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TOOTH FORMATION IN SUDANESE CHILDREN

By

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Abstract

The aim of this study was to describe the average age of permanent mandibular tooth formation in two groups of Sudanese children using a cross-sectional design following STROBE guidelines. The Northern groups are of Arab origin whilst the Western groups are predominantly Fur of African origin (Darfur). North Sudanese sample (844 males, 802 females) consisted of equally distributed, randomly selected healthy university students and school children, whilst the West Sudanese (848 males, 401 females) consisted of a convenience sample attending religious schools in camps for the internally displaced. Panoramic radiographs (2-23 years), with known date of birth were taken. Height and weight were also measured. Developing permanent mandibular teeth were staged from radiographs (Moorrees et al., 1963). The mean age of attainments were calculated using logistic regression and comparisons carried out on 331 tooth stages to determine gender and ethnic differences. Results showed that mean age of attainment of teeth was not significantly different between the genders within each ethnic group ($p>0.05$) for 135 out of 155 stage comparisons. The mean age of attainment of teeth was not significantly different between same sex groups in 113 out of 174 stage comparisons ($p>0.05$). The canine was more advanced in females compared to males while the reverse was true for some crown and root stages of third molars. Growth indicators showed that all groups are undernourished (z-scores =< -2). Severe malnutrition had minimal effect on tooth formation of M1 and M2 ($p>0.05$) in Northern females. The prevalence of hypodontia (excluding third molars) was 0.7% in the Western group and 2.6% in the Northern group. The pattern of hypodontia differed between groups.

In conclusion, the within group variation for most tooth stages is considerable while the differences between groups are small. The study represents the first radiographic investigation of permanent tooth formation in Africa.

Acknowledgements and Dedication

To those children with unimaginably tough lives who taught me the meaning perseverance against all odds,

and a broken country that soon will soon cease to be.

To my supervisors and mentors; but above all friends: Prof. Hector, Prof. Marcenes, and of course,

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To my grand-dad, parents, brothers, sister, nephews and nieces who are the pillars in my life.

To my true lifelong friends MW, SA, RS, MB, VM, EB and AY for supporting me through the trauma of it all, and assure you with a little help I will be normal again.

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Chapter One: INTRODUCTION

1.1 Overview

Applications of tooth formation extend widely into a number of disciplines. These include biological, anthropological, forensic and clinical applications. Despite recent advances in genetic and statistical fronts, clear gaps still exist in our knowledge regarding the variability of human tooth formation. Furthermore assessment of the literature revealed no universally accepted criteria for the study of tooth formation. Methods and statistical techniques are continuously undergoing modification and refinement with the aim of producing a more conclusive results. To complicate matters, ethical constraints on studying human subjects, classically done by the use of radiographs, have increasingly rendered longitudinal surveys more difficult to come by in recent times (National Radiological Protection Board Guidelines, 2001).

Dental development is a generalized term usually given to denote growth of the whole dentition and sometimes extended to include the teeth and surrounding jaws, such as exemplified by the study malocclusions. Tooth formation is part of overall dental development. Review of the literature reveals the common interchangeable use of the terms dental development, dental eruption and tooth formation, which are different biological entities. No current universally accepted definitions exist to clearly demarcate these processes. The number of studies on tooth eruption and tooth formation in relation to age estimation are relatively more prevalent in the literature than those on tooth formation. A number of reasons have contributed to the current state and these will be discussed in later sections.

The published literature also shows a clear gap in our knowledge of tooth formation in relation to African populations and has been referred to by many authors. Currently little clinical research data exists on tooth formation from African human populations.

Furthermore, the diverse techniques and statistical methods used in studying the extent variation in tooth formation render comparison between groups difficult and open inferential conclusions. Therefore it is also as important to appraise the current methods used in analyzing tooth form in addition to investigating the biological process itself. The full understanding of tooth formation is further limited by the small number of large inter-population studies and missing major human population data which has rendered much of the research on comparative tooth formation open to misinterpretation.

The following section reviews the relevant literature to radiographic tooth formation. The main focus for the literature review and the study has been tooth maturation rather than histology, eruption or age estimation which seem to be covered more extensively in the literature. All of these modalities are interlinked and are therefore covered briefly where they relate to tooth formation and the scope of this study.

1.1.1 Tooth Emergence (Eruption) and Tooth Formation

It is important at this stage to describe the process of tooth formation and differentiate it from clinical emergence both of which are interlinked stages of tooth development. Unfortunately the two are in common interchangeable use in the scientific literature. A number of publications describe tooth formation when in fact they have assessed tooth emergence.

Some investigators refer to clinical tooth emergence (eruption) as a dynamic process in which the tooth emerges through the alveolar soft tissues and into the oral cavity until it reaches its functional position and encompasses root development (Fanning, 1961; Garn et al., 1958). Similarly when the tooth emerges through the alveolar bone, the term alveolar emergence is sometimes used. Tooth emergence can be denoted clinically or radiographically in living individuals or skulls. Clinical emergence is presented in the literature in different ways when conducting studies. The definition is further complicated by the fact that teeth erupt without sometimes having completed root formation (Gowgiel, 1967).

A tooth can also be described as erupted if it had just pierced the alveolar soft tissues but not emerged more than 3mm (Gron, 1962). Others categorize eruption according to clinical stage in reference to the functional occlusion (Ekstrand et al., 2003; Feasby, 1981). Eruption is thought to be a poor indicator for health, age and tooth formation. For example, when the relationship between the number of erupted teeth and age and health in west Kenya was assessed no correlation between tooth emergence and age could be established. Age estimation could not therefore, be based on eruption and number of teeth alone (Towlson and Peck, 1990).

Tooth emergence can be affected by a number of environmental factors; nutrition, health, habits, early extraction of deciduous dentition amongst others (Liversidge, 1998). Tooth formation on the other hand describes the development of the tooth from its initial calcification stage through to crown mineralization, root formation and root apex closure. These processes are independent of emergence as evident in impacted teeth, and are thought to be less vulnerable to environmental influences (Garn et al., 1965).

The relative uniformity of tooth formation is more accurate in chronological age assessment than other maturational events such as eruption, puberty and skeletal maturity. This is a widely held view and has been reported by many investigators (Nolla, 1960; Saunders, 2000).

Despite the relative biologic stability of tooth formation, the definitive estimation of age of individuals from maturational without the due consideration of other growth systems should be viewed with care (Demirjian et al., 1985; Krogman and Iscan, 1986; Moorrees et al., 1963a; Tanner, 1962). To aid the translation of tooth formation into chronological age, a number of reference data has been published almost exclusively for Caucasian populations (Anderson et al., 1976; Liversidge et al., 2006; Moorrees et al., 1963a). Only few large non-Caucasian population studies with appropriate design have been published.

The main focus of this investigation is to assess tooth formation radiographically, however, invariably reference has to be made to age estimation and tooth emergence as they can yield useful information where a comparable study design was utilized.

1.1.2 The Study Setting

Sudan, currently the largest country in Africa, lies to the east of the continent, with a distinctive ethnic diversity and is set for a major north-south division on the 9th of July, 2011, with a very uncertain future (Figure 1).

Figure 1. The Map of the Sudan

Sudan being one the poorest countries in the world has an approximate population of 40 million. The World Health organization (WHO) estimates more than two thirds of the population to be under the age of 18 years. No accurate census figures exist due to the continued civil wars, systematic internal displacement of populations and political instability for almost 60 years.

The study was carried out in and around Khartoum, the capital of Sudan with an estimated population of 9 million. A number of camps for the internally displaced exist in the periphery of the capital.

The far North is populated by Nubian tribes who are fairly homogeneous with endogenous language and culture. Further South along the Nile corridor lies the capital Khartoum. The North of Sudan is inhabited by a mix of heterogeneous tribes such as Jaliaa, Bedairia and Shaigia who are thought to be of Arab origin and are united by religion, language and culture. The country has over 597 defined ethnic groups and over 400 spoken languages and countless unrelated cultures and religions. The capital is predominantly northern in both demographics and culture and is more affluent than other parts of the country. The country itself is rich in crude oil, agricultural resources as well as live stock. However, none of the wealth has filtered down due to continued civil conflicts and extreme corruption. The vast majority of the population lives in extreme poverty.

In part, this study was conducted on subjects originating from the western regions region of Darfur Figure 2, recently made famous by the vicious civil conflict. The region is roughly the size of France and has an estimated population of 5-6 million people with no accurate census. It is bordered by a number of central African states the closest both geographically and culturally being the Republic of Chad.

Figure 2. Map of the Western state of Darfur (Shaded).

The recent civil war in Darfur has displaced approximately 2 million from the West Sudanese region, mainly Fur in origin, many of whom have escaped to the capital Khartoum. The current estimate stands at 2.9 million internally displaced individuals, 1.7 million of whom are placed in and around camps for the internally displaced in Greater Khartoum (IDMC, 2010).

1.2 Aims and Objectives

The aim of this study is to:

1. Establish patterns in tooth formation in Sudanese children (Northern Sudanese and Western Sudanese).
2. Investigate the impact of gender, ethnic origin and malnutrition on tooth formation.

1.3 The Null Hypothesis

No difference exists in patterns of tooth formation between Northern and Western ethnic groups.

Chapter Two: LITERATURE REVIEW

2.1 Overview

The study of dental development can encompass a number of different areas: premineralization sequences, mineralization sequences, incremental patterns of enamel and dentine formation, clinical tooth emergence, gross mineralization patterns examined clinically, histological or radio-graphically. However, one of the biggest factors that impact on both timing and variability in dental development and tooth formation is that of genetics. In the following sections the basic tooth histology is introduced, followed by reviewing the methods used in assessing tooth formation. Inter-population studies will also be reviewed. Brief sections on hypodontia and general growth are also included but in the context of tooth formation.

2.2 The Histology of Tooth Formation

Tooth formation is a continuous biological process which begins with the formation of the cusp of the tooth and ends when the root apex closes. It is commonly divided into four broad histological stages: the bud stage, the cap, the bell stage, and finally maturation (Ten Cate, 1998). These will be covered briefly in the next sections.

2.2.1 The Developing Tooth Bud

The tooth bud (sometimes called the tooth germ) is an aggregate of cells that form the building blocks of the tooth. These cells are derived from the ectoderm of the first branchial arch and the ectomesenchyme of the neural crest. The tooth bud consists of the enamel organ, the dental papilla and the dental follicle (Ten Cate, 1998). The enamel organ comprises the inner and outer enamel epithela, stellate reticula as well as the stratum intermedia. These cells are precursors to ameloblasts which are responsible for the production of the reduced enamel epithelium. The growth of cervical loop (the junction between the inner and outer enamel epithelia) forms Hertwig's epithelial root sheath which is responsible for the overall root shape (Thesleff, 2003).

The dental papilla contains the dentine forming cells: odontoblasts. The shape of the crown of the tooth is determined by the junction between the inner enamel epithelium and the dental papilla. The pulp is formed from mesenchymal cells within the dental papilla (Marks, 1995).

The dental follicle is responsible for the production of osteoblasts which is responsible for laying and remodeling alveolar bone together with osteoclasts, fibroblasts have the primary responsibility for laying the periodontal ligament and cementoblasts which form the cementum of the tooth (Berkovitz et al., 2009).

2.2.2 Bud Stage

The bud stage which typically appears approximately in the 6th week of intra-uterine life and is characterized by the proliferation of epithelial cells into the alveolar mesenchyme (Ten Cate, 1998).

2.2.3 Cap Stage

The ectomesenchymal cells aggregate and starts taking the appearance of a cap, becoming the enamel organ. Ectomesenchymal cells (dental follicle) surround the enamel organ and limit the dental papilla. In broad terms the enamel organ is responsible for enamelogenesis, the dental papilla for dentinogenesis and pulp formation, and the dental follicle will produce alveolar supporting structures (Thesleff, 2003).

2.2.4 The Bell Stage

The bell stage is characterized by cellular differentiation of the dental organ which resembles a bell shape. The majority of its cells are stellate reticulae because of their star-shaped appearance (Ten Cate, 1998). The layers of the bell stage consists of dentine, enamel (formed by ameloblasts), inner enamel epithelium and stratum intermedium. The stage follows is defined by the initiation of the enamel organ (consisting of stellate reticulum) which is enclosed by the outer enamel epithelium layer.

Additionally the dental lamina disintegrates isolating the tooth from the epithelium of the oral cavity but will later rejoin after clinical tooth emergence (Ten Cate, 1998).

2.2.5 Crown Maturation Stage

This stage is characterized by the development of enamel and dentin and is referred to as crown maturation. At this point accelerated mitosis of the inner enamel epithelium ceases and amelogenesis starts. Enamel is layered incrementally to form the cusp tips. The inner enamel epithelial cells morph into columnar cells and their nuclei migrate away from the dental papilla towards the stratum intermedium. This is followed by differentiation of cells within the dental papilla into odontoblasts. (Ten Cate, 1998). The odontoblasts are responsible for the production of the organic matrix and eventually dentine.

After dentin formation begins, the cells of the inner enamel epithelium secrete an organic matrix against the dentin which mineralizes to form the enamel. Ameloblasts continue the process of enamel formation.

2.3 Clinical Tooth Formation

Tooth formation and development is a continuous process that starts in utero and continues after birth into adulthood.

Clinically many investigators separate these stages into a categorical scale in order to record them and are very useful in radiographic studies. Several methods, and many modifications, have been developed for the assessment of tooth formation over the past half a century. Modification of these methods and their applicability to different populations are constantly being produced (Chaillet et al., 2004; Demirjian, 1973; Fanning, 1961; Fanning and Brown, 1971; Garn et al., 1959; Gleiser and Hunt, 1955; Liversidge, 2010; Moorrees et al., 1963a; Nolla, 1960; Nystrom et al., 2007).

A number of studies have shown that tooth formation may be variable in different human groups (Chertkow, 1980; Fanning and Moorrees, 1969; Harris and McKee, 1990; Liversidge et al., 1999; Nanda and Chawla, 1966; Nystrom et al., 2007; Owsley and Jantz, 1983; Tompkins, 1996). However, it is important to note that different methods were used to reach this conclusion. Furthermore, some of the earlier studies have very low subject numbers, while others use methods aimed at age estimation together with the analyses that go with it to support their findings. It was previously assumed that similarities existed between populations of the same ethnic origin and it was assumed that differences existed between geographically and/or ethnically distinct populations (Smith, 1991).

Trends in the literature have proved somewhat difficult to gauge. Differences in the timing of tooth formation events between published studies can be marked and may be influenced by a number of factors such as their methods, sample size, statistical analysis and age distribution. These difficulties were highlighted by Smith (1991) and others who noted that the different studies did not lend themselves to easy comparability (Harris and McKee, 1990). A major factor is the non-Caucasian population data limited true inter-population populations between different ethnic groups. Additionally, all large studies on human populations almost exclusively represent samples of middle class European heritage or are of ethnic groups living in Western countries. Additionally, a number of studies are designed with the primary aim of assessing chronological age and do not lend themselves to easy comparability to tooth formation studies. However, increasingly studies with large sample sizes show similarities between groups (Liversidge et al., 2006), albeit in Caucasian groups.

The most prominent conclusion when comparing groups is the scarcity of organized inter-population studies on tooth formation, maturation and development in African populations and the great need to conduct such studies to bridge those missing links (Harris, 2007; Hillson, 1996; Liversidge et al., 2006).

However, a number of recent studies comparing tooth maturity events in relation to age of attainment from the international data sets, suggest that the true extent of geographical variation in permanent tooth formation is much less than previously thought (Liversidge et al., 2006; Nystrom et al., 2007).

These matters are further complicated by a sizable proportion of the literature being devoted to assessing the third molar formation and using this information to predict chronological age. This poses particular problems as the third molar is reported to have the greatest variability in the course of its formation between individuals and between groups (Arany et al., 2004; Liversidge, 2008b; Olze et al., 2005).

Earlier tooth formation references were constructed, usually without objective analysis of factors such as nutrition and growth, amongst others, that may influence conclusions on direct inter-population comparisons if indeed they are found to be confounding factors (Smith, 1991).

2.4 Clinical Methods for Evaluating Tooth Formation

The fact that each tooth follows a specific predetermined formation sequence has been known for many decades and has been associated to chronological age (Schour and Massler 1941). Forensic applications of dental age estimation are hence very useful in forensic science and anthropology (Sopher, 1976). Some methods were developed, in part, having these applications in mind but proved very useful in studying tooth maturation (Demirjian 1973).

For any system to be accurate it must be clearly defined as well as able to record the different stages of development. It must also be reproducible, discriminatory and reliable, with good intra- and inter-observer reliability (Demirjian and Goldstein, 1976; Liversidge et al., 2003).

Tooth formation is a continuous process and the precision of the recording system is markedly influenced by the number of stages within it. The ability to differentiate between two successive stages if they are only marginally different poses particular problems in analysis and comparative studies (Dhanjal et al., 2006; Levesque and Demirjian, 1980; Liversidge, 1998; Liversidge et al., 2003). Too many stages will increase inter-examiner reliability but decrease reproducibility. Too few will have the opposite effect.

Some methods rely on the definition of the stages of tooth formation of the completed crown or root formation according categorical scale that resembles that defined by the authors (Anderson et al., 1976; Moorrees et al., 1963a; 1963b).

Other methods rely on identifying stages of tooth formation based on the relative size within the stage, for example some stages of root development are defined as a proportion of the size of the root relative to the size of the crown (Demirjian, 1973).

The methods of tooth formation will be discussed in order of their historical standing each with modifications that may have been introduced to it by different researchers.

2.4.1 Logan and Kronfeld

Logan and Kronfeld recognized the failing of the methods available at the time and set out to carry out histological and radiographic examination of teeth on twenty five cadavers of children with cleft lip and palate (Logan and Kronfeld, 1933).

Interestingly they found no correlation between the degree of calcification and the age of the child. An important conclusion is that the lack of uniformity may be due to chronic illnesses prior to their death.

Despite the small sample size and uneven distribution this study provided a bench mark in tooth development.

2.4.2 Schour and Massler

This method used non invasive radiographic methods to produce an ‘atlas’ (Figure 3). A map of tooth formation that is related to chronological age is provided. Superimposition of an available radiograph on the atlas would provide an indication of the child’s chronological age (Schour and Massler, 1941).

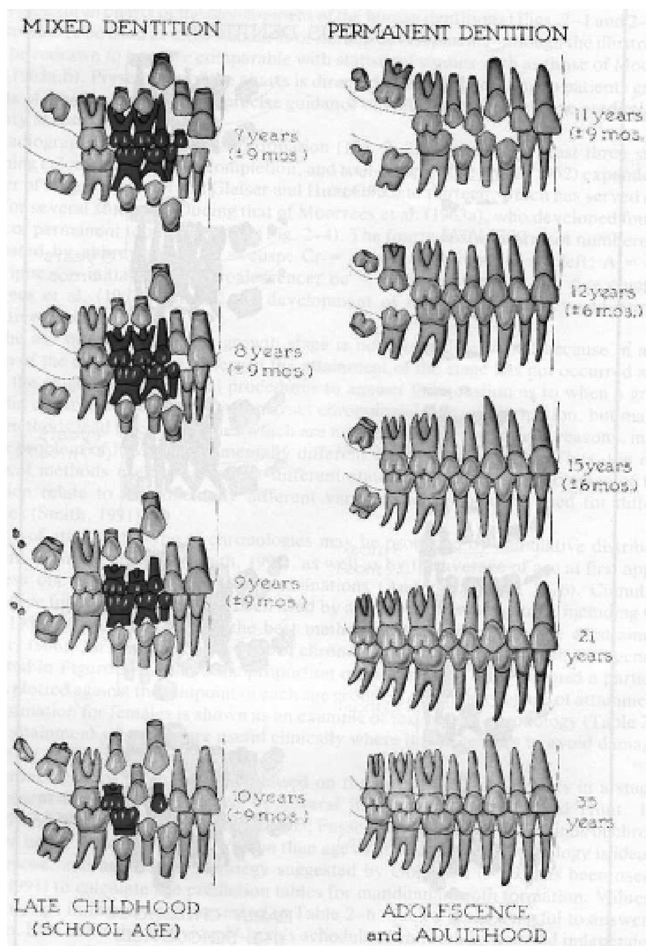


Figure 3. Diagrammatic representation of permanent tooth development.

(*Schour I, Massler M. The development of the human dentition. JADA 1941;28(7):1153-60. (All rights reserved. Reprinted by permission. Copyright © 1941 American Dental Association; Appendix A.1)*

The method is relatively simple and has wide applications, even today. Similar shortcomings of the study included the small and uneven sample size, and using Logan and Kronfled's (1933) sample of institutionalized children were subject to the same limitations. Due to the small sample size the Atlas combines males and females. A further important issue the atlas based on the mean age of children within a stage rather than mean age of attainment. This concept is explained and illustrated in section 3.9.3 later. Although there is small range around the mean, the small sample size introduces a marked effect on the standard error of the mean and renders statistical comparisons inapplicable and limits it use to the clinical setting. Again the study offers an insight into the relative autonomy of tooth formation compared to other growing systems.

2.4.3 Gleiser and Hunt

This is a bench mark longitudinal radiographic study of fifty subjects in which the authors assessed a maximum of sixteen stages every six months (Gleiser and Hunt, 1955). At the time the study highlighted a number of important new issues. A key find was the variability in the rate of tooth formation at different developmental stages. For example when third of the root is complete, there appears to be an increase in tooth formation rate. Statistically significant differences between boys and girls were demonstrated. However, they state that over the course of tooth formation the development rate is equal in both sexes. They hypothesize that this is due to the tooth size discrepancy between boys and girls rather than due to true formation rate. Interestingly this finding is reiterated the findings by other investigators despite similar limitations in the methodologies and sample size (Hurme, 1949).

Gleiser and Hunt also report that clinical emergence is an inaccurate predictor of chronological age and that tooth formation was more predictable. They charted the stage of development against chronological age for the first molar and showed that delays in skeletal maturity had little effect on the rate or tooth formation supporting the view of the stability of dental formation that is held today.

Despite the shortfall of using one tooth as an indicator for chronological age, the study will continue to play an important role simply in the light of ethical issues surrounding serial radiographic examination of children (National Radiological Protection Board Guidelines, 2001). A number of researchers adapted their methods to form their own and examples include Fanning (1961), Moorrees et al. (1963) and Haaviko (1970). A strength of their study was the inclusion of very young children enabling early stages of early forming teeth to be investigated.

2.4.4 Nolla

Nolla devised standards for ten stages of tooth formation based on her study of 3402 radiographs obtained serially of 25 boys and 25 girls. The material consisted of intra-oral and extra-oral radiographs of subjects aged between 2.1 and 23.3 years. The stages are summarized below:

0 No crypt
1 Presence of crypt
2 Initial calcification
3 One third crown completed
4 Two third crown completed
5 Crown almost completed
6 Crown completed
7 One third root completed
8 Two third root completed
9 Root almost open (open apex)
10 Root apex completed

The teeth were then scored according to their average stage of development. The scores were then added up to give an indication of tooth formation of all the teeth. Individual tooth scores proved useful in setting standards in individual tooth formation and the total sum of scores was used to identify overall dental maturity. An atlas is also provided to facilitate the reading of radiographs. Conversion tables were then constructed and direct comparisons made to them.

A shortcoming of this method is that the tables are not specific to an ethnic group. Additionally, Nolla's scores are assigned according to the relative length of the completed root in relation to age (i.e. by calculating the mean stage for a given age) and are likely to introduce inter-observer errors, which can have an impact on inter-population comparisons.

2.4.5 Fanning

This study is a longitudinal study of lateral skull radiographs every 3 months for the first year of life then every 6 months until the age of 11.5 years as well as on anterior occlusal radiographs from 4 -11 yrs every six months after Gleiser and Hunt (1955). The study sample consisted of 51 females and 49 males. Fifteen stages were assigned to denote different levels of tooth formation. Different teeth were staged differently; molars had twenty stages while incisors had only twelve stages (Figure 4).

The results describe the mean age of attainment of each stage on cumulative curves (recoding 10^{th}, 25^{th}, 75^{th} and 90^{th} percentiles) for each of the twenty stages of tooth formation.

Mean ages of attainment for each stage of development was reached by each tooth together with the 10th, 25th, 75th and 90th percentile bands were calculated. The study also suggested strong sexual dimorphism. Mean ages of stage attainment were calculated separately for males and females.

Despite the relatively large sample size the study is limited by factors such as the narrow age range of the children in the sample. The relatively high number of stages increased the inter-observer error significantly has proved a limiting factor in its use in clinical applications despite its role as a research tool.

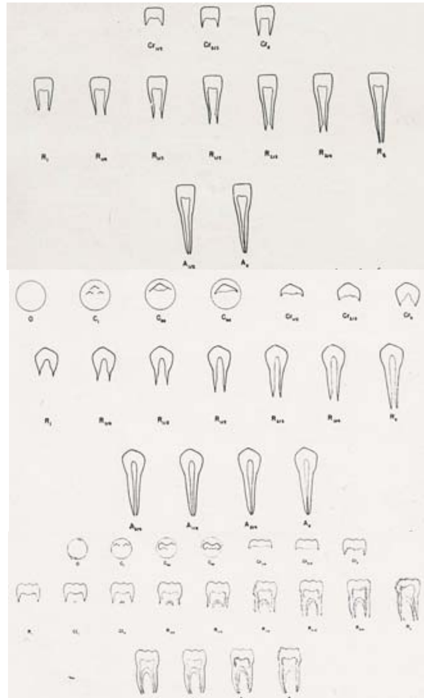
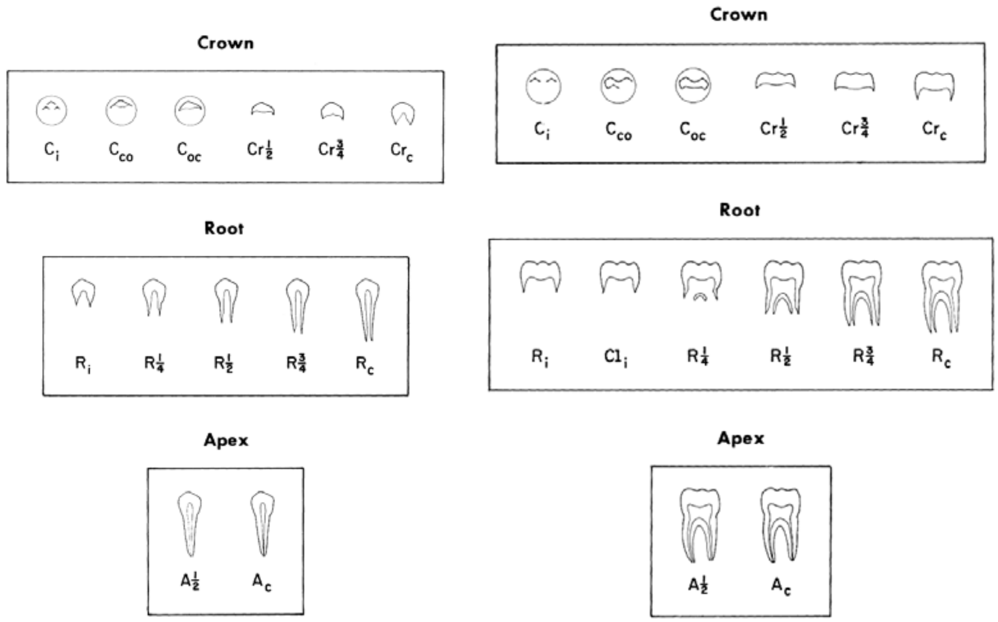


Figure 4. Fanning's twenty developmental stages assessing selected as standards for assessing the formation of incisors molars and premolars (1961).

2.4.6 Moorrees et al. (1963a)

This study builds on the earlier work of Fanning (1961) and aimed to tackle the shortcomings of the previous study. Moorrees and co-workers examined two different Caucasian samples; the first had 48 males and 51 females from zero to ten years of age and another sample with 136 male and 110 female subjects up to eighteen years of age. The method describes fourteen stages outlined in Figure 5.



TOOTH-FORMATION STAGES AND THEIR CODED SYMBOLS

Stage	Coded Symbol
Initial cusp formation	C_i
Coalescence of cusps	C_{co}
Cusp outline complete	C_{oc}
Crown ½ complete	$Cr_{.1/2}$
Crown ¾ complete	$Cr_{.3/4}$
Crown complete	$Cr_{.c}$
Initial root formation	R_i
Initial cleft formation	$Cl_{.i}$
Root length ¼	$R_{1/4}$
Root length ½	$R_{1/2}$
Root length ¾	$R_{3/4}$
Root length complete	R_c
Apex ½ closed	$A_{1/2}$
Apical closure complete	A_c

Figure 5. Stages of tooth development (Moorrees et al., 1963a).

The mean and standard deviation for the age of tooth formation stage was calculated at 50th percentile for each age group and was defined the number of children having passed a certain stage. The advantage of using this system over earlier systems is that it is close to the continuity similar to that seen growth without being cumbersome. Additionally there were relatively fewer stages. This made the technique more user-friendly and increased its reliability and rendered it in part more robust for comparison with other methods.

A disadvantage is that the method is tooth specific and does not take into account the overall dental development. Another disadvantage of the method is that it uses relative root length and which at times may be hard to judge and may be a source of errors either in age estimation or in overestimating variability. This may not have any significance in large groups being studies but where the method is being applied to a single tooth or individual, accuracy about establishing stages is crucial.

2.4.7 Modifications After Moorrees et al.

2.4.7.1 Wolanski

The above investigators assigned numerical categories to each developmental stage described by Moorrees et al. (1963a) and used the same sample to assess the sum of the scores of ten teeth. A final weighting was assigned to each case. Curves were then derived for the mean ages of attainment which enabled estimation of chronological age of a given individual. The purpose of the study was to elucidate the effects of environmental factors on the stages of development (Wolanski, 1966).

The method has the advantage of accounting for a lot more stages compared to other methods, for example Demirjian's and hence takes more into account the continuous process of development but does not overcome it. The method can introduce intra and inter observer errors due to the number of stages and hence thorough training is usually required. The method also has same shortcoming of the original Moorrees's method in that it assigns the same categories related to relative tooth development. All these factors limited the use of this modification.

2.4.7.2 Anderson, Thompson and Popovich (1976)

In this longitudinal study, Anderson et al. calculated the mean age of attainment together with standard deviation of the each tooth on both dental arches from serial radiographs of a group of 232 Caucasian children from the ages of 3 to 18 years. They used the stages described by Moorrees, Fanning and Hunt. They constructed a reference table for the mean age of attainment for each stage of each deciduous and permanent tooth. The idea is that the table can be used to estimate the chronological age based on comparing the stage of development of teeth. The strength of the study is that it allows a number of teeth to be used rather than the entire dentition which at times may not be a possibility. The system has the advantage also in clearly demarcating mean ages of developmental stages of a particular population and may therefore not be applicable in different populations (Anderson et al., 1976).

Additionally, mean stages of attainment for each stage of each tooth enable direct comparison of those teeth with the least variability in the course of their development such as first permanent molars and enable comparison between population groups.

2.4.8 Gustafson and Koch

Similar to objectives of Schour and Massler (1955) the investigators present an atlas to which comparisons can be made. It does not differentiate between the sexes (Gustafson and Koch, 1974).

The process of tooth development was divided into:

1– Initiation of mineralization

2– Crown completely formed

3– Tooth emergence

4– Tooth formation completed

This method had a number of disadvantages, the main is the difficulty of accurate identification of the third stage, which introduces quite marked intra and inter observer errors.

2.4.9 Demirjian et al.(1976)

Demirjian et al.'s method, and its modifications, relies on identifying eight stages of tooth development from 'A' through to 'H' (Figure 6). Maturity scores for each of these seven teeth to almost each of the 8 developmental stages are assigned after using matrix formulae to work out the tables.

It is a widely used system and has the advantage of being tested on a number of populations by many authors with very different and varying conclusions (Frucht et al., 2000; Liversidge et al., 2006; Nystrom et al., 2007). Some investigators claim that populations with similar genetic affinity, e.g. Caucasians, are similar in their tooth formation (Liversidge et al., 2006). Others on the other hand found that the system underestimates or overestimates tooth maturational events when tested against chronological age of different groups (Eid et al., 2002; Loevy, 1983; Willems et al., 2001).

A key difference from the method proposed by Moorrees et al.(1963a) is that the last stages of root formation are not defined according to proportions of the completed part, but according to size of the root relative to the size of the crown. However, there can be relatively high inter-examiner reliability errors (Levesque and Demirjian, 1980).

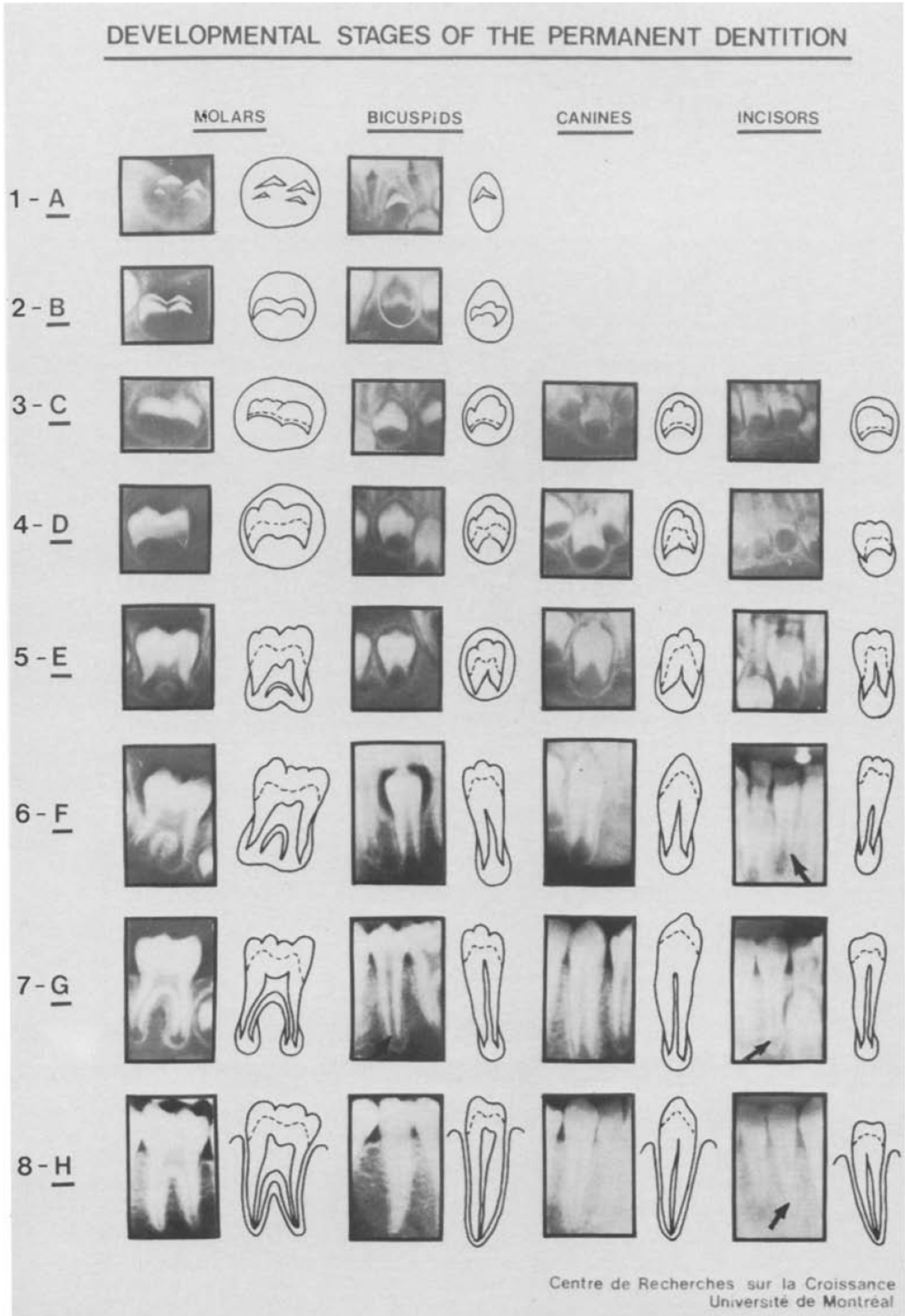


Figure 6. Diagrammatic illustration of Demirjian's *Stages.*

Description of the stages is outlined:

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 cemento-enamel 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 speckle.

E) i) 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.

ii) Molars:

a. Initial formation of the radicular bifurcation is seen in the form of either calcified points or a semi-lunar shape.

b. The root length is still less than the crown height.

F) i) Uniradicular teeth:

a. The walls of the pulp chamber now form a more or less isosceles triangles. The apex ends in a funnel shape.

b. The root length is equal to or greater than the crown height.

ii) Molars:

a. The calcified region of the bifurcation has developed down further 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) The walls of the root canal are now parallel and its apical end is still partially open (distal root on molars).

H) a. The apical end of the root canal is completely closed (distal root on molars).

b. The periodontal membrane has a uniform width around the root and the apex.

A major disadvantage of the system is that it is that is specific to French Canadian 2-15 year olds which limits its universal applicability. Another disadvantage is that it requires the presence of all mandibular teeth to calculate the maturity score. In forensic cases application or where there is tooth agenesis using the method poses some difficulty. The system was later modified to enable the four teeth instead of seven to be used. A revised self-weighted scores for each stage of each tooth for each sex was also published (Demirjian, 1976). These scores were more accurate predictors of age.

Despite a CD-ROM being published aid in the process of training but inter-examiner reliability continues to be an issue in direct comparative studies (Demirjian, 1993/94).

2.5 Variability of Tooth Formation

Evidence supporting the overriding genetic control of tooth formation has been known for many years (Garn et al., 1965). Individual variation has been noted by a number of investigators for tooth formation for a number of populations. The differences between individual are more marked than between standard ages for maturation in geographically or even racially distinct populations. Liversidge and co-workers , using meta-analysis, assessed samples obtained from Australia, Belgium, Canada, England, Finland, France, South Korea and Sweden and failed to find statistical differences in the stages of tooth formation between groups but also noted intra-group variability (Liversidge and

Speechly, 2001; Liversidge et al., 2006). Nystrom and co-workers reached the same conclusion about their large Finn sample when constructing maturity standards (Nystrom et al., 2007).

However, it should be remembered that tooth formation is an interactive continuous process, and that with correct use of explanatory measures can be used as biological predictor or indicator, similar to other growth systems (Cameron, 2004). It is also important to view tooth formation in the wider context of growth and maturity rather than a separate and independent distinct series of events.

When studying variability between ethnic groups it is important to recognize that variations may exist in tooth formation between different sexes within groups as well as between ethnic groups due to evolutionary patterns (Hillson, 1996); i.e. normal variability within a growth system. Additionally factors such as age, sex, ethnicity , health and diseases are known to have a contributory effects on the variability of eruption but the exact effect on tooth formation is remains unclear (Demirjian et al., 1985).

Hence, if standards on tooth formation of a population are to be constructed and used in a clinical or forensic setting, it is essential to evaluate all the factors that may impact on the biological processes including its relation to general growth and development (Moorrees et al., 1963a).

To accurately define the patterns of tooth formation on human groups it is important to understand other factors that may contribute to this variation. These may include gender variation, secular trends, nutritional and socio-economic conditions and individual tooth variation (e.g. third molar). These factors, along with two additional aspects of interest, namely hypodontia and the growth assessment in relation to malnutrition, will be reviewed in the next sections.

2.5.1 Sources of Variability in Tooth Formation

To establish whether true biological inter-population variability exists it is important to consider all the non-biological factors that may have an impact on interpreting the results. Smith (1991) expresses clearly the difficulty in interpreting inter-population comparisons due to different methodologies and statistical analysis used. For example modern clinical studies rely on cross-sectional data rather than longitudinal data but have the advantage of much bigger sample sizes enabling group comparisons. Another factor that may introduce variation could be related to the assessment of measurement error. One of the most important consequences of high measurement error is the increased likelihood of type II errors and can amplify the variance of the mean and reduce the magnitude of correlation and regression coefficients (Sokal, 1965). This may not be of significance in larger biological studies but where small samples are being considered would be detrimental.

In the case of tooth formation the measurement error can play a crucial part in assessing variability and therefore should be carefully assessed. Cross sectional studies with sufficient sample size are less affected by measurement error than growth data. The main source of error is related to the recall interval in longitudinal studies as well as the truncation of the observation period which may not span the whole span of the age range (Smith, 1991). This enables cross-sectional studies to be as valid in assessing the tooth formation provided sufficient sample size and appropriate design are used (Ulijaszek and Lourie, 1994).

A survey of some of published radiographic tooth formation studies, to mention but a few (e.g., Gleiser and Hunt, 1955; Nolla, 1960; Moorrees et al.,1963; Haavikko, 1970; Demirjian et al., 1973; Gustafson and Koch, 1974; Anderson et al., 1976; Demirjian and Levesque, 1980; Demirjian, 1986; Harris and McKee, 1990; Liversdige, 2008) reveal a wide range of mean crown formation times for human teeth and suggests there are differences between populations. On the other hand there is increasing evidence that different populations exhibit less variability than originally thought (Liversidge, 2010).

This is in part supported by histological findings that histological enamel formation timing is very similar in different ethnic groups (Reid and Dean, 2006).

No universally accepted view on the source of variability currently exists. For example, the effect of radiograph quality, choice of tooth formation assessment method and statistical techniques all have contributed to higher reported variation. The methods are continuously being refined with differing outcomes to those originally published using refined statistical techniques (Liversidge, 2010). A key issue is the lack of organized inter-population data that allow these comparisons to be repeated on populations of different ethnic groups.

Additionally whilst clinical radiographic studies can investigate longitudinal tooth development and mineralization (Tompkins, 1996), we are not able to differentiate between the different biological mechanisms that underlie tooth formation similar to those in African human studies (Dean et al., 1993).

Another source of variability is the study design. Many studies use non-cumulative growth statistics to calculate the mean age of children in stage, and despite its important in age estimation, has contributed to difficulties in comparing tooth formation between different groups. The mean age of children for a given stage is always more advanced than the mean age of stage attainment obtained by cumulative statistics (3.9.3 and Figure 7) and therefore the two are not comparable despite its common use in the literature.

2.5.2 Racial Influences and Inter-population Variation in Tooth Formation

The results of several studies have shown that direction in tooth formation can vary between populations and different ethnic groups when different teeth are considered (Harris and McKee, 1990; Liversidge, 2008b; Peiris et al., 2009; Tompkins, 1996). However, many these studies highlight the need for population-specific dental development standards for the accurate assessment of tooth formation.

A study found that South African black children were significantly advanced in the timing of the development of their third molars compared to a group of Caucasian children as well as another group of Bangladeshi children from London for almost all the stages of third molar development (Liversidge, 2008b).

Another study of mandibular teeth (except the third molar) in a large group of mostly Caucasian subjects suggests that no actual variation exists when large groups of children are studied using alternative statistical analysis (Liversidge, 2010).
Other investigators found a significant delay in the relative timing of calcification of mandibular third molars in Australian Caucasoids when compared to the Australian Aborigines (Fanning and Moorrees, 1969).

Somali children were also found to be advanced by approximately one year over British children who were in turn more advanced in tooth formation than the Canadian reference sample by up to 6 months (Davidson and Rodd, 2001).

In another study, maxillary incisors and mandibular second molars of Arikara Indians were found to be older by 0.5 to 1.1 years in their development than white Americans. Third molar development was advanced by more than 2 years and the authors concluded that systematic occurrence of these observations reflected more than just individual variability; and that tooth formation timing differences were closely linked to the differences in population. The study is characterized by the very small number size and no accurate age record but is quoted here as it was one of the earlier studies to document the independence of tooth formation but only from observation (Owsley and Jantz, 1983).

Harris found that American blacks were significantly more advanced in achieving each third molar tooth formation stage than American whites, but not in a uniform manner (Harris, 2007). The same finding was reported by (Liversidge, 2008b).

Furthermore, a study. on children living in San Francisco found that the Caucasian Americans were significantly advanced over those of Japanese and Chinese ancestry, but that the Japanese and Chinese children shared similar dental developmental patterns (Maki et al., 1999) despite using the mean age of children within a stage as an indicator to tooth formation.

Furthermore, some tooth formation assessment methods (mostly modifications of Demirjian's method) have been shown to overestimate chronological age considerably. For example, when different methods have been used on the same sample, results indicated that the Demirjian method overestimated the age by 0.75 and 0.61 years, while the Willems method overestimated the age by 0.55 and 0.41 years among boys and girls, respectively (Mani et al., 2008).

Another study carried out on German children found that when applying the standards of the Demirjian system, the dental ages of the group was not related to those found in the French Canadian reference sample (Frucht et al., 2000).

A study carried out on children in China found that the local children were retarded in their dental development by a mean of 11 months for males and 7 months for females when compared to the French-Canadian reference sample (Davis and Hagg, 1994).

Another study on Belgian children found that their dental ages were retarded compared to the French-Canadian reference sample by a mean of 0.4 years for males and 0.7 years for females (Willems et al., 2001). The findings of the latter group were supported by Chaillet et al., (2004) who suggested a modification to include polynomial functions which they felt was more accurate in estimating age for Belgian children in their sample.

In a study on Brazilian children, Eid et al. compared their sample to the French-Canadian children of Demirjian and found that both Brazilian males and females were 0.68 years and 0.62 years, respectively, more advanced in their dental maturity (Eid et al., 2002).

Other studies have revealed that in the confines of the same racial group within a defined population, there is statistically significant variability in tooth formation.
Nyström developed predictive tables based upon a sample from one region of Finland and applied them elsewhere in the same country (Nystrom and Ranta, 1988).

Not surprisingly a number of authors have concluded that population specific standards are required when using any system of age estimation based upon dental development.

The majority of these authors have suggested that the variations in tooth formation may be due, at least in part, to racial differences between the sample populations. This may result from different genetic ancestry with the heterogeneity of the populations being prevented, or at least restricted, by the geography. This effect may keep gene pools separate causing rates of development to vary between groups and consequently many authors concluded that genetic heritage is primarily responsible for the variation that exists. The variability between ethnic populations probably takes root from those of general growth studies, for example, studies on seventeen African populations have shown that stature is more closely related to tribal origin than geographic location (Eveleth and Tanner, 1990).

A number of studies have suggested that no clear pattern of racial differences in tooth formation actually exist (Liversidge, 2008 and Liversidge, 2010). This fact has been reported previously for skeletal growth by Habicht et al. who concluded that skeletal growth is not subject to racial influences. However the study itself had relatively poor design and could be a mere observation (Habicht et al., 1974). Despite this there is a widely held belief that genuine variation exists between distinctly different ethnic groups in a similar way ethnicity seems to affect other growth systems.

2.5.3 Tooth Formation Variation between Individuals

Biological variability is the norm rather than the exception. To establish meaningful biological differences between groups the intra-group variation should firstly be understood. The extent of intra-group variation can impact on study design, sample size and future applications of any findings. Garn et al. (1959) reported that variability can be as high as three times the standard deviation for some teeth. Demirjian and Levesque (1980) found that variation within groups could be considerable, as high as five stages in some age groups.

Even for early forming teeth Moorrees et al (1963a) found variation to be considerable. Haavikko (1974) also found that variation between the 10^{th} and 90^{th} percentile ranged from approximately 1 to 7 years.

Liversidge (2010) interpreted the results of her study in a way that highlights group similarities and that variation between groups is not biologically significant. Equally an effect could not be disregarded.

2.5.4 Secular Trends

Secular trends are known to have a direct impact on general growth. For example a, in South Africa, found that skeletal development in both white and black children was more advanced in 2001 than those living in 1962, but was more marked in black children signifying a removal of some growth constraints for the latter group (Hawley et al., 2009). Holtgrave et al. reported an acceleration dental maturation over the past few decades and attributed this to better living conditions and freedom from disease amongst other factors (Holtgrave et al., 1997), despite the limitation in the methodology used; in that they calculate the mean age of children in stage utilizing Nolla's method which in itself has been shown to have shortcomings (Smith, 91) A more recent study concluded that secular trends are important in the variability between children of the current decade and those who lived fifty years ago (Heuze and Cardoso, 2008). Others have reported definitive secular trends in their studies (Holtgrave et al., 1997; Nadler, 1998) and argued that one possible explanation for this trend is a consistent improvement in living conditions and overall growth improvements. This is in contrast to a very recent study, in which the investigator concluded that secular trends had little effect on tooth formation (Liversidge, 2010).

2.5.5 General Growth Assessment

The WHO has recently commissioned and produced a set of charts that have been universally accepted as standards in the assessment of different modalities of child growth from 0-59 months (WHO 2006). They also produced references for children from 5-19 years. For some time there has been consensus that genetic factors are thought to be more predominant in tooth formation over environmental influences. It is widely circulated that different ethnic populations in different parts of the world vary greatly, in not only in the final adult stature but also in the rate of maturation (Eveleth, 1978). Clear differences exist between populations both in terms of height and body weight. It is thought that height has a strong genetic component which makes it inappropriate to judge the health of the population despite the many environmental factors that exert influences on growth (Eveleth, 1975). The specific effect on growth has classically revolved around the impact of poverty and more recently on relative wealth, as in the example of obesity. Both nutrition and childhood infection are thought to be the greatest environmental factors affecting the height and weight within a population (Eveleth and Tanner, 1990). Ethnicity also seems to play a major role in the variation between populations even in relatively similar geographic locations (Reddy et al., 2008).

Overall growth assessment is an important indicator to health in individuals. This is usually assessed by means of growth charts. Their value resides in helping to determine the degree of how physiological needs for growth and development are met during the childhood period. Beyond their usefulness in assessing children's nutritional status, many governmental, non-governmental organizations health agencies use these tools to measure the general health of populations. Information is also commonly used to formulate and monitor health policies and interventions. Their usefulness can be applied to individuals or populations.

A number of published studies conducted on growth after major wars and environmental disasters demonstrated that the growth of these children was markedly affected by their living conditions (Dowell et al., 1995; Peck et al., 1981). Other studies of smaller clinic populations of refugee children did not find high prevalence rates of growth deficiency but detected high prevalence rates of other problems, such as intestinal parasites, anemia, dental abnormalities (Hayes et al., 1998; Hjern et al., 1998; Pinto et al., 2007; Tittle et al., 1982). Given the increasing diversity within populations, it is important to establish growth reference data to enable meaningful conclusions to be drawn in relation to health and of children. The references currently in common use are:

1. The Center for Disease Control and Prevention Reference (2-20 years); 2000
2. The World Health Organization Standards (0-59 months); 2006
3. The World Health Organization References (5-19 years); 2006

2.5.5.1 World Health Organization Standards and References

In many parts of the world growth charts are used to assess childe growth. The World Health Organization (WHO) Child Growth Standards date back to the early 1990s when a group of experts were appointed to evaluate and critically appraise the National Center for Health Statistics (NCHS) and World Health Organization (NCHS/WHO) growth reference that had been acknowledged internationally until then (WHO, 2006). However, the limitations of the NCHS/WHO reference have been documented (de Onis and Yip, 1996). The main reason for this was that the data used to construct the references covering birth to three years of age came from a longitudinal study of children of European ancestry from a single community in the USA. These children were measured every three months, which is inadequate to describe the rapid and changing rate of growth in early infancy. Also, the statistical methods available at the time of construction of the NCHS/WHO growth curves were too limited to correctly model the pattern and variability of growth (de Onis et al., 1997).

The initial phase of the expert group's work documented the deficiencies of the reference and led to a plan for developing new growth charts that would show how children should grow in all countries rather than merely describing how growth had taken place particular time (de Onis et al., 2004).

The World Health Organization conducted a Multicentre Growth Reference Study (MGRS), which was implemented between 1997 and 2003 (de Onis et al., 2004). The MGRS is unique in that it was designed to produce a standard rather than a reference. The MGRS data provide a solid foundation for developing a standard because they are based on healthy children living under conditions likely to favour achievement of their full genetic growth potential. Furthermore, the mothers of the children selected for the construction of the standards engaged in fundamental health-promoting practices, namely breastfeeding and non-smoking (de Onis et al., 2004).

A second feature of the study that makes it applicable as a basis for an internationally applicable standard is that it included children from a diverse set of countries: Brazil, Ghana, India, Norway, Oman and the USA in five continents. By selecting privileged, healthy populations the study reduced the impact of environmental variation. Assessment of differences in linear growth among the child populations of the MGRS shows a striking similarity among the six countries (de Onis et al., 2004). The remarkable similarity in growth during early childhood across human populations is consistent with genomic comparisons among diverse continental groups reporting a high degree of inter-population homogeneity (King and Motulsky, 2002). Of interest however is that the MGRS sample has considerable built-in ethnic and genetic variability in addition to the cultural variation in how children are nurtured, which further strengthens the standards' universal applicability and renders comparison between groups more valid for the purpose of our study.

In practical terms the values corresponding to specific z-scores (-2, -1.5,-1,-0.5,0, 0.5,1,1.5,2) provided by the WHO growth reference curves can be calculated and analyzed for the 5-19 year group. The following z-scores can be calculated:

1. BMI for age z-scores (5-19)

2. Height for age z-scores (5-19)

3. Weight for age z-scores (5-10)

For the 0-59 months age group the standard is a more precise measure of growth potential and the following z-scores for the following indicators is provided: length/height-for-age, weight-for-age, weight-for-age, weight-for-length, weight-for-height, body mass index-for-age (BMI-for-age), head circumference-for-age, arm circumference-for-age, triceps skin-fold-for-age, motor development milestones, sub-scapular skin-fold-for-age, weight velocity, length velocity and head circumference velocity (WHO/MGRS, 2006).

2.5.5.2 Centre for Disease Control and Prevention References

The Centre for Disease Control and Prevention for many years has been the most widely quoted reference both clinically and in the scientific literature. It describes the continuous growth of children from 2 to 19 years of age.

Growth charts have long been used in the assessment of growth by a number of different professionals. The CDC growth charts represent a series of percentile curves that illustrate the distribution of selected measurements representing growth in U.S. children. The 1977 growth charts were developed by the National Center for Health Statistics (NCHS) as a clinical tool for health professionals to assess the growth of children and have been widely since then. It was the initial intention that the CDC charts be revised periodically to allow for changes in the health of individuals. The charts remained as an international tool until recently and were recently revised in 2000 to produce the '2000 CDC' growth charts which had major modifications over the 1977 version.
The charts were constructed from the National Health and Nutrition Examination Survey (NHANES) which collected height and weight of children over a 40 year period as well as periodically collecting height and weight and other health information on the American population at large since the early 1960's (King and Motulsky, 2002).

BMI is the most commonly used approach to determine if adults are obese or underweight. The BMI growth charts are designed for use from 2 years of age.

These BMI-for-age charts were created for use in place of the 1977 weight-for-stature charts. BMI (wt/ht^2) is calculated from weight and height measurements and is used to judge whether an individual's weight is appropriate for their height.

BMI is increasingly being used in identifying children and population with growth problems by calculating z-scores children and populations to allow comparison to the CDC reference data. However it is note worthy to mention that growth charts are not intended to be used as a diagnostic measure of health and disease in individuals but are useful tools when an overall view is required. This is particularly helpful in research.

In practical terms the values corresponding to specific z-scores (-2, -1.5,-1,-0.5,0, 0.5,1,1.5,2) provided by the CDC growth curves can be calculated and analyzed for any given population. The curves for infants are: weight-for-age; length-for-age, weight-for-recumbent length; head circumference-for-age; and older children are: weight-for-stature; weight-for-age; stature-for-age; and BMI-for-age.

2.5.6 Age and Sex

Classically, it is thought that early stages of tooth development are less variable for both males and females, while the sexual dimorphism in tooth formation increases with age and tooth stage. Several studies have reported that for some teeth sexual differences exist in tooth formation (Demirjian and Levesque, 1980). Clinical Studies of the pattern of dental calcification reported that females were relatively more advanced than males (Fanning, 1961; Anderson et al.,1976; Moorrees et al., 1963a; Chaillet et al.2004, Liversidge, 2006). For example Gleiser and Hunt (1955) found that female permanent molars completed root formation approximately 4 months earlier than males. Nolla (1960) also reported that females were more advanced in their permanent tooth formation than males.

Garn et al. (1958) found that despite females being advanced in all stages of tooth development, males were more advanced in relation to the first molars. Thompson et al. (1975) reported that the previously reported differences were due to variability in tooth formation times was more marked in the root formation phase for girls.

Gleiser and Hunt (1955) suggested that although females have more advanced tooth formation than males for a given age the overall rate of microscopic formation exhibits less variability and differences can be attributed to tooth size rather than other frank biological differences. However no explanation is offered as to why tooth formation appears to vary between early and later forming teeth.

Demirjian and Levesque (1980) reported that the mean age for apex closure of the permanent first molar to be at 9.5 years for females and 8.8 years for males showing marked sexual dimorphism. A finding common with other studies is the similar tooth formation between males and females in early forming teeth. However for most other stages females were advanced over males by an average of 0.54 years over all the teeth. They also reported that females were advanced by almost 1 year in the formation of the canine tooth, signifying marked sexual dimorphism for that tooth.

Several age estimation studies using Demirjian's method have shown that girls were more advanced than boys in early forming teeth but less predictably was noted in later forming teeth especially in the crown formation phases (Chaillet et al., 2004 and Liversidge et al., 2006).

These results agree with earlier studies; e.g. Garn et al. (1958), Fanning (1961), Moorrees et al. (1963a) and Thompson et al. (1975) all reached similar conclusion. It is important however to consider that all these studies had relatively small sample sizes.

Several studies on the third molar have shown that there is clear dimorphism between males and females in different groups (Liversidge, 2008b; Olze et al., 2005). However there is marked variability in the formation in that tooth especially between different populations and different ethnic groups within a population and as of yet it is not clear whether the differences are partly due to ethnicity or variability of the formation of the tooth or even the methodologies analytical methods used.

2.5.7 Hypodontia

In the context of tooth formation hypodontia can be viewed as the most extreme in the continuum of the biology of dental development. For the purpose of this study the term hypodontia is used when up to five congenitally missing. Failure of six or more missing teeth is referred to as oligodontia. Brook related hypodontia to other several aspects of the developing dentition such as tooth size, tooth morphology, velocity of tooth formation and hyperdontia (Brook, 1984). All these modalities are interlinked and there is a cascade effect of one on the other, i.e. subjects with small teeth are more predisposed to tooth agenesis (Baum and Cohen, 1971).

Molecular studies show that Msx1 and Pax9 are co-expressed concurrently in dental mesenchyme during the early stages of tooth development. Genetic mutations in the homeodomain transcription factor MSX1 and the paired domain transcription factor PAX9 have been associated with hypodontia (Brook, 2009; Mostowska et al., 2003; Vastardis et al., 1996; Wang et al., 2009).

Other investigators have shown that mutations of double heterozygosis of the *Pax9*+/− or *Msx1*+/− gene had missing lower incisor; with incomplete penetrance, had missing third molars (Nakatomi et al., 2010).

On the clinical front, a meta-analysis of the prevalence of hypodontia of the permanent teeth (except third molars) has shown that no African study has met their inclusion criteria to enable meaningful comparisons (Polder et al., 2004). Of all the ethnic groups, hypodontia was more common in Australian Caucasians (7.6% for females and 5.5% for males). European Caucasian females had a prevalence of 6.3% compared to 4.6% in males. North American females had a prevalence of 4.6% and 3.2% in males. Females were 1.37 more likely to be affected than males. They report that despite the relative low numbers of reports from other ethnic groups, African Americans were very similar in the prevalence rates to North American Caucasians. Saudis had a prevalence of 2.2% in females and 2.7% in males. Many non-Caucasian studies were excluded because of issues with sampling methods and techniques as well as statistical analysis.

The overall distribution and order of hypodontia in all groups was comparable in both the maxilla and the mandible in all ethnic groups (overall prevalence for each jaw). However, a significant difference exists when the prevalence of each tooth type is considered separately between maxilla and mandible.

They also subdivided the more common clinical presentation of hypodontia into three categories; common, less common and rare:

1. Lower second premolar followed by upper second incisor followed by upper second premolar (Overall prevalence of 1.5 - 3.1%).
2. Lower first incisor followed by lower second incisor and upper first premolar followed by upper canine and lower second molar (Overall prevalence 0.1-0.3%)
3. Upper second molar and upper first molar followed by lower canine followed by lower first molar and lower first incisor (Overall prevalence 0.01-0.04%)

A study of American Blacks showed that hypodontia including third molar was significantly less than that in American Caucasians (Harris and Clark 2008). They showed that ethnicity has an impact on the prevalence of hypodontia. Groups with smaller tooth size are more predisposed to the prevalence of hypodontia. Brook et al. found that tooth size is affected by racial origin in 4 human populations with Caucasians, known to have relatively high prevalence of hypodontia amongst human populations, as having relatively smaller teeth than Chinese and also found greater size variation in later forming teeth (Brook et al., 2009).

This study will be limited to reporting hypodontia prevalence in the identified ethnic groups with future plans to expand the investigation.

2.5.8 Malnutrition and Tooth Formation

The effect of malnutrition on somatic growth is well documented (Tanner, 1962; Waterlow et al., 1977). Garn et al. (1965b) examined the effect of nutrition on dental development and reported a relatively low correlation between nutrition and dental development.

A study by Demirjian (1986) concluded that severe malnutrition does affect both skeletal development and to a lesser extent tooth formation. According to the study the correlation was low even for eruption. This is in contrast with other findings on malnutrition and eruption (Cameriere et al., 2007; Gaur et al., 2011).

The effect of malnutrition and conclusions on its effect on tooth formation has been by association when either examining secular trends of children living in different times and socio-economic conditions (Cardoso et al., 2010; Garn et al., 1965).

Variations in dental development usually examine socioeconomic conditions of which malnutrition is one factor, amongst others (Cardoso et al., 2010; Rosen and Baumwell, 1981).

Another study examined the effects of malnutrition and found that permanent teeth were more advanced in the group with mild to moderate malnutrition (Alvarez, 1995). They examined1481 Peruvian children (1-13 years) found to be chronically malnutrition prevalent. They concluded that malnutrition delayed tooth development although the focus was eruption rather than formation.

Other researchers whist investigating a small group of deprived Peruvian children reported that malnutrition did not significantly affect tooth formation (Cameriere et al., 2007).

Our knowledge of the effect of malnutrition from the literature amounts currently to a relationship of association and a link, although expressed, therefore still remains somewhat inconclusive and inferential.

Chapter Three: MATERIALS AND METHODS

3.1 Overview

The subjects for this study were recruited from and around Greater Khartoum, representing the age range between 3 and 23 years of age in two very different settings and two locations. Two dental schools were conveniently located Elrazi Dental School in southern Khartoum and Ribat National Hospital in the Centre of Khartoum. Following sample size calculation, ethical approval was sought and obtained. Agreement was sought and obtained to use the x-ray machine in both dental schools. After establishing the date of birth the children underwent clinical and radiographic examination. At times establishing the date of birth was not straight forward and was a cause of many delays. Children without consent or exact date of birth were omitted from the study but given verbal oral health information as there is a very strong sense of community as well as ethnic seclusion. Those selected were transported to the dental school with the OPG machine where the radiograph was taken. Clinical examination was performed together with height and weight measurements. Those with clinical findings were referred to the relevant department for treatment free of charge.

3.2 Ethical Approval

Ethical approval was applied for and obtained from El-Razi Dental College's Ethical Committee and the approval was granted prior to embarking upon the study and stipulated that participants should be treated at the dental school free of charge (Appendix A.4).

As outlined earlier Sudan is one of the poorest countries in the world and lacks basic services such as a continuous supply of water and electricity. Only 4 OPG machines exist in the country so far. The children are not likely to experience any form of serial examination of this nature in the near future, as the economic situation is rapidly deteriorating. Additionally, all of the Western Sudanese children had no contact with dentist previously and this was their first experience.

It was also the intention of this study to provide a base line for other disease, such as impacted teeth, cysts and other disease that can only be detected radiographically.

In fact even before the study had been completed a charity dental clinic had been installed on the back of preliminary findings. It is hoped that dental and growth findings will be of interest to policy advisors as this is the first study of its kind in the country.

Due to the political sensitivities, every care was taken not to offend or highlight ethnic categorization when subjects were chosen. In fact oral health education was administered to the whole group and only the selected children transported to the dental school. A couple of khalwas with mixed students declined segregating the students based on ethnicity and declined to participate. However, most were understanding and allowed access to the children and their families.

Another unexpected advantage is that when the children were back to their communities and schools, they felt they had a positive experience and that was an extremely effective health promotion exercise in an otherwise inaccessible area with extreme poverty and mistrust of government agents. Relatives of children became aware of what a dentist does through their children. This served as an effective tool for increasing patient numbers and diversity almost overnight contributing to training of dental students to the delight of the school authorities.

3.3 Sample Size Calculation

Sample size calculation for this study is based on a recent study by Liversidge (2008), which is a comparative study in two African populations.
The formula used for calculating the sample size is:

$$N = 4\,(s.d.)^2 / d^2$$ where,

N = sample size

s.d. = standard deviation

d = margin of error.

(At 95% confidence)

The difference in the means of the Cape Coloured group and the African group in stage is 0.83yrs for stage R1/4 of M3, while the standard deviation is $\pm$1.68yrs.

$$N = 4\,(1.68)^2 / (15.89-15.06)^2 = 17$$

The total sample size is therefore 17 subjects per age group per sex. The age range is 3-23 year olds and hence the minimum total sample size is 714.

3.4 Sample Selection

Subjects for the Northern Sudanese group were derived by multistage stratified clustered sampling of preschools, schools and universities three cities joining together to establish the Sudanese capital, Khartoum and are defined by the River Nile. The three different selected localities are: Khartoum city, city of Bahri and the city of Um Durman.

A list of both public and private schools was obtained from the Ministry of Education. Pre-schools are usually mixed for both sexes and are attended by children between the ages of 3-5 years. Mainstream schools are unisex and are attended by 5 to 18 years of age. All universities (except one which is all female) are mixed for both sexes and were targeted for males and females subjects between the ages of 18-22. All subjects had northern parents and grandparents descending from Northern tribes. After organizing the schools in a list and classes representing age groups, the table of random numbers was used to select the class children were chosen from. The headmasters where then approached and the children given consent letters to take to their parents. In addition to consenting the parent was asked to supply the date of births. Some of the schools were very organized and had dates of births especially for younger children whilst others had no record at all. Where there was a discrepancy in the date of birth the child was omitted and another was chosen.

The Western Sudanese group was selected from dedicated religious schools in or near camps for the internally displaced (khalwas). These consist of persons from western Sudan and predominantly the Darfur region where conflict still rages. They are of African ancestory with parents and grandparents originating from the region. This is reflected in their facial features which is indicative of their African origin. Although an attempt was made to choose subjects from the khalwas randomly many practical obstacle were encountered forcing a deviation from the original design. These include difficulties in access as well as the lack of organized information which necessitated convenience sampling. The ethnic choice of children was also an issue in mixed khalwas and schools. The lack of accurate records also limited the number of khalwas which could be approached. Some also had unsafe access and we were advised against approaching them. The children chosen were restricted to those day students living with either parent (mother or father) in the camps. Orphaned children tended to be kept in the khalwas full-time and there were no details on these children and hence were excluded from the study. Children were also excluded if the self-reported date of birth differed from that present in the khalwa records. It should be taken into account that many of the parents were illiterate, but this is the only way one could be sure. The schools themselves are heavily based on tribal heritage and religious education rather than secular education. In all, 8 major camps for the internally displaced are dotted around the capital. These consist of very basic shelters and very poor services. 9 khalwa's were targeted based on the ethnic origin of the children. Each khalwa has hundreds and sometimes thousands of children some living in the khalwa full time. Those with parents are allowed to leave at the end of the day or at weekends. Children of non-Darfur origin were excluded. There is a very strong tribal commitment to these khalwas and ethnic identification was relatively simple. In fact the displaced children tended to come from the same villages in Darfur.

Within the camps, the khalwas are organized around a very specific daily schedule and are run by a group of religious teachers who are selected by a local Sheikh but have to go through a loose governmental approval system. The majority of the teachers received university level education in religious studies but some had more basic levels of education. This was due to fact that the khalwas run by educated staff received more public funding. The teachers all unite under one Sheikh in a single jurisdiction. The Sheikh is usually responsible for maintaining a group of khalwas and operates independently of government control. There is usually very little communication between the two systems. The Sheikhs command enough power in the community to oversee the education of the children and fund those khalwas either directly from their own funds or indirectly through liaising with governmental and non-governmental agencies. However, very little public funding goes directly to the khalwas due to the mistrust between the displaced and the government agencies and the inaccessible demographic nature of the camps. The regional control was mostly with the Sheikhs and Imams. The khalwas can be viewed as community led initiatives.

Each subject included in the study had their date of birth, sex, and tribal origin recorded and/or village of origin. Where there was a question mark on the date of birth, i.e. conflict between parents, school or khalwa, and often there was, the child was excluded. The most common reason for our inability to establish the date of birth was usually that the mother had been displaced to a different camp, killed or missing. So a question was added to establish who was actually filling the information i.e. aunt, grandparents etc.

The inclusion criteria are that the children had to be between 3 and 22 years of age at the time the radiographs were obtained and should have no clinical genetic abnormality or long term debilitating illness.

For both the Northern and Western children of interest, consent letters were given to the children to take to their parents. A bus was then hired to transport the children to and from the dental school or khalwa, where they received a dental check up and the radiographs subsequently taken together with height and weight measurements (as will be described later). A subsequent appointment was organized if treatment was indicated. For the western children this was relatively easier as there were no time constraints due to the way their day is structured.

In all 844 Northern males were selected together with 802 Northern females. The Western Sudanese sample consisted of 848 males and 401 females.

3.5 Equipment and Setting

Permission was obtained from the Ribat National University's Dental College's authorities to undertake the research in their facilities. This college is situated in the centre of the capital with easy access. The dental school has no Panoramic (OPG) X-ray machine. The OPG machine (Siemens, Germany, 1988) was installed according to the specifications of the radiation safety guidelines and was checked by the chief radiographer to ensure the safe working environment of both the operator and the patient. The room was shielded with a 1mm mobile lead sheeting entirely to prevent any radiation leakage. Interestingly the room offered was in the horse's stable behind the dental school, and provided fantastic entertainment for the children in the long summer days.

Initially a dark room adjacent to where the Radiographic machine was placed had been used. However, as the room became unavailable an automatic second hand machine was purchased enable the entire process to be taken in a single room.

The location enabled the Northern Sudanese sample to be collected more easily.

For the Western Sudanese, the OPG machine and the radiographic facilities were moved to the south of the capital to Elrazi Dental School which is nearer to the majority of the camps for the displaced. This again enabled easy access to a very poor community. The main problem faced at this college was the transport as the children had to be transported large distances. Simple matters such as high summer temperatures (reaching 48 degrees Celsius) and lack of transport sometimes proved challenging.

The radiographs were examined in darkened room using a light box. Radiographs included had to be of sufficient quality to enable them to be read. Thirty radiographs were of poor quality and had to be disregarded.

The radiographs taken were then given a code and recorded on the radiograph with a marker pen on the right hand side end of the radiograph. Details of the patients' data including the name, origin, date of birth (both self reported and from records), origin and the date the radiographs had been taken were recorded on a separate form together with their height and weight.

Some patients (n=106) were excluded based on discrepancies in the date of birth or mistakes in identification.

Digital photographs were taken of all the radiographs after being placed on a viewing box in a darkened room and coded. The radiographs, initially organized by age, were grouped in one file randomized by a person other than the investigator to and given new codes with a reference to previous codes of identification unknown to the examiner. This was done to eliminate examiner bias with regard to age whilst staging tooth formation and to ensure blind reading of radiographs.

3.6 Assessment of Height and Weight

Weight and height were measured by using precision electronic scales (Seca 870, Seca, Hamburg, Germany) and stadiometres fixed to the wall of the radiography room.

Stature was measured on a calibrated and stable height gauge attached to a flat vertical pole with right angled head board. The subject was asked to stand straight, arms at the sides, and shoulders relaxed. The subject was then asked to look straight ahead so that the line of vision is perpendicular to the body or in the Frankfort horizontal plane position. The headpiece was then lowered onto the crown of the head with sufficient pressure to compress the hair. Hair ornaments, buns, braids, and the like are removed as necessary to obtain an accurate measurement. Stature is measured at maximum inspiration. The measurer's eye was level with the headpiece to avoid reading errors due to parallax.

Weight is taken on an accurate device with the child wearing minimal clothing. 0.1kg is subtracted from the reading. The weight and measures are calibrated weekly.

One hundered university dental students who were initially investigated had repeated measures of their height and weight one month later to assess intra-examiner reliability by intra-class correlation coefficient.

3.7 Calculation of Decimal Age

Age was converted to a decimal value using previously published methodology (Eveleth and Tanner, 1990). Decimal age calculation is undertaken by dividing the year into 10 rather than 12 months. Each date in the calendar is marked in terms of thousandths of the year. Thus for a radiograph taken on the 10/04/2008 for a child who's date of birth is 12/10/2004 the decimal age would be 3.49.

The system greatly facilitates the computing of velocities since the proportion of the year between two examinations is easily calculated.

Children are then grouped per age group, e.g., 5 year olds would include all those in the 5 to 5.99 decimal age range.

3.8 Examiner Training and Calibration

Training on the method after Moorrees, et al. (1963a) was done with a trained and calibrated examiner (Dr. H. Liversidge) until a degree of independent reading was possible. Training was then done on a number of radiographs to increase familiarity with the method.

3.8.1 Inter-Observer Error Assessment

The total observed variance in a population is a function of the true variance and of several sources of error, of which an important one is measurement error.

Inter-observer error was carried out on a random sample of 30 radiographs which were selected from those previously analyzed by Dr H. Liversidge. The same radiographs were then reassessed by the author and both assessments subjected to statistical analysis using the Kappa test to establish the level of agreement. The formula used is represented below:

K=(Po-Pe)/(1-Pe)

Where,

Po is the observed proportion of agreement.

Pe is the proportion of agreement that is expected due to chance alone.

A high Kappa value indicates a high level of agreement between

Examiners whilst a low kappa indicates a poor level of agreement. The kappa values used to estimate the level of agreement are (Landis and Koch, 1977):

<0 Poor

0 – 0.2 Slight

0.21 – 0.4 Fair

0.41 – 0.6 Moderate

0.61 – 0.8 Substantial

0.81 – 1.0 Almost Perfect

Inter-observer error of stage assessment was calculated using double readings of 30 radiographs. Cohen's Kappa was 0.86 indicating excellent agreement.

3.8.2 Intra-Observer Error Assessment

To assess the degree of intra-observer error a random sample of 90 OPGs were selected from those previously analysed by the author. These were re-analysed as described above, approximately 3 months after the initial analysis was undertaken. The kappa statistic was calculated for between the readings.

The stadiometre was also calibrated at the beginning of each session against a rod of known length. The scales were calibrated each session against a weight of known weight.

To enable the intra-observer assessment the last 10 radiographs of each 100 consecutive radiograph were read a month later. Cohen's kappa was calculated to determine intra-observer agreement. A Cohen's kappa of 0.91 indicated excellent intra-examiner reliability.

3.8.3 Rating Radiographs

Permanent teeth on the lower left mandible were rated in the following order: third molar M3, second molar (M2), first molar (M1),second premolar(P2), first premolar (P1), canine (C) , lower lateral incisor (I2), lower central incisor (I1).

Table 1 defines the stages and the abbreviated text used throughout this thesis.

Table 1. Definition and abbreviated text of stages used; after Liversidge (2008)**.**

Stage (numerical)	Stage (initials)	Stage (name)	Descriptive criteria
1	Cr	Crypt	Radiolucent area visible within alveolar bone.
2	Ci	Cusp tip initiation	One or more separate cusp tip(s) visible within crypt.
3	Cco	Cusp coalescence	Two or more cusp tips coalesced.
4	Coc	Crown outline	Crown outline, including marginal ridges. Enamel and dentine but less intense radio-opacity than full thickness.
5	C1/2	Crown one half	Thicker enamel of the crown occlusal surface radio-opaque with some dentine visible. Flat inferior dentine border.
6	C3/4	Crown three quarters	Full thickness occlusal enamel with considerable aproximal dentine at the contact points. Curved inferior border.
7	Cc	Crown complete	Aproximal enamel complete to neck of tooth. Roof of pulp chamber visible.
8	Ri	Root initial	Some root visible aproximally, but less than half crown height.
9	Rcl	Cleft	Beginning of root furcation visible as a dot or line.
10	R1/4	Root one quarter	Clear semilunar furcation visible. If taurodont, aproximal root length about half of crown height.
11	R1/2	Root one half	Root bifurcation more extensive. Aproximal root length equal to crown height. Distal root canal walls diverge with sharp edges.
12	R3/4	Root three quarters	Root length considerably more than crown height and root canal walls diverge.
13	Rc	Root complete	Walls of the distal root canal are parallel and full length with rounded/blunt edges.
14	A1/2	Apex half closed	Apex of distal root partially open. Periodontal ligament slightly wider at distal apex.
15	Ac	Apex closed	Distal apex appears closed, with uniform periodontal ligament width.

All teeth were scored from radiographs using the stages described after Moorrees et al. (1963a). An additional staging of the crypt was added to these stages making them 15 in total.

These are cusp tip initiation, coalescence of cusp tips, cusp outline completed, crown stages, crown complete, initiation of root, root cleft formation, root stages for single and multi-rooted teeth, root length complete, apex half closed and apex closed. An earlier score is always assigned where by the tooth is in between stages. If there are no sign of tooth calcification a zero rating is assigned.

3.8.4 Height and Weight Intra-Examiner Consistency

Intra-class correlation co-efficient was used to assess the level intra-examiner reliability. It was chosen to test the "consistency" or "repeatability" of height and weight measurements (Shrout and Fleiss, 1979). In this instance it was used to assess test-retest reliability and can be conceptualized as the ratio of between-groups variance to total variance.

3.9 Data Entry and Statistical Analysis

The Data was entered into a Microsoft Excel 2003 spreadsheet and then analyzed in SPSS for Windows (Version 13.0).

3.9.1 Probit Analysis

When biological responses are plotted against their causal stimuli (or logarithms of them) they often form a sigmoid curve. Sigmoid relationships can be linearized by transformations such as logit, probit and angular accumulations. For most systems the probit (normal sigmoid) and logit (logistic sigmoid) give the most closely fitting result. Logistic methods are useful in epidemiology because odds ratios can be determined easily from differences between the fitted logits but have not been reported much in tooth formation studies. Probit analysis is more commonly encountered. The formulae used for calculating the mean age of the 50% probability usually denotes the mean of that given parameter (LC50). This can easily be confused with the arithmetic mean which in tooth formation could denote children within a given stage rather than the actual mean of attainment (Finney, 1971).

The age LC50 denotes the mean age of attainment, indicates the average age of entering a stage and has biological importance. It does not take into account outliers at either extreme of the age distribution in the same way the arithmetic mean age within stage does. In practical examples the mean age of attainment (a term used in this report to denote mean age of entering a stage) is important in biological comparisons whilst the mean age of children within a stage is important in forensic applications where information on 'approximate age' is required and where available knowledge on outliers is a key factor.

The formula for probit analysis is:

$$Y' = \Phi^{-1}(p)$$

Y' is the probit transformed value (5 used to be added to avoid negative values in hand calculation).

p is the proportion (p = responders/total number).

inverse F (**p**) is the 100 * p % quantile from the standard distribution.

Logit

Odds = p/(1-p)

[**p** = proportional response, i.e. **r** out of **n** responded so **p = r/n**]

Logit = log odds = **log[p/(1-p)]**

The fitted model analysis and critical quantiles are then assessed by statistics for heterogeneity which follow a chi-square distribution. If the heterogeneity statistics are significant then the observed values deviate from the fitted curve too much for reliable inference to be made (Finney, 1971).

3.9.2 Descriptive Statistics of the Sample

Descriptive statistics of both Northern and Western groups are presented in age groups together with the frequencies. General description and frequencies of the groups are analysed together and presented in the results chapter together with more specific issues such as growth problems and hypodontia.

3.9.3 Calculation of Mean Age of Attainment

At this point it is important to differentiate between the mean age of stage attainment (sometimes referred to the mean age of stage entry) and the mean age of children within a particular stage. Figure 7 illustrates the difference between the two and although they are used interchangeably in the literature, they are essentially very different.

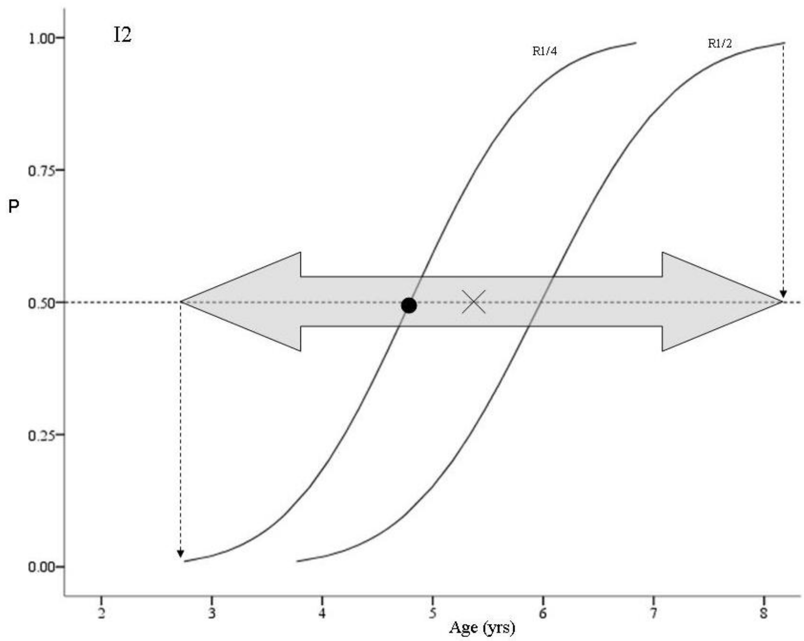


Figure 7. Smoothed cumulative curves for I2 for stages R1/4 showing the difference between age of attainment and the mean age of children within the stage (approximately)

⟺ *The observed age range for a stage*

X *Mean age of children within a stage; Mean (SD)=5.60 (1.09) yrs*

● *Mean age of attainment for the stage; Mean (SD)=4.80 (0.49) yrs*

Mean age of attainment of each dental maturational event (age when 50% of the sample had reached/passed that each stage) of each tooth was calculated using probit regression for males, females and ethnic group using one year age. This produces a cumulative distribution function assuming log normality. Smoothed curves give an indication of an estimate of age of attainment of a growth stage. Of note is that the mean age of children within a stage have been calculated and are presented in Appendix A.5.

3.9.4 Mean Age of Attainment Calculation (Logistic Regression)

Using Logistic regression the mean age of attainment, standard deviation and standard error were calculated (Liversidge, *in press*). The standard error was calculated using Fiellers theorem. To estimate the mean age of occurrence *m* using logistic regression:

$$logit(p) = a + bx$$

$$m=a/b$$

$$CVa=SE\ a/b$$

$$CVb=SE\ b/b$$

Estimates of standard error of *m*,

$SE\ (a/b)=sqrt\ (CVa^2 + CVb^2 - 2x\ CVa\ x\ CVb)*a/b$

Where CVa=SEa/a and CVb=SEb/b

a= intercept

b=coefficient

c=correlation of a and b

The 95% confidence interval for the mean was then calculated using the standard formula:

95% CI of the Mean = Mean $\pm$ (1.96 x Standard Error of Mean)

The student t-test was then used to establish whether sex and ethnicity differences existed. Odds ratio's were also calculated and presented in Appendix A.6 (Table A.6.1 to A.6.3).

3.9.5 Calculation of z-scores for Height and Weight

Epi Info software was used to calculate z-scores for a number of parameters using 2000 CDC reference. The parameters investigated were:

1. Weight for height to indicate wasting (thinness).
2. Height for age to indicate stunting and is indicative of the state of nutrition.
3. Weight for age which is an indication for the overall growth, which is directly dependent on weight for height and height for age.
4. BMI for age which indicates overall general development.

The z-scores are represented for each of the groups and data analyzed at the 2.3% cut off rate for the z-scores to describe the sample.

3.9.6 Hypodontia

When considering hypodontia it is important to take into account either the age after which a development to of the tooth is unlikely, or consider the stage of development of other teeth.

Agenesis in the context of formation of other teeth has only been recently available for third molars. There is very little chance of third molar developing after the root of the second molar is ¾ complete (Liversidge, 2008a). Given the large variability between individuals it is somewhat surprising to see reports of hypodontia in children as young as 3 and 4 years of age (Glenn, 1964).

The stage of development of the second molar was the determinant factor which confirmed the tooth congenitally missing. The third molar was therefore only included if the roots of the lower second molar were at least ¾ formed.

Chapter Four: RESULTS

4.1 Sample Age Distribution

The Northern Sudanese subject age groups were more evenly distributed than the Western Sudanese. This is evident in Figure 8 and Figure 9.

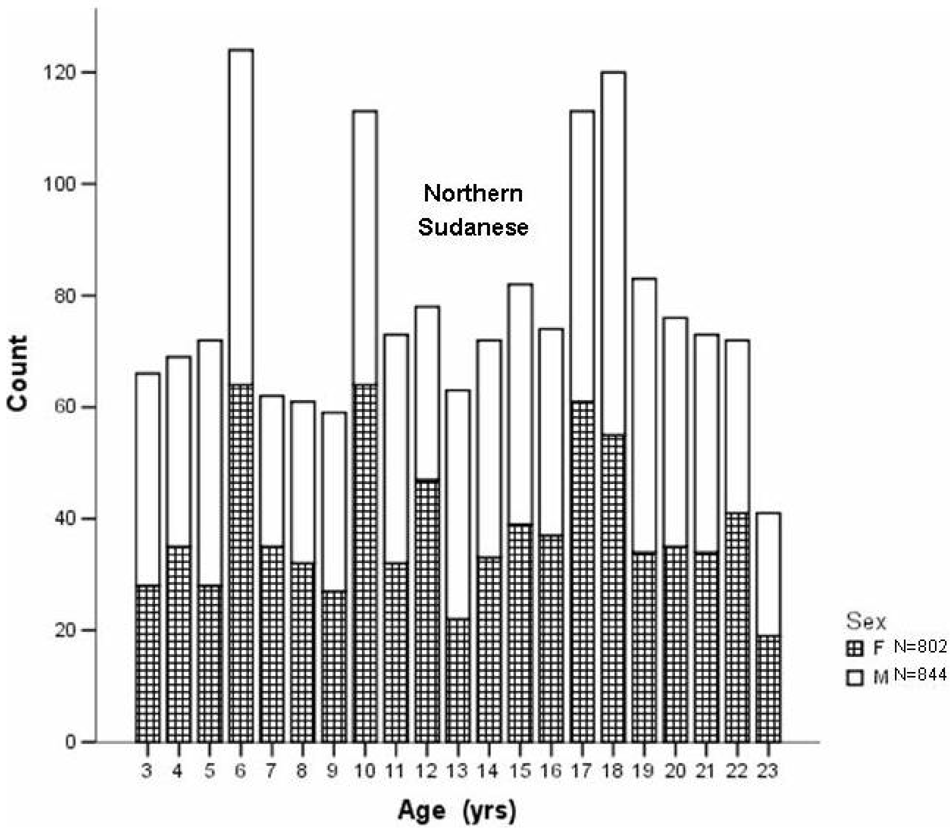


Figure 8. Age distribution of Northern Sudanese subjects.

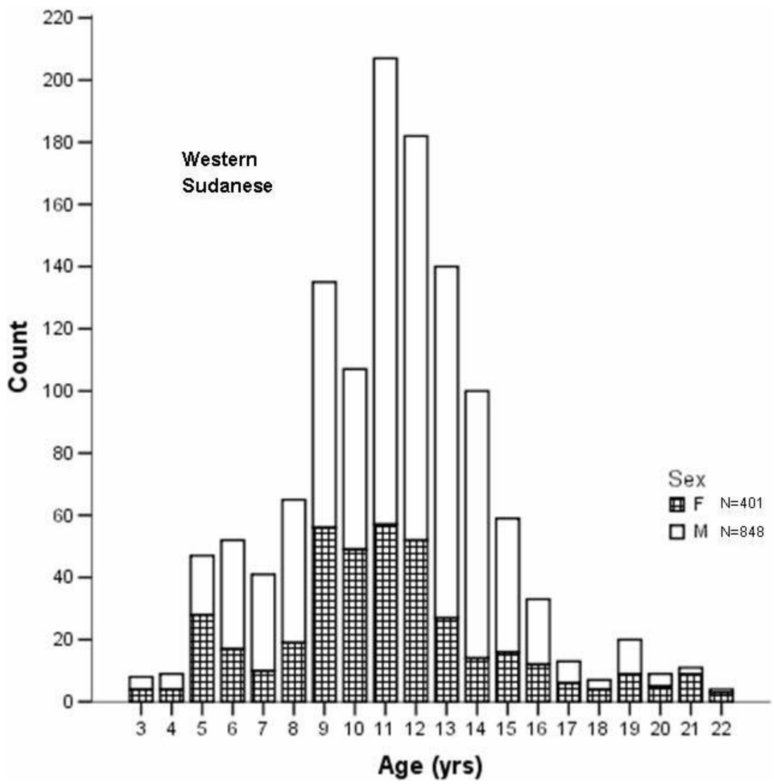


Figure 9. Age distribution of Western Sudanese subjects.

Logistical difficulties outlined earlier contributed to the uneven distribution of the Western Sudanese age groups Figure 9). This age group reflects the age distribution most available at the khalwas. It was very difficult to recruit older males and females as there was no one place of congregation.

4.2 Inter-Examiner and Intra-Examiner Reliability

Intra examiner reliability using Cohen's Kappa had a coefficient of 0.86 which is excellent agreement.

Intra-class coefficient for height and weight was 0.98 and 0.96 respectively.

4.3 Choice of Statistical Methods

Comparison of mean age of attainment of a stage using probit and logistic regression
As discussed in the previous section both cumulative distribution curves are used to establish the mean age attainment of tooth formation stages (Smith, 91; Liversidge, 2006). Logistic regression has not been reported much and the difference between using this method of statistical analysis versus others in common use has not been yet tested. Comparison of the two methods on the same stages of formation of the same tooth yields the same mean of stage attainment but has an effect on the confidence interval. In simpler terms the confidence interval is wider for means established with probit analysis due to tendency of the method to approximate the asymptotes and effectively use a cumulative curve of best fit (Figure 10).

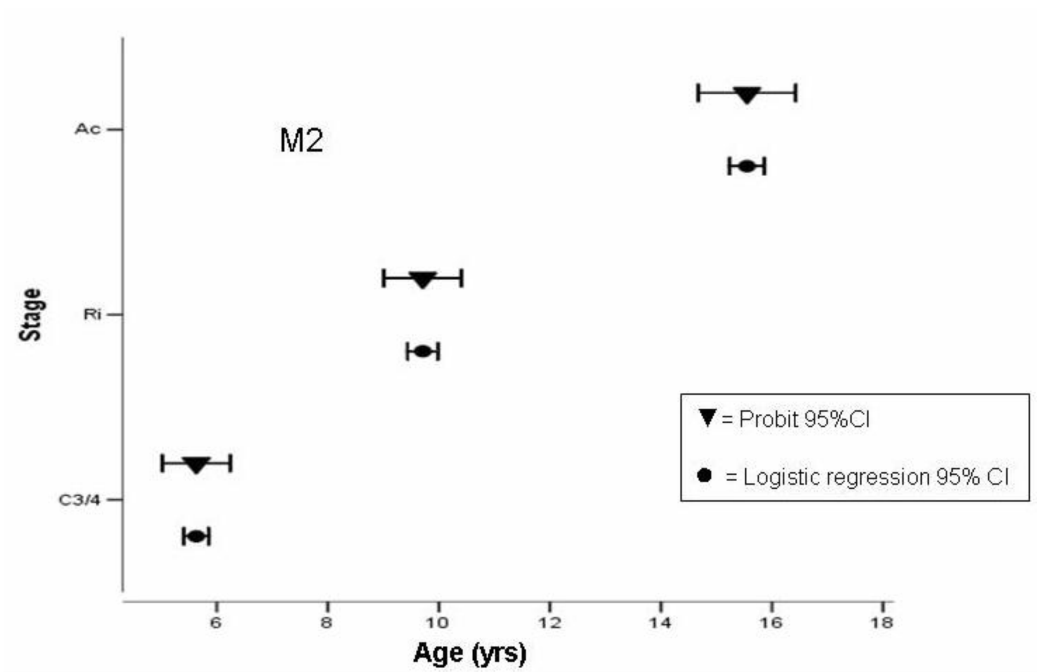


Figure 10. Comparison of mean age of attainment and 95% CI for the 15 stages of M2 in North females using probit analysis and binary logistic regression.

The direct impact is on both the standard deviation and the standard error and in turn the confidence interval (Figure 11).

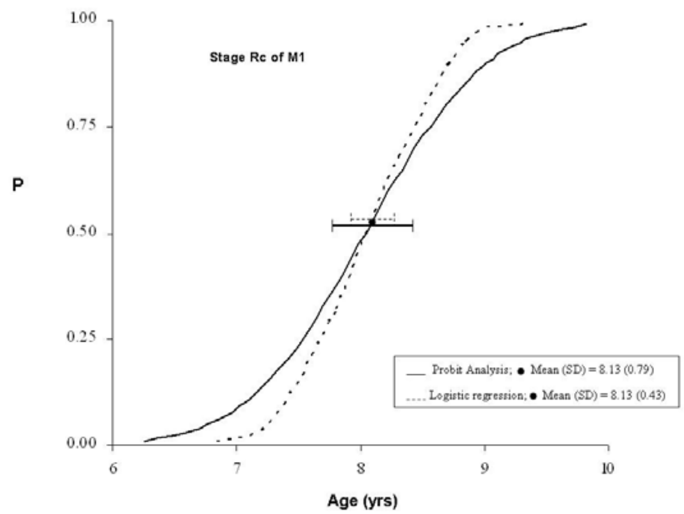


Figure 11. Smoothed cumulative curves for two statistical methods showing the effect on standard deviation and 95% CI for the same stage on the same tooth (Rc of M1).

Although the mean obtained is the same for M1 stage Rc (Figure 11) using the two statistical methods, the effect on 95% CI can be seen in Figure 10.

4.4 Mean Age of Attainment References for Tooth Formation Stages

One of the aims of this study is to produce reference data for the groups that were studied. The mean age of children having attained a stage is presented in Table 2 through to Table 9.

Table 2. Comparison of Mean ages of attainment in years for M3 stages in Northern Sudanese and Western Sudanese children using logistic regression.

	Northern Females (N=802)			**Western Females(N=401)**			**Northern Males(N=844)**			**Western Males(N=848)**		
Stage*	Mean(SD)	SEM	95% CI	Mean(SD)	SEM	95% CI	Mean(SD)	SEM	95% CI	Mean(SD)	SEM	95% CI
Cr	9.75 (2.23)	0.25	9.26-10.24	8.23 (0.93)	0.49	7.28-9.18	8.49 (1.13)	0.38	7.75-9.23	8.35 (1.47)	0.17	8.01 - 8.68
Ci	10.06 (1.03)	0.22	9.62-10.49	8.75 (1.14)	0.21	8.34-9.16	9.54 (2.08)	0.24	9.08 -10.01	8.45 (0.80)	0.22	8.02 - 8.89
Cco	10.79 (1.15)	0.21	10.38-11.20	9.50 (0.73)	0.19	9.13-9.87	9.72 (1.14)	0.26	9.21-10.23	9.45 (0.75)	0.14	9.17 - 9.73
Coc	10.82 (1.97)	0.23	10.38-11.27	10.11 (0.85)	0.17	9.79-10.44	10.17 (1.17)	0.23	9.72-10.62	10.29 (0.83)	0.12	10.05 - 10.53
C1/2	11.61 (1.23)	0.21	11.21-12.02	11.23 (0.82)	0.14	10.95-11.51	11.14 (0.96)	0.17	10.80-11.47	11.55 (0.90)	0.10	11.36 - 11.74
C3/4	13.29 (0.95)	0.17	12.96-13.61	12.26 (0.65)	0.12	12.02-12.51	12.44 (1.03)	0.18	12.09-12.79	13.30 (0.78)	0.09	13.12 - 13.48
Cc	14.15 (0.88)	0.17	13.82-14.47	13.04 (0.80)	0.17	12.71-13.36	13.72 (0.95)	0.17	13.40-14.05	14.04 (0.69)	0.09	13.85 - 14.23
Ri	14.88 (0.86)	0.16	14.56-15.20	14.23 (0.68)	0.18	13.86-14.59	14.76 (0.71)	0.14	14.48-15.03	14.58(0.65)	0.11	14.37 - 14.80
Rcl	15.28 (1.00)	0.17	14.95-15.62	15.25 (0.81)	0.23	14.80-15.71	15.10 (0.75)	0.14	14.83-15.37	14.91 (0.62)	0.10	14.71 - 15.12
R1/4	15.70 (1.18)	0.18	15.34-16.06	15.49 (0.67)	0.22	15.07-15.92	15.66 (0.76)	0.14	15.39-15.93	15.49 (0.75)	0.15	15.19 - 15.79
R1/2	16.69 (1.38)	0.18	16.34-17.05	16.27 (0.62)	0.23	15.82-16.72	16.62 (0.98)	0.15	16.32-16.91	17.26 (0.90)	0.31	16.64 - 17.87
R3/4	18.06 (1.51)	0.20	17.68-18.45	17.28 (0.47)	0.30	16.70-17.86	17.64 (1.12)	0.16	17.33-17.95	18.01 (0.75)	0.32	17.26-18.74
Rc	19.16 (1.32)	0.19	18.78-19.54	18.11 (0.55)	0.29	17.55-18.66	18.51 (1.03)	0.15	18.21-18.81	18.63 (0.66)	0.32	18.01-19.25
A1/2	19.57 (1.26)	0.18	19.21-19.93	18.44 (0.44)	0.20	17.95-18.93	18.89 (0.94)	0.17	18.55-19.22	19.05 (0.54)	0.31	18.44 -19.66
Ac	20.61 (1.07)	0.19	20.24-20.99	19.43 (0.74)	0.36	18.72-20.14	19.70 (0.85)	0.15	19.41-20.00	20.17 (0.81)	0.43	19.33-21.00

(Tooth stages = Table 1 (p.54); SD = standard deviation; SEM = standard error of mean; 95% CI = 95% confidence interval of mean)

*Please refer to Appendix A.5.1 for the number of individuals (*N*) used to for calculating the mean of each stage

Table 3. Comparison of Mean ages of attainment for M2 stages in Northern Sudanese and Western Sudanese children using logistic regression.

	Northern Females (N=802)			**Western Females(N=401)**			**Northern Males(n=844)**			**Western Males(n=848)**		
Stage	Mean(SD)	SEM	95%CI	Mean(SD)	SEM	95%CI	Mean (SD)	SEM	95%CI	Mean(SD)	SEM	95%CI
Ci	3.48 (0.12)	0.12	3.25-3.71				3.03(0.52)	0.22	2.59-3.47			
Cco	3.78 (0.31)	0.11	3.56-3.99	3.90(0.29)	0.27	3.36-4.44	3.44(0.52)	0.16	3.12-3.76			
Coc	3.98(0.38)	0.11	3.76-4.21	4.19(0.18)	0.13	3.81-4.57	3.86(0.42)	0.12	3.62-4.10	4.13(0.30)	0.24	3.67-4.60
C1/2	4.34(0.54)	0.14	4.07-4.61	4.66(0.53)	0.26	4.15-5.17	4.23(0.53)	0.13	3.97-4.49	4.79(0.49)	0.25	4.29-5.28
C3/4	5.63(0.49)	0.12	5.41-5.86	5.58(0.63)	0.09	5.39-5.76	5.40(0.65)	0.13	5.15-5.65	5.88(0.59)	0.18	5.52-6.23
Cc	7.61(0.83)	0.15	7.32-7.90	7.52(0.63)	0.19	7.14-7.90	7.33(1.01)	0.16	7.00-7.65	7.59(0.66)	0.13	7.33-7.85
Ri	9.71 (0.76)	0.14	9.44-9.98	9.00(0.45)	0.13	8.76-9.25	9.76(0.84)	0.15	9.47-10.04	9.44(0.35)	0.07	9.29-9.58
Rcl	10.49(0.72)	0.25	10.24-10.74	9.84(0.36)	0.09	9.65-10.02	10.29(0.69)	0.13	10.04-10.54	10.14(0.37)	0.08	9.98-10.29
R1/4	11.24(0.89)	0.29	10.95-11.53	10.79(0.40)	0.08	10.62-10.95	11.23(0.67)	0.12	10.99-11.47	10.83(0.34)	0.05	10.72-10.93
R1/2	12.07(0.66)	0.13	11.81-12.33	11.60(0.27)	0.08	11.45-11.75	11.79(0.62)	0.13	11.53-12.05	11.82(0.36)	0.05	11.73-11.92
R3/4	12.87(0.68)	0.14	12.60-13.14	12.33(0.35)	0.08	12.17-12.49	12.54(0.65)	0.14	12.27-12.80	12.49(0.33)	0.05	12.39-12.59
Rc	13.44(0.65)	0.13	13.18-13.69	13.09(0.31)	0.10	12.89-13.28	13.50(0.60)	0.12	13.27-13.74	13.29(0.42)	0.05	13.19-13.39
A1/2	14.01(0.62)	0.14	13.74-14.29	14.30(0.62)	0.19	13.93-14.67	14.29(0.57)	0.13	14.04-14.55	14.08(0.56)	0.08	13.92-14.23
Ac	15.55(0.92)	0.16	15.24-15.85	15.62(0.60)	0.22	15.19-16.06	15.32(0.55)	0.11	15.10-15.53	15.57(0.70)	0.15	15.28-15.86

(See Table 2 for abbreviations)

*Please refer to Appendix A.5.2 for the number of individuals (*N*) used to for calculating the mean of each stage

Table 4. Comparison of Mean ages of attainment for M1 stages in Northern Sudanese and Western Sudanese children using logistic regression.

	Northern Females (N=802)			**Western Females(N=401)**			**Northern Males(N=844)**			**Western Males(N=848)**		
Sage	Mean(SD)	SEM	95%CI	Mean(SD)	SEM	95%CI	Mean (SD)	SEM	95%CI	Mean(SD)	SEM	95%CI
C3/4	2.43(0.40)	0.76	0.95-3.92									
Cc	2.97(0.57)	0.34	2.30-3.65				2.66(0.82)	0.39	1.89-3.43			
Ri	4.07(0.23)	0.09	3.90-4.25	3.14(0.82)	0.90	1.37-4.91	4.18(0.42)	0.12	3.95-4.41	4.01(0.18)	0.15	3.71-4.32
Rcl	4.20(0.21)	0.08	4.04-4.37	4.33(0.50)	0.32	3.71-4.96	4.51(0.34)	0.10	4.31-4.71	4.58(0.41)	0.25	4.10-5.07
R1/4	5.22(0.31)	0.10	5.03-5.41	4.86(0.32)	0.17	4.53-5.19	5.23(0.32)	0.09	5.06-5.41	5.39(0.13)	0.09	5.20-5.57
R1/2	6.00(0.41)	0.10	5.80-6.20	6.09(0.15)	0.09	5.92-6.27	6.08(0.47)	0.10	5.88-6.27	6.12(0.23)	0.09	5.94-6.30
R3/4	6.63(0.49)	0.99	6.70-7.57	6.80(0.31)	0.15	6.51-7.09	7.06(0.47)	0.11	6.84-7.28	6.81(0.57)	0.13	6.55-7.08
Rc	8.13(0.43)	0.12	7.91-8.36	7.75(0.32)	0.16	7.44-8.06	8.54(0.31)	0.09	8.35-8.73	7.69(0.54)	0.12	7.47-7.92
A1/2	9.50(0.41)	0.12	9.27-9.73	9.16(0.53)	0.13	8.89-9.42	9.69(0.41)	0.11	9.47-9.91	8.99(0.43)	0.09	8.81-9.17
Ac	11.02(1.02)	0.16	10.71-11.32	10.27(0.45)	0.10	10.08-10.47	10.86(0.74)	0.14	10.59-11.13	10.11(0.48)	0.08	9.95-10.28

(See Table 2 for abbreviations)

*Please refer to Appendix A.5.3 for the number of individuals (*N*) used to for calculating the mean of each stage

Table 5. Comparison of Mean ages of attainment for P2 stages in Northern Sudanese and Western Sudanese children using logistic regression.

	Northern Females (N=802)			**Western Females(N=401)**			**Northern Males(N=844)**			**Western Males(N=848)**		
Stage	Mean(SD)	SEM	95%CI	Mean(SD)	SEM	95%CI	Mean (SD)	SEM	95%CI	Mean(SD)	SEM	95%CI
Cco	3.40(0.70)	0.28	2.85-3.96				3.36(0.50)	0.19	2.99-3.72	2.89(0.73)	1.11	0.70-5.09
Coc	3.98(0.52)	0.16	3.66-4.30	3.31(0.45)	0.90	1.55-5.08	3.57(0.40)	0.14	3.30-3.82	4.15(0.39)	0.23	3.69-4.60
C1/2	4.26(0.56)	0.16	3.95-4.57	3.88(0.47)	1.02	1.88-5.88	4.15(0.45)	0.13	3.90-4.40	4.20(0.47)	0.35	3.51-4.89
C3/4	5.25(0.59)	0.14	4.99-5.52	5.24(0.96)	1.05	3.71-7.31	5.43(1.47)	0..91	3.64-7.21	5.16(0.65)	0.27	4.64-5.68
Cc	6.50(0.71)	0.13	6.24-6.76	6.75(0.63)	0.20	6.36-7.13	6.15(0.82)	0.14	5.88-6.43	6.85(0.61)	0.14	6.58-7.12
Ri	8.73(0.97)	0.16	8.42-9.04	8.16(0.67)	0.19	7.80-8.53	8.75(0.81)	0.13	8.86-9.49	8.45(0.50)	0.11	8.24-8.66
R1/4	10.32(0.99)	0.16	10.00-10.63	9.54(0.54)	0.11	9.32-9.76	10.32(0.74)	0.13	10.06-10.59	9.78(0.54)	0.09	9.61-9.96
R1/2	11.24(0.95)	0.15	10.94-11.53	10.58(0.58)	0.11	10.37-10.80	11.12(0.80)	0.15	10.83-11.40	10.79(0.53)	0.08	10.64-10.94
R3/4	12.25(0.89)	0.16	11.94-12.55	11.80(0.43)	0.10	11.61-11.99	11.90(0.87)	0.15	11.60-12.20	11.82(0.64)	0.07	11.68-11.96
Rc	12.93(0.57)	0.14	12.61-13.13	12.70(0.46)	0.11	12.48-12.92	12.93(0.67)	0.22	12.50-13.37	12.87(0.60)	0.08	12.73-13.02
A1/2	13.63(0.54)	0.13	13.38-13.88	14.25(0.41)	0.17	13.92-14.58	13.93(0.63)	0.12	13.69-14.17	14.05(0.60)	0.08	13.90-14.20
Ac	14.59(0.67)	0.14	14.31-14.86	14.09(0.57)	0.71	12.70-15.48	14.36(0.45)	0.12	14.13-14.60	15.03(0.70)	0.13	14.78-15.28

(See Table 2 for abbreviations)

*Please refer to Appendix A.5.4 for the number of individuals (*N*) used to for calculating the mean of each stage

Table 6. Comparison of Mean ages of attainment for P1 stages in Northern Sudanese and Western Sudanese children using logistic regression.

	Northern Females (N=802)			Western Females(N=401)			Northern Males(N=844)			Western Males(N=848)		
Stage	Mean(SD)	SEM	95%CI	Mean(SD)	SEM	95%CI	Mean(SD)	SEM	95%CI	Mean(SD)	SEM	95%CI
Coc							3.05(0.27)	0.14	2.79-3.32			
C1/2	2.99(0.65)	0.35	2.31-3.68				4.14(0.27)	0.13	3.88-4.40			
C3/4	4.20(0.50)	0.13	3.93-4.46	3.66(0.67)	0.59	2.51-480	5.29(0.51)	0.11	5.06-5.51	3.93(0.76)	0.48	2.98-4.87
Cc	5.47(0.44)	0.11	5.26-5.69	4.97(0.78)	0.28	4.41-5.52	6.23(0.75)	0.13	5.97-6.48	5.23(0.69)	0.26	4.72-5.73
Ri	6.54(0.64)	0.12	6.30-6.78	6.82(0.65)	0.20	6.43-7.20	7.37(0.82)	0.16	6.06-9.69	6.99(0.63)	0.14	6.71-7.26
R1/4	9.06(0.95)	0.16	8.75-9.37	8.32(0.64)	0.18	7.97-8.66	9.03(0.80)	0.15	8.74-9.33	8.66(0.46)	0.10	8.46-8.86
R1/2	10.41(0.94)	0.15	10.12-10.70	9.70(0.54)	0.11	9.49-9.91	10.64(0.64)	0.12	10.39-10.88	10.00(0.51)	0.09	9.82-10.18
R3/4	11.41(0.92)	0.15	11.11-11.71	10.82(0.55)	0.10	10.63-11.01	11.28(0.64)	0.13	11.04-11.53	10.97(0.56)	0.07	10.83-11.12
Rc	12.52(0.72)	0.14	12.24-12.80	11.97(0.40)	0.10	11.77-12.16	12.06(0.74)	0.13	11.80-12.32	12.07(0.62)	0.07	11.93-12.21
A1/2	13.00(0.64)	0.13	12.75-13.25	12.95(0.51)	0.12	12.72-13.17	12.91(0.67)	0.14	12.64-13.18	13.13(0.60)	0.08	12.98-13.28
Ac	13.51(0.56)	0.32	12.88-14.15	13.94(0.53)	0.16	13.61-14.26	13.67(0.70)	0.13	13.42-13.93	13.90(0.48)	0.08	13.75-14.05

(See Table 2 for abbreviations)

*Please refer to Appendix A.5.5 for the number of individuals (*N*) used to for calculating the mean of each stage

Table 7. Comparison of Mean ages of attainment for C stages in Northern Sudanese and Western Sudanese children using logistic regression.

	Northern Females (N=802)			**Western Females(N=401)**			**Northern Males(N=844)**			**Western Males(N=848)**		
Stage	Mean(SD)	SEM	95%CI	Mean(SD)	SEM	95%CI	Mean (SD)	SEM	95%CI	Mean(SD)	SEM	95%CI
C3/4							2.97(0.49)	0.23	2.52-3.42			
Cc	3.93(0.55)	0.16	3.62-4.24	3.56(0.10)	1.22	1.18-5.95	4.05(0.67)	0.16	3.73-4.37	4.52(0.36)	0.24	4.05-4.98
Ri	5.18(0.48)	0.12	4.95-5.41	5.08(0.74)	0.26	4.57-5.58	5.21(0.61)	0.13	4.96-5.46	5.51(0.44)	0.18	5.16-5.86
R1/4	6.69(0.79)	0.14	6.42-6.96	6.90(0.51)	0.18	6.54-7.25	7.33(0.84)	0.15	7.04-7.63	7.09(0.59)	0.13	6.83-7.35
R1/2	9.04(1.01)	0.16	8.72-9.36	8.19(0.60)	0.40	7.39-8.89	9.75(0.70)	0.14	9.48-10.02	8.80(0.48)	0.10	8.60-9.00
R3/4	10.28(0.93)	0.15	9.98-10.58	8.89(0.65)	0.18	8.54-9.24	10.69(0.79)	0.14	10.41-10.96	9.99(0.51)	0.08	9.83-10.15
Rc	11.63(0.98)	0.16	11.32-11.94	10.83(0.55)	0.10	10.64-11.02	11.71(0.65)	0.20	11.33-12.10	11.36(0.64)	0.08	11.20-11.52
A1/2	12.67(0.82)	0.15	12.39-12.96	12.65(0.53)	0.11	12.44-12.87	13.15(0.74)	0.15	12.87-13.44	13.30(0.66)	0.08	13.14-13.45
Ac	13.49(0.68)	0.14	13.21-13.77	13.95(0.54)	0.40	13.16-14.78	13.86(0.61)	0.12	13.62-14.10	14.11(0.57)	0.08	13.95-14.26

(See Table 2 for abbreviations)

*Please refer to Appendix A.5.6 for the number of individuals (*N*) used to for calculating the mean of each stage

Table 8. Comparison of Mean ages of attainment for I2 stages in Northern Sudanese and Western Sudanese children using logistic regression.

	Northern Females (N=802)			Western Females(N=401)			Northern Males(N=844)			Western Males(N=848)		
Stage	Mean(SD)	SEM	95%CI	Mean(SD)	SEM	95%CI	Mean (SD)	SEM	95%CI	Mean(SD)	SEM	95%CI
Cc							2.40(0.37)	0.60	1.23-3.53			
Ri	3.61(0.46)	0.16	3.29-3.93	3.83(0.34)	0.33	3.19-4.47	3.27(0.65)	0.22	2.843.70			
R1/4	4.80(0.49)	0.12	4.56-5.04	4.63(00.40)	0.23	4.17-5.09	4.78(0.59)	0.13	4.52-5.03	4.77(0.50)	0.26	4.27-5.28
R1/2	5.97(0.51)	0.11	5.75-6.19	5.88(0.46)	0.15	5.58-6.18	5.90(0.63)	0.13	5.63-6.16	5.40(0.69)	0.23	4.95-5.86
R3/4	7.16(0.71)	0.13	6.90-7.42	7.09(0.41)	0.17	6.76-7.43	7.41(0.64)	0.14	7.15-7.687	6.70(0.61)	0.14	6.42-6.98
Rc	8.48(0.80)	0.15	8.20-8.77	8.17(0.46)	0.16	7.85-8.48	8.37(0.69)	0.14	8.09-8.66	8.28(0.55)	0.11	8.06-8.50
A1/2	9.01(0.48)	0.16	8.77-9.25	8.82(0..63)	0.13	8.52-9.12	9.62(0.59)	0.10	9.36-9.87	8.57(0.43)	0.13	8.38-8.75
Ac	9.36(0.59)	0.14	9.11-9.60	9.15(0.62)	0.16	8.87-9.43	9.35(0.39)	0.09	9.05-9.66	9.12(0.41)	0.12	8.95-9.25

(See Table 2 for abbreviations)

*Please refer to Appendix A.5.7 for the number of individuals (*N*) used to for calculating the mean of each stage

Table 9. Comparison of Mean ages of attainment for I1 stages in Northern Sudanese and Western Sudanese children using logistic regression.

	Northern Females (N=802)			**Western Females(N=401)**			**Northern Males(N=844)**			**Western Males(N=848)**		
Stage	Mean(SD)	SEM	95%CI	Mean(SD)	SEM	95%CI	Mean (SD)	SEM	95%CI	Mean(SD)	SEM	95%CI
Ri	3.04(0.42)	0.28	2.49-3.58									
R1/4	4.00(0.43)	0.12	3.75-4.24	3.83(0.34)	0.33	3.19-4.47	3.58(0.72)	0.20	3.18-3.98	3.46(0.75)	0.62	2.24-4.68
R1/2	5.15(0.48)	0.12	4.92-5.38	4.83(0.29)	0.92	3.02-6.63	5.02(0.57)	0.13	4.77-5.27	4.77(0.50)	0.26	4.27-5.28
R3/4	6.17(0.61)	0.12	5.93-6.41	5.88(0.46)	0.15	5.58-6.18	6.16(0.64)	0.12	5.92-6.39	5.40(0.69)	0.23	4.95-5.86
Rc	7.32(0.63)	0.12	7.08-7.56	7.10(0.41)	0.17	6.76-7.43	7.68(0.71)	0.14	7.40-7.96	6.75(0.59)	0.14	6.47-7.02
A1/2	7.69(0..71)	0.14	7.42-7.96	7.73(0.46)	0.17	7.40-8.07	6.87(0..42)	0.10	6.68-7.07	7.40(0.32)	0.09	7.23-7.58
Ac	8.25(0.62)	0.13	7.99-8.51	8.30(0.35)	0.15	8.01-8.59	7.76(0..48)	0.11	7.54-7.98	7.48(0.55)	0.10	7.29-7.67

(See Table 2 for abbreviations)

*Please refer to Appendix A.5.8 for the number of individuals (*N*) used to for calculating the mean of each stage

4.5 Formation Patterns and In Stage Analysis

Having presented mean ages of attainment, it is useful to examine the relationship between stages, variability and within stage patterns.

The numerical values for the estimate mean age of attainment are shown from Table 2 to Table 9 in section 4.4. Additionally the mean age of children is presented in Appendix 2.

Only some results are illustrated. Presenting all stages of all teeth in all the ethnic groups may be practically excessive given the constraints of this thesis. Therefore, selected stages were chosen for the purpose of presentation.

As a guide, previous findings of other investigators were used to identify teeth and stages of interest. For example, teeth M3 and P2 were chosen due to the frequent variability in the course of their formation. C is known to have sexual dimorphism both its formations as well as its tooth size. I2, M1 are known to be less variable, whilst M2 (a late forming tooth) is exposed to more environmental factors over its formation, such as nutritional status.

Smoothed curves (Figure 12 to Figure 35) provide a visual way to view results and establish trends in tooth formation. They also illustrate the minimum age and age when all individuals have reached that stage. This is important in age estimation but out of the remit of this study but presented for completion in Appendix 2. It is important to remember however that the curves are smoothed for illustrative purposes. In real terms cross-sectional tooth formation does not behave in such a linear form. Statistical methods using logistic regression are used for more precise calculations of mean age.

Figure 12 to Figure 35 illustrate the smoothed cumulative curves for selected stages in some permanent teeth. Illustrating results in this way yields a number of useful points. Statistical assessment of mean differences will be presented in section 4.6. The graphs also give an approximation of the mean ages of attainment. For more accurate assessment of means, SDs and 95% confidence intervals in section 4.4 (Table 2 to Table 9). An example is illustrated in Figure 12, where the mean age for stage Cr of M3 is 8.49 yrs (arrowed) for males.

Figure 12 to Figure 35 show the extent of variation between males and females as well as between groups. On the whole Western females are more advanced whilst Northern males are more advanced for the selected stages.

Other gender similarities can be seen in a number of teeth, namely, M1 (Figure 20 and Figure 21), P2 (Figure 24 and Figure 25), and I2 (Figure 33 and Figure 34).

Sexual dimorphism is evident in the canine with females being more advanced in both groups over their male counter parts for almost all stages (Figure 28 and Figure 29). Inter-ethnic canine comparisons show relative variability between the groups (Figure 30 and Figure 31).

A less clear picture emerges when groups are analysed. Similarities in mean ages are more predominant on crown stages while more variation is observed on the selected root stages between groups is M3 (Figure 14 and Figure 15), M2 (Figure 18 and Figure 19), M1 (Figure 22 and Figure 23) P2 (Figure 26 and Figure 27) and I2 (Figure 32 and Figure 33). The curves also yield information about the children within a stage. Appendices A.5.1 through to A.5.8 presents the mean ages for the children within a stage together with the range of age it was seen for the mandibular teeth..

4.5.1 Third Molar

4.5.1.1 Gender Comparisons

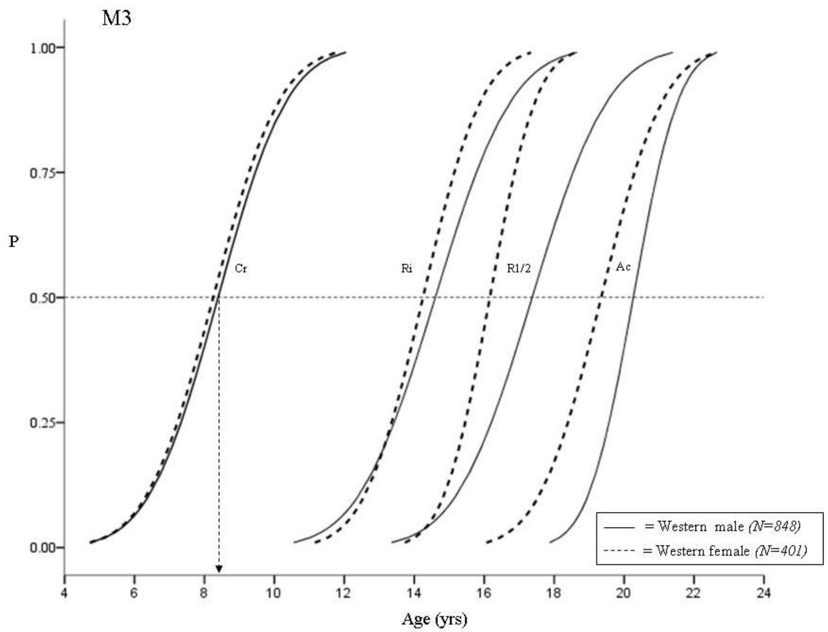


Figure 12. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M3 for Western males and Western females.

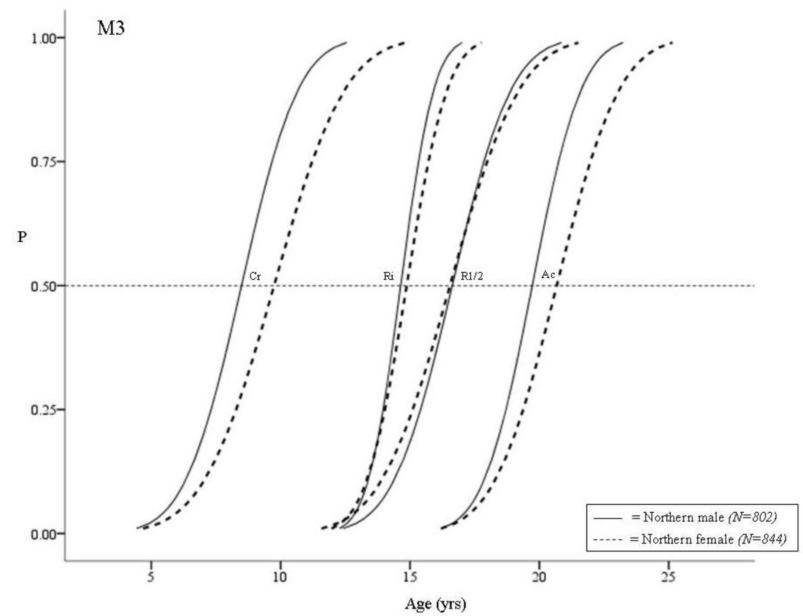


Figure 13. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M3 for Northern males and Northern females.

4.5.1.2 Ethnic Comparisons

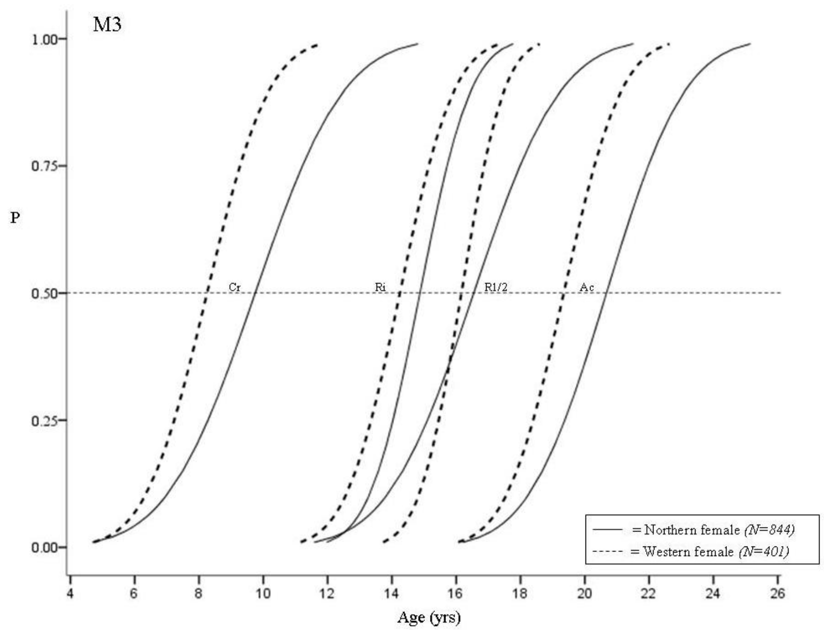


Figure 14. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M3 for Northern females and Western females.

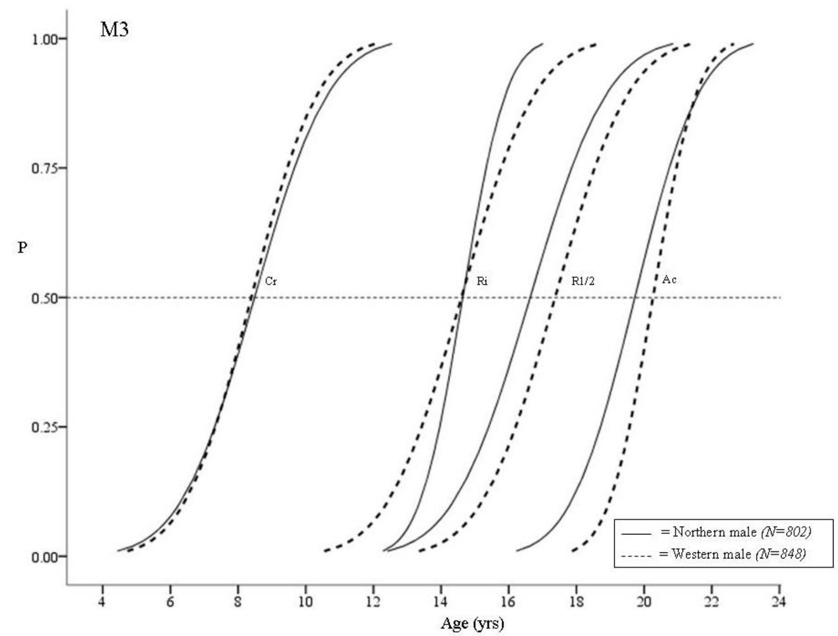


Figure 15. Smoothed cumulative curves comparing the mean age of attainment for some selected stages of M3 for Northern males and Western males.

4.5.2 Second Molar

4.5.2.1 Gender Comparisons

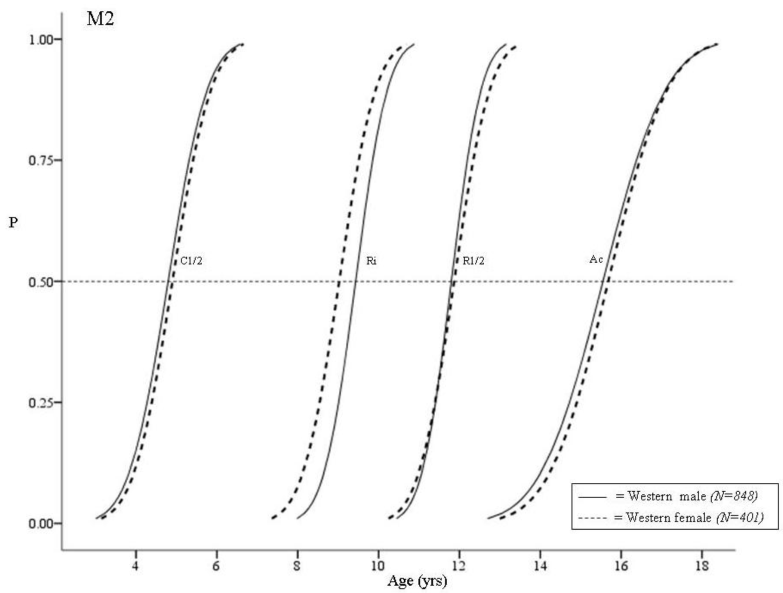


Figure 16. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M2 for Western males and Western females.

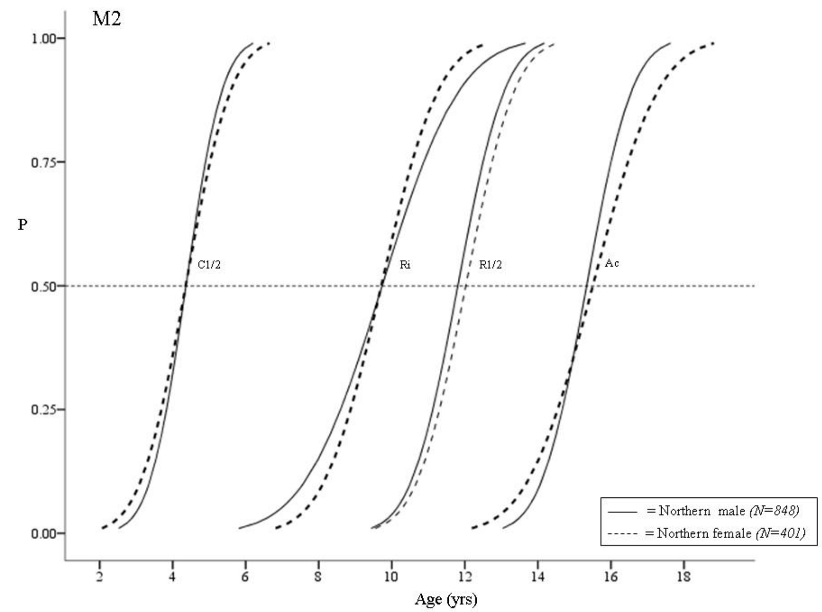


Figure 17. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M2 for Northern males and Northern females.

4.5.2.2 Ethnic Comparisons

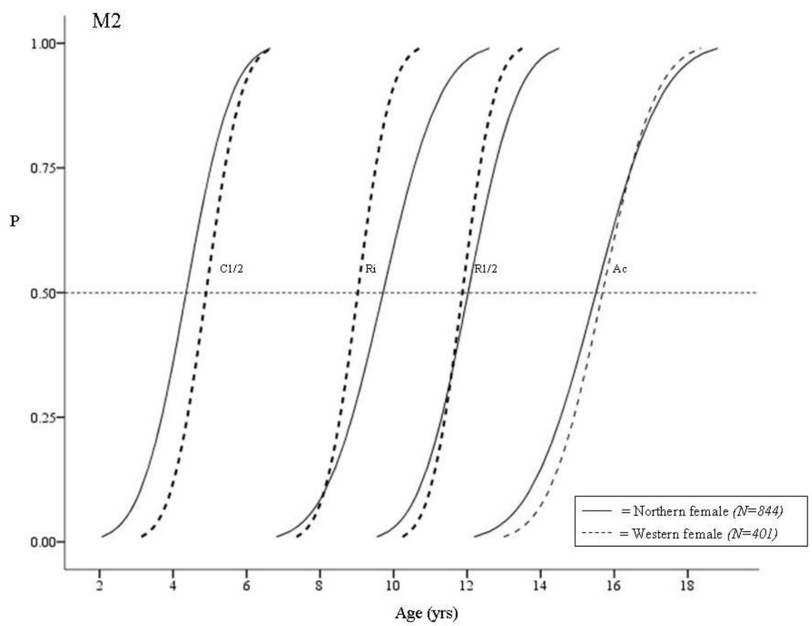


Figure 18. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M2 for Northern females and Western females.

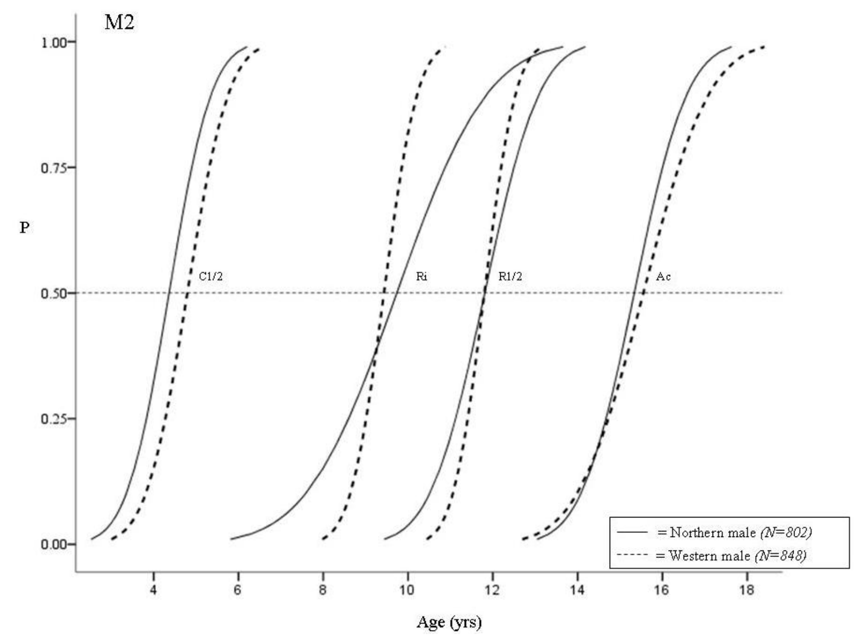


Figure 19. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M2 for Northern males and Western males.

4.5.3 First Molar

4.5.3.1 Gender Comparisons

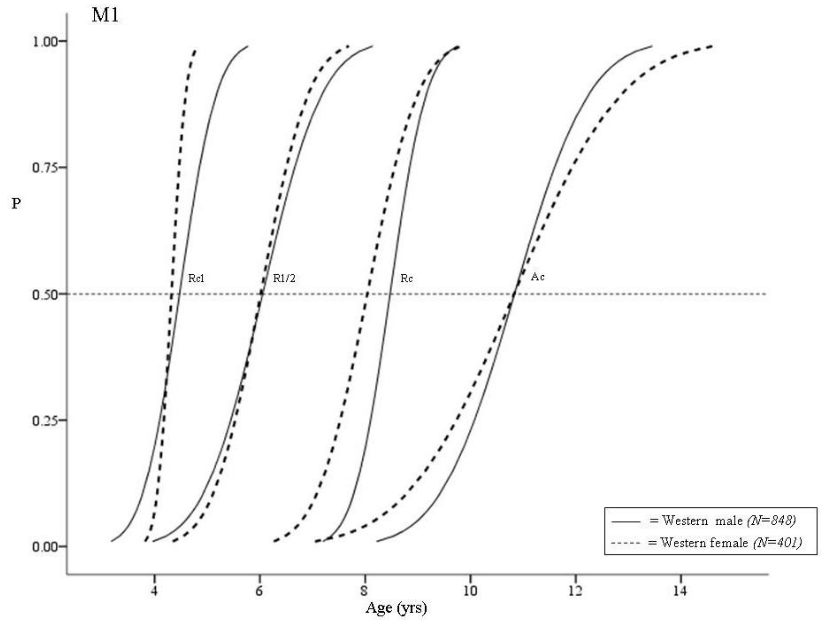


Figure 20. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M1 for Western males and Western females.

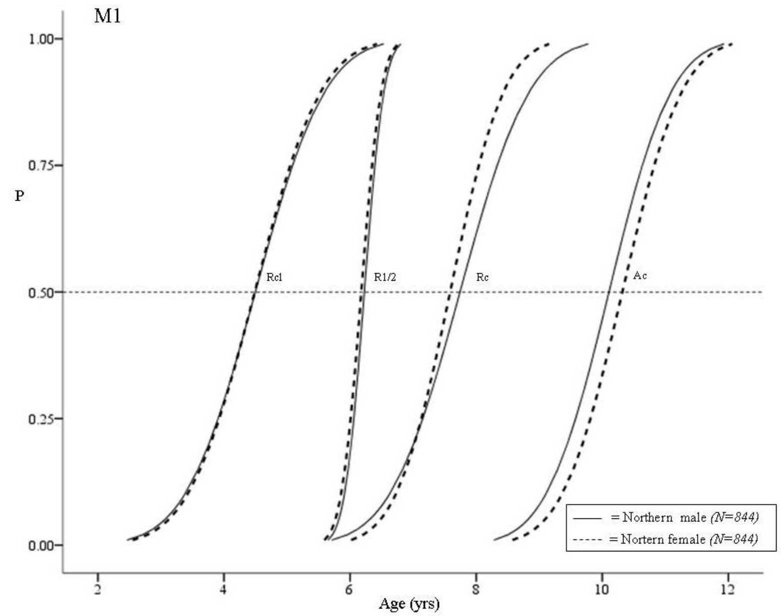


Figure 21. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M1 for Northern males and Northern females.

4.5.3.2 Ethnic Comparisons

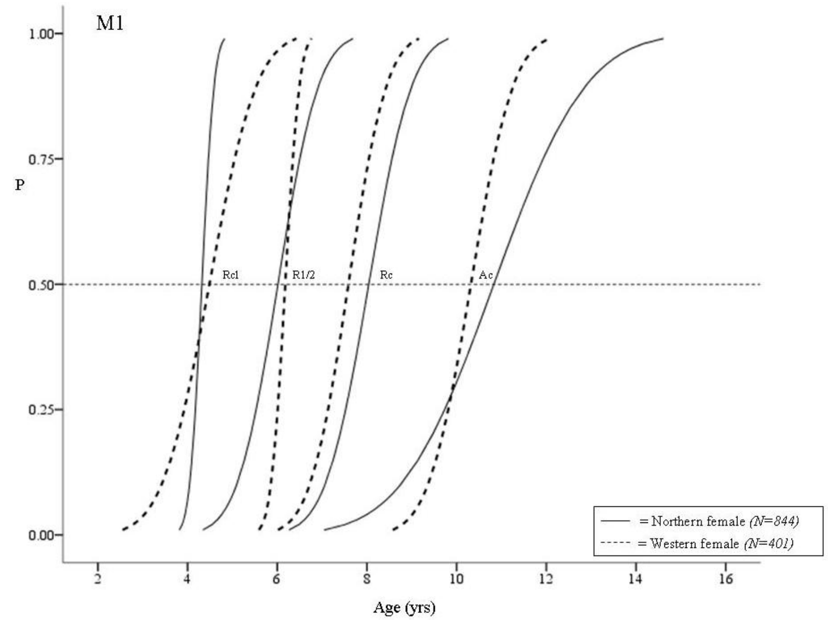


Figure 22. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M1 for Northern males and Western females.

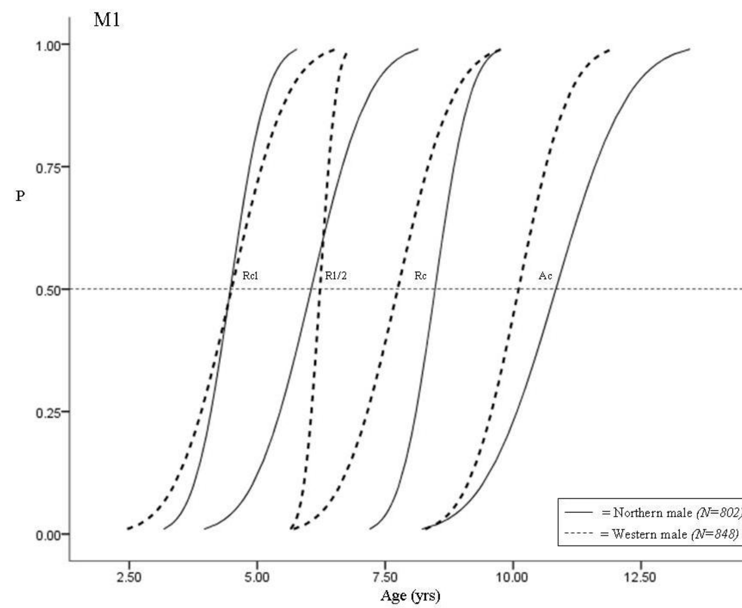


Figure 23. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M1 for Northern males and Western males.

4.5.4 Second Premolar

4.5.4.1 Gender Comparisons

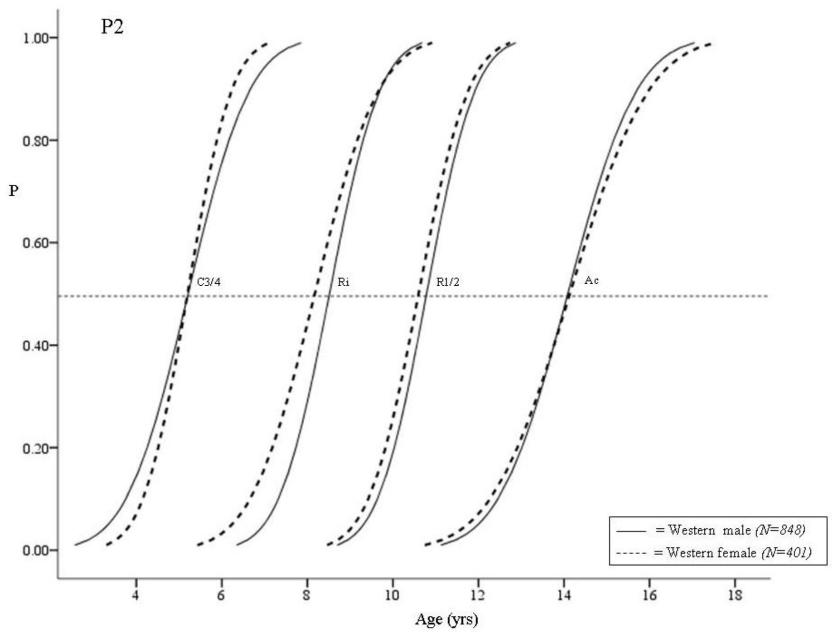


Figure 24. Smoothed cumulative curves comparing the mean age of attainment for selected stages of P2 for Western males and Western females.

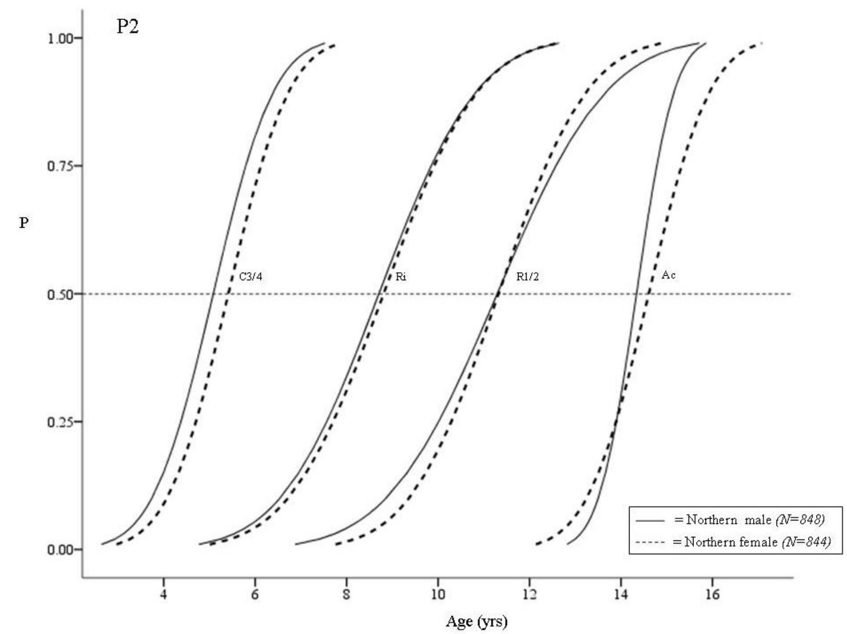


Figure 25. Smoothed cumulative curves comparing the mean age of attainment for selected stages of P2 for Northern males and Northern females.

4.5.4.2 Ethnic Comparisons

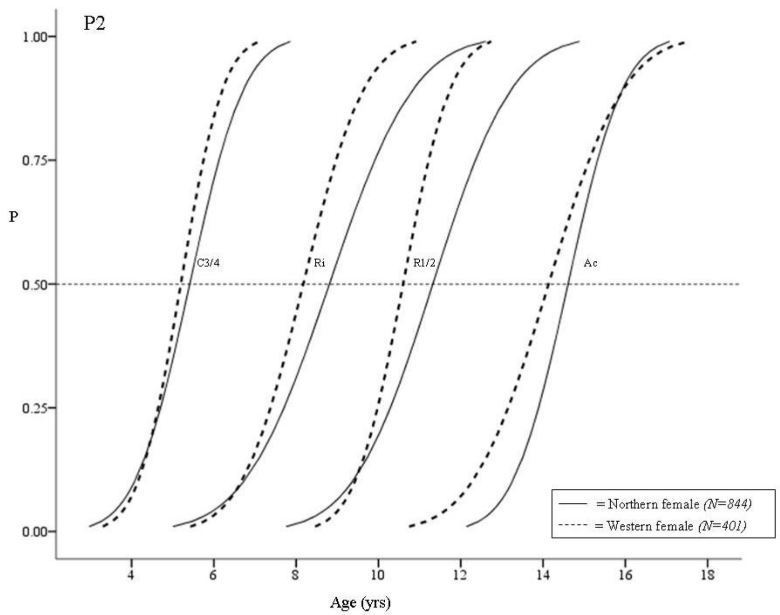


Figure 26. Smoothed cumulative curves comparing the mean age of attainment for selected stages of P2 for Northern females and Western females.

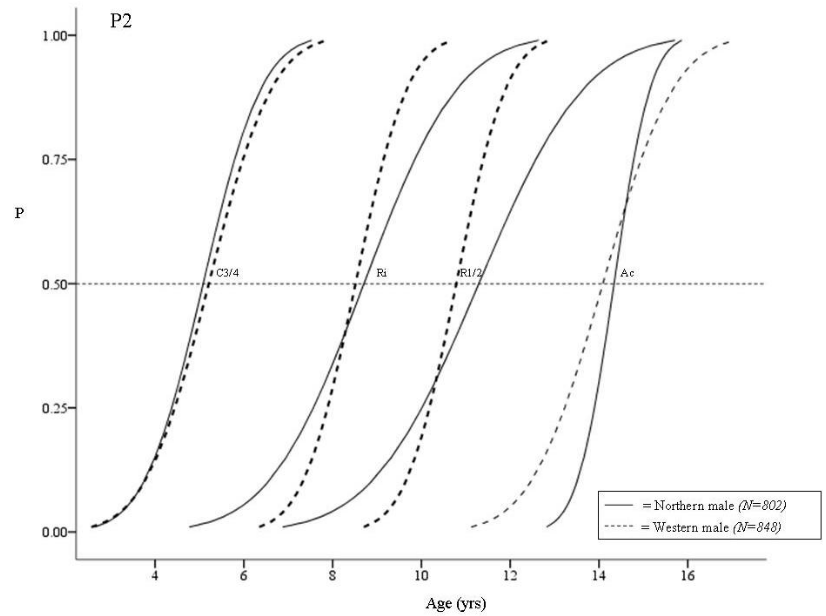


Figure 27. Smoothed cumulative curves comparing the mean age of attainment for selected stages of P2 for Northern males and Western males.

4.5.5 Canine

4.5.5.1 Gender Comparisons

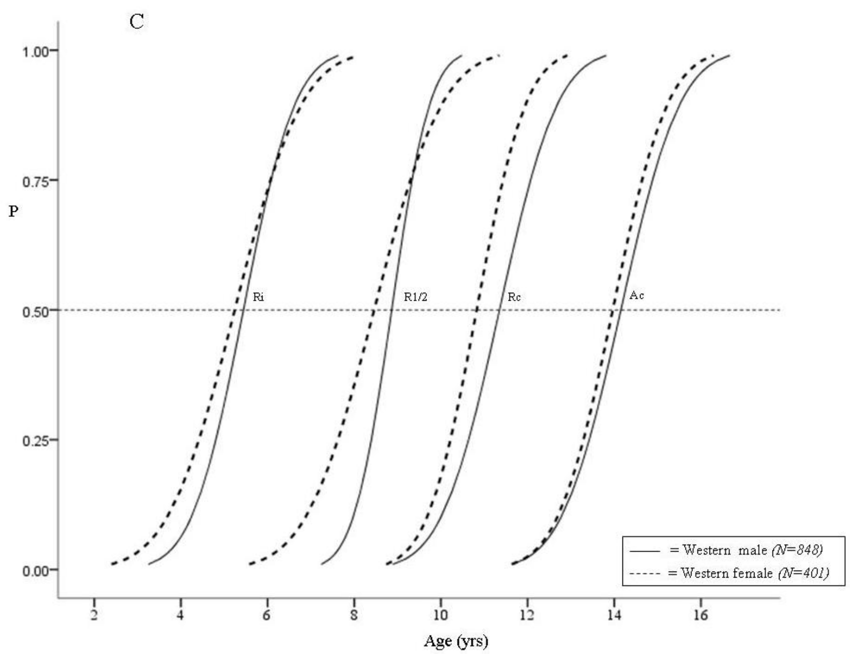


Figure 28. Smoothed cumulative curves comparing the mean age of attainment for selected stages of C for Western males and Western females.

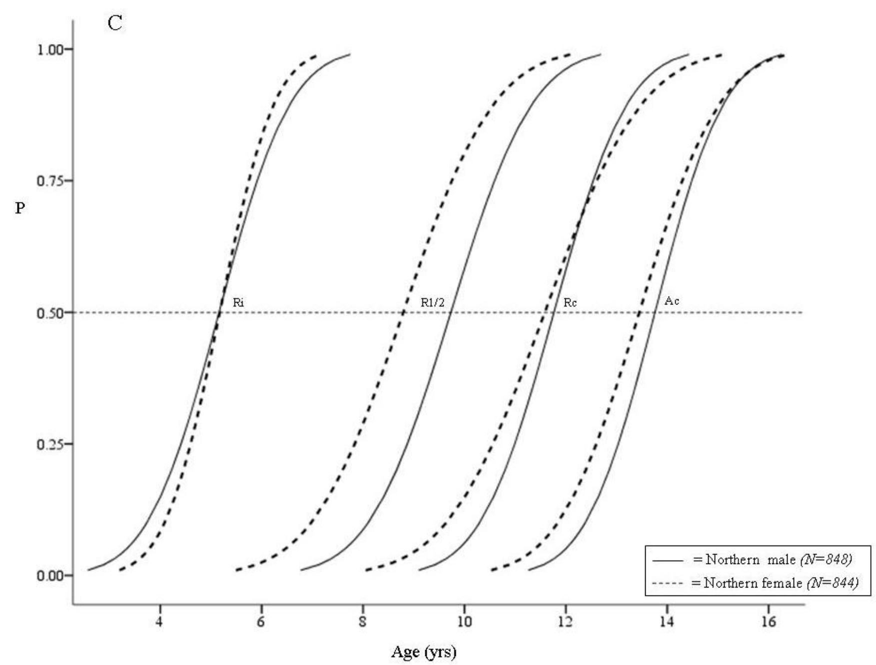


Figure 29. Smoothed cumulative curves comparing the mean age of attainment for selected stages of C for Northern males and Northern females.

4.5.5.2 Ethnic Comparisons

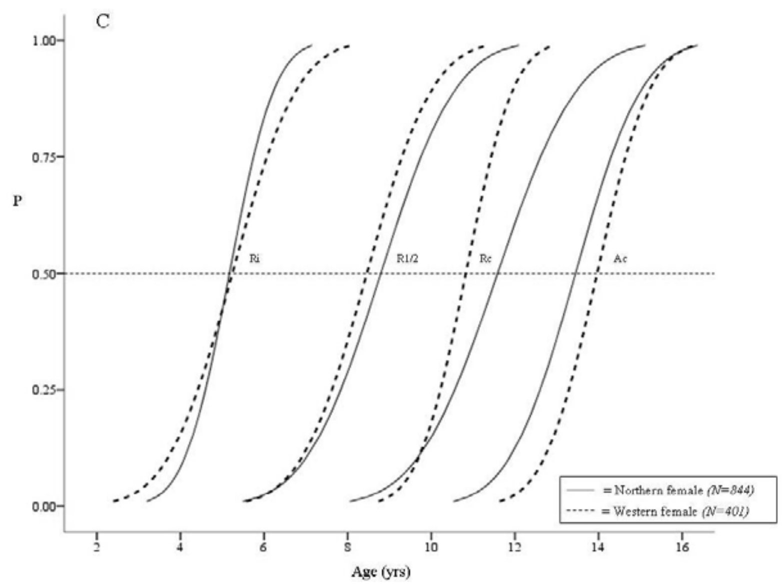


Figure 30. Smoothed cumulative curves comparing the mean age of attainment for selected stages of M1 for Northern females and Western females.

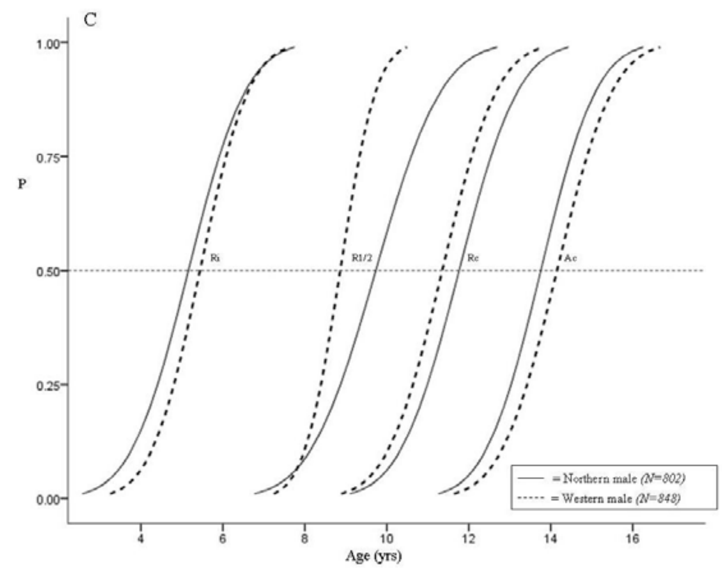


Figure 31. Smoothed cumulative curves comparing the mean age of attainment for selected stages of C for Northern males and Western males.

4.5.6 Lateral Incisor

4.5.6.1 Gender Comparisons

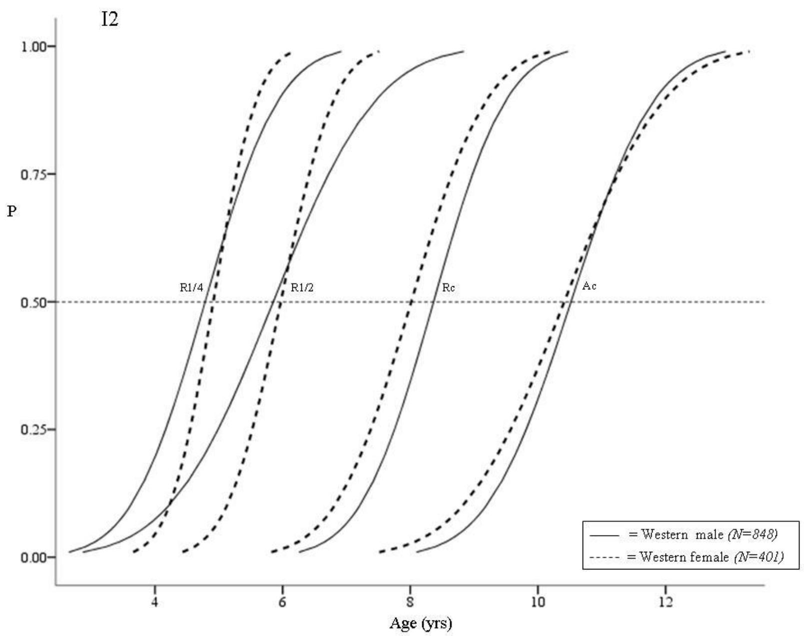


Figure 32. Smoothed cumulative curves comparing the mean age of attainment for selected stages of I2 for Western males and Western females.

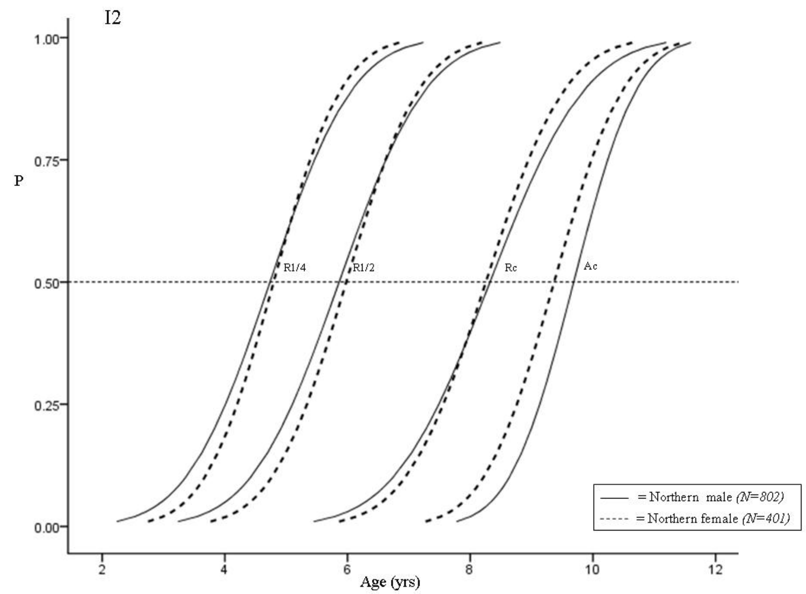


Figure 33. Smoothed cumulative curves comparing the mean age of attainment for selected stages of I2 for Northern males and Northern females.

4.5.6.2 Ethnic Comparisons

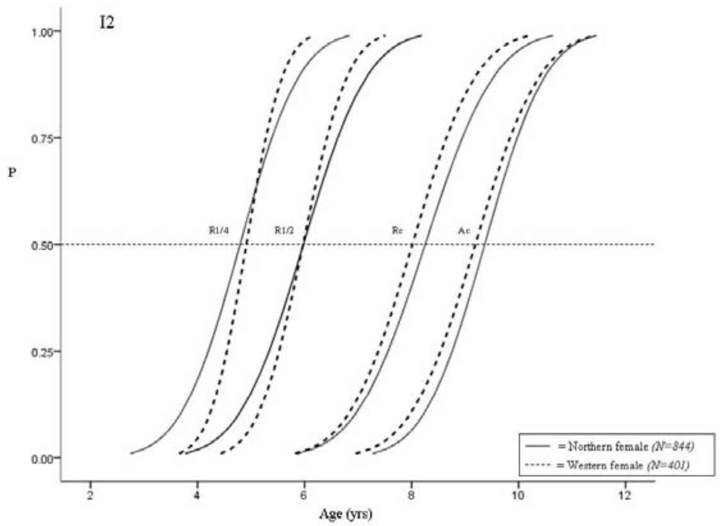


Figure 34. Smoothed cumulative curves comparing the mean age of attainment for selected stages of I2 for Northern females and Western females.

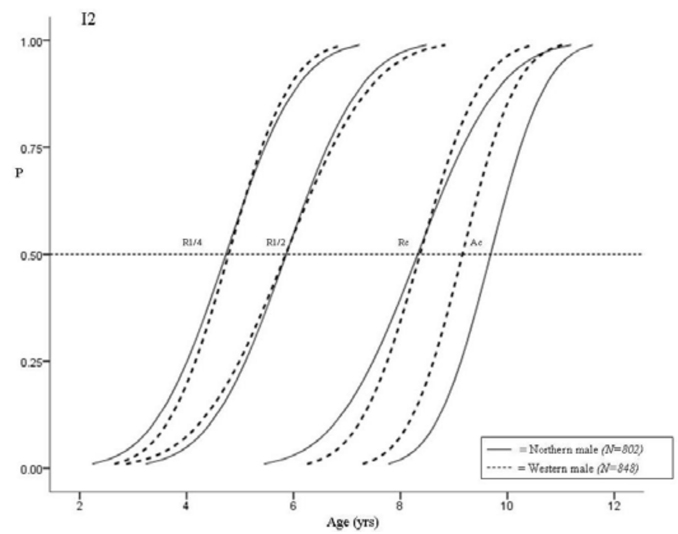


Figure 35. Smoothed cumulative curves comparing the mean age of attainment for selected stages of I2 for Northern males and Western males.

4.6 Group Comparisons

4.6.1 Inter-Ethnic Mean Age Comparisons

Figure 36 provides a summary of interethnic stage comparisons for males and females of different ethnic origin. The odds ratios and their 95% confidence intervals are presented in Appendix A.6.1 and A.6.2.

Male Comparisons	I_1	I_2	C	P_1	P_2	M_1	M_2	M_3
Cr							.	↑**
Ci							.	↑**
Cco					.		.	↑**
Coc				.	.		.	↓**
C1/2				.	.		.	.
C3/4	.	.	.	.	.		.	↓*
Cc	.	.	.	.	.	.	.	.
Ri	.	.	.	↑**	↑*	.	↑*	.
Rcl						.	.	.
R1/4	.	.	.	↑**	↑**	.	↑**	.
R1/2	.	↑*	↑**	↑**	↑*	.		↓*
R3/4	↑**	↑**	↑**	↑*	.	.	.	↑*
Rc	↑**	.	↑*	.	.	↑**	.	.
A1/2	.	↑*	.	.	.	↑**	.	.
Ac	↑*	↑**	.	.	↓**	↑**	.	.

Female Comparisons	I_1	I_2	C	P_1	P_2	M_1	M_2	M_3
Cr							.	↑**
Ci							.	↑**
Cco							.	↑**
Coc				.	↑*		.	↑**
C1/2				.	↑*		.	↑**
C3/4	.	.	.	.	↑*		.	↑**
Cc	.	.	.	.	.	.	.	.
Ri	.	.	.	.	↑**	.	↑**	.
Rcl						.	↑**	.
R1/4	.	.	.	↑*	↑**	.	.	.
R1/2	↑*	.	↑**	↑**	↑**	.	↑*	.
R3/4	.	.	↑**	↑*	.	.	.	.
Rc	.	.	↑**	↑*	.	↑*	.	.
A1/2	↑**	↑*	.	.	↓*	.	.	.
Ac	↑*	↑**	.	.	.	↑**	.	↑*

Figure 36 Summary of inter-ethnic male (N=87) and female (N=86) mean age of attainment stage comparisons.

- ■ Stage assessed
- . Indicates no difference exists in mean age entering tooth stage
- * indicates significant difference in mean age entering tooth stage ($p<0.05$).
- ** Indicates highly significant difference in mean age entering tooth stage ($p<0.001$).
- ↑ Indicates advanced mean age of entering a particular tooth stage for the non-reference group *(Northern group is reference)*
- ↓ Indicates an delayed mean age of entering a particular tooth stage for the non-reference group *(Northern group is reference)*

Figure 36 shows that there is a general tendency of Western males to be more advanced in some stages of tooth formation than Northern males.

When 87 stages were compared for males from the two ethnic groups, 30 (34.48%) had significant differences with the Western group being more advanced. An exception was M3 which had two crown stages (Coc and C3/4) and one root stage (R1/2) that were more advanced in the western group (Figure 36).

Of the 15 stage comparisons assessed for M3, seven were significantly more advanced in with Western males, namely stages Cr, Ci, Cco and Coc (0.14, 1.09, 0.27and 0.12 years respectively) with a high level of significance ($p<0.001$) and stages C3/4, R1/2 and R3/4 (0.78, 0.90 and 0.75 respectively) showing significant differences ($p<0.05$).

Of the 12 stage comparisons assessed for M 2 two comparisons, namely; stages Ri (0.32 years; $p<0.05$) and R1/4 (0.40; $p<0.001$) were more advanced in Western males.

For M1, of the 8 stage comparisons assessed 3 stage comparisons (Rc, A1/2 and Ac) showed high levels of significant differences ($p<0.001$) with Western males being more advanced for all three stages by 0.85, 0.70 and 0.75 years respectively.

Of the 7 stage comparisons assessed for P2, four comparisons showed levels of significance. Stages Ri and R1/2 were significantly advanced in Western males by 0.30 and 0.33 years respectively ($p<0.05$) whilst stage R1/4 was advanced by 0.54 years ($p<0.001$). An exception was stage Ac which that the Northern male group was more advanced in the mean of attainment by 0.67 years ($p<0.001$).

P1 had a total of 7 stage comparisons with 4/7 stages more advanced in Western males. The stages are Ri, R1/4 and R1/2 with a difference of 2.38, 0.36 and 0.31 years respectively ($p<0.001$). Stage R3/4 was advanced in the same group by 0.59 years ($p<0.05$).

Of the 8 stage comparisons assessed for C 3 comparisons namely; stages R1/2 and R3/4 were more advanced in Western males by 0.95 and 0.75 years respectively ($p<0.001$) and stage Rc by 0.58 years ($p<0.05$).

In all I2 had 6 stage comparisons assessed of which 4 were more advanced in Western males, namely; stages R3/4 and Ac and were advanced by 0.71 and 0.72 years ($p<0.001$). Stages R1/2 and A1/2 were more advanced by 0.50 and 0.36 years respectively ($p<0.05$).

The story is somewhat similar for I1. Of the 6 stage comparisons assessed 3 comparisons were more advanced in Western males; namely stages R3/4 and Rc by 0.76 and0.93 respectively ($p<0.001$). Stage Ac was advanced by 0.40 years ($p<0.05$).

When female inter-ethnic comparisons were considered 30/86 (36.1%) were more advanced in Western females. Of these 13 had high levels of significance ($0<0.001$). One stage in P2 (stage A1/2) was more advanced in Northern females.

Of all the teeth M3 was the most advanced in mostly in crown stages. The stage comparisons assessed 7/15 were more advanced in Western females. Stages Cr, Ci, Cco, Cc, C1/2 and C3/4 were advanced by 0.43, 0.34, 0.37 and 0.53 years respectively ($p<0.001$) whilst Ac a significantly was more advanced by 0.30 ($p<0.05$).

M2 showed relatively less ethnic variation between females than did M3. Of the 13 stage comparisons assessed 3 stages; namely, Ri and Rcl were more advanced in Western females by 0.71 and 0.65 ($p<0.001$) whilst stage R1/2 was more advanced by 0.47 years ($p<0.05$).

For M1 only 2/8 stages were more advanced in Western females. Stage Rc was more advanced by 0.65 years ($p<0.05$) and stage Ac by 0.47 years ($p<0.001$).

P2 had a relatively high stage comparison differences with 7/11 (63.6%) most of which were more advanced for western females. Stages Ri, R1/4 and R1/2 were advanced by 0.57, 0.78 and 0.66 years ($p<0.001$) whilst stages Coc, C1/2 and C3/4 were advanced in Western females by 0.67, 0.38 and 0.38 years respectively ($p<0.05$). Stage A1/2 did not follow the trend and was more advanced in Northern females by 0.62 years ($p<0.05$).

Of the 10 stage comparisons assessed for P1, 4 were more advanced in Western females. Stage R1/2 was advanced by 0.71 years ($p<0.001$) and stages R1/4, R3/4 and Rc were more advanced by 0.73, 0.59 and 0.55 years respectively ($p<0.05$).

For C 3/8 stage comparisons, namely stages R1/2, R3/4 and Rc were more advanced by 0.85, 1.39 and 0.80 ($p<0.001$).

I2 had 2/7 stages comparisons in the root stages more advanced in Western females. Stage A1/2 was more advanced by 0.48 ($p<0.05$), whilst stage Ac was advanced by 1.09 ($p<0.001$).

I1 showed a similar trend to I2. The Western females were more advanced by 0.64 years for stage A1/2 ($p<0.001$), 0.32 year for stage R1/2 and 0.45 years for stage ($p<0.05$).

4.6.2 Intra-Ethnic Gender Mean Age Comparisons

Within each group male female comparisons were carried out to determine if differences between the sexes exist.

For the Northern group (male-female), differences were detected in 13/81 (16.1%) stage comparisons, of which 12 were significant (p< 0.05) and 1 highly significant (p< 0.001); Figure 37. The odds ratios and their 95% confidence intervals are presented in Appendix A.6.3.

	I_1	I_2	C	P_1	P_2	M_1	M_2	M_3
Cr								.
Ci							.	.
Cco					.		.	.
Coc					.		.	↓*
C1/2				.	.		.	↓*
C3/4				.	.		.	↓*
Cc			.	.	.	.	.	.
Ri		.	.	.	.	.	.	.
Rcl						.	.	.
R1/4	.	.	↑*	.	.	.	.	.
R1/2	.	.	↑*	.	.	.	.	.
R3/4	.	.	↑*	.	.	↑*	.	.
Rc	.	.	.	↓*	.	.	.	↓*
A1/2	.	.	↑*	.	.	.	.	↓*
Ac	.	.	↑*	.	.	.	.	↓**

Figure 37. Gender comparisons of mean age of stage attainment in the Northern group (N=81).

■ Stage assessed
. Indicates no difference exists in mean age entering tooth stage
* indicates significant difference in mean age entering tooth stage (p<0.05).
** Indicates highly significant difference in mean age entering tooth stage (p<0.001).
↑ Indicates a delayed mean age of entering a particular tooth stage for the non-reference group *(Males are reference group)*
↓ Indicates an advanced mean age of entering a particular tooth stage for the non-reference group *(Males are reference group)*

The bulk of mean age differences between Northern males and females were seen on two teeth (C and M3). Females were more advanced in root stage formation of C, whilst males were more advanced in some crown and some root stages of M3.

Of the stage comparisons assessed for M3 6/15 were more advanced in Northern males. Stages Coc, C1/2, C3/4, Rc and A1/2 were advanced by 0.65, 0.47, 0.85, 0.65 and 0.68 years ($p<0.05$) and stage Ac was advanced by 0.91 years ($p<0.001$).

Of the 9 stage comparisons assessed for M1, one stage was more advanced in Northern females by 0.43 years ($p<0.05$).

For P1, 1/10 stage comparison (Rc) was more advanced in advanced in Northern females by 0.46 years ($p<0.05$).

C had the 10 stages assessed, 5 of which were more advanced in Northern females; namely R1/4, R1/2, R3/4, A1/2 and Ac and were advanced by 0.64, 0.71, 0.41, 0.48 and 0.37 ($p<0.05$).

When the Western group is considered females were more advanced in 10/77 (12.99%) stage comparisons, 8 of which were significant ($p<0.05$) and 2 highly significant ($p<0.001$). Three teeth were specifically affected; M3, M2, and C. Nearly all root stages were advanced in Western females for C Figure 38. The odds ratios and their 95% confidence intervals are presented in Appendix A.6.3.

	I_1	I_2	C	P_1	P_2	M_1	M_2	M_3
Cr								.
Ci								.
Cco								.
Coc					.		.	.
C1/2				.	.		.	.
C3/4				.	.		.	↑**
Cc			.	.	.		.	↑**
Ri			.	.	.	.	↑*	↑*
Rcl						.	↑*	.
R1/4	.	.	.	.	.	.	.	.
R1/2	.	.	↑*	↑*	.	.	↑*	.
R3/4	.	.	↑*	.	.	.	.	.
Rc	.	.	↑**	.	.	.	.	.
A1/2	.	.	↑**	.	.	.	.	.
Ac	.	.	.	.	.	.	.	.

Figure 38. Gender comparisons of mean age of stage attainment in the Western group (N=77)

■ Stage assessed
. Indicates no difference exists in mean age entering tooth stage
* indicates significant difference in mean age entering tooth stage ($p<0.05$).
** Indicates highly significant difference in mean age entering tooth stage ($p<0.001$).
↑ Indicates a delayed mean age of entering a particular tooth stage for the non-reference group *(Males are reference group)*
↓ Indicates an advanced mean age of entering a particular tooth stage for the non-reference group *(Males are reference group)*

Of the 15 stages comparisons assessed for M3, 3 stages were more advanced in Western females; namely, stage Ri by 1.04 years ($p<0.05$), C3/4 and Cc by 1.00 and 0.35 years respectively ($p<0.001$).

M2 had 3/12 stage comparisons and Western females were more advanced in stages Ri, Rcl and R1/2 by 0.44, 0.30 and 0.22 years respectively ($p<0.05$).

For C, Western females were more advanced in 4/8 root stages. The stages were R1/2, R3/4, Rc and A1/2 and were advanced by 0.61, 1.1, 0.53 and 0.65 respectively ($p<0.05$).

4.7 Analysis Height and Weight

As explained earlier the both references published by the Center for disease Control and prevention and the WHO references will be used together with the standards published by the WHO for children less than 60 months of age. The purpose of this exercise is to make meaningful comparison about each group. Of the two references each has its advantages and disadvantages but both will be used for the purpose of this study. As discussed previously a z-score of -2 or less signifies the extreme in growth and nutritional status when compared to standards and references and can be gauged by different growth indicators.

4.7.1 Center of Disease Control and Prevention References

4.7.1.1 Body Mass Index z-Scores

BMIz denotes thinness and can be used as a clinical measure to establish the relative well being of individuals, groups and populations (Cole et al., 2007).

Using the CDC references to analyze both groups it can be seen that 26.10% of the group had z-score less than -2 Table 10 which is on the lower extreme of the BMI z-scores and signifies grade 3 thinness (CDC, 2000). It is noteworthy to mention that the World Health Organization denotes a population as exhibiting signs and growth problems and malnutrition if more than 2.3% of the population exhibits z-scores under -2. Grade 1 and 2 thinness is exhibited by 50.52% of the entire group (-2 > z-score > 0). The overall picture reflects advanced thinness in the whole Sudanese child population.

Table 10. Summary of frequency BMI z-scores using the CDC reference for males and females in two ethnic groups (N=2583).

	BMIz				
	z <-2	-2> z >0	0< z <+2	z>+2	N
Northern females	135	338	182	14	669
Western females	111	229	39	2	381
Northern males	219	333	130	21	703
Western males	210	536	76	2	833

When the groups are considered separately, the Northern female group exhibited 21.18% grade 2 thinness (z < -2); Figure 39. This is illustrated in Table above. Grades 1 and 2 thinness was seen in 51% of the group (-2 > z-scores >0).

The Western female group had 29.1% with grade 3 thinness whilst 60.10% presented with grade 1 and 2 thinness.

In Northern males 31% had grade 3 thinness and 47.37% grade 1 and 2 thinness. The Western males exhibited 25.21% grade 3 thinness and 64.35% grade 1 and 2 thinness.

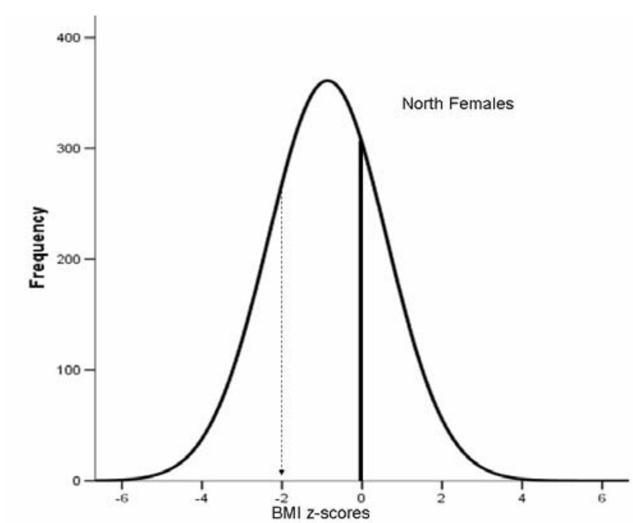


Figure 39. Normal distribution with cut-off (arrow) for BMIz for Northern females showing prevalence of malnutrition

4.7.1.2 Height for Age z-Scores

The entire group exhibited 16.03% z-scores less than -2. This signifies growth stunting and malnutrition according to the height for age index that is common to the entire group (Table 11). The Western males were most affected with 26.75% exhibiting growth stunting and severe malnutrition. The Western females were closely behind with 22.31%. The Northern group is relatively less affected with 7.1% of the male group and 8.5% of the female group exhibiting signs of stunting and malnutrition. As with other indices the cut-off was $z < -2$. It can be concluded that all groups had stunted growth and that malnutrition was prevalent.

Table 11. Summary of frequency height for age z-scores using the CDC reference for males and females in two ethnic groups (N=2583).

	HA z-score				
	z < -2	-2> z >0	0< z <+2	z>+2	N
Northern Females	57	773	225	14	669
Western females	85	232	61	3	381
Northern males	50	369	250	34	703
Western males	222	525	81	2	830

4.7.1.3 Weight for Age z-scores

Overall 28.23% of the group had $z < -2$ scores, indicating that growth is faltering in all groups, The Western female and males were particularly affected with 37.27% and 38.74% respectively, exhibiting z-scores less than the cut-off of -2 (Table 12 and Table 13). Again although affected, but to a lesser degree, both Northern females and males exhibited growth faltering (18.68% and 20.06 respectively).

Table 12. Summary of frequency weight for age z-scores using the CDC reference for males and females in two ethnic groups (N=2579).

	WA z-score				
	z <-2	-2> z >0	0< z <+2	z>+2	N
Northern Females	125	353	174	17	669
Western females	142	207	29	3	381
Northern males	141	383	154	21	703
Western males	320	459	46	1	826

4.7.2 World Health Organization

4.7.2.1 Body Mass Index z-Scores

Using the WHO growth references and standards and using the cut-off of 2.3% for both Northern and Western groups can be shown to display signs of thinness as well as being considerably underweight. Approximately 25 % of adults in both groups display z-scores of less than -2, an indication of malnutrition across the all groups. Nearly 5% in both groups display wasting (Table 13 and Table 14).

Table 13. Percentage of distribution of BMI z-scores for children more than 60 months in two ethnic groups compared to the WHO reference**.**

	BMI z-score					BMIz mean	BMIz SD	N
	<-3	<-2	>+1	>+2	>+3			
Northern group	5.0%	19.1%	12.4%	4.7%	1.6%	-0.73	1.50	1174
Western group	4.3%	20.2%	3.3%	0.60%	0%	-1.17	1.08	1162

Table 14. Percentage of distribution of BMI z-scores for children between 35 and 60 months in two ethnic groups compared to the WHO standard.

	BMI z-score					BMIz mean	BMIz SD	N
	<-3	<-2	>+1	>+2	>+3			
Northern group	7.5%	24.6%	5.2%	2.2%	2.2%	-1.01	1.47	134
Western group	11.1%	27.8%	5.6%	0%	0%	-1.37	1.38	18

4.7.2.2 Height for Age z-Scores

Both groups over 60 months exhibited stunting of growth according to height for age z-scores with 6.8% of the Northern group and 21.5% of the Western group being below the cut-off point (Table 15 and Table 16). The standard for HAz could not be as the number of individuals below 60 months was too small to make any inferences (Western group N=18).

Table 15. Percentage of distribution of height for age z-scores for children more than 60 months in two ethnic groups compared to the WHO reference**.**

	HA z-score					HAz mean	HAz SD	N
	<-3	<-2	>+1	>+2	>+3			
Northern group	1.1%	5.7%	13.3%	2.8%	0.60%	-0.17	1.15	1178
Western group	3.5%	18.0%	4.2%	0.3%	0.10%	-0.99	1.16	1163

Table 16. Distribution of children's (35-60 months) height for age z-scores two ethnic groups compared to the WHO standard.

	HAZscore		HAZmean	HAZ SD	N
	<-3	<-2			
Northern group	0.08%	1.5%	0.99	1.48	133
Western group	0%	0%	0.58	1.65	18

4.7.2.3 Weight for Age z-Scores

Weight for age z-scores indicate that of the Northern and Western groups 21.3% and 17.4% respectively were below the cut-off point and were underweight

(Table 17 and Table 18) while 4.8% of the Northern group exhibited severe underweight compared with 3.5% of the Western groups. Of the Northern (<60 months) group 4.4% were underweight, whilst the number of individuals were too small to make inferences about the Western (<60 months) group (N=18).

Table 17. Percentage of distribution of weight for age z-scores for children more than 60 months in two ethnic groups compared to the WHO reference.

	WA z-score					WAz mean	WAz SD	N
	<-3	<-2	>+1	>+2	>+3			
Northern group	4.8%	16.5%	8.6%	1.2%	0.20%	-0.74	1.25	419
Western group	3.5%	13.9%	1.7%	0.2%	0%	-1.03	1.00	403

Table 18. Percentage of distribution of weight for age z-scores for children between 35 and 60 months in two ethnic groups compared to the WHO standard.

	WA z-score		WAz mean	WAz SD	N
	<-3	<-2			
Northern group	0.7%	3.7%	-0.04	1.09	136
Western group	0%	0%	-0.53	1.01	18

4.7.2.4 Weight for Height z-Scores

Both Northern and the Western groups, over 60 months, had prevalent wasting with 25.6% and 38.9% respectively of each group being affected. Of note is that 11.1% of the Western group exhibited severe forms of wasting (Table 19).

Table 19. Percentage of distribution of weight for height z-scores for children more than 60 months in two ethnic groups compared to the WHO reference

	WH z-score					WHz mean	WHz SD	N
	<-3	<-2	>+1	>+2	>+3			
Northern group	5.3%	20.3%	5.3%	2.3%	2.30%	-0.192	1.41	133
Western group	11.1%	27.8%	5.6%	0%	0%	-1.33	1.34	18

4.7.3 Summary

The height for age z-score analysis indicated the stunting of both groups with the Western group more affected. The results are supported by weight for age z-score analysis which also showed that both groups are underweight with the problem being marginally more prevalent in the Northern group.

The weight for height z-scores indicated that wasting was common in all groups but more prevalent and markedly more severe in Western male and female groups.

The combination of height for age and weight for age z-score results show that both groups are stunted and underweight. Again the Western groups especially the females seem to be affected the most.

When weight for age z-scores and weight for height z-scores are considered together the results show that the Western groups are more underweight and show exhibit more severe forms of wasting than the Northern groups.

Combining the results of HA z-scores and WA z-scores, on the other hand, indicated that both groups have stunted growth. The Western group has a higher prevalence of stunting as well as severe forms of being underweight.

4.8 Tooth Formation and Malnutrition

The impact of malnutrition was tested by examining tooth stage development in against overall thinness, stunting of growth and being underweight (BMIz) by selecting those children with z-scores below the WHO cut-off ($z < -2$), and comparing them to groups above this cut-off point. The BMIz was chosen as the main factor to test tooth formation against. This is due to the fact that it takes into account the height and weight of individuals simultaneously. Low height for age z-scores indicate stunting whilst low weight of age scores indicate wasting as discussed in previous sections. Both would need to be investigated if BMI z-scores were shown to impact on tooth stage formation.

4.8.1 The Effect of Malnutrition on M2

Comparison of mean ages was carried out in groups identified have BMIz of -2 or under, signifying severe malnutrition, and compared with those who had BMIz of 0 or more. Of note, the entire group was undernourished and therefore had more children in it than the healthy group. The stages chose were (C1/2, Ri and R1/2 and Ac) for M2.

The test group consisting individuals with -2 z-scores or less was tested against two control groups. The first control group consisted of those with z-scores equal or higher than -1 and the second control group consisted of those with z-scores of 0 and above. Initially the effects of malnutrition were tested on females to eliminate variation introduced by sexual dimorphism (Figure 40 and Figure 41). The results show no statistically significant differences between the groups ($p<0.05$).

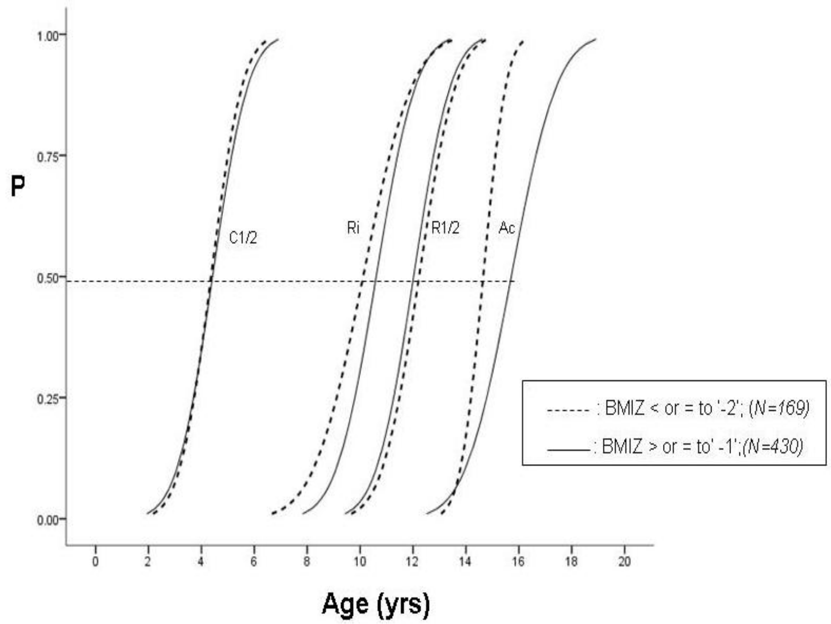


Figure 40. Cumulative curve of mean of age of attainment of some stages for tooth M2 for Northern females comparing children with malnutrition (BMIz<=-2) and those just above the cut-off (BMIz >= -1).

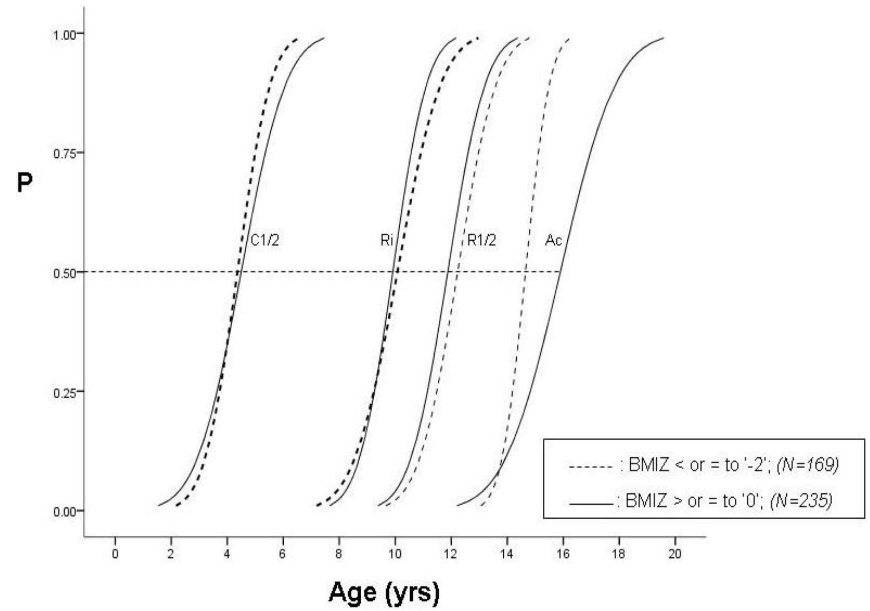


Figure 41. Cumulative curve of mean of age of attainment of some stages for tooth M2 for Northern females comparing children with malnutrition (BMIz<=-2) and normal females (BMIz >= 0).

The same test was repeated with the combination of males and females to increase the sample size given that boys and girls within each ethnic group. Additionally most groups are similar in their nutritional backgrounds. This was to ensure that there are enough children per group to allow for more robust analysis (Figure 42 and Figure 43). Of note is the similarity of mean of age of attainment of these chosen stages. Additionally some variability, indicated by the slope of the graph is also seen. No significant differences were detected any of the formation stages for any of the groups ($p>0.05$).

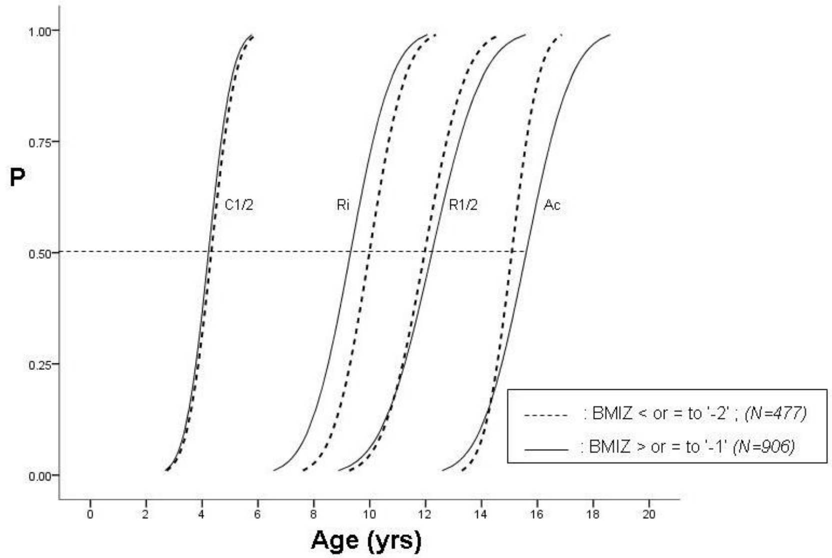


Figure 42. Cumulative curve of mean of age of attainment of some stages for tooth M2 for Northern males and females comparing children with malnutrition (BMIz <= -2) and those just above the cut-off (BMIz >= -1).

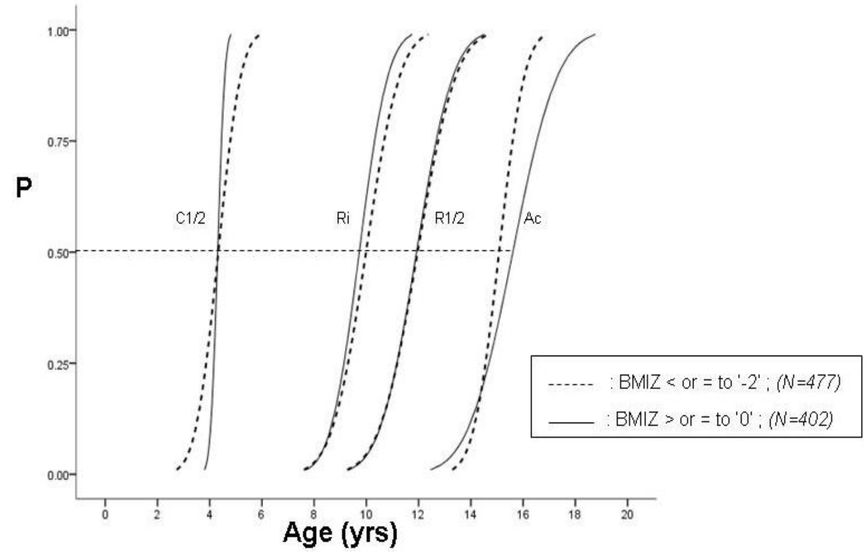


Figure 43. Cumulative curve of mean of age of attainment of some stages for tooth M2 for Northern males and females comparing children with malnutrition (BMIz <= -2) and those just above the cut-off (BMIz >= 0).

4.8.2 The Effect of Malnutrition on M1

The effect of malnutrition was further tested by examining the mean stage of attainment of stages of some stages (Rcl,R1/2, Rc and R1/2) of tooth M1 by comparing the group with malnutrition with control groups out lined earlier; (those with z-score of -2 or below against others).

M1 being an early forming tooth would adjust for the variability that is often seen with late forming teeth e.g. M3 and P2. Again the same groups of children with no sign of overall development problems were used as controls. The smoothed graphs presented in (Figure 44 and Figure 45) show very similar patterns to those seen in M2, suggesting minimal effect of malnutrition on tooth formation. Stage Ac shows some variability between groups due to the relatively small numbers of children in those groups ($p=0.048$), but only just. No differences were detected in the other formation stages in other groups.

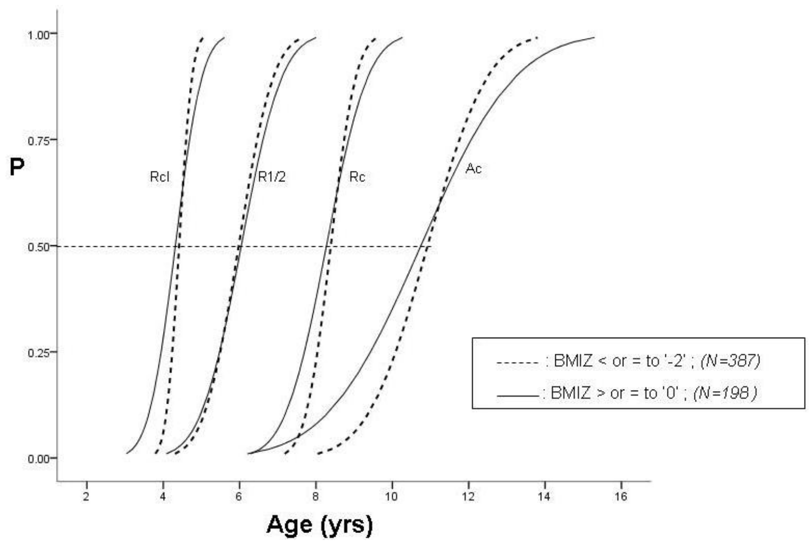


Figure 44. Cumulative curve of mean of age of attainment of some stages for tooth M1 for Northern males and females comparing children with malnutrition (BMIz <= -2) and normal children (BMIz >= 0).

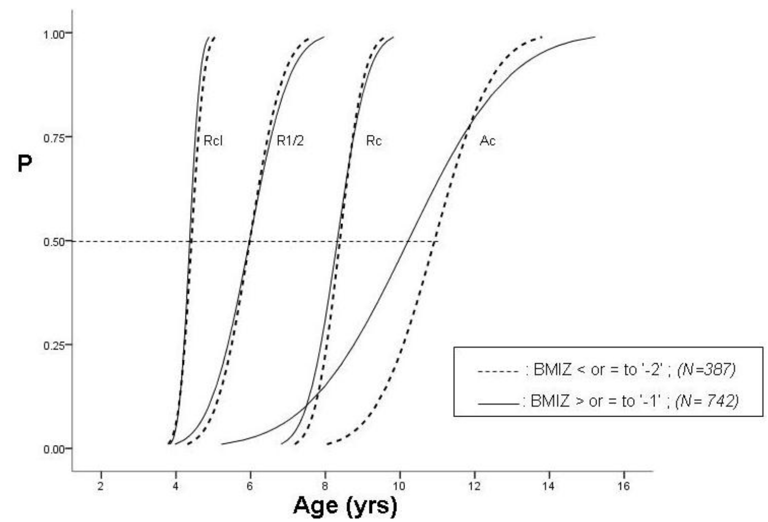


Figure 45. Cumulative curve of mean of age of attainment of some stages for tooth M1 for Northern males and females comparing children with malnutrition (BMIz <= -2) and those just above the cut-off (BMIz >= -1).

4.9 Hypodontia

4.9.1 Hypodontia (Excluding Third Molars)

The impact of hypodontia on tooth formation may be of some relevance due to the recent genetic advances that have made on this front. This section reports the prevalence of hypodontia in both groups. Table 20 summarised the prevalence of hypodontia in both groups.

Table 20. Frequency of hypodontia in two Sudanese ethnic groups.

	All teeth (except M3)	M3	Total
Western group(N=578)	4 (0.7%)	76 (13.2%)	80
Northern group (N=947)	25 (2.6%)	142 (15.0%)	167

** 1 child presented with oligodontia in Northern Sudanese.*

In the Northern group 947of 1646 subjects met the inclusion criterion of reaching or passing stage R3/4 of M2. This is shown in

Table 21. Of the Western group 578 of 1249 reached or passed the same stage and had a mean age of 14.63 years (SD2.42). The age is of importance as it could be indicative of possible previous extractions, mainly due to caries.

When M3 is excluded the northern group had 50 congenitally missing teeth (CMT) in a total of 25 children; in other words a ratio of 2 CMT per child. The Western group had 8 congenitally missing teeth in 4 subjects and a ratio 2 CMTs.

Table 21. Combined frequency of hypodontia for both sexes in Northern and Western subjects in both jaws.

	Northern Group N=947 subjects		Western Group N=578 subjects	
Tooth	Mandible *n*	Maxilla *n*	Mandible *n*	Maxilla *N*
M3	134	189	97	98
M2	2	2		
M1	1			
P2	18	10	6	
P1	1	2		
C	1	4		
I 2		2		
I1	7		2	

N = Number of individuals with missing teeth
n = Number of missing teeth.

As hypodontia may involve one or more teeth it is important to establish the frequency of those affected by more severe forms hypodontia (i.e. oligodontia). The pattern of frequency of proportion of missing teeth is presented in Figure 46. Of note, one subject had severe oligodontia and was missing 10 teeth in the Northern group and although reported in frequency was not represented graphically.

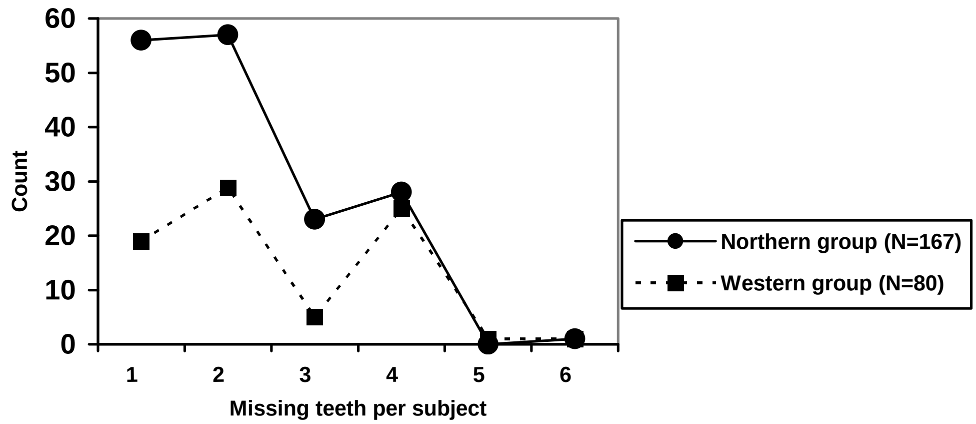


Figure 46. Distribution of hypodontia of by number of missing teeth in two Sudanese ethnic groups.

Despite higher frequency observed in the Northern group, as a proportion the two groups exhibit similar pattern. The most common pattern is when 2 teeth are missing per person followed by 1 missing tooth. Both these patterns were by far the commonest, accounting for 60.1% and 67.5% occurrence in the Western and Northern groups respectively (Figure 46).

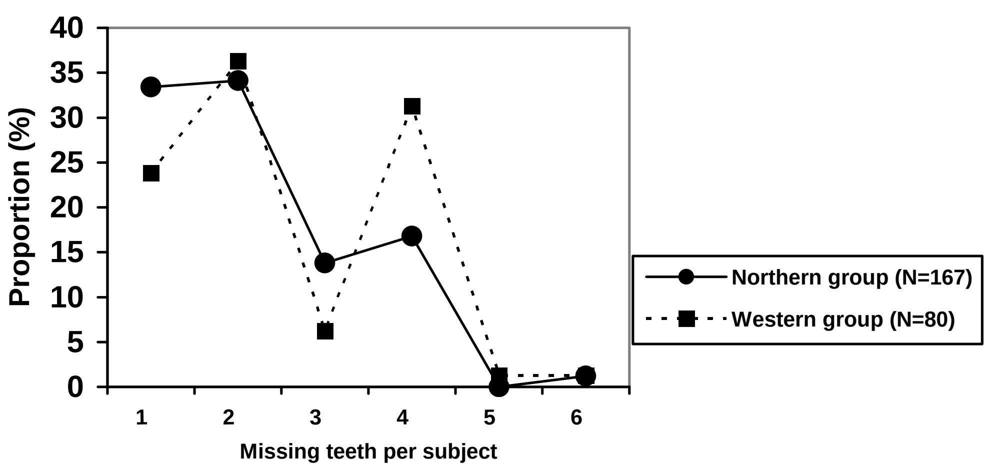


Figure 47. Proportion of total hypodontia in two Sudanese ethnic groups.

4.9.2 Third Molar Agenesis

The third molar accounted for 84. 2% and 94.6 % for the Northern and Western groups respectively, of the total observed hypodontia in each group.

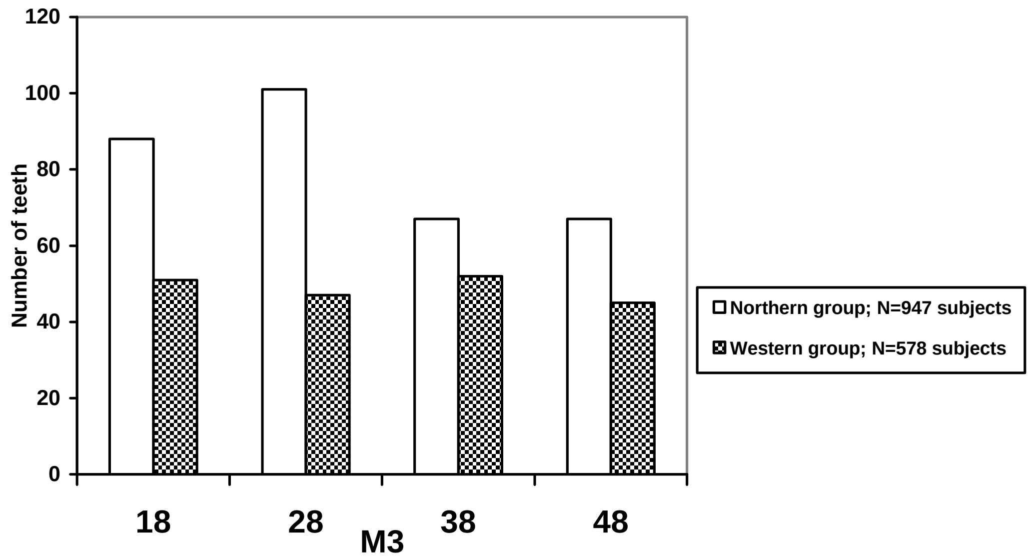


Figure 48. Frequency of M3 agenesis in two ethnic groups.

The frequency (Figure 48) was higher in the Northern groups but the proportion (Figure 49) very similar.

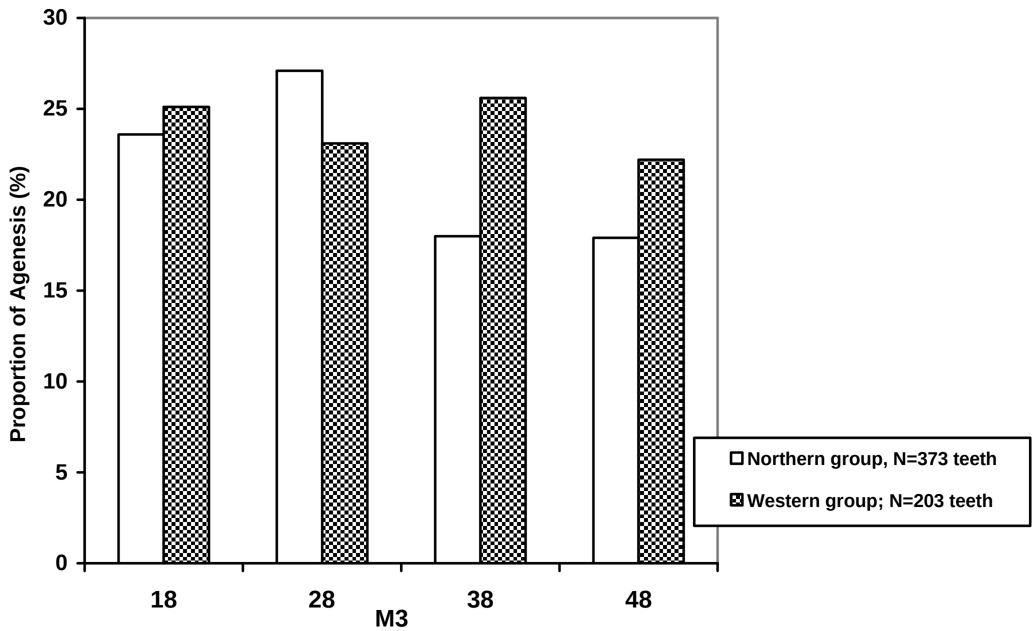


Figure 49. Proportion of hypodontia of M3 as a proportion of the total observed in each group.

4.9.3 Summary of Hypodontia

In the Northern group the prevalence hypodontia; excluding M3, was of 2.6%. When third molars are included the northern group had an occurrence of 2.23 CMT per child and when they were excluded the rate was 2 CMT per child, and are low prevalence rates. Hypodontia of M3 had a prevalence of 13.2%.

The western group had hypodontia a prevalence of 0.7% (excluding the third molar) and a rate of 13.2% for M3. Each child had approximately 2.53 CMT for all teeth including third molars and a rate of 2 CMT excluding them.

In both groups the most common combination of missing teeth is one or two per individual.

Chapter Five: Discussion

5.1 Principal Findings

1. Mean age of entering each MFH stage for two distinct populations is presented.

The appropriateness of comparing non-Caucasian groups with established European or North American tooth formation such as those devised by Demirjian et al. 1973, Gusatafson and Koch (1974), Moorrees et al. (1963a & b), Schour and Massler (1941) as well as Haavikko (1970) amongst others, has long been under question. Many investigators argued for the case of population specific references to enable meaningful interpopulation comparisons to be made (Davis and Hagg, 1994; Harris and McKee, 1990; McKenna et al., 2002).

Smith (1991) also clearly highlighted the need for more non-Caucasian tooth formation standards to enable meaningful comparative studies to be made. To date these are scarce and non-existant in the Eastern part of Africa. This is the first study to produce a reference for two ethnic groups endogenous to the region and one of a handful of randomized population surveys reported in the literature.

This study shows that variation between individuals within a group is considerable and group differences are small, therefore population references can only be used as biological indicators but have low precision at the individual level, e.g. age estimation applications amongst others. The need for population specific references for the purpose of identifying major differences between human populations has been contested by Liversidge (2010). This study also suggests that references for tooth formation may continue to highlight differences at the biological level and trends in tooth formation rather than clinically specific tools. The study also shows the extent of the true variation in tooth formation at the population level. This is important as many investigators have stressed the need for population specific reference. The results presented show that the use of such references will be limited by the extent of variability of individuals within them. These findings are particularly important in applications where a single child is being aged, as in the example of forensic or archaeological applications. This study also shows that applying biological references in clinical situations amounts to no more than informed guesses in relation to age estimation of a single child or a group of children. However, it is imperative that more population based studies, unified in their methods, be carried out to clarify the extent of variability.

2. Ethnic differences exist between the North and the West Groups.

Although the study highlights that more similarities exist between the sexes than between the groups, it is important to differentiate as to whether this is due to secular trends or evolutionary change. Tompkins (1996) hypothesized that the variability in tooth formation may be associated with either space in the posterior jaw or may be due to genetically inbuilt differences to do with the timing of tooth formation that may be population specific. The literature does not reveal a consensus as to whether tooth formation and skeletal maturity are dependent and to what extent. This is important due to reported differences in growth of ethnic groups (Cole, 2007).

The main ethnic difference noted was in the pattern of hypdontia in the different groups (being lower in the western group) rather than frequency, in contrast to tooth formation. Due to the study design the Northern group can be considered representative. The rates of hypodontia are amongst the lowest reported, confirming that some differences between our two groups and other reported populations do exist.

On the other hand, tooth formation confirms that both groups cannot be defined as having more advanced or delayed tooth formation.

3. The variability of overlap in the formation of different teeth is marked.

From an age estimation point of view, it has long been argued that population specific standards for different teeth need to be constructed to improve age estimation of individuals. From the biological aspect it is unlikely that the high variation within this population based study would render a population specific standard of identifying children of unknown age more accurate. This view is supported by other investigators (Liversidge et al., 1999; Thevissen et al., 2010). Despite this fact, subtle variations between ethnic groups or populations have considerable biological importance. Stated in another way, one should consider the similarities rather than the differences between ethnic groups, given the high variability within each population. This holds true especially for the third molar which exhibits the highest variability in relation to formation time and yet is used commonly in studies of age estimation and is commonly used in forensic odontology. This view is supported by Reid and Dean (2006) in their histological study of enamel formation concluded that variability was somewhat less than expected both within and between the populations. This fact is reiterated by looking at both molars and anterior teeth. However, clear differences exist in late forming teeth, both in individuals and between groups.

4. Early forming teeth tend to be similar (e.g. M1 and I2) whilst late forming teeth are highly variable (e.g. P2 and M3). Root stages appear to be more variable than crown formation.

A consistent finding is that the formation of early forming teeth appears to be less variable. Others types of study e.g. histological and on tooth size support the view that crowns of early forming stages of early forming teeth, i.e. M1 and I2 have are uniform in their formation times between the sexes as well as between groups (Brook et al., 2009; Reid and Dean, 2006). It is not clear as to why some teeth are so clearly demarcated in the timing of their formation (e.g. M1 and I2) whilst others vary considerably over the time (e.g. P2 and M3). It is may be that earlier forming teeth are under tighter genetic control.

However, establishing the cause of variation may be a difficult question to answer from this study. The study also complicates the issue of tooth prediction formation. Comparisons between populations are scarce, and when the information is available the different methods of assessment and analysis are not usually comparable.

5. No clear pattern for sex differences exist except for C and some root stages of M3. M3 has high variability amongst individuals within the same group.

Some teeth showed sexual dimorphism consistently namely the canine and the third molar. This fact was also shown by Garn et al. who found clear dimorphism of the canine with girls advanced to boys (Garn et al., 1959). This study is in agreement with findings that girls are consistently more advanced in all groups. One hypothesis is that the canine tooth being having greater dimensions in males take longer in the course of its formation (Garn et al., 1967). Histologic studies suggest that the time taken to lay enamel and/or the absolute measure of dentine may be responsible for the dimorphism noted (Schwartz and Dean, 2001; Schwartz and Dean, 2005). This study also agrees with those findings of investigators who have reported sexual dimorphism on other parameters such at tooth shape and size (Garn et al., 1967; Saunders et al., 2007). On average the advancement in this study is six months rather than one year, previously reported by Garn et al. (1967) for the canine. The results of this study do not support the theory of 'the canine field effect' on sexual dimorphism that first premolars and second incisors are more dimorphic than other further adjacent teeth (Garn and Swindler, 1966). The study also supports the widely quoted study by Demirjian & Levesque (1980) who reported in their sample, girls are more advanced by almost one year for some teeth with the biggest difference seen in the canine. However, they also report that in general girls where more advanced by 0.54 in their overall dental maturation. This study fails to support their view that girls are advanced for all teeth. Why sexual patterning still exists in the canine is yet to be explained despite some theories such as the field theory amongst others (Garn et al., 1966).

There has been much discussion on the development of third molars in the literature. Some investigators reported sexual dimorphism (Kasper et al., 2009; Prieto et al., 2005), whilst others, for example Garn et al. (1962) do not demonstrate an appreciable difference in M3 formation between males and females. Other investigators suggest that sexual dimorphism exists especially in root stages (Caldas et al., 2010; Meinl et al., 2007). However mean age of children in a stage was used in some of these studies which again makes some of these findings speculative. Similar findings are reported in this study with the difference that in the northern group, males were more advanced than the females. The finding that sexual dimorphism between males and the females in the West group is opposite to that of the North group (with some crown and some root stages showing significant differences) is in agreement to an international comparison which reported that males were more advanced than females except for a black South African group; where females where advanced (Liversidge, 2008b). This was also a consistent finding in African Americans (Harris and McKee, 1990).

In the current study only some root stages appear to be significantly different between the two groups, supporting the fact that variability between individuals may have an important part to play in late forming teeth and increases with age.

6. Extreme malnutrition has little effect on tooth formation

The evidence for the effect of malnutrition on tooth formation has been so far inconclusive and largely inconclusive. Non-human studies show that malnutrition has an effect and usually retards tooth development (DiOrio et al., 1973; Luke et al., 1979). Keller et al. reported that dental formation was retarded in children with growth deficiencies and various metabolic endocrine and metabolic disease (Keller, 1970).

Other investigators did not agree. For example Van Erum et al. assessed the craniofacial growth of 48 subjects with short stature found that tooth formation to be independent of general growth and bone age (Van Erum et al., 1998). This fact is reiterated by Naidoo et al. who despite showing that a correlation exists between tooth formation and skeletal development, also report that extreme developmental abnormalities such as caused by foetal alcohol syndrome, do not consistently retard tooth formation (Naidoo et al., 2006). Poor nutrition may not be directly related to retarding tooth development in children with poor nutritional status (Fleshman, 2000). On the other hand, Hilgers et al .found accelerated dental development in obese boys, but not girls (Hilgers et al., 2006). The latter study should be viewed with caution given the low number of obese subjects examined using Demirjian's method (n=18). Our results suggest that malnutrition does not influence the process of tooth formation. Variation between individuals subject to systematic malnutrition and those whose growth is normal are comparable. The second molar is representative as it is a late forming tooth and is more likely to be affected by extremes in environmental pressures and was therefore investigated in this study. The first molar was also chosen to test the effects of malnutrition on early forming teeth. The study confirms the independence of the tooth formation of extreme under-nourishment at the population level.

5.2 The Development Process and Choice of Groups

In 1991, Smith clarified the difficulties in undertaking comparative tooth formation research and highlighted methodological, statistical and interpretation difficulties. To overcome these issues a randomly selected group was selected from very different two distinct ethnic groups, namely, a group of African decent (Fur tribe) and those originating in the North of Sudan, mainly of Arab or Nubian decent. The two groups lend themselves easily for comparison for a number of factors. Despite sharing the same geographical country, both groups have very different anthropologic migratory patterns. Physical appearance is one of the main differentiating features.

In the medieval period the northern part of the country was dominated anthropologically by Nubian Kingdoms and later served as a corridor for migratory patterns from North East Africa and the Arab world (Edwards, 2003).

Darfur however remained secluded from the northern part and no substantial evidence of significant anthropological interactions existed between the Nubian and Darfur Kingdoms. This has continued to modern times and into the post colonial period. To date very little interaction exists and this has been a source of tension between the two regions and has been for many centuries (O'Fahey, 1996).

All this evidence points to racial differences and two distinct gene pools from which each group descends with very little interaction despite sharing relatively close geographical locations. There is also international interest in the Sudan as it has offered the only corridor into sub-Saharan contact into Africa through what is know as ‘the Nile corridor’ with many tribes to the west and South that are genetically and culturally distinct (Edwards, 2003). The unique opportunities which the Nile corridor offered for trans-Saharan contacts ensures the special interest of this region as a zone of interaction between diverse cultural traditions in sub- Saharan Africa, Egypt, the Mediterranean world, and elsewhere, across millennia.

In this study, apart from the distinct morphological features, the Northern tribes are grouped together because of their linguistic and geopolitical similarities. The Darfur tribes were also grouped together along the same lines.

5.3 The Validity of Methods

Although the North group was randomly selected sample, the same does not hold true for the Western sample. This is of relevance as we are comparing the two groups and making references. Some age groups in the West demographic, for example older females, are under-represented due to social and logistical factors. After their teenage years females are expected to either stay at home or work as often they have to provide for the their families. There is no education to speak off and access was very difficult indeed as females above the age of 15 had to leave either their home or place of work which was not feasible.

Additionally despite controlling for sex and growth, the North groups came from relatively higher socio-economic classes and establishing the exact age was less problematic. This was not the case for the western group especially the females.

Two statistical techniques are presented and compared probit analysis with log-percentiles (Finney, 1971) and logistic regression (Smith, 1991). Although both yield the same mean age of attainment, the standard error and hence the 95% confidence interval for the mean was significantly narrower around the mean for logistic regression. In practical terms this fact improves the chances of assessing true biological variability but further complicates the issue of age estimation. This is important as both techniques utilize cumulative techniques and despite being robust enough to accommodate relatively small sample numbers, logistic regression was found to be more precise in estimating the curve of best fit, especially with larger sample sizes.

5.4 Strength of the Study

The data collected represents the first population based study of an African population from infancy to adult hood. Many logistic problems such as access, red tape, language barriers political instability, technical difficulties had to be overcome for data collection. As discussed earlier the two groups represent two distinct populations.

The Northern group is completely randomized and representative of their population with equally distributed age groups. This enables some teeth to be studied from the initial stages of crown formation to the root completion stages. The Western group is thought to be relatively representative due to migratory patterns (UNHCR, 2010). This holds true when comparing the groups to those presented in the literature both from the sample size and sample location point of view.

The data from the two groups was collected using the same methodological and statistical techniques rendering statistical comparison more meaningful; however, difficulties still exist in comparing our results directly with previous studies using other techniques.

The fact that the study was conducted by one examiner and using the same radiographic and statistical techniques reduces bias considerably in a development system known to be affected by intra-population variation. The number of tooth stages used (after Moorrees et. al. 1963a) has several advantages as explained by Smith (1991) as well as Fanning and Brown (1971). Fewer stages would improve reliability but decrease specificity, which may have an impact on reporting variability leading to a less accurate record of biological events. On the other hand, too many stages would improve specificity and reduce reliability rendering comparison with other studies difficult. The methods used are established and well documented in a number of other studies enabling some comparisons to be made.

The strongest contribution of this study is that it describes tooth formation and hypodontia in two populations not previously studied. It also makes available a valuable data set that may prove useful in later studies due to the fact that the subjects where randomly selected. An additional strength of the study is the fact that the data collected for hypodontia makes available a missing population with strict inclusion criteria and biological indicators for assessment rather than chronological age.

5.5 Weaknesses of the Study

Ideally, a longitudinal study design would be more appropriate in assessing tooth formation over time. However, given ethical and logistic constraints, well designed radiographic longitudinal studies with a sufficient number of subjects are unlikely.

Additionally, there is missing information on the full maturational events of all teeth. This would require very young children (below 2 years of age) and is practically difficult. An example is the first molar, which has been shown to be less variable in its course than other teeth especially for crown formation (Read and Dean, 2006). This fact may have an impact when presenting comparative inter-population data. Furthermore, to complicate matters, where age groups are available, not all stages of all teeth are present due to the practicalities of recruiting specific subjects from different ethnic groups.

In general terms, as variability in any growth parameter increases with time this study has highlighted more similarities than differences between ethnic groups. The cause and extent of variation between populations is difficult to establish due limitations in the current methods use, but they are constantly being refined. Results from this study and some published literature on the ethnic effects appear to be conflicting. Liversidge (2010) goes some way to shed light on variability but had only sufficiently large sample size for the Caucasian groups and concluded that there is marked variability within a populations and ethnic differences, although present, are only contributory rather than the main cause. It may very well be that it is the extent of variability between individuals rather than ethnic effects that are of utmost importance.

As stated earlier the Western group was conveniently obtained with low numbers in some age groups. Additionally the study does not take into account other factors such as crown and root length, or tooth size and shape. This may be of particular importance especially when related to sexual dimorphism. The logistical problems encountered such as transport and electricity supplies, which may not always readily available, together with the long distances the children had to travel to have the x-ray taken meant that one had to accept the age groups. Although a randomly selected sample across all age groups would have strengthened the study. This was not practically possible.

Methodological weaknesses include the nature of the radiographs themselves. Although excellent for detecting missing and unerupted teeth, OPG radiographs can be less specific for anterior labial segments due to the superimposition of the vertebral column and this may have led to reading errors especially during later root stages.

5.6 Comparison with Other Groups

The most common application of tooth formation and age reference tables is age estimation. Demirjian's (1973) method is the most commonly used but does not lend itself to direct comparison with mean ages obtained by other methods except for some stages, for example stage Ac, and this limits easy inter-population comparisons.

As demonstrated earlier the method used in the protocol has recently been published and therefore may yield different results if used on previously published non-European data.

Previous hypothesis has been put forward that later stages of root maturation in late developing teeth may be more influenced by non-genetic factors. The issue of the environmental effect on tooth movement although relatively small has not been taken into account although may be an important biological question.

Garn et al.s study strength lies in its serial design (Garn et al., 1958). However, the mean ages published do not lend themselves to statistical testing, as only the mean is reported with no standard error. The numerical values are shown in Appendix A.7.1. When groups of means are visualized a picture of relative similarity emerges. The graph does not reveal any trend of one being consistently more advanced than the other (Figure 50). The trend is repeated for both males and females (Figure 51).

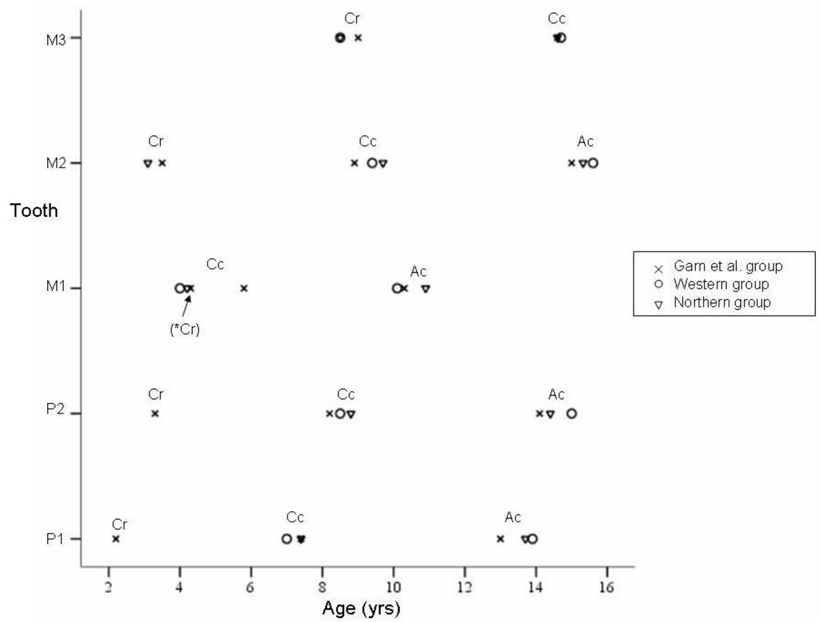


Figure 50. Comparison of mean of attainment for males between Garn et al.(1958); (using available data), Western and Northern Sudanese groups showing relative similarity.

** indicates stage overlap.*

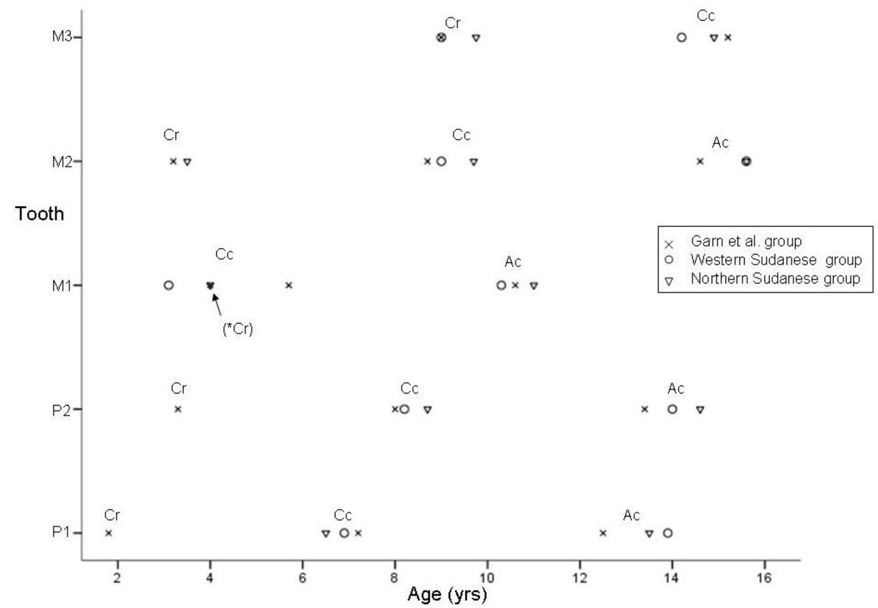


Figure 51. Comparison of mean of attainment for females between Garn et al.(1958); (using available data), Western and Northern Sudanese groups showing relative similarity.

** indicates stage overlap.*

Comparison with Liversidge et al. (2008) Caucasian group clarifies the variation between human groups. Although their study uses Demirjian's method for age estimation, stage H is similar to that of stage Ac used in this methodology. Given that the both the mean and the standard errors are published; variance can be examined. This is illustrated in the graph below. The original numerical figures are presented in Appendix A.7.2. Two teeth, namely M1 and M2 are used to illustrate this point. Figure 52 illustrates the relative overlap of confidence intervals between females from the Western, Northern and Liversidge et al. groups despite the diverse ethnic origins indicating relative similarity.

Males are mostly similar for the chosen stages (Figure 53), favouring the opinion that the similarity between populations is considerable.
As discussed earlier many investigators have reported variability between populations for the third molar. To try to shed light on these findings, confidence intervals were used to compare groups to see if this fact holds true when the same methodology and statistical analysis is used. Data from this study was compared to that of a Caucasian group published by Liversidge (2008). The original numerical figures are presented in Appendix A.7.3.

Even the most variable tooth, namely M3 exhibits signs of similarity between human groups (Figure 54 and Figure 55).

Although there appears to be some individual stages that appear to be different the overall picture is that there are more similarities than there are differences.
The above figures also very clearly demonstrates the short fall of using references for the purpose of comparing individuals in purposes of identification or establishing chronological age.

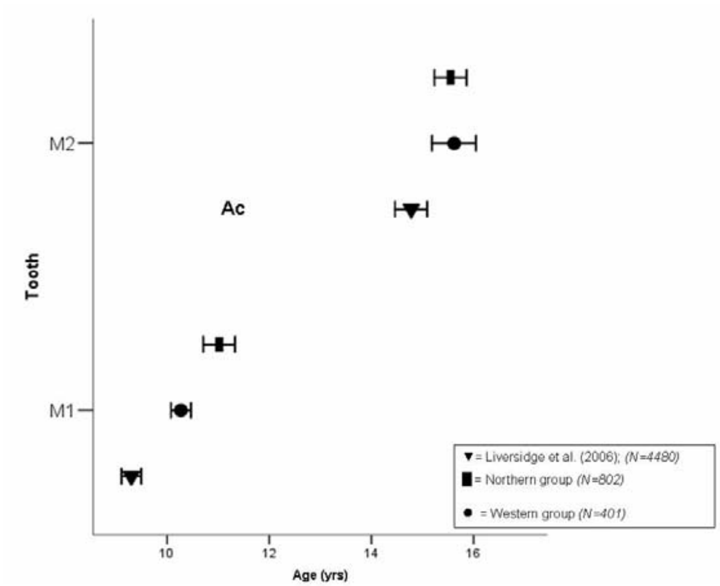


Figure 52. 95% confidence interval of mean of attaining stage Ac for teeth M1 and M2 for females from 3 ethnic groups.

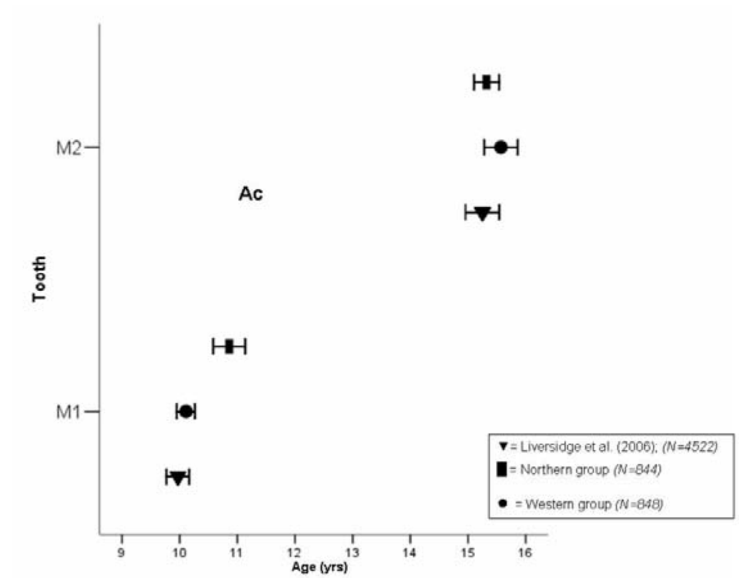


Figure 53. 95% confidence interval of mean of attaining stage Ac for teeth M1 and M2 for males from 3 ethnic groups. Overlap indicates no significant difference between the groups.

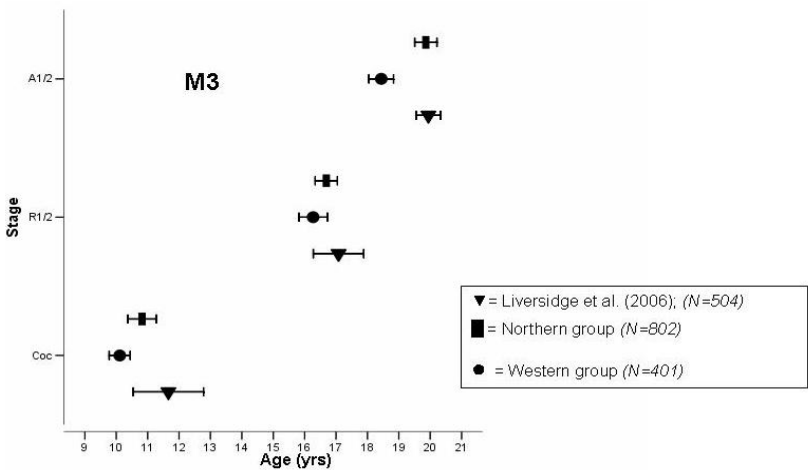


Figure 54. Graph to compare 95% confidence intervals of mean of age of attainment of some stages of tooth M3 for females from three ethnic groups**.**

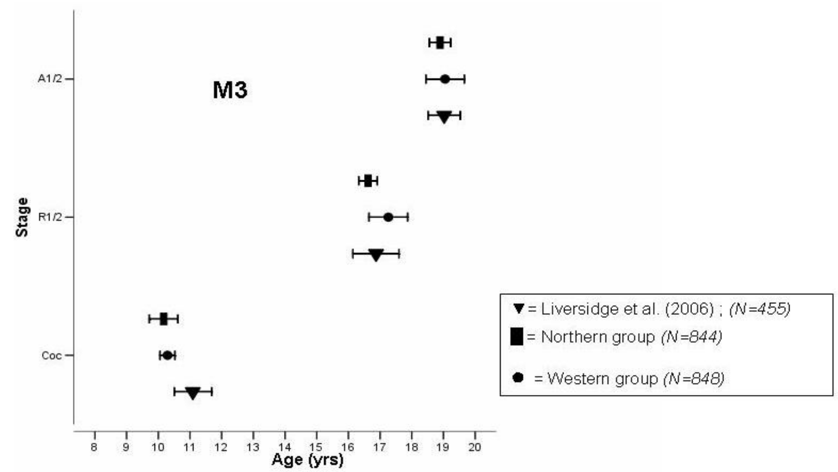


Figure 55. Graph to compare 95% confidence intervals of mean of age of attainment of some stages of tooth M3 for males from three ethnic groups.

5.7 Unanswered Questions

Using a well constructed population based study has left a number of questions unanswered, for example;

1. Is the pattern of overlap between stages subject to similar variability as was seen in tooth formation?
2. Can stage formation be influenced by the particular overall pattern of tooth development?
3. Why does sexual patterning in the canine and some root stages of M3 exist and why are most studies united in these findings?
4. Why are early forming teeth less variable?
5. Does the formation of crown or root development have an impact on the timing of tooth eruption?
6. Why does physical maturity, chronic malnutrition and overall growth problems affect tooth emergence but not tooth formation?
7. Why does hypodontia prevalence relatively low in both groups?

The study sheds light on the non-urgent need of constructing tooth formation references, especially for the purpose of aging an individual child even if they are specific to their ethnic group, due to inter-population variability. The study also further complicates the issue of age estimation based on the formation of one tooth or multiple teeth for the purpose of forensic identification. A commonly used tooth for age estimation is the M3, which unfortunately has a rather large variability as demonstrated in this study.

5.8 Meaning and Application of the Study

The present study (which is relatively large, randomized, with evenly distributed age groups spanning the formation of most permanent teeth) shows that tooth formation is mostly similar in two different ethnic groups and highlights very little ethnic variability. This suggests the need for a new approach for the study of differences between human populations than the ones that currently exist. It is true that subtle variations exist between ethnic groups but it is also true that no particular pattern exists between the groups. This is relevant on the basis of trying to establish the extent of variability. Despite this fact, the study establishes a reference for the two groups predominantly for future research, rather than an indication of chronological age applications. The age range of stage attainment is of particular interest in age estimation, especially the minimum and maximum ages, which are likely to have forensic applications and are tabulated in Appendix A.5A.5.1 to A.5.8).

In comparing groups and reviewing the literature, this study dispels reasons for assuming that African girls' teeth' mature faster than boys, despite evidence that both their skeletal and somatic development is indeed more advanced. It also dispels the fact that the dentition of African children matures faster than other groups.

The study also shows that intra-group variation contributes significantly to the overall variability in tooth formation. For many years the focus was to study and identify population differences rather than similarities. It is my view that this opinion has been based on a few non-representative samples and does not hold true. The non-uniform use of statistical methods has also had a major role to play and was sometimes applied incorrectly to the study of tooth formation. Having to dispel this belief means that large international studies will have to be coordinated and many are underway. I am aware that one factor to consider is that the two groups may, in part due to their geographical affinity, represent the same population. Even if this were to hold true the variability between individuals is still marked enough to draw the same conclusions for the teeth studied. Therefore the age range should also be broadened to include all teeth before references and comparisons can be considered valid.

The study also shows that definitive sex patterning exists in two teeth; the canine and third molar. This may turn out to be of importance for identification purposes, such as in the case of human remains.

Additionally, the results of this study may prove useful in setting a baseline for evolutionary and secular trends in tooth formation in the future or from archaeological material.

East African populations do not necessarily achieve tooth formation stages earlier or later than other populations, and it appears the prevalence of hypodontia in both groups is comparable and very low compared to other human groups. It is important to note that the pattern of hypodontia is different in both groups.

The study will also be of interest to those investigating hypodontia as this is the first prevalence study to emerge from East Africa.

Extreme systematic malnutrition does not seem to affect tooth development and connot be used as an indicator for under-nourishment. The study also has implications for anthropologists as increasingly human remains of historic importance are being excavated from the northern part of Sudan.

5.9 Further Studies

In the attempt to answer the question whether ethnic groups vary in their tooth formation invariably more questions than answers came to light.

Immediate research potential for this data set includes, some of which are underway:

1. Examining the overlap of tooth stage formations, i.e. do they always follow the same pattern?
2. Reporting further on the pattern of hypodontia in the groups.
3. Reporting on the incidence of impacted canines.

5.10 Conclusions

1. The mean age of attainment of each stage (after Moorrees et al. 1963a) was described over the course of individual tooth formation in two previously unstudied randomly selected Sudanese ethnic groups.

2. More overall similarities than differences exist between the different stages of tooth formation for M2, M1, P2, I2 and I1 between Northern Sudanese and Western Sudanese groups.

3. The age of stage attainment for each tooth is more variable between individuals within the same group than between the ethnic groups.

4. The time taken for crown formation in early forming teeth is similar across ethnic groups and is independent of gender.

5. Gender differences exist; with the mean age of stage attainment being advanced in females of both ethnic groups for C and later root stages of the M3 confirming sexual dimorphism.

6. The average time for overall tooth formation (per tooth) between males and females for each tooth of different ethnic origin is very similar, supporting the view that the within-group variation is more important than overall variation between groups.

7. Where variation existed, it tended to be in the mean age of attainment of root formation stages of later forming teeth and this variation increased with age. The tooth with most variability was the M3.

8. The study also provides evidence that severe systematic malnutrition has little impact on the timing of tooth formation.

9. The study also reports for the first time the low prevalence in Northern and Western Sudanese groups of non-M3 hypodontia (2.6% and 0.7% respectively) and for M3 (15.0% and 13.2% respectively).

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April 26, 2011

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Mr. Fadil Elamin
April 26, 2011
Page 2

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Title: Age Variation of Formation Stages for Ten Permanent Teeth

Author: Coenraad F.A. Moorrees, Elizabeth A. Fanning, Edward E. Hunt, jr

Publication: Journal of Dental Research

Publisher: Sage Publications

Date: 11/01/1963

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A.3. Copyright Permission for Figure 6.

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SAGE

Title: Sexual Differences in Dental Development and Prediction of Emergence
Author: A. Demirjian, G.-Y. Levesque
Publication: Journal of Dental Research
Publisher: Sage Publications
Date: 07/01/1980

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A.4. Ethical Approval.

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كلية الرازي للعلوم الطبية والتقنية

Elrazi College of Medical &Technological Sciences

01/11/2006

Dear Dr. Elamin,

After careful consideration of your application for ethical approval of the study entitled: "Tooth Formation in Sudanese Children"

Supervisors: H. Liversige and Prof. M. Hector.

We are pleased to inform you that the Ethical Committee has approved the study with the condition that all identified disease should be treated free of charge in the Department Of Paediatric Dentistry and Orthodontics free of charge.

Kind Regards

Prof. FROUK M. ELAMIN
Secretary to The Ethical Committee
& Academic Affairs

Prof. ABDALLAA A. DAFFALLA
Chairman, to the ethical committee
& Dean of Faculty of Medicine

El-Azhari - Block 2 - Khartoum - Sudan
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A.5. Tables of Mean Age of Children Within a Tooth Formation Stage

A.5.1. Mean age of children within a stage for M3.

M3	Northern females						Western females						Northern male						Western male					
Stage	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N
Cr	9.20	1.69	0.270	6.39	12.69	39	9.44	1.28	0.285	6.48	12.00	20	9.30	1.76	0.326	6.27	12.42	29	9.09	1.37	0.216	5.06	11.77	40
Ci	10.60	1.32	0.287	8.57	12.84	21	9.72	1.10	0.228	7.60	11.96	23	10.42	1.89	0.379	6.67	14.86	25	9.84	1.43	0.231	8.02	12.85	38
Cco	11.38	1.36	0.291	8.80	14.59	22	10.60	1.27	0.260	8.45	13.79	24	11.00	1.50	0.434	8.89	13.18	12	10.80	1.28	0.173	8.36	14.67	55
Coc	11.94	1.33	0.265	9.36	14.54	25	10.88	0.86	0.129	9.06	12.89	44	10.64	1.19	0.233	8.87	13.77	26	11.57	1.17	0.110	8.05	15.22	113
C1/2	12.13	2.41	0.335	5.92	20.44	52	11.41	1.19	0.178	9.29	14.06	45	12.17	1.57	0.236	9.19	15.05	44	12.33	1.21	0.089	9.12	16.27	183
C3/4	13.59	1.84	0.348	10.52	19.21	28	12.62	1.54	0.302	10.00	16.13	26	13.06	1.94	0.290	10.11	19.38	45	13.16	1.25	0.144	10.67	18.55	75
Cc	14.61	1.85	0.369	11.43	20.61	25	12.88	1.03	0.175	11.26	15.85	35	13.67	1.51	0.248	10.64	18.51	37	13.73	1.32	0.190	11.36	16.79	48
Ri	16.27	2.56	0.641	12.45	20.19	16	14.53	1.42	0.336	12.27	17.36	18	15.25	1.82	0.505	12.13	18.36	13	14.13	0.89	0.182	12.32	16.20	24
Rcl	17.10	1.82	0.442	14.48	22.55	17	14.54	1.12	0.336	13.10	15.95	11	15.53	1.74	0.371	13.15	19.25	22	14.95	0.81	0.159	13.67	17.70	26
R1/4	17.07	1.91	0.302	14.28	22.34	40	15.42	1.03	0.421	14.38	17.30	6	16.95	1.48	0.232	14.90	20.58	41	15.19	1.00	0.143	12.87	18.19	49
R1/2	17.68	2.03	0.274	14.16	22.77	55	16.04	0.99	0.299	14.31	17.98	11	17.70	1.56	0.230	14.63	21.82	46	15.87	2.07	0.574	13.84	21.33	13
R3/4	17.80	1.85	0.279	14.52	22.37	44	17.64	1.62	0.723	16.35	20.26	5	17.80	1.46	0.231	14.97	21.31	40	15.94	1.29	0.489	14.43	17.59	7
Rc	18.50	1.89	0.473	15.72	22.14	16	17.46	0.66	0.465	16.99	17.92	2	18.04	1.52	0.358	16.01	20.72	18	17.70	2.18	1.259	16.34	20.22	3
A1/2	19.15	1.61	0.257	16.08	23.96	39	19.69	0.84	0.344	18.26	20.68	6	18.86	1.49	0.242	15.89	22.82	38	19.90	0.65	0.265	19.14	20.93	6
Ac	21.46	1.58	0.153	17.44	23.96	106	20.78	1.23	0.262	18.44	22.34	22	21.19	1.66	0.136	17.07	23.94	149	20.16	1.05	0.348	19.41	22.02	9

A.5.2. Mean age of children within a stage for M2.

	Northern females						Western females						Northern male						Western male					
Stage	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N
Cr	3.5	0.26	0.073	3.00	3.82	13							3.41	0.74	0.197	3.00	5.86	14						
Ci	3.89	0.50	0.165	3.16	4.80	9	3.93	0.48	0.279	3.49	4.45	3	3.86	0.34	0.106	3.42	4.54	10	3.60	0.26	0.150	3.44	3.90	3
Cco	4.24	0.54	0.203	3.59	4.87	7							3.61	0.43	0.137	3.11	4.56	10	4.47	0.41	0.290	4.18	4.76	2
Coc	4.79	0.96	0.266	3.81	6.78	13	5.27	0.42	0.148	4.76	5.89	8	4.60	0.95	0.262	3.26	6.76	13	5.26	0.94	0.360	3.90	6.79	7
C1/2	5.13	0.86	0.129	3.03	7.20	44	5.59	0.93	0.239	4.09	7.92	15	5.25	1.04	0.154	3.03	7.76	45	6.10	1.14	0.250	4.11	8.20	21
C3/4	6.91	1.34	0.146	3.55	10.73	84	6.71	1.22	0.213	5.03	9.07	33	6.72	1.56	0.174	3.04	10.86	80	7.25	0.95	0.126	5.16	9.83	57
Cc	8.58	1.69	0.190	5.94	12.84	79	8.50	1.29	0.228	5.39	10.40	32	8.32	1.77	0.187	5.41	11.95	89	8.53	1.10	0.119	6.10	10.92	86
Ri	10.10	1.26	0.223	7.14	12.80	32	9.56	0.76	0.129	7.27	10.99	35	9.42	1.68	0.376	6.11	12.89	20	9.87	0.75	0.109	8.06	11.29	48
Rcl	11.37	1.28	0.231	9.17	12.99	31	10.38	0.48	0.067	9.55	11.66	51	10.72	1.25	0.202	7.77	12.95	38	10.62	0.60	0.082	9.08	11.99	53
R1/4	10.90	0.82	0.139	9.19	12.70	35	11.04	0.83	0.123	9.06	12.55	45	11.27	1.12	0.239	9.83	13.70	22	11.49	0.65	0.059	9.12	12.98	120
R1/2	12.43	2.12	0.386	10.36	20.44	30	12.00	0.67	0.109	11.17	13.90	38	12.21	1.09	0.207	10.52	14.86	28	12.11	0.77	0.082	10.53	14.27	89
R3/4	12.91	1.04	0.233	11.19	15.08	20	12.48	0.40	0.071	12.00	13.79	32	12.94	1.38	0.231	11.10	16.73	36	12.96	0.66	0.067	12.00	16.29	99
Rc	13.57	1.28	0.287	11.37	16.25	20	13.84	1.06	0.201	12.26	16.05	28	13.87	0.95	0.171	11.70	16.53	31	13.74	0.90	0.102	12.17	16.49	77
A1/2	15.47	1.81	0.238	13.19	21.40	58	14.49	1.14	0.232	12.01	16.99	24	14.79	1.09	0.170	13.10	17.39	41	14.39	1.08	0.108	12.32	18.55	100
Ac	19.19	2.40	0.133	14.02	23.96	327	18.46	2.48	0.334	14.31	22.34	55	19.18	2.36	0.123	14.05	23.94	366	16.50	2.16	0.233	14.00	22.02	86

A.5.3. Mean age of children within a stage for M1.

Stage	Northern females						Western females						Northern male						Western male					
M1	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N
Coc													5.38	0.16	0.115	5.26	5.49	2						
C1/2	3.51	0.49	0.345	3.16	3.85	2							4.47	1.35	0.780	3.47	6.01	3						
C3/4	3.87	0.53	0.188	3.00	4.87	8							3.49	0.52	0.172	3.00	4.50	9						
Cc	3.65	0.43	0.098	3.03	4.90	19	4.87	1.01	0.507	3.49	5.80	4	3.66	0.52	0.099	3.03	4.79	28	3.75	0.36	0.180	3.44	4.18	4
Ri	4.09	0.56	0.278	3.46	4.78	4	4.36	0.65	0.327	3.68	5.22	4	4.11	0.69	0.219	3.21	5.24	10	4.94	0.28	0.128	4.54	5.27	5
Rcl	4.79	0.47	0.080	4.03	5.90	34	4.68	0.71	0.355	3.86	5.46	4	4.97	0.53	0.102	4.16	5.99	27	4.83	0.64	0.242	3.90	5.50	7
R1/4	5.94	0.77	0.139	4.06	7.70	31	5.42	0.38	0.075	5.00	6.44	25	5.97	0.72	0.113	4.47	7.64	41	5.96	0.49	0.118	5.11	6.83	17
R1/2	6.51	0.81	0.145	4.28	7.97	31	6.59	0.41	0.124	6.01	7.27	11	6.46	0.67	0.099	5.10	7.86	46	7.08	0.72	0.139	6.01	8.79	27
R3/4	7.14	0.76	0.098	5.90	9.60	61	7.30	0.61	0.169	6.05	8.08	13	7.46	0.99	0.144	5.86	9.73	48	7.44	0.67	0.118	6.21	8.65	32
Rc	8.84	1.02	0.152	6.32	12.45	45	9.12	1.13	0.181	6.48	12.08	39	9.32	0.84	0.132	8.16	12.20	40	8.44	1.20	0.158	6.10	10.97	58
A1/2	11.14	1.92	0.238	7.00	20.14	65	9.86	0.87	0.121	8.74	14.32	51	10.78	1.29	0.184	9.00	14.97	49	9.80	1.06	0.123	8.11	16.16	75
Ac	17.11	3.71	0.166	10.00	23.96	498	13.59	3.14	0.199	9.29	22.34	249	17.20	3.61	0.156	10.05	23.94	538	13.15	2.08	0.083	8.12	22.02	623

A.5.4. Mean age of children within a stage for P2.

	Northern females						Western females						Northern male						Western male					
Stage	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N
Cr	3.77	0.44	0.167	3.38	4.55	7							3.46	0.44	0.134	3.06	4.54	11	4.91	0.21	0.150	4.76	5.06	2
Ci	4.33	1.12	0.336	3.00	7.18	11							3.84	0.36	0.136	3.21	4.28	7						
Cco	3.76	0.57	0.202	3.16	4.85	8							3.02	0.02	0.012	3.00	3.04	3	3.68	0.31	0.220	3.46	3.90	2
Coc	4.66	1.11	0.392	3.03	6.72	8	4.74	1.50	1.060	3.68	5.80	2	4.18	0.66	0.166	3.33	5.80	16						
C1/2	5.12	0.92	0.165	3.24	7.20	31	5.31	0.48	0.146	4.35	6.01	11	5.08	1.04	0.182	3.03	7.77	33	5.81	1.33	0.368	4.11	8.95	13
C3/4	6.23	1.31	0.185	4.21	10.68	51	6.04	1.08	0.197	4.09	8.54	30	6.11	1.29	0.188	3.48	9.81	47	6.59	0.89	0.139	4.56	7.99	41
Cc	7.84	1.63	0.175	5.50	12.58	86	7.99	1.49	0.287	5.03	10.40	27	7.38	1.57	0.162	4.47	11.12	94	7.82	1.63	0.148	6.00	11.21	62
Ri	9.67	1.73	0.223	6.54	12.96	60	9.23	1.23	0.190	6.05	11.84	42	9.34	1.74	0.227	5.76	12.92	59	9.45	1.05	0.119	7.02	11.86	79
R1/4	10.62	1.50	0.246	6.40	12.99	37	10.29	0.76	0.105	9.06	11.96	52	10.99	1.48	0.267	8.22	13.93	31	10.58	0.96	0.107	8.90	12.30	80
R1/2	11.50	1.54	0.243	7.14	15.08	40	11.03	0.95	0.117	8.84	13.34	65	11.75	1.40	0.256	9.68	14.86	30	11.69	0.98	0.091	9.08	14.25	117
R3/4	11.97	1.69	0.313	5.92	14.54	29	12.11	0.72	0.112	10.32	13.94	42	11.88	1.31	0.211	7.82	14.64	39	12.30	1.08	0.095	9.32	16.30	128
Rc	12.50	1.31	0.280	11.00	15.14	22	13.09	0.90	0.131	11.06	15.49	47	13.41	1.27	0.206	11.10	16.54	38	13.23	0.96	0.085	10.94	16.27	128
A1/2	14.45	1.31	0.228	11.72	18.85	33	15.17	1.00	0.257	13.76	16.99	15	13.46	0.97	0.236	11.67	15.89	17	14.22	1.18	0.140	11.48	18.55	72
Ac	18.84	2.59	0.136	11.73	23.96	361	17.98	2.71	0.347	12.02	22.34	61	18.80	2.58	0.128	13.15	23.94	402	15.96	2.12	0.196	13.08	22.02	117

A.5.5. Mean age of children within a stage for P1.

	Northern females						Western females						Northern male						Western male					
Stage	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N
Ci	3.58	0.28	0.200	3.38	3.78	2																		
Cco	3.93	0.63	0.281	3.00	4.55	5							3.12	0.50	0.035	3.08	3.15	2						
Coc	3.41	0.28	0.124	3.16	3.85	5							3.25	0.24	092	3.00	3.68	7						
C1/2	4.05	0.63	0.129	3.03	5.96	24	4.70	1.10	0.493	3.49	5.89	5	4.01	1.00	0.171	3.00	8.04	34	4.46	0.77	0.312	3.44	5.46	6
C3/4	4.92	0.80	0.123	3.24	6.46	42	5.41	0.88	0.236	3.68	7.09	14	4.95	0.88	0.136	3.03	6.76	42	5.67	1.04	0.277	3.90	7.31	14
Cc	6.39	1.21	0.176	4.28	10.68	47	6.04	1.14	0.211	4.09	8.54	29	6.21	1.14	0.173	4.00	10.00	43	6.69	0.94	0.140	4.56	8.95	45
Ri	8.04	1.62	0.164	5.90	12.58	97	8.02	1.45	0.270	5.03	10.62	29	7.48	1.54	0.151	4.47	11.12	104	7.91	1.10	0.135	6.00	9.78	66
R1/4	9.80	1.84	0.253	6.40	12.96	53	9.39	1.19	0.176	6.05	11.84	46	9.71	1.54	0.197	8.86	10.56	61	9.64	0.98	0.106	7.16	11.89	85
R1/2	10.85	1.29	0.201	7.32	13.50	41	10.40	0.80	0.106	9.06	12.57	57	9.72	1.10	0.216	5.96	12.92	26	10.89	1.08	0.117	8.90	14.32	86
R3/4	11.38	1.48	0.220	5.92	15.08	45	11.20	0.94	0.120	8.84	13.94	62	10.92	1.19	0.217	9.31	12.95	30	11.78	1.04	0.092	9.12	16.29	128
Rc	12.25	0.78	0.183	10.68	13.54	18	12.37	0.77	0.119	11.06	15.12	42	11.91	1.24	0.220	9.55	13.85	32	12.52	0.94	0.083	10.06	15.28	128
A1/2	12.85	1.43	0.337	11.03	15.56	18	13.10	0.87	0.160	11.47	15.49	30	12.27	1.39	0.257	10.48	14.64	29	13.12	0.77	0.081	11.17	15.26	89
Ac	18.43	2.84	0.141	11.00	23.96	402	17.01	2.86	0.309	12.13	22.34	86	13.44	2.82	0.136	11.22	16.50	431	15.31	1.95	0.138	11.48	22.02	199

A.5.6. Mean age of children within a stage for C.

	Northern females						Western females						Northern male						Western male					
Stage	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N
C1/2	4.40	1.37	0.791	3.38	5.96	3							3.47	0.37	0.103	3.00	4.16	13						
C3/4	3.95	0.54	0.106	3.16	5.27	26	4.49	0.93	0.414	3.49	5.89	5	4.22	0.94	0.168	3.06	6.35	31	4.38	0.73	0.276	3.44	5.46	7
Cc	4.70	0.87	0.139	3.00	6.46	39	5.43	0.84	0.218	3.68	7.09	15	4.90	1.06	0.168	3.00	6.76	40	5.60	0.85	0.226	4.18	7.25	14
Ri	6.38	1.43	0.183	3.64	10.68	61	5.92	0.97	0.180	4.09	8.10	29	6.40	1.25	0.134	3.48	10.43	87	6.82	0.91	0.151	5.11	8.95	48
R1/4	8.08	1.69	0.177	5.84	12.69	91	8.08	1.24	0.253	5.43	10.62	24	8.37	1.72	0.184	5.41	12.58	87	8.11	1.16	0.138	6.10	11.62	71
R1/2	9.57	1.81	0.258	5.90	12.96	49	9.47	1.22	0.182	6.05	11.84	45	10.49	1.46	0.240	6.27	12.95	37	9.68	0.97	0.111	7.16	11.86	76
R3/4	11.04	1.45	0.196	7.14	15.08	55	10.26	0.88	0.111	7.60	12.51	62	10.88	1.21	0.189	9.00	13.85	41	11.16	1.12	0.097	8.90	16.29	132
Rc	11.57	1.46	0.227	5.92	15.56	41	11.59	1.06	0.111	8.84	15.12	91	12.57	1.15	0.157	10.48	14.99	54	12.35	1.07	0.071	9.12	16.30	229
A1/2	12.51	1.65	0.302	7.32	15.35	30	12.92	0.87	0.134	11.43	15.49	42	13.12	1.31	0.252	10.74	16.54	27	13.28	1.02	1.098	10.98	15.98	87
Ac	18.40	2.87	0.143	11.00	23.96	403	17.01	2.87	0.310	12.13	22.34	86	18.55	2.75	0.134	11.05	23.94	424	15.39	1.98	0.147	11.48	22.02	183

A.5.7. Mean age of children within a stage for I2.

	Northern females						Western females						Northern male						Western male					
Stage	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N
C3/4	3.64	0.25	0.180	13.46	3.82	2							3.28	0.35	0.203	3.01	3.68							
Cc	3.79	0.42	0.100	3.19	4.78	18	4.04	0.65	0.376	3.49	4.76	2	3.81	0.80	0.180	3.00	5.81	3	5.26	0.28	0.200	5.06	5.46	2
Ri	4.49	0.81	0.137	3.00	6.46	35	4.86	0.70	0.265	3.68	5.46	3	4.44	0.95	0.144	3.00	6.37	20	6.22	0.96	0.310	4.54	7.99	9
R1/4	5.60	1.09	0.165	3.24	7.84	44	5.48	0.58	0.124	4.09	6.85	7	5.59	1.04	0.152	3.11	7.31	44	6.22	1.14	0.320	4.54	7.99	13
R1/2	6.81	0,89	0.122	4.87	8.85	53	6.34	0.90	0.201	5.00	7.92	22	6.54	1.07	0.133	4.00	8.87	47	6.55	1.00	0.177	4.11	8.61	32
R3/4	7.89	1.64	0.232	4.32	12.11	50	7.98	0.85	0.200	6.48	9.87	20	7.88	1.38	0.240	5.86	10.76	65	7.72	1.13	0.146	5.78	9.78	60
Rc	9.69	1.72	0.229	6.35	12.86	56	9.62	1.19	0.175	6.05	13.94	18	9.51	1.80	0.238	5.49	12.94	33	9.29	1.14	0.132	7.04	11.94	74
A1/2	10.91	1.54	0.192	6.92	15.08	64	10.31	1.05	0.135	7.60	12.57	46	10.37	1.04	0.146	8.66	12.94	57	10.49	1.15	0.136	7.16	13.31	72
Ac	17.35	3.64	0.166	5.92	23.96	479	13.85	3.21	0.210	9.29	22.34	60	17.39	3.49	0.153	17.39	23.94	51	13.28	2.08	0.086	8.12	22.02	585

A.5.8. Mean age of children within a stage for I1.

	Northern females						Western females						Northern male						Western male					
Stage	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N	Mean	SD	SEM	Min.	Max.	N
Cc	3.63	0.22	0.082	3.38	3.38	7							4.37	1.12	0.458	3.16	5.81	6						
Ri	3.95	0.53	0.129	3.00	4.80	17	4.04	0.65	0.376	3.49	4.76	3	3.75	0.67	0.139	3.00	5.80	23	5.18	0.77	0.385	4.18	6.01	4
R1/4	4.77	0.94	0.153	3.16	7.20	38	4.76	0.70	0.249	3.68	5.46	8	4.57	1.06	0.160	3.00	7.16	44	4.66	0.94	0.358	3.44	5.99	7
R1/2	6.04	1.17	0.180	4.22	8.69	42	5.54	0.51	0.110	5.02	6.85	21	5.81	1.09	0.154	3.11	9.00	50	6.22	1.14	0.317	4.54	7.99	13
R3/4	6.74	0.82	0.116	4.32	8.85	50	6.34	0.90	0.201	5.00	7.92	20	6.83	1.16	0.147	4.00	10.43	62	6.53	0.99	0.173	4.11	8.61	33
Rc	8.22	1.51	0.211	6.29	12.11	51	7.95	0.89	0.223	6.48	9.87	16	7.98	1.33	0.246	5.86	10.72	29	7.78	1.10	0.141	6.17	9.78	61
A1/2	9.85	1.88	0.271	6.35	15.08	48	9.49	1.02	0.147	6.05	11.56	48	9.56	1.81	0.246	5.49	12.95	54	9.28	1.11	0.131	7.04	11.94	72
Ac	16.59	4.03	0.173	5.92	23.96	543	13.12	3.23	0.191	7.60	22.34	284	16.79	3.87	0.162	8.93	23.94	573	12.98	2.18	0.085	7.16	22.02	657

A.6. Odds Ratios for Mean age of Attainment Comparison

A.6.1. Table showing the odds ratios for the stages that were significantly different between male groups of different ethnic origin.

	Males				
Tooth	Stage	OR	95% CI		p value
M3	Cr	0.417	0.305	0.572	p<0.001
	Ci	0.408	0.296	0.560	p<0.001
	Cco	0.387	0.281	0.533	P<0.001
	Coc	0.515	0.376	0.706	p<0.001
	C3/4	1.489	1.069	2.074	p<0.05
	R1/2	2.206	1.275	3.818	p<0.05
	R3/4	2.171	1.163	4.053	p<0.05
M2	Ri	0.460	0.272	0.780	p<0.05
	R1/4	0.369	0.213	0.638	p<0.001
M1	Rc	0.204	0.104	0.403	p<0.001
	A1/2	0.221	0.119	0.412	p<0.001
	Ac	0.250	0.152	0.413	p<0.001
P2	Ri	0.535	0.335	0.856	p<0.05
	R1/4	0.380	0.239	0.603	p<0.001
	R1/2	0.513	0.328	0.800	p<0.05
	Ac	2.441	1.501	3.971	P<0.001
P1	Ri	2.855	1.564	5.210	p<0.05
	R1/4	0.458	0.265	0.791	p<0.001
	R1/2	0.305	0.182	0.512	p<0.001
	R3/4	0.586	0.363	0.914	p<0.05
C	R1/2	0.169	0.094	0.304	p<0.001
	R3/4	0.292	0.177	0.408	p<0.001
	Rc	0.576	0.364	0.910	p<0.05
I2	R1/2	0.505	0.259	0.985	p<0.001
	R3/4	0.319	0.176	0.578	p<0.05
	A1/2	0.524	0.324	0.849	p<0.05
	Ac	0.317	0.197	0.508	p<0.001
I1	R3/4	0.335	0.173	0.649	p<0.001
	Rc	0.232	0.128	0.419	p<0.001
	Ac	0.481	0.295	0.786	p<0.05

A.6.2. Table showing the odds ratios for the significantly different stages between female groups of different ethnic origin.

	Females				
Tooth	Stage	OR	95% CI		p value
M3	Cr	0.427	0.298	0.611	p<0.001
	Ci	0.342	0.237	0.493	p<0.001
	Cco	0.365	0.253	0.527	p<0.001
	Coc	0.386	0.267	0.557	p<0.001
	C1/2	0.526	0.362	0.764	p<0.001
	C3/4	0.378	0.242	0.591	p<0.001
	Ac	0.298	0.127	0.697	p<0.05
M2	Ri	0.250	0.138	0.451	p<0.001
	Rcl	0.274	0.155	0.484	p<0.001
	R1/2	0.421	0.228	0.777	p<0.05
M1	Rc	0.337	0.151	0.754	p<0.05
	Ac	0.363	0.228	0.580	p<0.001
P2	Coc	0.301	0.123	0.737	p<0.05
	C1/2	0.344	0.155	0.766	p<0.05
	C3/4	0.503	0.274	0.923	p<0.05
	Ri	0.381	0.236	0.614	p<0.001
	R1/4	0.321	0.206	0.500	p<0.001
	R1/2	0.436	0.283	0.673	p<0.001
	A1/2	2.740	1.443	5.208	p<0.05
P1	R1/4	.0329	0.190	0.570	p<0.05
	R1/2	0.340	0.208	0.557	p<0.001
	R3/4	0.463	0.286	0.749	p<0.05
	Rc	0.435	0.243	0.778	p<0.05
C	R1/2	0.299	0.173	0.517	p<0.001
	R/34	0.359	0.221	0.584	p<0.001
	Rc	0.371	0.230	0.599	p<0.001
I2	A1/2	0.503	0.314	0.849	p<0.05
	Ac	0.420	0.269	0.658	p<0.001
I1	R1/2	0.335	0.129	0.875	p<0.05
	A1/2	0.305	0.160	0.582	p<0.001
	Ac	0.500	0.310	0.807	p<0.05

A.6.3. Table showing the odds ratios for the gender differences.

Tooth	Stage	OR	95%CI		p value
	Northern Group				
M3	Coc	0.715	0.516	0.992	P<0.05
	C1/2	0.715	0.511	0.999	P<0.05
	C3/4	0.596	0.418	0.852	P<0.05
	Rc	0.588	0.402	0.859	P<0.05
	A1/2	0.562	0.378	0.837	p<0.05
	Ac	0.421	0.267	0.663	p<0.001
M1	R3/4	2.002	1.185	3.384	p<0.05
P1	Rc	0.530	0.311	0.903	p<0.05
C	R1/4	2.187	1.337	3.577	p<0.05
	R1/2	2.189	1.336	3.589	p<0.05
	R3/4	1.578	0.982	2.534	p<0.05
	A1/2	1.845	1.093	3.112	p<0.05
	Ac	1.775	0.993	3.173	p<0.05
	Western group				
M3	C3/4	3.127	2.055	4.758	p<0.001
	Cc	3.751	2.347	5.995	p<0.001
	Ri	1.876	1.085	3.245	p<0.05
M2	Ri	2.637	1.359	5.115	p<0.05
	Rcl	2.285	1.242	4.204	p<0.05
	R1/2	1.958	1.122	3.418	p<0.05
P1	R1/2	1.747	1.045	2.923	p<0.05
C	R1/2	2.483	1.266	4.872	p<0.05
	R3/4	1.933	1.163	3.213	p<0.05
	Rc	2.442	1.566	3.822	p<0.001
	A1/2	2.558	1.582	4.136	p<0.001

A.7. Comparison With other Published Studies

A.7.1. Garn et al. (1958)

Table showing mean age comparison between three groups (Western Sudanese, Northern Sudanese and Garn et al. 1958).

	Boys								
Stage	Cr			Cc			Ac		
Group	Garn	West	North	Garn	West	North	Garn	West	North
P1	2.2			7.4	7.0	7.4	13.0	13.9	13.7
P2	3.3			8.2	8.5	8.8	14.1	15.0	14.4
M1	4.3			5.8	4.0	4.2	10.3	10.1	10.9
M2	3.5		3.1	8.9	9.4	9.7	15.0	15.6	15.32
M3	9.0	8.5	8.5	14.6	14.7	14.6			
	Girls								
Stage	Cr			Cc			Ac		
Group	Garn	West	North	Garn	West	North	Garn	West	North
P1	1.8			7.2	6.9	6.5	12.5	13.9	13.5
P2	3.3			8.0	8.2	8.7	13.4	14.0	14.6
M1	4.0			5.7	3.1	4.0	10.6	10.3	11.0
M2	3.2		3.5	8.7	9.0	9.7	14.6	15.6	15.6
M3	9.0	9.0	9.75	15.2	14.2	14.9			

A.7.2. Liversidge et al. (2006)

Comparison of mean age for stage Ac in different teeth between teeth of Northern (N=1646), Western Sudanese (N=1249) and White Children (N=9002) data published Liversidge et al. (2006).

	Males						Females					
	Liversidge	SEM	Western	SEM	Northern	SEM	Liversidge	SEM	Western	SEM	Northern	SEM
I1	8.34	0.11	9.59	0.09	10.00	0.15	7.90	0.11	9.48	0.14	9.93	0.15
I2	9.33	0.10	10.51	0.09	11.23	0.13	8.81	0.11	10.43	0.12	11.52	0.17
C	13.23	0.12	14.11	0.08	13.86	0.12	11.72	0.10	13.95	0.40	13.49	0.14
P1	13.04	0.12	13.90	0.08	13.67	0.13	12.24	0.11	13.94	0.16	13.51	0.32
P2	14.12	0.14	15.03	0.13	14.36	0.12	13.47	0.14	14.09	0.71	14.59	0.14
M1	9.97	0.10	10.11	0.08	10.86	0.14	9.30	0.10	10.27	0.10	11.02	0.16
M2	15.25	0.15	15.57	0.15	15.32	0.11	14.78	0.16	15.62	0.22	15.55	0.16

A.7.3. Liversidge et al. (2008)

Table 22. M3 comparison of mean age for selected stages of Northern (N=1646), Western Sudanese (N=1249) and White Children (N=959) data published by Liversidge et al. (2008).

	Boys						Girls					
	Liversidge	SEM	West	SEM	North	SEM	Liversidge	SEM	West	SEM	North	SEM
Crypt	9.54	0.32	8.35	0.17	8.49	0.38	8.63	0.39	8.23	0.49	9.75	0.25
Ci	9.49	0.31	8.45	0.22	9.54	0.24	9.11	0.21	8.75	0.21	10.06	0.22
Cco	10.30	0.32	9.45	0.14	9.72	0.26	10.28	0.37	9.50	0.19	10.79	0.21
Coc	11.09	0.30	10.29	0.12	10.17	0.23	11.66	0.57	10.11	0.17	10.82	0.23
C1/2	12.54	0.30	11.55	0.10	11.14	0.17	11.97	0.31	11.23	0.14	11.61	0.21
C3/4	12.80	0.35	13.30	0.09	12.44	0.18	13.79	0.36	12.26	0.12	13.29	0.17
Cc	14.49	0.43	14.04	0.09	13.72	0.17	15.42	0.74	13.04	0.17	14.15	0.17
Ri	15.16	0.36	14.58	0.11	14.76	0.14	15.43	0.53	14.23	0.18	14.88	0.16
Rcl	15.02	0.51	14.91	0.10	15.10	0.14	15.92	0.71	15.25	0.23	15.28	0.17
R1/4	15.39	0.30	15.49	0.15	15.66	0.14	16.90	0.31	15.49	0.22	15.70	0.18
R1/2	16.87	0.37	17.26	0.31	16.62	0.15	17.08	0.41	16.27	0.23	16.69	0.18
R3/4	17.62	0.30	15.48	0.32	17.64	0.16	18.39	0.35	17.28	0.30	18.06	0.20
Rc	17.73	0.45	18.63	0.32	18.51	0.15	19.71	0.38	18.11	0.29	19.16	0.19
A1/2	19.02	0.26	19.05	0.31	18.89	0.17	19.94	0.20	18.44	0.20	19.86	0.18