada

- Developed extensive knowledge of tooth and jaw structure, pathology, and dental radiology which has contributed greatly to my abilities as a Physical Anthropologist.

UWO Zooarchaeology Laboratory – *Volunteer Bone Processor*
December 2006 – June 2007
London, Ontario, Canada

- Helped clean and identify animal bones for the reference collection in the University of Western Ontario's new zooarchaeology laboratory.

WLU Archaeological Field School – *Archaeological Apprentice*
June 2006 – July 2006
Madaba, Jordan

- Excavated a Nabataean housing complex on the Wadi Ath-Thamad Project led by Dr. P.M. Michèle Daviau (Wilfrid Laurier University, Canada). Cleaned, processed, sketched and recorded artifacts, architectural elements and other archaeological features. Helped to write the final archaeological report.

GRANTS

Bioarchaeology of Ancient Egypt 2019 conference travel grant (750USD)
Institute of Ancient History at the University of Basel travel grant (500€)
Robert Hathaway Ontario Graduate Scholarship 2015 ($5000)
Ontario Graduate Scholarship 2014-2015 ($15000)
Western Graduate Student Conference Travel Award 2014 ($250)
Ontario Graduate Scholarship 2013-2014 ($15000)
Swansea University Postgraduate International Excellence Scholarship 2008 (2000GBP)
University of Western Ontario Dean's Honor List 2006 ($0)
Western Scholar Award 2002 ($750)

FUNDRAISING

Over $10,000 raised for the Paleo-oncology Research Organization to date.

LANGUAGES

Fluent in English; Conversational knowledge of French and Arabic; Basic understanding of Spanish.

PROFESSIONAL MEMBERSHIPS (past and present)

AAPA (American Association of Physical Anthropologists)
ACF (Ancient Cancer Foundation) – *Founding Executive Member; Executive Director*
ARCE (American Research Centre in Egypt)
CAPA (Canadian Association for Physical Anthropology)
DAA (Dental Anthropology Association)
EEF (Egyptologists' Electronic Forum)

EES (Egypt Exploration Society)
ESHO (European Society for the History of Oncology)
GTA Union (Graduate Teaching Assistant's Union, University of Western Ontario)
IADR (International Association for Dental Research)
IAPO (International Association for Paleodontology)
ICOM (International Council of Museums)
OMA (Ontario Museum Association)
PPA (Paleopathology Association)
PRO (Paleo-oncology Research Organization) – *Founding Executive Member; Executive Director*
WAGS (Western Anthropology Graduate Society, University of Western Ontario)