International Erasmus Mundus Master in

QUATERNARY AND PREHISTORY

Testing Geometric Morphometrics versus Traditional Methodologies on Mandibular Morphology and Dental Eruption for Age Estimation in Modern Human

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ABSTRACT

Dentition can provide an important indication of age, health, and developmental disorders of an organism and thus is widely used in paleo sciences for skeletal identification. However, traditional methods of estimating age based on dental eruption involve subjective assessments of tooth development and can be prone to error. In this study, a geometric morphometric approach has been developed and tested to predict age with greater accuracy than traditional methods. The study focuses on the correlation between the permanent teeth eruption (from crown completion to its eruption) and the modification in the mandible shape. Using two three-dimensional landmark configurations, the osteological features of the mandible and mandibular dentition (incisor, canine, premolar and first molar only) have been tracked on individuals from age 4 years to 14 years separately. Principal component analysis of the morphometric data is used to identify the groups of individuals based on mandibular form variation and dental growth pattern. The covariation between the developing teeth and mandibular form is then observed through linear regression. The main aim of the study is to identify the maximum correlation between phases of dental formation and mandibular alteration. The benefit of this research is to test the protocol in a well-known reference collection (in terms of sex and age-at-death) in order to be able in the future to apply the same protocol to fossil remains and then have better accuracy in age-at-death determination.

Keywords: dental formation, mandibular morpho-variation, geometric morphometrics, age estimation, principal component analysis.

CHAPTER 1

INTRODUCTION

1.1. Research Overview

Human biological maturation is not synchronised with its chronological age. That is to say, while some parts of the body start developing early in the growth, others may start after a long time. Scholars have tried to find if there is a relation between the ontogeny of distinct parts of the human body and if a relation can be established with the chronological age (Bromage, 1987; Demisch & Wartmann, 1956; Hagg & Taranger, 1985; Liliequist & Lundberg, 1971; Miles, 1963; Moorrees et al., 1963; B. Smith, 1991a; Tomes, 1923). In this respect, dental ontogeny is incredibly important as it is one of the key maturity indicators not just for living individuals but also for skeletal remains. It is easily accessible physically in comparison to other osteological maturity indicators and thus can be observed at regular intervals (Gleiser & Hunt Jr., 1955; Moorrees et al., 1963; Nolla, 1952; Smith, 1991). Through years of scientific endeavour, it has been found that age ranges can be assigned to particular stages of dental development. This is incredibly important in various scientific fields like medicine, forensic, legal, archaeological as well as anthropological and paleoanthropological studies.

It should be noted that dental remains are probably the skeletal remains most commonly found in an archaeological context, and often the most abundant. Dental tissue is regarded as one of the most resilient tissues of the human body. They are formed of a hard covering called enamel which is mostly composed of 98% inorganic material (Hillson, 2005). Because of this, they can undergo prolonged periods of sedimentary context with limited damage. In anthropology, the question of individual identification is important for skeletal remains. Teeth are also important because they provide a key indication of biological development (Cameron, 2002). The incremental layers of enamel formation have been studied for age estimation in the field of forensic and paleoanthropological studies (Beynon & Wood, 1987; Schour & Hoffman, 1939). However, the methods of analysis of such microscopic structure require prohibitive cost equipment which are not easily available. Instead, some of the widely used methodologies for age estimation rely on visual observation of histological or radiographic data. These methodologies have been developing over centuries to provide a relatively accurate measure of age estimation. It is seen that some scholars even apply these anthologies in paleoanthropological studies for the estimation of the age of hominins.

However, there are several problems regarding the methodologies and their level of accuracy. The current work is an exploratory study of the methods of age estimation based on dental eruption. Using geometric morphometrics, a new protocol for age estimation has been tested which utilises mandibular shape deformation and dental eruption as a combined configuration and checks the reliability of the proxy for age estimation.

1.2. Research problem

There is a close relation between each of the maturity indicators as they are part of the growth process of humans. Scholars have highlighted that a close relationship exists between mandibular ontogeny and dental development; however, the specificities of this aspect of growth need to be studied more (Coquerelle et al., 2010; Franklin et al., 2008; Krarup et al., 2005). Age estimation methods are therefore mainly based on single maturity indicators like dental development, somatic maturation, etc. but are seen to either underestimate or overestimate the age of individuals (). Having a technique of age estimation with high accuracy is especially essential in anthropological, paleoanthropological and forensic studies as it helps in the identification of skeletal remains. It requires an age estimation methodology which can be adapted to different ethnicities, sexes or species. The hypothesis underlying the current study is to ascertain whether the combined assessment of mandibular morphological changes and dental eruption stages can provide a proxy for age estimation of high accuracy and tackle the issues of adaptability in fossil remains. The current work explores the concept of combining two maturity indicators, that is dental eruption and mandibular deformation during pre-pubertal growth (post-natal to age 13 years approximately). It aims to explore a new protocol for age estimation by exploring the complete configuration of mandible and dental development as well as separate and check the degree of relation with age and allometric changes that each aspect can be indicated.

1.3. Aims and Objective

The current study aims to assess the impact of the dental eruption on the ontogenic growth of the mandible. It draws the argument of the relationship between biological age and chronological age through the observation of dental development. The objective of the study is to apply geometric morphometrics to observe the morphological deformation of mandibles during dental development. Using statistical analysis, the study aims to conduct a qualitative assessment of the shape deformation in the morphospace, based on the observation of an integrated configuration of mandibular and dental growth against a pre-selected sample. It also aims to check the accuracy of the method as a new proxy for age estimation.

The work has a further scope of implication for age estimation of fossil hominins with a higher accuracy. In paleoanthropological studies, the rate of dental development of humans and other living primates is compared to estimate the growth rate among extinct hominins (Cunha et al., 2009; M. C. Dean & Wood, 1981; Harvati, 2000). The aim of the study is to test the reliability of dental eruption along with mandibular morphology as a growth indicator without dependency on the growth rate of different species. Theis provides a scope of application to species with unknown rates of growth like for example extinct hominins and estimates the age of skeletal remains.

1.4. Conceptual framework

The thesis is comprised of 6 Chapters including the current one, that provide an introduction to the study, along with a brief discussion of the aims and objective, study overview, research hypothesis and its significance and limitations. Chapter 2 of State of the Art will provide a comprehensive overview of the existing literature and current knowledge relevant to the research topic: starting from a brief introduction to dental and mandibular anatomy, human ontogeny and growth as well as important concepts like dental age, maturity indicators and other. The chapter also discusses the age estimation methodologies that have been formulated over the years based on dental development. It particularly highlights the methods based on statistical analysis based on the classification of Smith (1991). The chapter will include a critical analysis of previous research, methodologies, and findings related to age estimation techniques to establish a solid foundation for the research by highlighting gaps, inconsistencies, and potential avenues for further exploration.

Chapter 3 will outline the research methodology employed in the study. It will describe the research design, including whether it is experimental, observational, or analytical. The chapter will detail the process of data collection, including the selection of study participants, criteria for inclusion, and ethical considerations. It provides information regarding sample preparation, how the group classification is developed and the landmark configuration that will be applied to the prepared samples. It also gives a brief introduction to the statistical methodology used for the data analysis, such as statistical tests or software tools, will also be presented.

In chapter 4, the research findings will be presented. First, the analysis of the sample will be conducted using the traditional methodologies of AlQahtani et al. (2010), Demirjian et al. (1973), Gleiser & Hunt Jr. (1955) and Logan & Kronfeld (1933). Next, the analysis-based new classification will be done using statistical software and 3-dimensional geometric morphometrics. Any patterns, trends, or correlations identified from the data analysis will be highlighted.

Chapter 5, discussion, will interpret and contextualize the results obtained from the research. It will begin by restating the research hypothesis and objectives, and then proceed to address each research question or hypothesis individually. The findings will be critically analysed concerning the existing literature reviewed in Chapter 2. Strengths and limitations of the study's methodology will be discussed, including potential sources of bias or error. The implications of the research findings within the broader context of age estimation techniques and their applications will be explored.

Lastly, the thesis includes chapter 6 comprising the conclusion that can be drawn from this work. It is to summarise the study and reiterate its significance. It will discuss the potential contributions to the field, suggest areas for future research, and emphasize the importance of the combined approach to age estimation using mandibular morphological changes and dental eruption stages.

CHAPTER 2

STATE OF THE ART

2.1. Physiological changes of modern human

2.1.1. Ontogenic changes in the human skeletal system from juvenile to subadult

Growth and development are ongoing processes among living organisms. In modern humans, the rate or velocity of growth is never homogeneous. According to Cameron (2002), following birth, two distinct phases of growth spurt, during which are increases in growth rate can be seen. The first occurs at 6–8 years which is known as the juvenile growth spurt while the second takes place around 11–18 years and is called the adolescent growth spurt (Figure 2.1). The growth velocity is measured based on the changes in the height. The term was first coined by Tanner (JM Tanner, 1985).

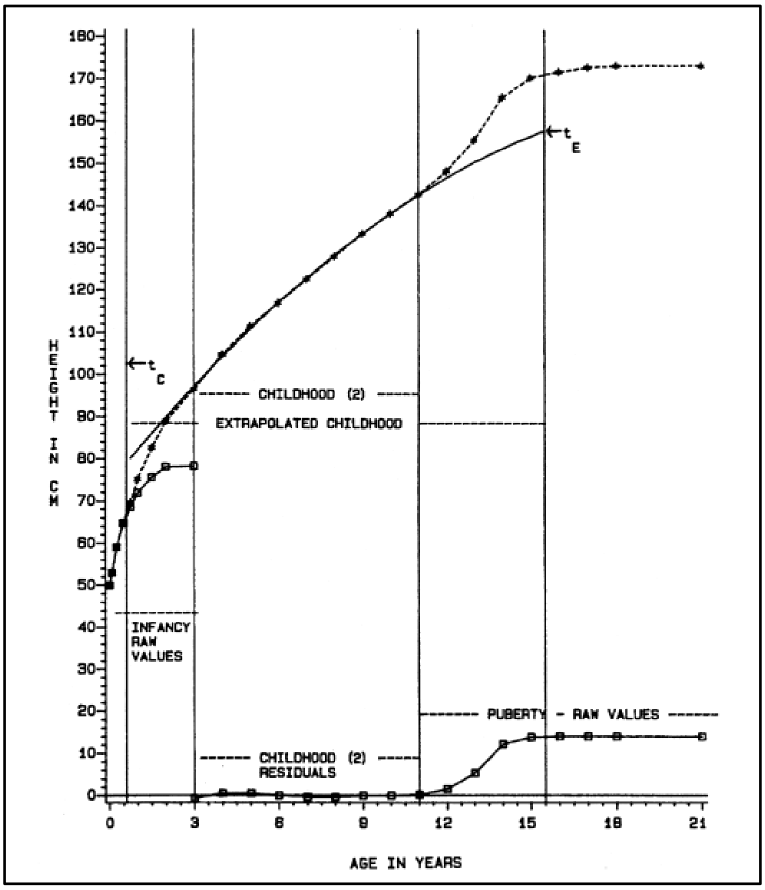

Figure 2.1: The three phases of postnatal growth, according to the ICP model (Cameron, 2002)

The juvenile growth spurt is characterised by the growth of height and body fat. However, no sexual difference is noticed during this phase of the growth spurt. It has also been pointed out in the works of JM Tanner (1985) and Cameron (2002), that there is a possibility of minor difference in timing depending on the sex but no difference in terms of magnitude when it comes to growth during the preadolescent stage. However, the rate of growth is slower in comparison to infancy and adolescence.

The process of growth and development takes almost 20 years for modern humans to complete. It is controlled by the endocrine system like the hypothalamus, as well as external environmental factors. In general, the subject of growth and maturity is rather complex and still unsolved by researchers. There are several growth reference charts to help estimate the 'normal' standard rate and time and growth. According to Cameron (2002), one of the earliest works is of a French scholar, De Montbeillard whose growth chart laid the foundation of many later works.

2.1. 2. Concept of biological and chronological age: Dental Age as a maturity indicator

According to Smith (1991), dental age can be defined as the time based on the time assessment between formation and eruption of teeth. Dental age is a methodology used in clinical as well as forensic sciences. It creates a comparison between the skeletal development of an individual with its physiological development. That is to say, since dental development is an indicator of biological or physiological age, it can be compared with chronological age which is indicated throughout the skeletal and structural development of an individual (Smith, 1991). There are many assessments of dental age as a standard for human growth and development. Firstly, dental development is resistant to nutritional effects. It also has a lower coefficient of variation in comparison to skeletal development (Garn et al., 1959; Garn & Lewis, 1957). Therefore, the correlation between dental growth and the relative weight, and stature of an individual is consistently low. It also does not show any effect due to environmental changes. It is also very less affected by the abnormalities related to endocrinopathies or developmental insults affecting sexual maturation, stature, bone age, etc (Maber et al., 2006). The cause of the variation is yet unclear.

Since dental development is a prime parameter for biological age assessment, it is very useful in clinical as well as anthropological studies. It tends to be the least affected tissue which helps in not only clinical assessment for assessing physiological age without intervention in normal growth but also forensic and anthropological studies for assessment of age which is an important unknown variable (Smith, 1991). Looking into the importance of the dental age, many scholars have produced different methodologies to approach the problem of estimating the dental age of an individual with the most accuracy.

2.2. Dental formation and methods of age estimation

2.2.1. Dental anatomy and process of formation

Teeth are mainly divided into three parts: the crown, the neck and the root (Figure 2.2). The crown is the flat, top visible part of the tooth which protrudes above the alveolar bone. It is covered with enamel which is a hard calcified material. The narrow part of the tooth that anchors into the alveolus of the mandible or maxilla and helps in the fixed position of the tooth is the root. Roots can have more than one root which can either be completely fused or remain partially or completely bifurcated. The bifurcates roots are known as secondary roots. The fusion of roots is often discernible by shallow developmental grooves. Lastly, the cementoenamel junction (CEJ) of the crown and the root is called the neck. It is a cervical line and is visible on many tooth specimens. The neck and the root of the tooth are covered with cementum while the crown is covered with enamel tissue. Inside the root, the pulp cavity which holds the nerve and blood vessels is present.

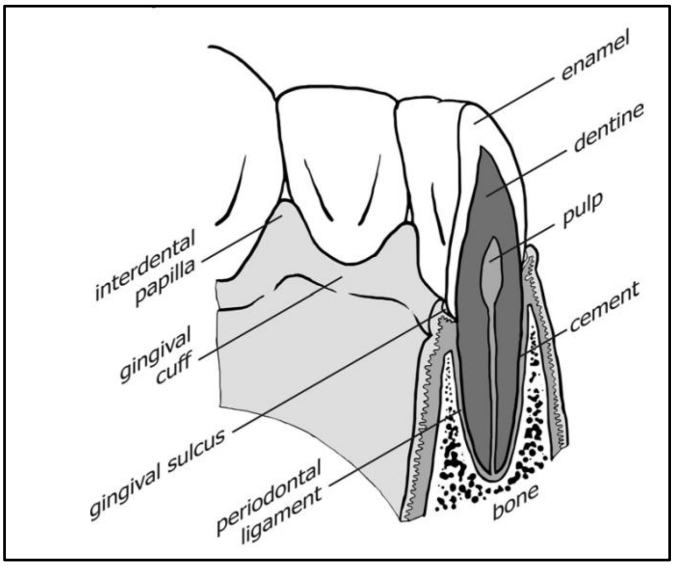

Figure 2.2:: Parts of tooth (Hillson, 2005)

The tooth is composed of inorganic as well as organic dental tissues. The inorganic components are mainly calcium phosphate $Ca_{10}(PO_4)_6X_2$ in the form of apatite (Hillson, 2005). Besides calcium (Ca^{2+}) and phosphate ($PO^{2-}{}_4$) ions, hydroxyl (OH^-) and fluoride (F^-) can also be found in the inorganic composition of dental tissue. Sometimes sodium (Na+), strontium (Sr+), orthophosphate (HPO2− 4), carbonate (CO2− 3) or hydrogen carbonate (HCO− 3), chloride (Cl−) are found in dental tissue due to their substitution from drinking water. The other types of dental tissue found are composed of organic elements. Collagen, enamel protein and other substances are the main types of organic material found in the composition of dental tissue.

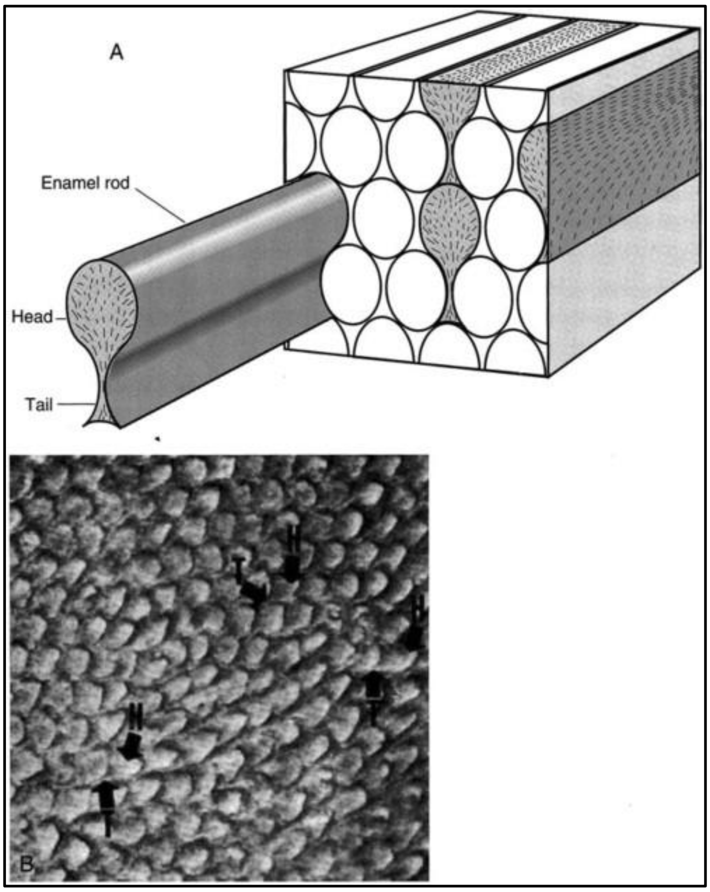

Figure 2.3: Crystalline structure of enamel (Hillson, 2005)

Enamel is a unique type of mammalian tissue as it is almost entirely composed of inorganic material (96%) and a very minute level of organic material (less than 1%) along with the presence of some water. The crystalline structures, forming the prismatic sheath create boundaries for the organic material (Figure 2.3). It is an acellular structure and is formed by the internal enamel epithelium. This layer of closely linked sheet of cells is called ameloblast cells which are narrow and cylindrical in structure. These cells contact together to form the layer called enamel and the process of formation is called amelogenesis. Tomes (1923) studied the different phases of amelogenesis in the 20^{th} century. His work led to the discovery of different stages of amelogenesis in different parts of the crown. Tomes studied the difference in size and shape of the cells during the process in multiple species. During amelogenesis it is sent at the cells are flat and have a protuberance which is called ‘ruffle’. The enamel development process after the formation of the ‘ruffles’ is known as Tomes’ process (Hillson, 2005). The entire process of amelogenesis can be divided into two stages: matric production and maturation. Matrix production is the process in which the organic material is in the form of thin ribbon-like crystallites which are embedded in the matrix. The crystallites are organised into bundles of simple prismatic shapes with variations in the width (4–12 µm). The composition of the matrix after this stage is one-third mineral, with one-third protein and one-third water. The matrix formation takes almost one year to be completed by each ameloblast cell. The protein and water are then removed during the maturation process. The crystallites which are embedded in the matrix increase in size during this stage of amelogenesis which leads to the development of a mature enamel. The Tomes process begins after a few days of matrix production and then ends before enamel maturation. In the enamel maturation phase, the ameloblast forms the ruffle cell walls which then lead to the removal of proteins. The maturation process slows down during the tooth eruption phase.

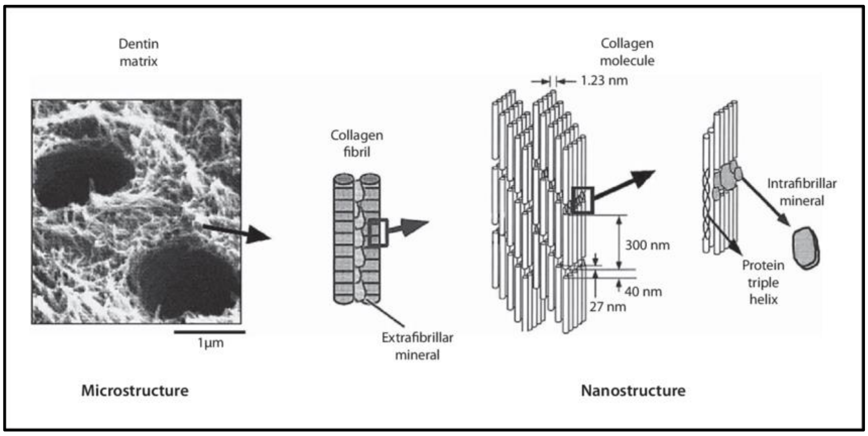

Figure 2.4: Dentine structure (Hillson, 2005)

Dentine is the dense bone-like material found in all mammalian teeth (White et al., 2011). It is calcified and extends to the socket in the inner part of the alveolar bone of the jaw. Dentin (or dentine) is the tissue that forms the core of the tooth. This tissue has no vascular supply but is supported by the vascular system in the pulp and is lined on the inner surface (the walls of the pulp cavity) by odontoblasts, and dentin-producing cells (Figure 2.4). The mineral composition of dentine is 72% inorganic material, 18% collagen and 2% other organic material. The main composition of dentine is collagen with minor presence of crystallites (20-100mm) and some amorphous calcium phosphate. It is formed of odontoblast cells which are living tissue unlike the enamel. Dentine formation takes place in two phases: formation of predentine and then seeding of crystallites. The formation of predentine is the phase of secretion of organic matrix next to the layer of odontoblast cells. For the process of mineralization, the seeing of crystallites takes place in the microscopic cavities called the matrix vessels. After the seeding, calcospherites are formed which are small spherical bodies of mineralised dentine. The most dominant feature of the structure of dentine is the dentinal tubules which extend from the pulp cavity to the enamel-dentine junction (EDJ). Dentine is almost 2-3 µm near the pulp chamber and the EDJ, it is less than 1 µm.

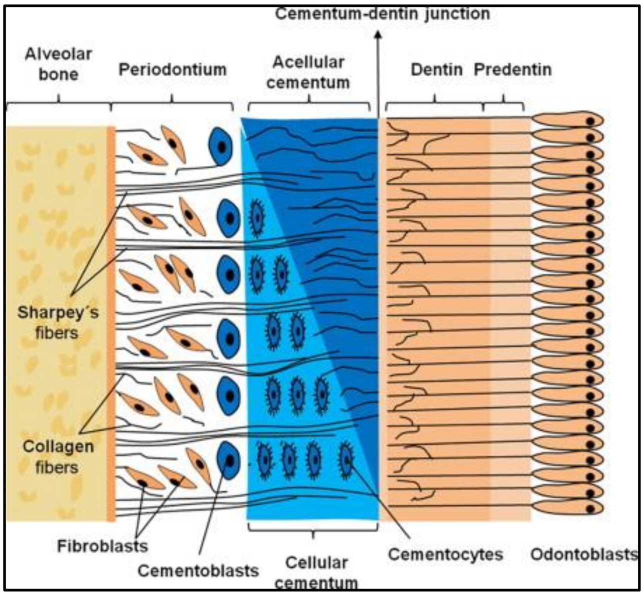

Figure 2.5: Cementum formation (Matalová et al., 2015)

Cementum is a hard, mineralised tissue which is highly variable in nature. It is 20 µm in thickness and covers just the toot or both, the root and crown in mammals. Cementum has a chemical composition similar to bones with 70% inorganic material, 21% collagen and 1% organic material. It is more porous than bones serves to anchor the periodontal ligament and helps in holding the tooth inside the alveolar socket (Figure 2.5). The layer of cementum is composed of organic material which is first manufactured and later mineralised. It is formed by cementoblast cells on the edge of the periodontal ligament and then is formed on the surface of enamel and dentine (Figure 2.5). When a cementoblast cell gets entrapped within the cementum, it develops the tissue called cemetocytes. The space occupied by the cemetocytes cells is known as lacunae (7-20 µm in diameter). They are irregular in shape and extend from the cell wall to the cementum surface forming tunnels called canaliculae (Figure 2.5). The density of lacunae and cementum varies greatly depending on the layering structure. According to Barbakow et al. (1981), cemetocytes can be classified into four major categories: Afibilar cement, extrinsic cement, mixed fibre cement and intrinsic cement. Afibilar cement is heavily mineralised and is devoid of cemetocytes or collagen fibres. It forced the ground structure of cementum. Extrinsic fibres (6-7 µm in diameter) of the cementum are perpendicular to the growing surface. They are fully mineralised but present without the cemetocytes. During rapid growth of cementum, the extrinsic cells are less mineralised while during slower cementum growth, it mineralises fast. These differences can be noticed through the presence of a pit at the end of extrinsic fibres because of the unmineralized cores. The cementoblast cells also manufacture intrinsic fibres (1-2 µm in diameter) which form a patch of organised series of fibre with similar orientation. Mixed fibre cement is formed of both extrinsic and intrinsic collagen fibres although cemetocytes may not be present in them.

According to Ten Cate (1985), dental formation begins from the embryo stage of an individual. In the head region of the embryo, the epithelium cells form a layer of tissue called mesenchyme that eventually forms the bones and cartilage of the jaw. This layer is called the dental laminae. Eventually, the proliferation of epithelium cells on the edge of dental lamina forms the tooth germs. The earliest stage of tooth germ formation is the bud stage during which the oral epithelium thickens and invaginates into the underlying mesenchyme, forming a dental papilla. The dental papilla is responsible for dentine and pulp formation. After the bud stage, the cap stage begins during which the tooth bud develops further and gives rise to an enamel organ which later deposits on the enamel (Figure 2.6). The growth of the bud like organise also causes indentation of the side of the dental laminae. These indentations grow to enter the bell stage of dental growth during which deposition of the hard tissue takes place. During this stage, the enamel organ differentiates into four distinct layers: the inner enamel epithelium, stratum intermedium, stellate reticulum, and outer enamel epithelium. The dental papilla also differentiates into odontoblasts, which secrete dentin. The next stage of dental growth is the apposition stage during which the enamel and dentin are secreted and deposited onto the tooth surface. The tooth continues to mature, and the enamel and dentin mineralize.

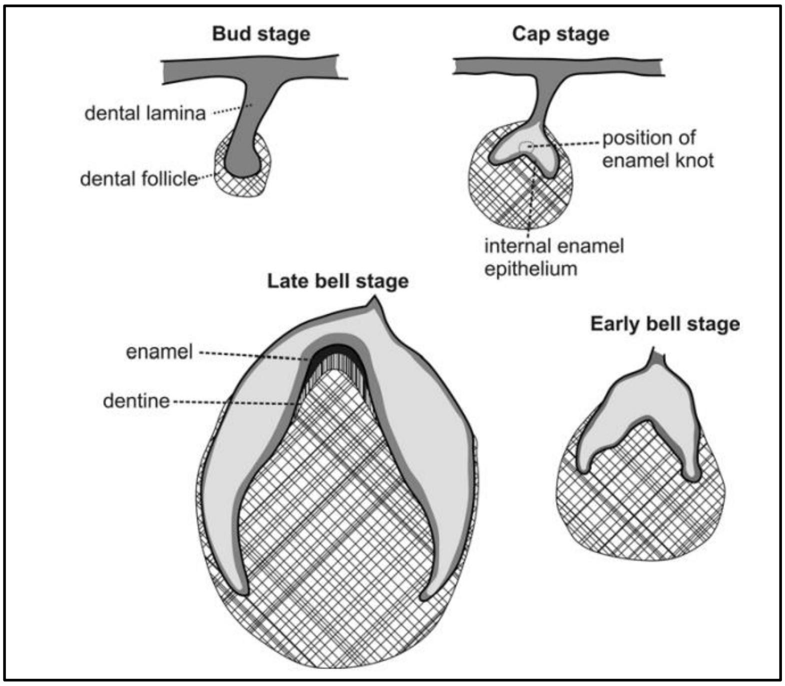

Figure 2.6: Development of tooth germs (Hillson, 2005)

Some of the tooth structures go through a continuous process of formation. Dentine is seen to form appositional tissues continuously. This leads to the formation of a secondary dentine which lines the pulp chamber. According to Marquez et al. (1986), secondary dentine formation is initiated by the subsequent process of dentinogenetic. The secondary dentine is seen to have a large variation depending on the biological response, masticatory stress and fluctuating temperatures (Philippas & Applebaum, 1968; Rösing & Kvaal, 1998). The deposition of secondary dentine in turn is seen to affect the area of the pulp cavity. Gustafson (1950) has suggested a 6 criteria methodology for a linear measurement of the reduction of the size of the pulp cavity due to secondary dentine deposition. Such measurement has also been devised by Woods et al. (1990) and (Rösing & Kvaal, 1998).

Another change that can be identified in the dental composition with the development of age is the transparency of the root. This event was first described by Tomes (1923) where the root of a tooth undergoes more calcification in association with sclerosis of the dentinal tubules. This results in a reduction in the diameter of the dentine tubule and decreased fracture resistance.

Cementum, which is formed in layers of primary and secondary acellular deposition has a layered appearance due to the altered mineral crystal orientations that reflect a cyclic annual formation pattern (Cool et al., 2002).

There is also age-related discolouration of a tooth due to the degradation of the organic components of the dental hard tissue. Ten Cate et al. (1977) in their study, estimated the chronological age of individuals based on the assessment of the colour densitometry. In this study, a clear relationship between the root colour and chronological age of an individual was recognised.

As evident from the above discussion, the dental components undergo chemical change with the passage of age of an individual. According to Bhussry & Emmel (1955), the nitrogen content in the tooth is found to increase which is thus causing a change in the colour of the teeth. There is also an increase in the amount of metal ions (Fe, Cu, Sn, and Pb) that enter the surface of the enamel through saliva and is commonly found in older individuals. The calcium content is also increased mostly in the dentine. This process of increase of calcium composition in the dentine is advocated by the fusion state of calcospherites. The calcium content increases while the amount of phosphate remains the same. This occurrence has been observed using Energy-dispersive X-ray analysis and has been correlated with chronological age (Atsu et al., 1998). The aspartic acid content in the dental tissue also undergoes alteration through age. The tissues of teeth which are metabolically inactive or brady trophic undergo racemization of aspartic acid. It's effected by temperature, pH, humidity and other factors.

Lastly, dental tissues show the assimilation of various minerals like fluoride over time. These depositions are secondary and mostly affected by the level of concentration in drinking water, food, and dietary supplement intake by an individual. The dentine has a higher intake capacity of fluoride than enamel and is mostly deposited near the pulp chamber.

All these various changes that take place in dentition have been correlated with chronological age. Scholars have devised methodologies for the estimation of age based on the above-mentioned changes. In the following section of the thesis, a discussion of these methodologies has been made in further detail.

2.2.2. Stages and sequence of dental eruption

The process of tooth formation can also be divided into four chronological phases: crown initiation, crown completion, eruption and tooth formation (Hillson, 2005). There are occlusal projections on the crown known as cusps (White et al., 2011). During the calcification of the enamel cap in the crypt (a hollow chamber in the alveolar bone where the tooth formation takes place), the process begins at the cuspal apices and then proceeds to the root. The eruption of the tooth begins after the completion of tooth formation and before complete root formation. It is the movement of the tooth from the jaw in the oral cavity at a functional position and this process continues a subsequent movement thereafter (Liversidge, 2015). This process includes the development of dental follicles, resorption of alveolar bone and formation of the root along the gingivae to the occlusion plane. During the pre-eruption phase, small changes in the crown can be seen. However, the movement towards the axial direction following the gubernacular canal begins during the active eruption phase. It includes an increase in root length, development of the periodontal ligament, and epithelial attachment as well as development of the dental pulp, cementum on the root surface, alveolar bone remodelling, and growth of the jaws. The active phase of eruption can be seen in the following stages: intra-osseus (mandibular developing tooth is below the alveolar bone) and supra-osseus (incisal or occlusal surface has penetrated through the alveolar bone margin), supra-gingival or prejunctional, and functional phases. The retraction of gingivae is followed by the passive eruption phase which mainly occurs after the adolescent growth spurt (Liversidge, 2015).

During the active phase of eruption, the root growth continues however, the completion of root apices occurs at the last part of dental development (White et al., 2011). The initial process of root formation begins with the reabsorption of the bony crypt in the region of dental laminae (deciduous teeth and permanent molars) or gubernacular canal (successional teeth). The root formation also controls the rate and direction of eruption. During the eruption of permanent dentition, especially for the incisors and canines, the reabsorption of bone and widening of the gubernacular canal allows the tooth to follow the direction of the deciduous precursor. The dental follicle on the permanent tooth crown allows absorption of the root of the fallen deciduous teeth after which the permanent incisors move in a vertical direction until the deciduous incisors are exfoliated. There are different theories describing the mechanism of tooth eruption. According to Liversidge (2015), fibres in the periodontal ligament play an important role in the forward movement of the tooth as they aid in the osteoclastic resorption ad widening of the gubernacular canal. It has been pointed out by Krarup et al. (2005) that in the case of permanent dentition, while the direction of the eruption of mandibular premolars and canine are vertical, molars erupt in a lingual direction.

It can be seen that the process of dental eruption is continuous and that is why scholars like Moorrees et al. (1963) have mentioned how it is difficult to separate stages within this process. Therefore, one of the widely used classifications is based on the work of Liversidge et al. (2004) which is also seen to be applied to the London Atlas of Human Tooth Development and Eruption (AlQahtani et al., 2010). According to her work, there are four stages of dental eruption: unerupted teeth (U), alveolar eruption (AE), partial eruption (PE), and complete eruption (E) (Figure 2.7). The first stage is associated with the tooth remaining inside the alveolar process. The stage of alveolar eruption is also known as clinical eruption. In this stage, the cusp of the tooth is at the alveolar level. The partial eruption stage is when the cusp of the tooth can be seen from the lateral view and is in between the alveolar level and the occlusal level. Finally, the last stage of complete eruption is when the tooth attains its final occlusal limit.

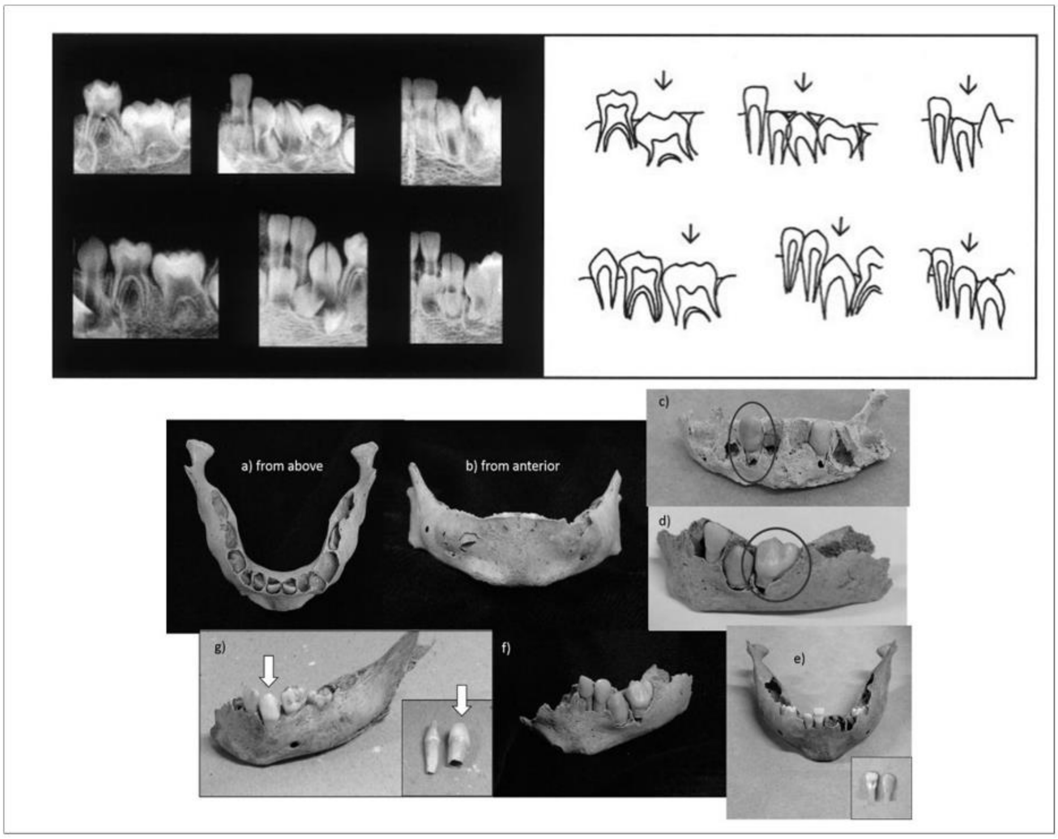

Figure 2.7: Stages of dental eruption according to Liversidge. Examples of alveolar eruption of mandibular deciduous central incisors (a, b,c), first molar (d), partial eruption of mandibular central incisor and alveolar eruption of lateral incisor (e), partial eruption of mandibular incisors and first molar, canine un-erupted (f) and canine partially erupted (arrowed) with lateral incisor and first molar fully erupted (g). (Liversidge et al., 2004)

There is also a pattern of dental formation. The teeth follow a sequential order of eruption. There are several hypotheses opined by scholars in order to understand the patterning and differentiation of teeth (White et al., 2011). According to Smith & Garn (1987) sequence of permanent teeth differs slightly between jaws; that is, first molar (M1) central incisor (I1), lateral incisor (I2), first and second premolar [P1 C P2], second molar (M2) and third molar (M3) in the maxilla and [M1 I1] I2 [C P1] [P2 M2] M3 in the mandible where the frequent reversal of sequence is due to polymorphism (Polymorphism is the condition where there is a reverse in the sequence between phase I and phase II eruption of dentition).

2.2.3. Background of dental age estimation and its application in anthropology

The dental formation in modern humans has been studied in the field of forensic and clinical research for over a century. In clinical practice, the age and timing of dental formation among modern humans are used for surgical accuracy, pathological identifications, etc. In forensic studies, 'dental age' is widely used to estimate the age (at death) of an individual (Dirkmaat, 2012). Such criteria for age estimation are also used in anthropological studies which are not just restricted to modern humans but also primates and extinct apes. Researchers have thus created several methodologies to precisely estimate the dental age. There have also been studies on the accuracy and adaptability of the methodologies. Age estimation can be made based on dental eruption, dental calcification, attrition, root translucency and cement annulation. According to Irish & Scott (2015), methodologies of age estimation can be made on several types of data. The histological data are usually made by visual observation of several individuals over a period, creating longitudinal data or among different individuals collecting in single until time, that is cross-sectional data. Radiographic data is also used. The radiographic data can also be collected longitudinally or cross sectionally depending on the researcher. For the radiographic data, X-Ray devices and CT scans are generally used.

It is important to note that these methods provide approximate age estimates and have limitations, especially in individuals with dental anomalies, certain diseases, or significant dental treatments. Therefore, age estimation based on teeth is usually used in conjunction with other methods for a more accurate assessment.

The earliest evidence of methodological study on dental age estimation based on eruption is found in the work of Schour & Hoffman (1939) and Gustafson (1950). In 1941, Schour & Hoffman (1939) developed one of the first comprehensive atlases on dental development and eruption (Figure 2.8). Their landmark publication provided detailed information on the sequence and timing of tooth eruption based on radiographic observations. This atlas served as a foundation for subsequent studies on dental development and age estimation.

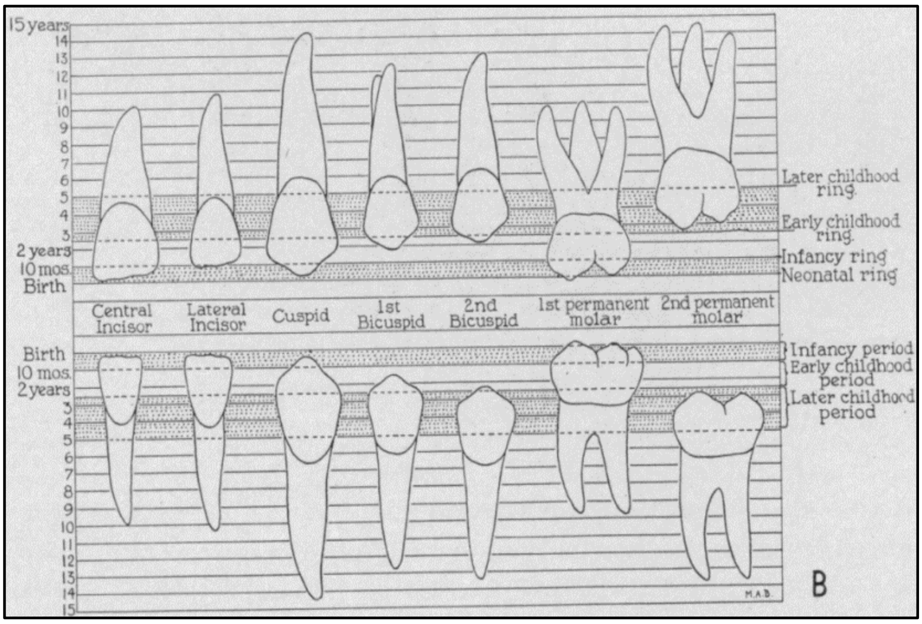

Figure 2.8: Tooth formation of permanent dentition Schour & Hoffman (1939)

In the 1950s, Gustafson proposed a method of age estimation based on dental eruption combined with tooth wear. He developed a scoring system that assessed the degree of tooth eruption and wear on specific teeth, primarily molars. This method was widely used in forensic contexts and provided an initial framework for age estimation based on dental characteristics. One of the most widely used methodologies of dental age estimation is Demirjian's method. Demirjian et al. (1973) developed a more comprehensive and systematic method of age estimation based on dental development. Their method utilized dental radiographs to assess the stages of tooth mineralization and eruption. They established reference standards and developed scoring systems for different teeth, allowing for more precise age estimation. The London Atlas of Human Tooth Development and Eruption by (AlQahtani et al. 2010) also provided an extensive collection of radiographs and detailed information on the stages of dental development and eruption in a diverse population. White et al. (2011) combined the models of Gustafson and Kotch (1974) and Anderson et al. (1976) to create a pictographic chart of deciduous and permanent dental eruptions (Figure 2.9). It served as a valuable resource for age estimation studies and became widely referenced in forensic odontology as it is one of the most recent and extensive research available currently.

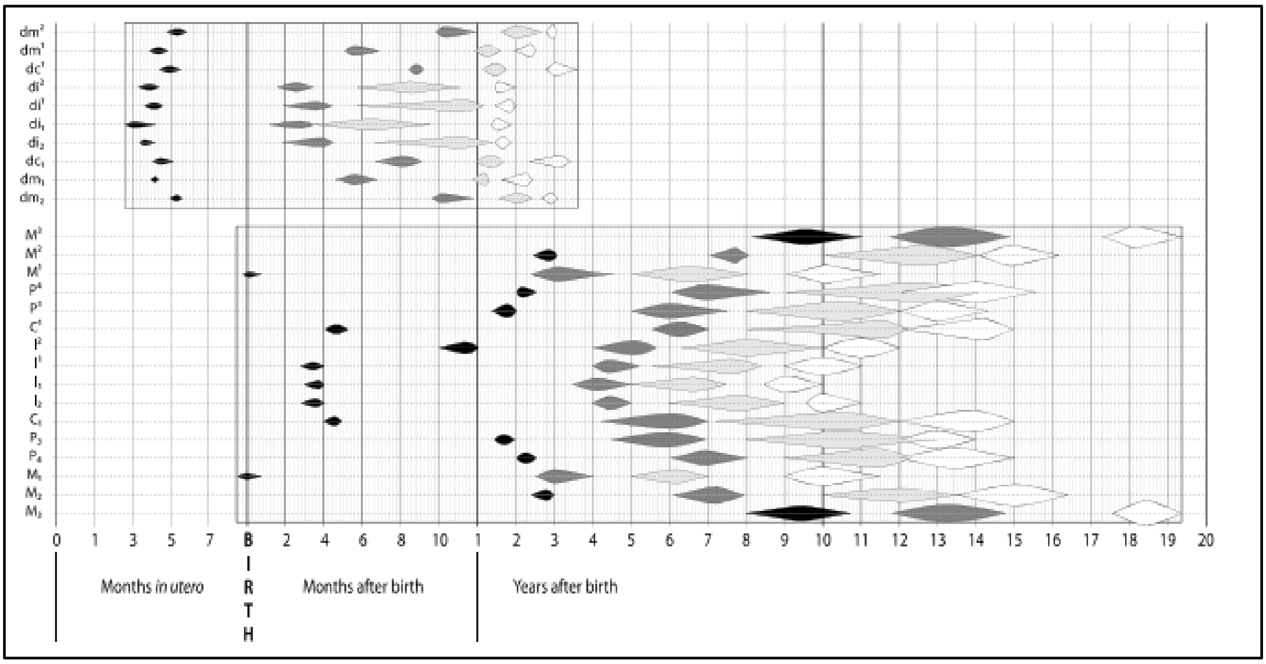

Figure 2.9: Variation of dental development based on Gustafson and Kotch (1974) and Anderson (1976) (White et al., 2011)

The studies on stages of dental formation can be said to have mainly taken place in the 20th century although there are few works where some literature on dental formation and timing is seen to have been mentioned. The studies can be roughly classified into two phases. The initial phase was where the work was done on smaller samples and was based on both dissection and observation of enamel. The publications found based on these studies were mostly charts and pictographs which had a lot of discrepancies. It is seen that the sample used in these studies was small, there was a lot of artistic rendering and less scientific background in the drawing of the pictographs and the works do not attest to a methodology or description of the basis of their observation. The work of Legros & Magitot (1880) was mostly concerned with the prenatal development of dental tissues and structure (Figure 2.10). Published in 1880 as separate articles by both scholars (later it was published together as a book in 1881), was widely acclaimed among English-speaking audiences but failed to reach other people due to the lack of translation.

CHRONOLOGY OF THE DENTAL FOLLICLE IN MAN.

	Designation of the Follicles.	Place of origin of the epithelial cord.	Period at which the enamel-organ first appears.	Period at which the dental bulb appears.	Time of the appearance of the follicular wall.	Closing of the follicle and rupture of the cord.	Periods at which the dentine-cap first appears.	Periods at which the teeth are erupted.	Periods at which the teeth are normally shed.
Temp'ry Dentition.	Inf. cent. incis.							6th month.	7th year.
	Sup. cent. incis.							10th month.	7½ years.
	Inf. lat. incis..						16th week	16th month.	8th year.
	Sup. lat. incis..							20th month.	
	Inf. cuspids ...	Epithelial lamina.	From 7th to 8th week.	9th week.	10th week.	Beginni'g of the 4th mo.		30th to the 32d month.	12th year.
	Sup. cuspids...								
	1st inf. molars.							24th month.	10th year.
	1st sup. molars.						17th week	26th month.	10½ years.
	2d inf. molars .							28th month.	11th year.
	2d sup. molars.							30th month.	11½ years
Permanent Dentition.	Inf. cent. incis.	Cord of the correspond'g temporary teeth.						7th year.	
	Sup. cent. incis.								
	Inf. lat. incis...							8½ years.	
	Sup. lat. incis..								
	Inf. cuspids ...		About the 16th week.	20th week	21st week.	9th month.	1st month aft. birth.	11 to 12 years.	
	Sup. cuspids ..								
	1st inf. bicusp.	Cord of the correspond'g temporary molars.						9 to 10 years.	
	1st sup. bicusp.								
	2d inf. bicusp..							11th year.	
	2d sup. bicusp.								
	1st inf. molars .	Epithelial lamina.	15th week.	17th week.	18th week.	20th week.	6th mo. of fœtal life.	From 5 to 6 years.	
	1st sup. molars.								
	2d inf. molars..	Cord of the 1st perm'nt molars	3d mo. after birth.	1st year.	1st year.	1st year.	3d year.	From 12 to 13 years.	
	2d sup. molars.								
	3d inf. molars..	Cord of the 2d perm'nt molars	3d year.	After the 6th year.	After the 6th year.	After the 6th year.	12th year.	From 18 to 25 years.	
	3d sup. molars.								

Figure 2.10: Chronology of the dental follicle in man (Legros & Magitot, 1880)

The second phase of studies on dental formation is more extensive. Around the 1960s, the researchers started taking into consideration a large sample size pertaining not only to a single community but also to different ethnicities. More varieties of methodologies were tested and were primarily based on statistical calculation. The main objective of this extensive collection and analysis of data was to create a solution to the problem of dental schedules (Smith, 1991). The research conducted during this time can be said to have laid a strong foundation for all kinds of forensic and anthropological studies on age estimation through dental remains which are still used today. Mention should be made of the works of Moorrees et al. (1963), Demirjian et al. (1973), Nolla (1952), Hurme (1948) and Fass (1969). Hurme (1948) unpublished work on a variation of dental eruption (Figure 2.11) was an important step towards understanding the clinical emergence of dentition. The classification of dental formation in 14 stages created by Moorrees et al. (1963) has laid the foundation for the work of successive researchers (Figure 2.12). The classification created by Nolla (1952) comprises 11 stages while the classification of Demirjian et al. (1973) consists of 8 stages. Nonetheless, the three major stages of dental formation are the beginning of calcification, crown completion and root completion as identified by (Smith, 1991).

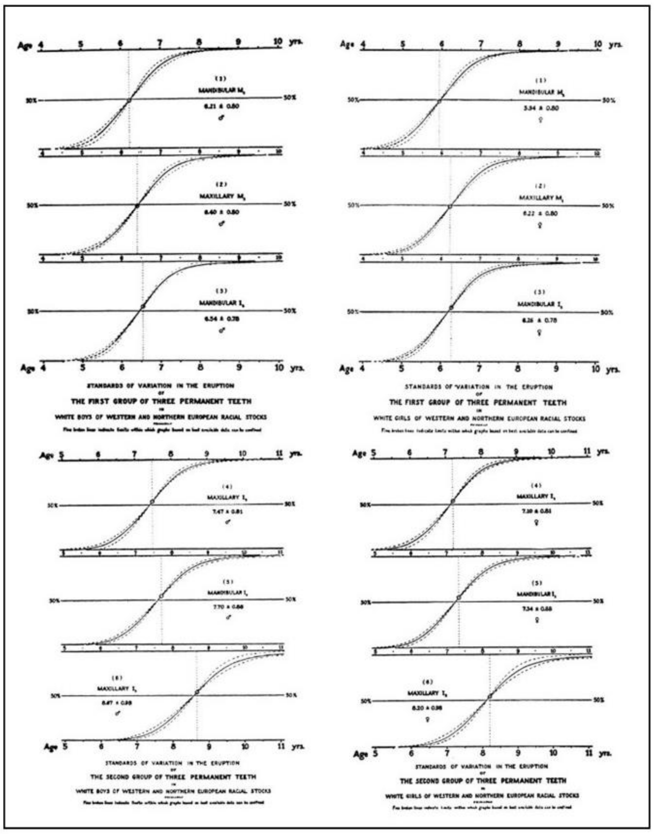

Figure 2.11:: Standard of variation in Dental variation by Hurme (1948)

Another development that has been noticed in the studies of human dental formation is the study of data from different ethnicities. Scholars like Tompkins (1996), Adserias-Garriga & Tejada (2020); Halcrow et al. (2007); Hernández et al. (2008) have compared the period between stages of dental growth from children belonging to different ethnicities and found significant differences. For example, in the study of Tompkins (1996), a comparison of the stage of calcification of M3 among black southern Africans, white French-Canadians, and prehistoric Native Americans has been made. The result showed a remarkable delay in the attainment of the dental stage among the French Canadians compared to the Africans. There have also been studies of age estimation based on the growth of the third molar (Demisch et al., 1956; Mincer et al., 1993; Olze et al., 2010). With the invention of modern technology, several other methods of age estimation have been invented. Scholars like Bhussry & Emmel (1955); Panchbhai (2014); and Schour & Hoffman (1939) have used radiographs from synchrotrons to study the enable biomineralization to estimate the age of an individual. In recent years, work has also been made to evaluate the applicability of the old methodologies. Some of the researches that have been successfully able to highlight the variation in the different standards of age estimation are Adserias-Garriga (2019); Blenkin & Evans (2010); Corral et al. (2010); Cruz-Landeira et al. (2010); Feijóo et al. (2012); González-Colmenares et al. (2007); Jacometti et al. (2023); Kermani et al. (2019); Liversidge et al. (2010); Maber et al. (2006); Mohammed et al. (2014)

The standards of age estimation are seen to be highly useful in the fields of forensic anthropology, physical anthropology, and palaeoanthropology since they help in the determination of the age of skeletal remains. In the field of palaeoanthropology, the standards of dental formation are also associated with the understanding of differing ontogenic rates among hominins. Shea (1981) in his work is seen to have used the standards of Krogman (1969) and Ashton and Zuckerman (1950). In the methods of age estimation of the Tuang Child, it is seen that Conroy & Vannier (1987) use the standards of Moorrees et al. (1963), Gustafson (1950) and Massler et al. (1941) for comparing the dental development rate of the *Australopithecus afarensis* with modern human. These standards have been repeatedly used in the age estimation of other fossil hominins as well (Beynon & Dean, 1988; Bromage, 1987; Bromage & Dean, 1985; C. Dean et al., 2001; M. Dean et al., 1986; M. C. Dean & Liversidge, 2015). In the work of Lampl et al. (1993) and Mann et al. (1990), a methodology that is widely used for age estimation in paleoanthropological studies, the use of the standards of Moorrees et al. (1963); Haavikko (1974) and Gustafson (1950) is recognised. It can be seen that the studies done on dental development in the mid-twentieth century are widely used in the field of palaeoanthropology as a standard for modern human dental development.

2.2.4. Classification of statistical methodologies according to Smith (1991)

The accurate time of dental formation is an unattainable variable since it cannot be observed. For this reason, there has been the synthesis of various methodologies of calculation time of dental formation. Smith has reviewed and compared this literature and has created a classification of the statistically based methodologies of tooth formation based on radiographic data. In this research work, Smith's categorization has been identified as the most recent work of its kind and thus has been followed in this thesis. According to his classification, there are three major types of methodologies: Age of Attainment, Age Prediction and Maturity Assessment.

Age of Attainment

The ontogenic methods of the age of attainment are based on the observation of the time when a tooth attains a certain predefined stage within mineralisation to complete formation. Two main methodologies fall under this category; namely, the method of Cumulative Distribution or Probit Analysis and Average of Age at First noted appearance Less One-Half Interval between examination (Smith, 1991). These two methods were widely applied in the 1950s-80s by scholars like Demirjian et al. (1973); Anderson et al. (1976); Fanning & Brown (1971); Garn & Lewis (1957); Moorrees et al. (1963).

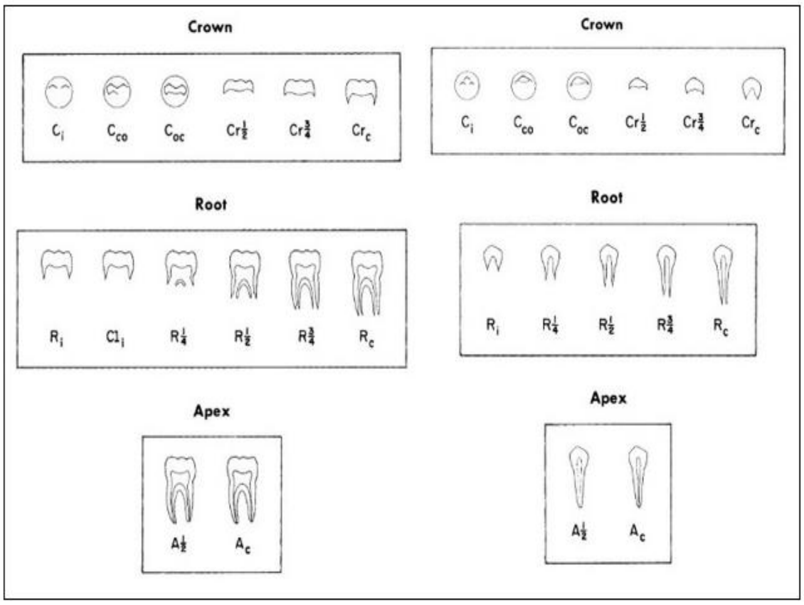

Figure 2.12:: Stages of tooth formation (Moorrees et al., 1963)

According to JM Tanner (1985), the Cumulative distribution method is one of the best methods for the determination of age of attainment in a developing adult. It takes into consideration the age of attainment of a certain dental formation stage by at least 50% of the subjects. Based on the data, central tendency and dispersion can be analysed of the whole sample. In the methodology, Hillson (2005) has pointed out that in cumulative distribution methods, the graphical representations are prepared for each developmental stage of each tooth where the horizontal axis represents the ages at which the examinations took place while the vertical axis represents the percentage of individuals in the study group, so it totals 100%. In graph is plotted according to youngest individual to eldest individual from left to right and the height on the vertical axis represents the cumulative percentage of individuals in the study group that attained the stage at their plotted age. According to Hillson (2005), the result from this analysis produces a ‘s’-shaped graph which he calls a ‘lazy S’ shaped curve, starting with a gentle gradient, then passing through a steep section and finally settling back towards a gentle slope. Hilson also points out that this methodology automatically helps in the visualisation of median and quartiles. This is useful for defining the age range. According to Hurme (1948), this methodology also is a more suitable approach for dealing with the true nature of the data and not missing observations based on variability. However, since the methodology required at least 50% of its specimen to attain the dental stage, which in some cases had several problems of observation biases, Finney 1939 created the Probit analysis which was then incorporated into this methodology. Probits are probability Units which are plotted against logarithms of age (Hillson, 2005). It is seen that by applying parametric methods like probit analysis, the parameters of the data can be estimated as well as assumptions, estimation of mean and variance and comparison with other samples can be performed (Smith, 1991). According to Hillson (2005), the probit analysis is a vigorous methodology and is not too sensitive to irregularities in the observation spread in terms of age groups. This however creates biases in the division between the gap of time between two stages ads using this methodology, the gap is always equal which is not the case in the actual scenario. Thus, scholars like Spearman-Kärber have formed an alternative method where no assumptions are made about the distribution of the underlying data (Hamilton et al., 1977; Finney, 1978).

The methodology was first used in the work of Garn & Lewis (1957). In his work, “Variability of Tooth Formation” he and his colleagues estimate variability for 3 stages of formation of the mandibular premolar and molar teeth. The study also investigates longitudinal data of secondary dentition using the dental formation chart created by Logan & Kronfeld (1933) but criticises the small age range and how it had an age discrepancy of 3 to 4 years. The work of Moorrees et al. (1963) is well known using the data from the Fels Research Institute in Yellow Springs, Ohio. In the study, probit analysis using the assumption of lognormality was applied. Fanning & Brown (1971) also used the same data as Moorrees et al. (1963) but instead of applying probit analysis, they calculated simple percentile from the cumulative distribution function.

This methodology can be used for both longitudinal and cross-sectional data. However, longitudinal data is seen to have been preferred in the studies of Kent et al. (1978); Moorrees et al. (1981); Smith & Garn (1987). Haaviko in his work published as "Tooth Formation Age Estimated on a Few Selected Teeth a Simple Method for Clinical Use" in 1970 used cross-sectional data (Haavikko, 1970) (Figure 2.13). Also, Demirjian and Levesque used cross-sectional data along with longitudinal data in their work which was published in 1980 (Demirjian & Levesque, 1980).

Chronological sequence of alveolar and clinical eruption and formation of teeth, according to age medians, in years

Upper											
Boys						Girls					
Alveolar eruption		Clinical eruption		Tooth formation A_c-stage		Alveolar eruption		Clinical eruption		Tooth formation A_c-stage	
Tooth seq.	Age medians	Tooth seq.	Age medians	Tooth seq.	Age medians	Tooth seq.	Age medians	Tooth seq.	Age medians	Tooth seq.	Age medians
M_1	5.3	M_1	6.4	(I_1	9.8	M_1	5.3	M_1	6.4	M_1	9.2
I_1	6.2	I_1	6.9	M_1)	9.8	I_1	6.1	I_1	6.7	I_1	9.3
I_2	7.3	I_2	8.3	I_2	10.8	I_2	7.0	I_2	7.8	I_2	9.6
P_1	9.8	P_1	10.2	P_1	13.3	P_1	9.0	P_1	9.6	P_1	12.6
P_2	11.1	P_2	11.4	C	13.6	C	9.3	P_2	10.2	C	12.7
C	11.2	C	12.1	P_2	14.0	P_2	9.5	C	10.6	P_2	13.4
M_2	11.4	M_2	12.8	M_2	16.2	M_2	10.3	M_2	12.4	M_2	15.1
M_3	17.7			M_3	19.5	M_3	17.2			M_3	19.6
Lower											
M_1	5.3	M_1	6.3	I_1	8.0	M_1	5.0	I_1	6.2	I_1	8.0
I_1	5.9	I_1	6.3	I_2	9.6	I_1	5.8	M_1	6.3	M_1	9.2
I_2	6.9	I_2	7.3	M_1	9.8	I_2	6.5	I_2	6.8	I_2	9.0
P_1	9.6	P_1	10.3	P_1	12.8	C	8.8	C	9.2	C	11.5
C	9.8	C	10.4	C	13.2	P_1	9.1	P_1	9.6	P_1	12.1
P_2	10.3	P_2	11.1	P_2	13.8	P_2	9.2	P_2	10.1	P_2	12.8
M_2	10.8	M_2	12.2	M_2	15.7	M_2	9.9	M_2	11.4	M_2	14.7
M_3	18.1			M_3	20.4	M_3	17.7			M_3	20.8

Figure 2.13: Chronology of dental eruption (Haavikko, 1974)

Although Cumulative distribution can be applied to both longitudinal and cross-sectional data, this methodology is dependent on the sample size. The limited number of observations creates a large margin of discrepancy in ae of attainment from one stage to another Smith (1991). Therefore, for this methodology, having more data helps in estimating proper dispersion.

Another problem of this methodology in terms of modern humans is the slow rate of growth. This makes the process of observation more difficult. However, for non-human primates, where the growth rate is accelerant in comparison to humans, this approach is used as seen in the work of Shea (1981).

According to Smith, Cumulative Distribution is the best way of establishing the chronology of tooth formation. The method can be used explicitly for estimating the age of attainment of a growth stage. The method is also not affected by sample age structure, and it uses cumulative data.

The other method of estimating the age of attainment is through the average age of first noted age of appearance one-half the interval between examinations. This methodology was created as a remedy to the problem of the previously discussed methodology. That is to say, the kind of observation required for tracking daily or hourly development of dentition for the Cumulative Distribution function is humanly not possible for individuals with a slow rate of growth. The method subtracts one-half of the length of the interval between examinations from the age at first appearance.

According to Dahlberg & Menegaz-Bock (1958) took into consideration the length of interval between examinations and the age of attainment of a particular stage was calculated taking into consideration the average age of first recorded appearance sums over small and large positive error. This method was applicable for longitudinal data but when compared to studied using cumulative distribution function using cross-sectional data, there was an error of older age for emergence. This issue was later rectified by subtraction of one. Half of the time interval between examinations. Using this methodology, researchers were able to rectify the problem of the interval between examinations for studies using longitudinal data and standardise a time of 6 months to 1 year.

The issue of proper estimation of interval time was not only affecting the age estimation of humans but also non-human primates. That is why, this methodology was widely used to revise the technique of age estimation for non-human primates. In terms of modern humans, according to Smith (1991), Anderson et al. (1976) applied this methodology in this research. The study faced problems of missing multiple stages in between observations which led to the change of time interval from one-third to two-third.

It is important to note that, this methodology is only applicable for longitudinal data, and it deals with the issue of rectification of observation interval. Thus, this methodology has not been applied to any study using cross-sectional data.

Age prediction

In contrast to the methods of Age of Attainment, the method of Age prediction uses the age of an individual as a variable and sorts the sample individually by stages of development. There are two main types of this methodology as identified by Smith (1991); Mean Age of Subject and Mean of current stage of Attainment.

As seen in the earlier methodology, there is a problem in estimating the age associated with different stages of dental formation since the observations were not adequate to precisely locate the moments of developmental changes. Although this issue was solved for longitudinal data by subtracting the one-half of average time of interval between examinations, the problem remained for studies using cross-sectional data. It is important to note that, cross-sectional data became important to be taken into consideration for creating of standardised reference index of age estimation due to the difference in human growth among different populations. The studies from 1960-1980 are seen to focus on the dental variability among the human population as seen in the work done by Demirjian et al. (1973; Nolla (1952). The methodology of 'Average Age of subject in a Stage' is opined to be appropriate for studies where the dental sage is known as the prediction of age is the goal as seen in the case of demographic and forensic studies Smith (1991).

However, one of the issues of this methodology is that it is sensitive to the age structure of a sample Gates (1966); and Hayes & Mantel (1958). Especially, if the sample record is a fossil hominin, whose representation of subjects from all ages is absent, then it affects the results of the study. It requires the estimation of the first to 100th percentile of the age of attainment of the growth stage in a relatively flat distribution. This has been noted in the work of Goldstein in 1979 but it is important to note that such conditions are not typically present in the studies of child growth (Demirjian et al., 1973).

This methodology was first popularly used in the work of Gleiser & Hunt Jr. (1955) The research published under the title "The Permanent Mandibular first Molar: Its Calcification, Eruption and Decay" where 15 arbitrary stages of the dental eruption were chosen and then correlated with the chronological age of the samples. The said methodology has also been used in the work of Demisch et al. (1956), Haatja (1965), Faas (1969) and Trodden (1982). One of the noticeable aspects of this methodology being used in the earlier research is that every scholar modified the technique according to their precise need. While the work of Gleiser & Hunt Jr. (1955) focuses on the first molar of longitudinal data, Demisch and Wartman applied the methodology for the study of the third molar in the cross-sectional sample (Demisch et al., 1956).

Smith produces another methodology for the prediction of age since the accuracy of the predictor for the previous method is sensitive age distribution in the selected sample. Smith (1991) proposes his methodology based on the suggestions of Goldstein (1979). According to Goldstein (1979), in order to show the age at a particular stage of an individual, the mid-point between the mean age of attainment and the current age of the subject should be selected. This gives the data an equal variance. Regression can also be performed on the age using a linearised summary score of tooth formation (Goldstein, 1979). Smith (1991) applies the suggestion of Goldstein and compares the methodology with the work of Moorrees et al. (1963). In his work, Smith calculates the mean of each stage and the attainment of the next consecutive stage. The age opposite to a stage is the representation of the midpoint between the age of appearance of that stage and the next. Each tooth must be analysed separately and then results are assigned to dental ages (Smith, 1991). Besides Smith, 1991, this methodology has also been used by Leinonen et al. (1972) using cross-sectional data.

This methodology's main advantage is its flexibility as it can be applied to fragmentary material found in archaeological and forensic contexts. However, the methodology is restricted to use during the developmental stages of dentition. That is to say, once the teeth reach complete maturity, it cannot be analysed to predict age. This is because the time between its last transition stage is unknown.

Maturity Assessment

The methodology of maturity assessment is popularly used for clinical studies. It is used to assess the overall maturity of a single individual and its difference from the reference group. This methodology was used for comparing dental formation of different populations in the Twentieth century and is still widely used by researchers. The two types of methodology that fall under this category are the Mean formation Stage for the Subject Age group and the Maturity Scale.

The methodology of the Mean formation Stage for the Subject Age group encounters the problem of creating an average of subject age as seen in the previous two methodologies. Thus, to tackle the issue, scholars like Nolla (1952), Nanda et al. 1966) and Lilliequist & Lundberg (1971) are seen to create a system based on the mean of the stage of dental portion for an age group. This method shares similarities with the method of the age of attainment as it deals with the stage of dentition as the variable instead of age. Thus, it is not widely popular for forensic and archaeological studies, for which the method of Age prediction is sued. However, this methodology is highly popular for anthropological work since it allows the comparison of diverse populations as seen in the work of Nolla (1952) Nanda et al. 1966) and others. One of the key factors of this system is that the stages of dentition are normally distributed. They are ordinal measures designated by integers and the time lapse between stages may or may not be unequal.

The main problem faced using this methodology is the calculation of standard mean and variance as the assumption of normal distribution of the stages creates bias in the analysis. This issue has been tackled in the work of Nyström et al. (1986) where the researchers used the ordinal stages without their average and by giving percentile or mode to each age group. Thus, the scale created using this methodology is not comparable with other systems (Smith, 1991).

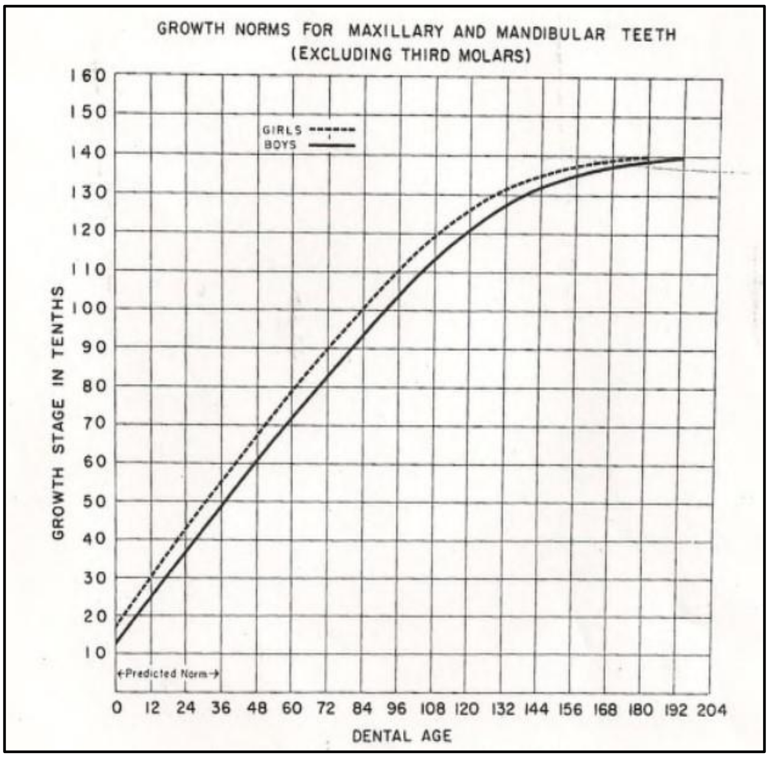

Figure 2.14: Maturity scale of dental formation Nolla (1952)

The most renowned work using this methodology Is the research conducted by Nolla (1952) (**Error! Reference source not found.**). In the study, Nolla (1952) assigned observational values on the growth level which is in the middle of the growth stage and the next level. The given values were then plotted graphically to norm developmental curves for each tooth and dental age values. According to the critique of Smith (1991), this system of creating a secondary curve for defining the mean stage can be applied to solve regression equations backwards for independent variables.

The other methodology of maturity assessment is the usage of the maturity scale. The methodology of Demirjian et al. (1973) (Figure 2.15) is one of the most widely used techniques in the field of anthropology and clinical studies where the researcher deals with the living population.

The maturity scales are predesigned evaluations with have been created to eradicate the complexities of the calculation of mean age or mean stage. The maturity scales are central tendency in stage for age. Age is an ordinal variable that needs to be transformed into scores and linearized concerning chronological time. It is seen that using these scores helps to achieve the closest range of variables for the determination of age from a set of sample dentitions. Tanner (1985) further developed a maturity scale for tooth formation in parallel to wrist ossification. There are several maturity scales produced by researchers like Wolanski (1966), Moorrees et al. (1963), Fanning and Brown (1971) and Demirjian et al. (1973).

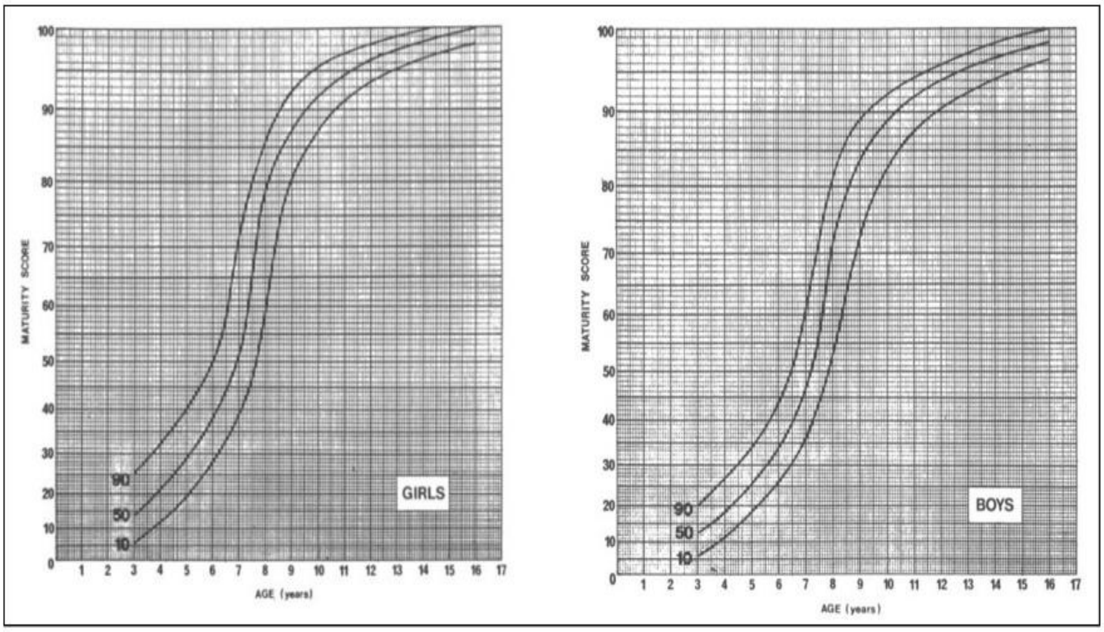

Figure 2.15: Dental Maturity percentile by Demirjian (Demirjian et al., 1973)

It is important to note that maturity scales have been prepared for clinical use where the age of the individuals is already known. This kind of methodology is particularly not suited for age prediction as the scoring cannot be created when there is missing data in the sample. Thus, in order to use this methodology for paleoanthropological or archaeological use, the scoring system has to be separately calibrated for each tooth which seems rather impractical.

2.3. Mandibular anatomy and ontogenic integration

2.3.1. Mandibular anatomy

The human mandible comprises the body corpus and the ascending ramus. The primary function of the mandible is to aid in masticatory activities. It provides a surface for the insertion of muscles required for mastication. The lower dental arcade is also located in the mandible (White et al., 2011). In Figure 2.16 and Figure 2.17, the anatomical parts of the mandible have been shown.

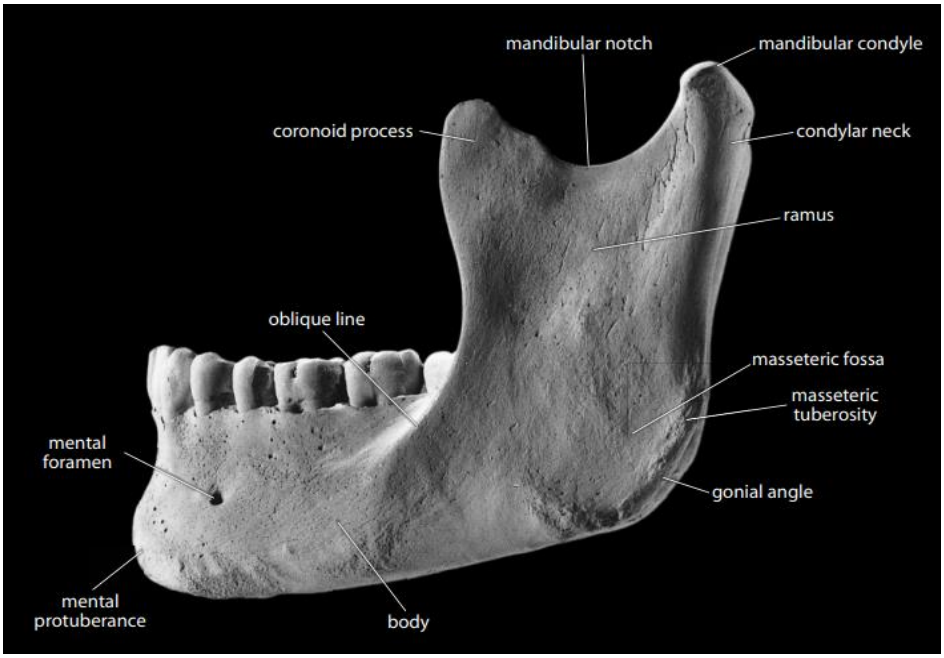

Figure 2.16: Human mandible (lateral view) (White et al., 2011)

The body corpus is a horizontal, curved part of the mandible that forms the chin and supports the teeth is called the body. It provides the foundation for the lower dental arch. It is thick and bony, and the teeth are implanted in the body. The mandibular body is made of dense bone which is resistant to destruction (White et al., 2011). The body also contains the alveolar process comprised of alveoli to which the root of the tooth is attached. The alveolar body also contains a large foramen on the lateral surface below the premolar region. This is mental foramen which can also be multiple at times. The mental foramen helps in the transmission of nerves and blood vessels (White et al., 2011). The oblique line, which is a weak eminence, passes from the rear end of the mental foramen to the anterior edge of the ramus process. The extra molar sulcus is the retromolar trigone a triangular-shaped area located behind the last molar tooth in the oral cavity, specifically the third molar or wisdom tooth. This area is found in the posterior (back) part of the mouth, adjacent to the mandibular ramus and the buccinator muscle attaches here. The next part of the mandibular body is the mylohyoid line where the mylohyoid muscle attaches. It passes obliquely crossing the medial surface of the corpus, beginning near the alveolar margin at the last molar position and diminishing as it runs anteroinferiorly (White et al., 2011). The mandibular body also contains submandibular fossa, sublingual fossa and digastric fossa each of which serves attachment to a specific nerve or muscle.

In the frontal part of the mandibular corpus, the mandibular symphysis and the mental protuberance or eminence are present. The mandibular symphysis is the anterior region in between canines. The symphysis is unfused till the age of 1 year among modern humans. The mental eminence on the other hand is the triangular eminence, or bony chin, at the base of the corpus in the anterior symphyseal region. It is separated from the alveolar margins of the incisors by a pronounced incurvation or “mental sulcus” (White et al., 2011).

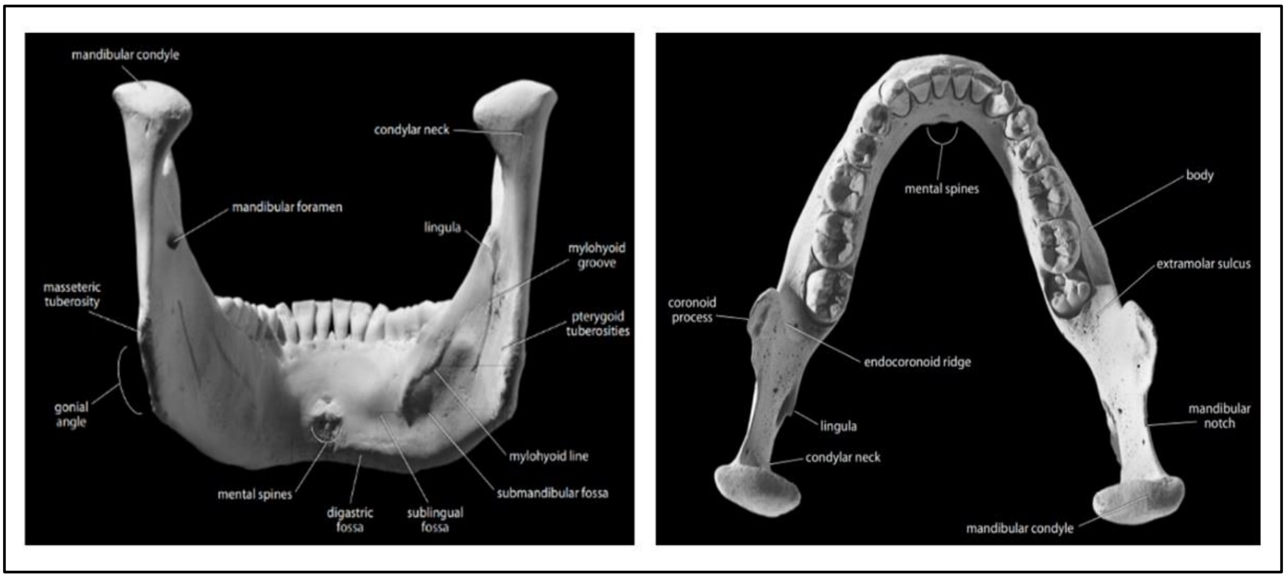

Figure 2.17: Human mandible (posterior and occlusal view) (White et al., 2011)

The ascending ramus is the vertical, upward extension from the posterior end of the body is termed the ramus. The junction of the body and ramus is referred to as the angle of the mandible. It is considerably thinner than the mandibular corpus. It comprises oof condyle neck, coronoid process, mandibular notch and gonion. The condylar neck is situated right below the condyle, towards the front and lower side (White et al., 2011). The anteromedial part of the neck, just beneath the condyle's articulating surface, hosts the attachment point for the head of the lateral pterygoid muscle. This connection occurs within the pterygoid fovea. The coronoid process is the thin triangular structure whose anterior border is thick and convex while the posterior edge is concave. It receives the insertion of the temporalis muscle. The mandibular notch is the insertion between the condyle and coronoid process. Lastly, the gonion is the posteroinferior corner of the mandible. It is rounded and proves the attachment to the masseter muscle. The point where the muscle attaches is called masseteric tuberosity.

2.3.2. Ontogenic development of the mandibular corpus

The mandibular corpus is seen to undergo morphological changes due to the ontogenic process. Development of the mandible can be noticed from the prenatal stage up to post-adolescence. In the period of infancy, the mandible is influenced by the eruption of the deciduous teeth. The angle between the ramus and the mandibular corpus is also more obtuse. However, the mandible starts to grow in size with the beginning eruption of permanent dentition. It is to be noted that the size and number of permanent dentition are more than deciduous dentition. Therefore, to adjust the whole dental arcade, the mandibular process increases by elongation. The condyle also seems to become elongated and develop articulation to adjust better with the temporal bone. This development has been suggested due to the adaption of more complex masticatory activities by the children as they begin eating more complex food than breast milk and other liquids. In the work of Polanski (2011), it is seen that the ascending ramus and nonalveolar portions of the corpus have a close integration throughout the ontogenic process. He suggests the process of dental eruption as well as masticatory activities that place in the later stages of ontogenesis are the reasons behind the morphological changes of these regions of the mandible. During adolescence, there is an important development in the frontal part of the mandible. The symphysis becomes fused, and the mental eminence also becomes more pronounced. During this time, morphological differences based on sexual dimorphism are noticed in the mandibular complex. According to Coquerelle et al. (2011), that shape dimorphism already exists by birth which is concentrated at the ramus and the mental region. However, the sex difference decreases quickly between the ages of 4 years and 14 years. From puberty to adulthood, males are characterized by a continuation of allometric shape changes while the female ontogenetic trajectory does not follow an allometric direction after puberty. In the post-adolescent period, the changes are mainly noticed in terms of bone density due to ageing, hormonal shifts or lifestyle changes.

CHAPTER 3

RESEARCH METHODOLOGY AND APPROACH

3.1. Research design

This chapter addresses the criteria of sample collection and the methodological configuration curated for the estimation of the age of modern human individuals using geometric morphometrics. Starting from data collection, in this research work, an online database of Computed tomography scans (CT scans) of modern human individuals has been accessed for the data collection if the present work. The second step of the research methodology has been the sample preparation and application of 3D landmarks. Each of the steps has been further described in detail in the chapter. The chapter also places an argument regarding the classification of samples for statistical analysis. The main aim of the research methodology is the extraction of three-dimensional information regarding the changes and development of mandibular complex and dentition separately to compare them in terms of shape variation according to the preformed classification. The method also includes multivariate analysis, that is, Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) for the visualisation of shape variation and estimation of the accuracy of the standard grouping that has been created. It is a quantitative research methodology in which a deductive principle has been applied to find the correlation between dental eruption and morphological changes in the mandible. The chapter also validates the ethical consideration of the research that has been conducted.

3.2. Data collection

The sample selection for this study has been done based on certain criteria that must be complimentary to the research analysis. CT scans of modern human individuals have been collected from the New Mexico Decedent Image Database (NMDID) (Edgar et al. 2020). This database allows researchers to study the medical tomography of modern humans for research and study purposes. It also provides information on the individuals' sex, age, ancestry, environmental history, medical history, medications, and substance usage. Since the analysis is focused on individuals in the phase of mixed dentition, individuals within the age group of 4 years to 13 years have been considered for this study. The sample collection has been made on radiographic data collected cross-sectionally. Scans of both male and female individuals have been collected at regular intervals. That is to say, from the age of 4 years to 6 years, an age interval of three months has been maintained for the sampling of one male and one female. Similarly, an age interval of 6 months has been maintained for individuals between the age of 6 years to 13 years. In total, there are 25 male and 23 female individuals (see Annex A.1). It is to be noted that, individuals with any pathological abnormality or signs of accidental trauma have been excluded to minimise any abnormalities outside the normal skeletal growth process.

3.3. Sample Preparation

The CT scans collected from the NMDID have been rendered using 3D Slicer (Fedorov et al. 2012), a free, open-source software for visualization, processing, segmentation, registration, and analysis of medical, biomedical, and other 3D images and meshes; and planning and navigating image-guided procedures (Fedorov et al. 2012). The NMDID provides multiple types of Unenhanced Thin Bone CT (medical imaging performed without using contrast media or dye) and Thin Bone CT (Temporal bone CT are the vertical crest consisting of bone or a thin layer of arachnoid tissue that may have an osseous component medially) scans of the head, thorax, legs as well as whole human body. The scans of the head have been preferred due to better resolution although in cases where such scans are not available, whole-body scans have been used instead. The resolution of the whole-body scans was generally lower than the scans of the head and upper body, but the cusps were detectable on the 3D mesh. The focus area which is the mandible with the mandibular dentition has been cropped using the crop tool of 3D Slicer as seen in Figure 3.1.

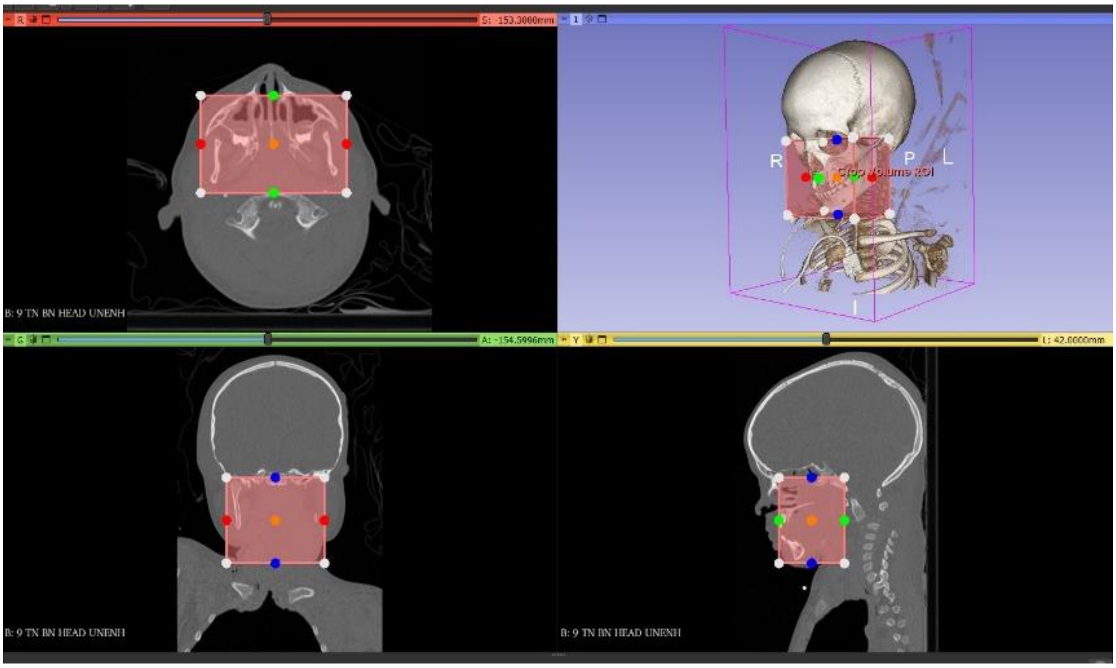

Figure 3.1: Preparation of CT scan of the individual in 3D slicer (individual id: 167431)

After the selection of the scans, the 3D mesh of the mandible and dentition of each individual was produced. The segmentation editor tool of the 3D Slicer has been used to make the models individually as seen in Figure 3.2. The density threshold for the model mandible is between 300 Hunsfield Unit (HU) to 1800 HU while the density threshold for the dentition model is 1800 HU to 2400 HU. The files are exported in ".nrrd" format. The 3d mesh models help in easier accessibility to each anatomical part of the mandible and the dentition and clear visibility. Since the study also includes unerupted teeth, the aspect of clear visibility was highly important. 3D Slicer allowed the creation of the model without compromising any anatomical part including the cusps of the dentition.

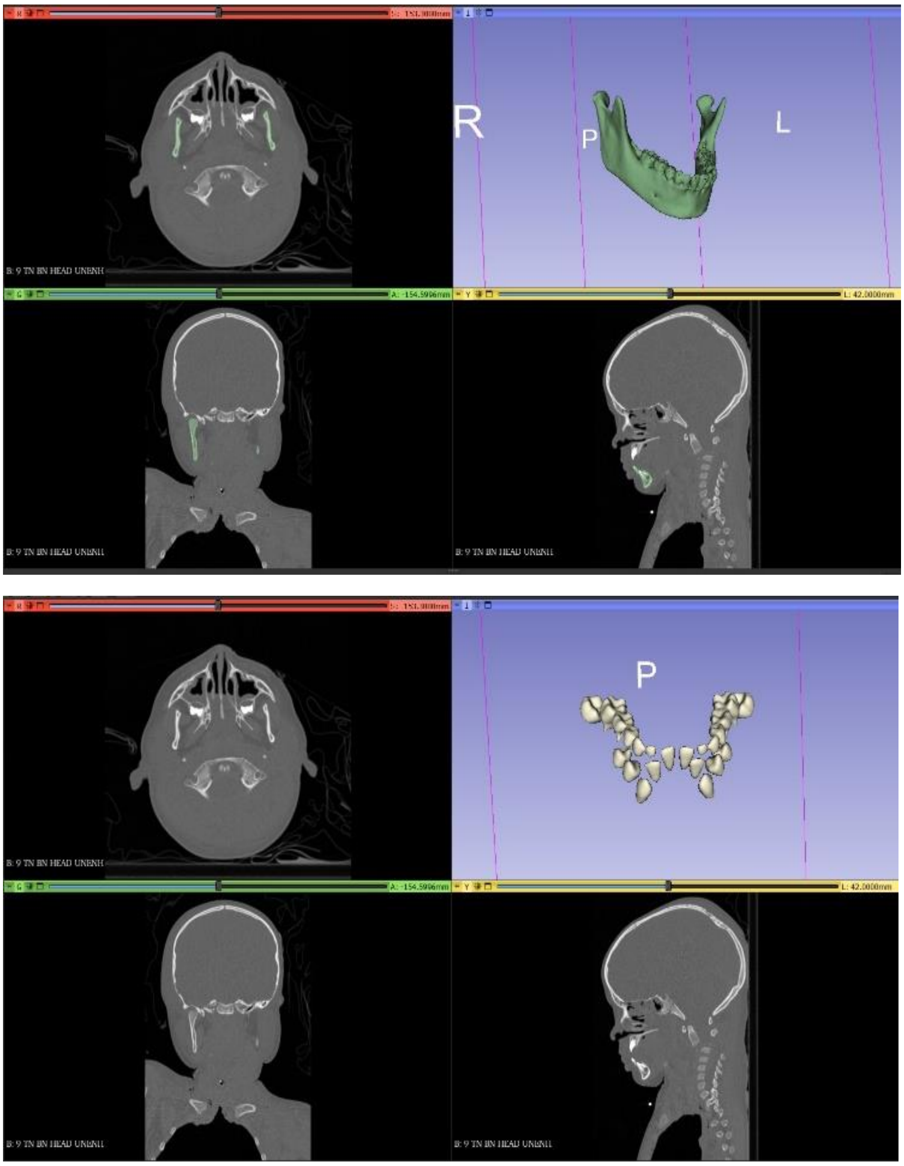

Figure 3.2: 3D mesh model of mandibular dental arcade and mandible of the individual in 3D slicer (individual id: 167431)

3.3. Group formation

Classification of individuals is necessary for performing multivariate analyses like CVA. As discussed in the literature, there are various standards for the classification of dental formation and eruption in association with age. First, an approach to test the different standards of dental formation and dental eruption for age estimation has been made on the sample collection. For this analysis, the standards found in the literature of Logan & Kronfeld (1933), Gleiser & Hunt Jr (1955), (Demirjian & Levesque (1980)and AlQahtani et al. (2010) have been applied against the sample collection. Although these standards are widely used for the estimation of age, they are mainly focused on dental formation. Only Gleiser & Hunt Jr. (1955) included some standards on dental eruption but it does not sufficiently apply to a larger age range. Therefore, in this study, a new standard for age estimation based on stages of dental eruption is proposed. The standard has been formed through observation of the mandibular dental arcade except second and third molar. The classification has been based on the definitions of Liversidge et al., (2004) on the four stages of dental eruption: unerupted teeth (U), alveolar eruption (AE), partial eruption (PE), and complete eruption (E). The eruption stages are based on the cusp position in the lateral view of the mandible. Therefore, the stage of unerupted teeth is when the cusp is below the alveolar level. The alveolar eruption stage is when the cusp of the tooth is at the alveolar level. The partial eruption stage is when the cusp of the tooth can be seen from the lateral view and is in between the alveolar level and the occlusal level. Finally, the last stage of complete eruption is when the tooth attains its final occlusal limit. Based on the definitions, observation has been made on the degree of the dental eruption of each tooth of the individuals (for details, see annexe A.1.). Based on the result of the observation five groups have been produced as seen in the table (3.1). The statistical analysis that has been conducted based on this division is to estimate the impact of the dental eruption on the ontogeny of modern humans along with testing the accuracy of the classification.

Table 3.1: Group categorized according to the dental eruption of individual

No	Group	Description
1	Unerupted	Molar, Incisor, Canine and Premolar are under the alveolar level.
2	Molar and Incisor partial eruption	Molar 1 and Incisors 1 or 2 are at the alveolar level or halfway between the alveolar bone and the occlusal level. Canine and premolars are under the alveolar level.
3	Molar and Incisor complete eruption	Molar 1 and both the Incisors 1 and 2 are at the occlusal level. The canine and the premolars are under the alveolar level
4	Premolar, canine partial eruption	Molar 1 and both the Incisors 1 and 2 are at the occlusal level. Canine and Premolar 3 or 4 are at the alveolar level or halfway between the alveolar bone and the occlusal level
5	Complete eruption	All the Molar, Premolar, Canine and Inscisor are at the occlusal level.

3.5. Landmark Configuration

The landmark configurations that have been used here are mainly of two types: type I and type II landmarks. According to Bookstein (1997), type I landmarks can be described as ‘discrete juxtaposition of tissues'. They can signify relative growth that is immediately adjacent or at a distance in any geometrical direction. The type II landmarks are the 'maxima curvature or other local morphogenetic processes'. These landmarks are used as points of application of real biomechanical forces. According to Bookstein (1997), the tips of the teeth can be considered a type II landmark. Landmarks are found to be highly useful for multivariate analysis as they provide the exact coordinates of biological points on several individuals which then can be compared on a virtual 3 dimensional plane. Therefore, in this study, the landmark variables have been used to mark the growth and development of dentition as well as the shape deformation of the mandibular complex.

For the dentition, the landmark configuration consists of Type II landmarks. The landmarks have been placed on the cusp or cuspid of each tooth (table 3.2). For the M1, the landmark has been placed on the meso-lingual cusp since it is the most pronounced cusp of the 1st mandibular molar. For the premolars, the landmarks have been placed on the buccal cusp. For canines, the landmark has been positioned on the cuspid while for the incisors, the landmark has been positioned in the approximate middle of the occlusal surface (incisor ridge). In total, 12 landmarks have been positioned both on the left and right of the oral cavity as seen in Figure 3.3.

Table 3.2:: Dental Landmark configuration

Count	Tooth	Landmark type	Description
1	R-M1	2	The metaconid or the mesiolingual cusp on the right first lower molar.
2	R-P4	2	The buccal cusp of the right second lower premolar
3	R-P3	2	The buccal cusp of the right first lower premolar
4	R-C	2	The cusp of the right lower canine
5	R-I2	2	The middle of the incisal edge of the right second lower incisor
6	R-I1	2	The middle of the incisal edge of right first lower incisor
7	L-M1	2	The metaconid or the mesiolingual cusp on the left first lower molar.
8	L-P4	2	The buccal cusp of left second lower premolar
9	L-P3	2	The buccal cusp of left first lower premolar
10	L-C	2	The cusp of left lower canine
11	L-I2	2	The middle of the incisal edge of left second lower incisor
12	L-I1	2	The middle of the incisal edge of left first lower incisor

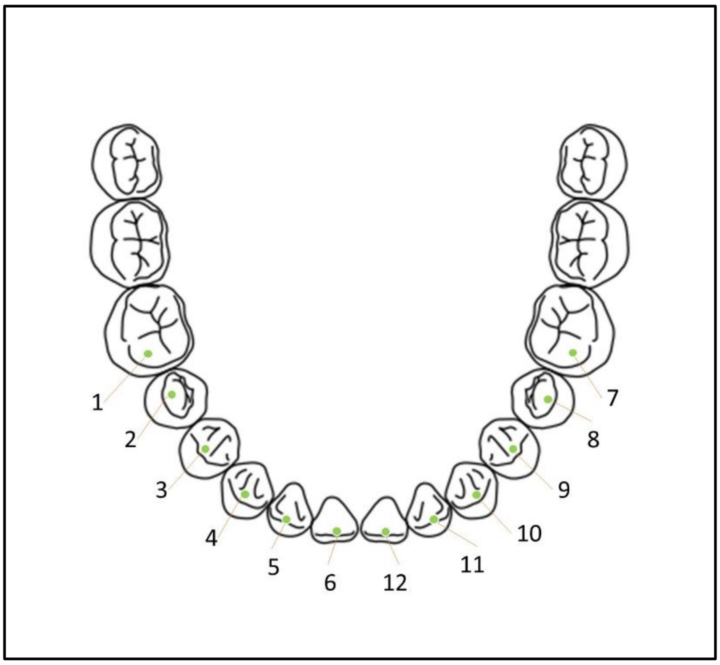

Figure 3.3: Dental landmark configuration

For the mandible, both Type I and Type II landmarks have been used. The configuration by Bastir et al. (2007); Rosas & Bastir (2004) as been followed (table 3.3). According to the configuration, the following are the Type I landmarks: left and right mandibular canine, left and right conoid process, left and right mandibular foramen, infradentale, chin, foramen genioglossus and internal infradentale. The type II landmarks are mental foramen, inferior basal border, preangular notch, ramus flexure, condylion, mandibular notch, posterior alveolar process limit, B-point and gnathion.

Table 3.3: Mandibular Landmark configuration

Count	Tooth	Landmark type	Description
1	Canine- right	2	Mesial to right lower canine, alveolar border
2	mental foramen- right	2	Foramen located on the lateral corpus surface, near mid-corpus, below the premolar region
3	inferior basal border-right	2	Posterior beginning of the inferior basal border
4	pre-angular notch-right	2	The notch on the inferior basal border before the gonion
5	gonion- right	1	point along the rounded posteroinferior corner of the mandible between the ramus and the body
6	ramus flexure- right	2	Concave mandibular notch that separates the condyle and coronoid process
7	condylion- right	2	the most medial point on the right mandibular condyle
8	mandibular notch-right	2	Maximum flexion of curvature
9	coronoid process-right	1	Anterior-superior tip of coronoid process

10	anterior ramus- right	2	Anterior point of minimal ramus breadth
11	posterior alveolar process limit- right	2	End of superior alveolar border and ramus process at the end of dental arcade
12	mandibular foramen- right	1	the most inferior point on the margin of the mandibular mental foramen
13	canine- left	2	Mesial to left lower canine, alveolar border
14	mental foramen- left	2	Foramen located on the lateral corpus surface, near mid-corpus, below the premolar region
15	inferior basal border-left	2	Posterior beginning of the inferior basal border
16	pre-angular notch-left	2	The notch on the inferior basal border before the gonion
17	gonion- left	1	point along the rounded posteroinferior corner of the mandible between the ramus and the body
18	ramus flexure- left	2	Concave mandibular notch that separates the condyle and coronoid process
19	condylion- left	2	the most medial point on the left mandibular condyle
20	mandibular notch-left	2	Maximum flexion of curvature
21	coronoid process-left	1	Anterior-superior tip of coronoid process
22	anterior ramus- left	2	Anterior point of minimal ramus breadth
23	posterior alveolar process limit- left	2	End of superior alveolar border and ramus process at the end of dental arcade
24	mandibular foramen- left	1	the most inferior point on the margin of the mandibular mental foramen
25	infradentale	1	midline point at the superior tip of the septum between the mandibular central incisor
26	B-point	2	The deepest point at the mandibular symphysis curvature
27	menton	1	the triangular eminence, or bony chin, at the base of the corpus in the anterior symphyseal region
28	gnathion	2	most inferior midline points on the mandible.
29	foramen genioglossum	1	Foramen for genioglossus muscle in the inner surface of thew mandible
30	internal infradentale	1	The midline points at the superior tip of the septum between the mandibular central incisor on the inner surface of the mandible

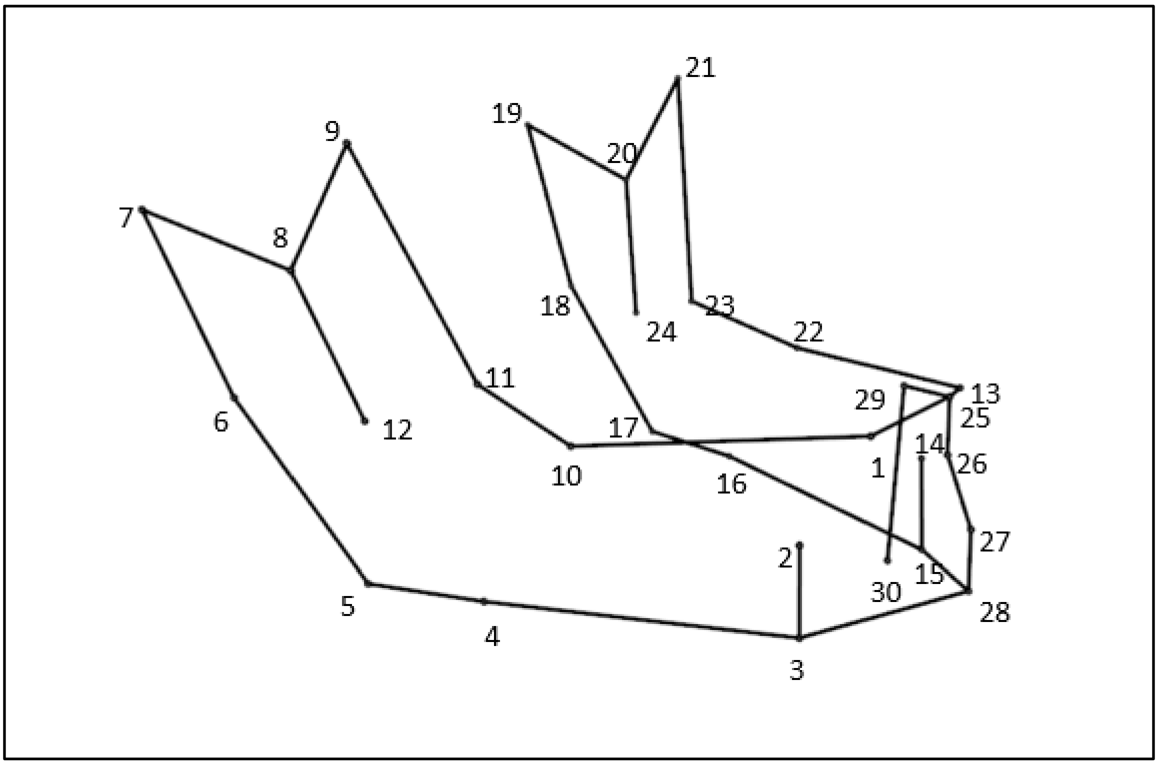

Figure 3.4: Mandibular landmark configuration

A repeatability test has been conducted to check the accuracy of the landmark digitalisation of the observer. To check the precision and repeatability of 3D coordinate data, a routine measured by superimposing datasets so that a particular parameter is minimized has been adapted. The landmarks have been positioned on 10 individuals repeatedly for 3 times and the configuration has then been obtained to perform GPA analysis. Using the GPA coordinates, a PCA analysis of the sample has been performed to check the distribution of the individuals concerning PC1 and PC2. The results of the error test have been described in the Chapter 4.3.1.

3.6. Statistical Analysis

Multivariate statistical analysis has been conducted in this study. According to Slice, (2006), traditional morphometric methods prove to be inadequate to provide information necessary to reconstruct the spatial relationship among structures since the measurements are not defined. Using geometric morphometrics, the high dimensional data that is acquired can provide a multivariate analysis opportunity. Three-dimensional landmark data from analogous anatomical points of both the mandible and the dentition have been collected and used as input to perform the Principal

Component Analysis using the R statistical software (R Core Team, 2022). The results then have been compared in terms of their PCA scores in order to understand the shape variation among the individuals. Then, CVA analysis was conducted to check the accuracy of the grouping performed based on dental eruption.

PCA is primarily used in paleoanthropological studies for data reduction or observation of group-specific patterns. When the variables are strongly related, it helps in providing strong results of multivariate analysis (Schillaci & Gunz, 2013). Such analysis can be useful in this case scenario to visualise the patterns of grouping among individuals based on their dental growth pattern and mandibular deformation and helps in the identification of large-scale trends in data. It is a standard multivariate technique which not only operates as an exploratory tool but also allows to identification of outliers in a dataset. The study has tried to combine the result of PCA with the shape variation of the mandible in order to observe the effective relation between the two in the morphospace.

The PCA has been conducted on the landmark coordinates after applying the Generalized Procrustes Analysis. The PCA analysis is visualized through a 3Dimensonal plot of the PC1, PC2 and PC3 using R. The GPA coordinates have also been plotted against age to check the relationship between the PC scores with age. Lastly, the allometric relation of the principal components has also been visualised. After the PCA analysis, a CVA analysis has also been performed. CVA is a form of multivariate analysis that helps in visualising discrepancies among groups. It helps in separating two or more prior groups. The discriminant functions are based on linear relations similar to Principal component analysis (Schillaci & Gunz, 2013).

For the statistical analysis, R software has been used. With the help of the R studio interface, which is an integrated development environment for R (RStudio Team, 2020), an interactive script has been prepared to perform the GPA on the landmark and then conduct the multivariate analysis of the acquired data. The R studio version 4.3 has been used. Along with the pre-installed packages, Arothron package 2.0.5 has been installed for the analysis. Beside Arothron, the following libraries have been operated which provide access to various functions mentioned further. The libraries operated are Arothron (Profico et al., 2021), Morpho (Schlager, 2017), geomorphic (Adams et al., 2016; Baken et al., 2021; Collyer & Adams, 2018), ggplot2 (Wickham et al., 2016), rgl (Murdoch et al., 2023), Rvcg (Schlager et al., 2017) and geometry (Habel et al., 2022) (table 3.4).

Table 3.4: R library applied for the analysis

R Library	Description
Arothron	It is a tool for geometric morphometric analysis which is meant to process and analyse digital models of skeletal elements (Profico et al., 2021).
Morpho	It is a toolbox created for Morphometric calculations. It has operations for Semi landmarks which helps in importing, exporting and manipulating 3D-surface meshes and semi-automated placement of surface landmarks. (Schlager, 2017)
geomorphic	It is a package which has functions that can read, manipulate, and digitize landmark data. It is used to generate shape variables via Procrustes analysis for points, curves and surface data. The tool is also useful for performing statistical analyses of shape variation as it provides graphical depictions of shapes and patterns of shape variation. (Adams et al., 2016; Baken et al., 2021; Collyer & Adams, 2018)
ggplot2	It is a system for creating graphics which is based on "The Grammar of Graphics". Based on the given commands of what graphical primitives to use, it can create detailed maps on chosen aesthetics. (Wickham et al., 2016)
rgl	RGL is a 3D real-time rendering system for R where multiple windows are managed at a time. (Murdoch et al., 2023)
Rvcg	It helps to provide meshing functionality from vcglib (meshlab) for R. (Schlager et al., 2017)
geometry	It forms the ‘Qhull’ library (Habel et al., 2022)

After the setup of necessary libraries and file destination, the landmark configuration of each individual that has been obtained in the ‘cvs’ format has been converted into an array. Then mandible and dental configurations were combined forming a new array. Any outliers have been identified using the ‘find. outliers’ function from package Morpho (Schlager, 2017). Two individuals, (id: 11261 and 11242) were found to be outliers and have been removed due to their extreme morphological disproportion of the mandible with the rest of the sample which can be due to pathological reasons not identified earlier.

To perform the GPA, the ‘Morpho’ package (Schlager, 2017) has been used. Categorical variables are created based on dental eruption groups, age, sex and name/id and colour have been set to visualize each group.

To perform the PCA analysis Morpho package is required. To visualise the groups distributed along the Principal components, the group hulls have been visualised using the ‘convhulln’ function from the ‘geometry’ package (Habel et al., 2022).

The shape variation of the maximum and minimum on each PC score has been created using the ‘restoreShapes’ function of the ‘Morpho’ package (Schlager, 2017) and then 3 dimensionally visualised.

For Canonical variate analysis (CVA) analysis, again the ‘Morpho’ package (Schlager, 2017) has been used. The typicality probabilities of the CVA scores have been calculated using the ‘typprob’ function of the Morpho package.

3.7. Ethical consideration

The study has been conducted with careful attention being paid to the ethical conduct of research work. No individual has been harmed or illegally exploited in the conduct of the study. The sample collection has been made with complete permission granted from the New Mexico Decedent database committee. The analytic software used has been legally obtained from open-access sources. In terms of literary sources, access has been legally sourced through the institution, The University of Ferrara as well as open access sources on Google Scholar. The study acknowledges the role of every entity directly or indirectly linked to the conduct of the work. The confidentiality and anonymity of entities have been maintained and only been disclosed upon receiving permission.

CHAPTER 4

RESULTS

4.1. Introduction

The chapter presents the results of the analysis discussed previously in Chapter 3. First, an approach to test the different standards of dental formation and dental eruption for age estimation has been made on the sample collection. The standards found in the literature of Logan & Kronfeld (1933), Gleiser & Hunt Jr (1955), Demirjian & Levesque (1980) and AlQahtani et al. (2010) have been applied to assess the sample collection. Following this section, 4.3. discusses the results from the geometric morphometrics protocol. Firstly the results from the error test have been shared after which the discussion of the PCA analysis in terms of shape variation. It mentions the different results observed in terms of the complete configuration of combined mandibular and dental data as well as each of them separately. The section follows a discussion of the allometric relation which is an important ontogenic association. Finally, in order to check the accuracy of the categorization that has been created for this study, CVA analysis has been performed. The CVA discusses the probability of affinity of each individual to their respective group classification.

4.2. Implementation of traditional methodologies of age estimation based on dental formation

4.2.1. Kronfeld (1933)

The standard of dental formation proposed by Logan & Kronfeld, (1933) in their study focuses on the crown formation and root formation period among people (for further detail, see annexe A.2). When the standard is applied to the sample, the following observation is made on each group (full detail in annexe A.3).

From GROUP 1 one individual, female and 61-months-old has started crown completion of the first premolar. The rest of the individuals in the group can be seen to process crown completion of both lateral and central incisors.

In GROUP 2 crown completion of the first and second premolar, canine and the lateral and central incisor is seen to have a place for the individuals except one male individual of 91 months old. Two individuals, male and female aged 57 and 53 months respectively are seen to be undergoing crown completion of their central and lateral incisors. Then, four individuals, 3 male and 1 female are in the phase of crown completion of their first premolar. The rest of the 6 individuals are seen to undergo crown completion of the second premolar and canine simultaneously.

GROUP 3 includes one individual, a female of 84 months of age who is seen to undergo crown completion of the second premolar and canine. Among the rest of the individuals in the group, two male and two female from ages 109 months to 120 months is seen to have begun the process of root completion in the first molar. Root completion of the central and lateral incisor is also seen for two individuals in GROUP 3: male and female respectively of age 109 months and 120 months.

For GROUP 4, the age estimation table of Kronfeld indicates two individuals in ongoing root completion of their first molar. Out of them, one is a male of 118 months and the other is a female of 107 months. Root completion of the first premolar and canine also began for two male individuals in the group of age 144 and 148 months respectively.

Lastly, GROUP 5 indicates individuals with ongoing root completion of second premolar and canine. Four individuals, two male and two female are indicated to go through the root completion.

4.2.2. Gleiser (1955)

The study of Gleiser & Hunt Jr (1955) is based on the first mandibular molar among modern humans. The research proposes a standard of age estimation based on dental formation as well as dental eruption (for further detail, see annexe A.4). Based on this methodology, the following observation has been made on the sample collection as per the group classification (full details in the annexe A.5).

The GROUP 1 individuals are in the stage of minimal root formation expect one female individual 61 months old who has root formation began in the first molar by one-fourth.

The GROUP 2 individuals are seen to indicate different stages of root formation starting from minimal formation to terminal divergence of the root canal. That is to say, two individual, male and female of 57 and 53 months old indicates minimal root formation. One male individual of 63 months old indicates one-fourth root formation, two female and male of 71 and 70 months old indicates one-third root formation and three individuals halve half of the root formation at age 72, 78 and 83 months. The former is male and the latter two are females. Then there is one female and one male with two-third root formation at age 88 and 80 months respectively. They also indicate the stage of clinical emergence One male individual of age 74 months has three-fourth root formation. Only one male individual in the group of 91 months of age has terminal divergence in the root canal.

In GROUP 3 there are two individuals, one female of 101 months of age and one male of 94 months of age who also have the stage of terminal divergence of root canal. There is one female of 84 months whose root is formed by two-third.

In GROUP 4 one female individual of 99 months of age is in the stage of terminal divergence of the root canal and one female individual of 107 months of age is in the stage of terminal convergence of the root canal.

4.2.3. Demirjian (1980)

According to the study (Demirjian & Levesque, 1980), there are 8 stages of dental formation (see annexe A.6) based on which maturity scores can be indicated. In the following section, the dentition of the sample has been implemented against these stages and the groups as been observed as follows (full details in Annexe A.7). His work also indicates the clinical age of dental eruption (Annexe A.6) which have not been possible to identify among the current sample due to absence of longitudinal data.

In GROUP 1, stages A to E indicate dental development. The first molar of the individuals from GROUP 1 has a stage E with one male individual of 48 months having stage D and one female individual of age 61 having stage F. Both the incisor of the individuals in this group are all stage E except two male individual of age 48 and 51 months whose lateral incisor are in stage D. The second premolar of the individuals are in between stage A to C with two individual, male and female of both 48 months having stage A; three individuals, 1 female and 2 males having stage B and the rest having stage C. In the case of the first premolar and canine, all the individuals expect three, 1 female and 2 males of ages 48 and 51 have stage C in the former. For the later, only 1 female of age 61 months has stage E. Rest of the individuals show stage D for the first premolar and canine.

In GROUP 2, individuals have stages B to G in their dental development. For the first molar, stages E, F and G are indicated. Similar stages are also indicated for the central and lateral incisors. The premolars and canine mostly indicate stages D and E except for one female individual of age 53 months indicating stage B for the second premolar.

In GROUP 3, it can be seen that the individuals have transitioned into stage G in the case of the first molar with three individuals, 1 male of 148 months and 2 females of 120 and 123 months age indicating stage H. For the incisor, the individuals indicate stages G and H with one exception of the female of 84 months indicating stage F for the lateral incisor. The premolars are seen to indicate stage E till the age of 113 months and 109 months for the second and first premolar respectively. Any individuals in the group older than the mentioned age indicate stage F except for one female of age 123 who has stage G in the first premolar. The canine indicates stage F with two exceptions of females of 120 and 123 months of age having stage G

In GROUP 4, all the males expect one of 118 months of age to attain stage H for the first molar. The females indicate stage G. Stage H is also indicated for the central incisor as well as lateral incisor except 2 females of 99 and 107 months having stage G for the later. The individuals between ages 129 and 148 are seen to have attained stage G for the second premolar and canine.

The rest indicate stage F with the exception of 1 female of 99 months having stage F indicated for the second premolar. In terms of the first premolar, only 2 individuals, both males of age 144 and 148 months have stage G. The individuals between 118 and 136 months, that is 3 males and 1 female indicate stage F and the rest in the stage E.

In GROUP 5, all the individuals indicate stage H for the first molar and both the incisors. The individual between 134 to 145 months of age indicates stage G for both the canine and the premolars. Stage G continues for the rest of the male individuals while for the females, one indicates stage H for the canine while another of 153 months indicates stage H for both the first premolar and canine.

4.2.4. London Atlas of Human Tooth development and Eruption (2010).

The London Atlas by AlQahtani et al., (2010) indicates both crown and root formation stages divided based on the model of (Moorrees et al., 1963). There are 14 stages of dental formation along with clinical age of emergence of mandibular dentition (see annexe A.8) which have been applied to the sample (full details in annexe A.9) and observed in terms of the groups formed.

In GROUP 1, we see the development of a crown among most of the dentition. 2 males and two females aged 51 months and 48 months are in the stage of crown half completed with dentine formation while the rest indicate root less than crown length for first molar. The same 4 individuals also indicate crown half completed with dentine formation for the lateral incisor while they indicate three-fourth crown completion for the central incisor. The initial root formation with diverse edge stages is indicated by the method of the rest of the individuals in the group for the incisors. The same individual is seen to have the three-fourth crown completion stage for the canine while the crown half is completed with the dentine formation stage for the first premolar. The stage they attend for the second premolar is cusp outline completion. Initial cusp formation for the first premolar is seen for the 2 males and two females aged 51 months and 48 months. These individuals also indicate the cusp outline completion stage for the canine.

In GROUP 2, we see the initiation of root development. The root length less than the crown length stage is indicated for the first molar and the incisor of the individuals. There are 5 male and 1 female between 57 to 72 months of age who shows this stage for the first molar. Individuals between age 70 to 72 months, that is 2 male, and 1 female has the same stage for both the incisor. 2 female and 2 male of age 78, 83 and 80 and 84 respectively continues to have this stage for the lateral incisor only. In the central incisor as well as the first molar, they indicate the stage of root length equal to crown length. Lastly, there is 2 individual, 1 male of 91 months and 1 female of 88 months who has the former stage indicated for the canine. The stage of a quarter of the root length developed with diverge end is indicated for the first molar and lateral incisor of these two individuals whereas they indicate root completion for the central incisor. Only 1 female individual of age 53 months indicates the stage crown half completed with dentine formation for the first molar and lateral incisor and crown three quarter completed for the central incisor. It also indicated the crown initiation stage for the first premolar whereas the canine of the individuals is indicated with the stage of cusp outline completion. The same stage is seen for two male individuals of age 57 and 63 months for the second premolar. These two individuals have crown half completed with dentine formation for the first premolar and crown three-fourth completed for the canine. 2 male and 1 female aged 70, 72 and 71 months respectively also has the same stage for the first and second premolar. The canine of these individuals indicates the stage of the crown completed with a defined pulp roof. The stage of crown completed with a defined pulp roof is also seen for the first and second premolars the 2 females and 2 males between the ages 78 to 84 months. In terms of their canine, the stage indicated is root initiation.

In GROUP 3, all the individuals indicate apex completion for the central incisor expect 1 female of 84 months and 1 male of 94 months. The stage of apex completion is also seen for the lateral incisor and first molar of the male induvial with age 126 months in this group. All the individuals expect the formally described also indicate the stage of apex half closed with diverging root end and wide Periodontal ligament space (PDL) for the lateral incisor. This stage is seen for the first molar of 2 females, 120 and 123 months of age and 1 male 122 months of age in the group. These three individuals have the stage of root length equal to crown length for the lateral incisor, canine and first premolar and root length equal to crown length for the second premolar. 4 males and 2 females between the ages 101 and 113 are seen to have the stage of root length less than the crown length for the first premolar and canine. They also have the stage of three-quarters of the root length developed with a diverge end for the first molar and root initiation stage for the second premolar. The 94 months old male individuals in the group have similar dental formation stages except for the second premolar which indicates the stage of crown completion.

GROUP 4 is seen to have individuals with apex completion attained in the first molar, central and lateral incisor of the 1 female and 4 male individuals between the ages 129 and 148 months as well as one more male and female of age 107 and 108 months but only indicated for the central incisor. The male has the stage of apex half closed with diverging root end and wide PDL for the first molar and root length equal to the crown length of the lateral incisor while the female individuals have the stage of apex half closed with diverging root end and wide PDL for the lateral incisor and the stage of three-fourth root completion for the first molar. 3 individuals in the group, 1 female of 129 months of age and 2 males of 131 and 136 months of age respectively are seen to have the stage of root length equal to crown length for the premolars and three-fourth root length completion for the canine. The last two individuals in the group, that is two males aged 144 and 148 months are seen to indicate this stage for both the premolar sand canine. Lastly, among the younger individuals if the group, the stage of root length less than crown length is indicated for the second premolar of age 118 male, first premolar of age 107 female and canine of age 99 and 107 female. The canine and the first premolar of the male is seen to indicate the stage of root length equal to crown length whereas the female of 99 months age is seen to indicate root initiation stage for the first premolar and crown completion for the second premolar.

In GROUP 5, most of the teeth have finished root completion. The first molar and both the incisor, the central and lateral are seen to indicate the stage of apex completion among all the individuals. 3 females and 1 male between 137 and 145 months of age are seen to have three-fourth root completion indicated for the canine and both the premolars. Then, there are 2 female and 1 male of age 151,153 and 155 months respectively which indicate the stage of root completion in the premolars and apex half closed with diverging root end and wide PDL for the canine. Lastly, the youngest individual of the group, that is 134 months age female is seen to have root length equal to crown length for the premolars and three-fourth root completion for the canine indicated as per the methodology.

4.3. Geometric Morphometric approach for age estimation

4.3.1. Observer efficiency of landmark digitalisation

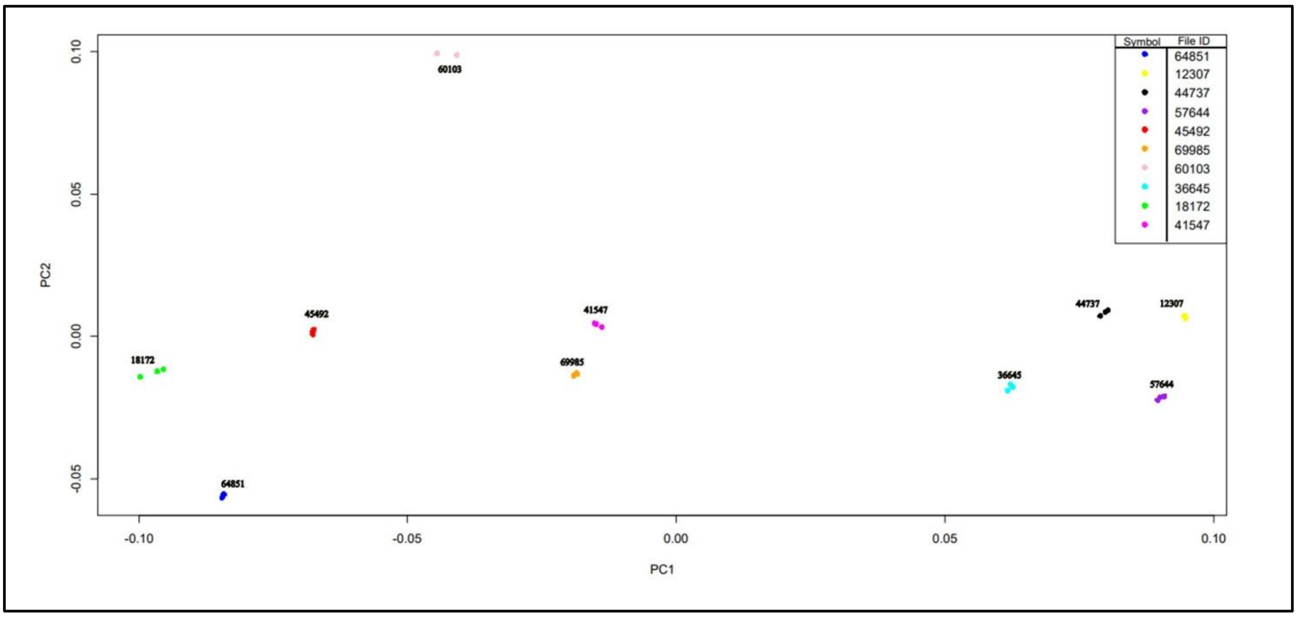

Figure 4.1: PCA analysis of the repeated landmark configurations of the 10 individuals

Figure 4.1 represents the PCA analysis of the GPA coordinates of 10 individuals on which 3 repetitions of landmarks have been plotted (for information on the individuals, see annexe A.10). The 'x' axis represents the PC1 and the 'y' axis represents the PC2. The plot indicates an overlap between the repeated landmark configuration of the individuals. No overlap can be seen among different individuals. The visual representation of the graph indicates that on repeating the landmark digitalisation on the same individuals, the same result is obtained. Therefore, the landmark digitalisation has been made successfully accurate in terms of its placement and the distribution of the individuals is mainly affected by the morphological variation of the mandible and dentition.

4.3.2. Principal Component Analysis and Shape Variation of complete configuration

In the following section, PCA analysis has been conducted on Procrustes residuals of 48 individuals, male and female, distributed in 5 groups that have been previously mentioned in Chapter 3. The combined configuration of the landmarks of the mandible and dentition has been used. The results allow the examination of the morphological variability of the groups of individuals and assess the variables contributing to the most observed differences. First, the relation between the first and second principal component has been described and then the second and third principal component has been spoken about. Also, the relation of the PC with the age of the individuals has been made. Lastly, the allometric relation of the principal components has also been discussed.

PC1 vs PC2

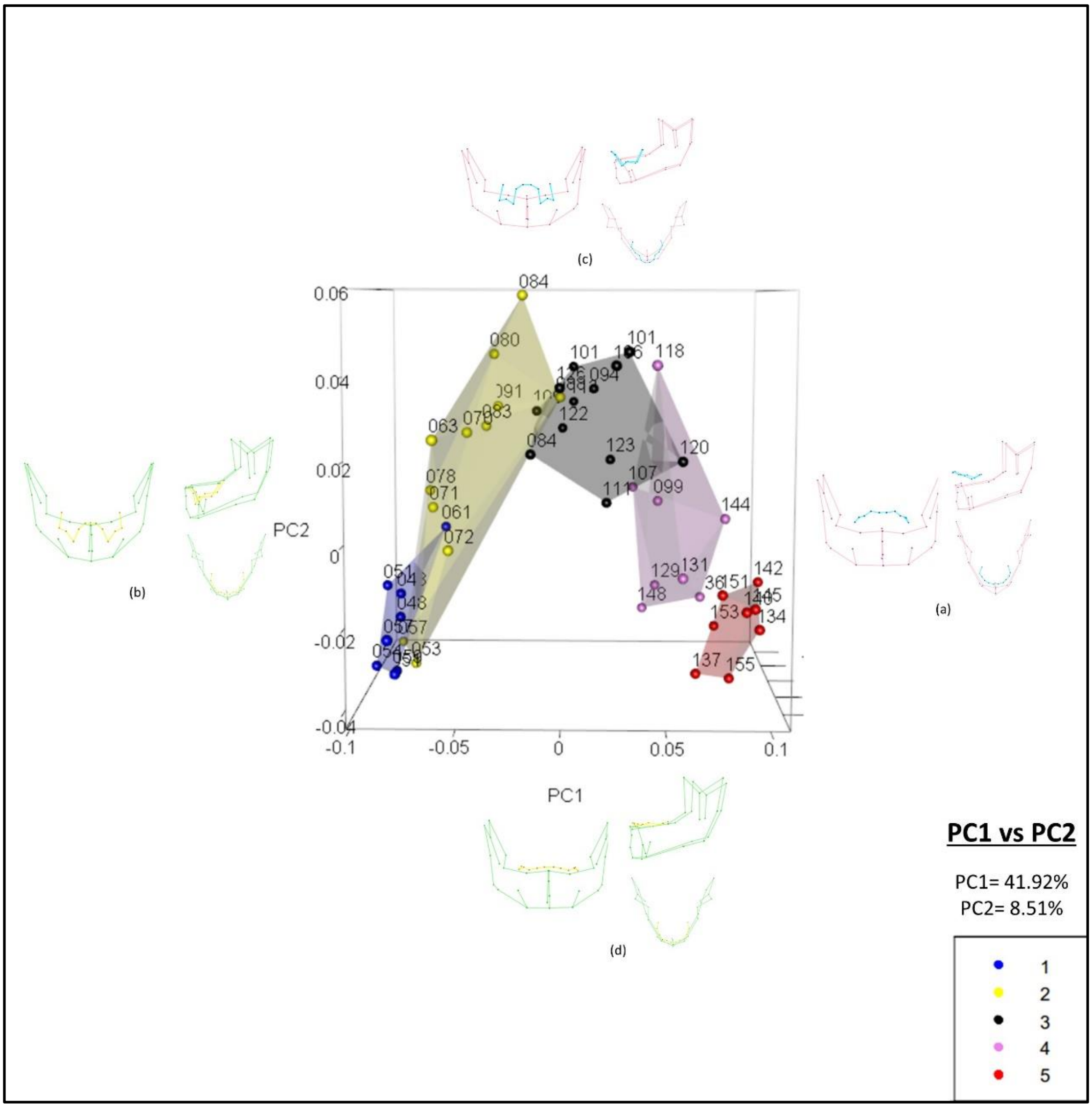

Figure 4.2: PC1 vs. PC2 of complete configuration. (a): maximum shape variation on PC1; (b): minimum shape variation on PC1; (c) maximum shape variation on PC2; (d): minimum shape variation on PC2

Figure 4.2 represents the distribution of sample groups in relation to PC1 and PC2. The 'x' axis represents the PC1, and the 'y' axis represents the PC2. The individuals in the graph have been named according to their respective ages (in months) and have been represented by the colour of the group they belong. The cumulative variance of PC1 and PC2 is 50.43% of which PC1 is 41.92% and PC2 is 8.51% (see annexe A.11). The shape variation along the PC1 and PC2 can also be seen. Figure 4.2 (a) represents the maximum shape variation of PC1 while (b) represents the minimum. Similarly, (c) is the maximum shape variation on PC2 and (d) is the minimum. In all the figures, the 3-three-dimensional constructed model of the mandible and dental arcade from the frontal, lateral and occlusal view based on landmark configuration has been shown. The mandibular configuration in green colour with yellow colour dental configuration (figure b;c) indicates minimum shape variation and the mandibular configuration in red colour with cyan blue coloured dental configuration (figure a;d) represents the maximum configuration respectively. For the maximum variation of PC1, it is seen that all the teeth are in a position of complete eruption except the second premolar while in the case of the sample indicating minimum shape variation, all the teeth are unerupted except the first molar and central incisor which are in the position of alveolar eruption. An important projection of the mandibular symphysis can be seen which indicates the development of chin in the figure (a) while, the symphysis is rather flat in figure (b). The inferior mandibular base is seen to be more straight in figure (a) in comparison to figure (b). The mental foramen can be seen to be positioned posteriorly in terms of the beginning of the inferior basal border while it is almost parallel for figure (a). The preangular notch is prominent in figure (b) while not so important in figure (a). In general, the mandibular body of figure (a) is seen to be more parallel with a more convergent ramus while in figure (b) it is more divergent.

The difference in the shape variation in PC2 is less evident. In figure (c), the first molar, lateral and central incisors are seen to be fully erupted while in figure (d), all the teeth are in position of alveolar eruption. There is no other major morphological difference between Figure (c) and figure (d).

In terms of the distribution of the groups, in the graphs, it can be seen that other than GROUP 5, there is a slight overlap among all the other groups. Along the PC1, the distribution of the individuals is from young to mature. Most of the individuals younger than 100 months are seen to be on the negative PC score while the individuals older than 100 months fall along the positive score on PC1. In terms of PC2, GROUP 1 and GROUP 5 have low PC scores in comparison to GROUP 2, GROUP 3 and GROUP 4. The individual with the maximum PC2 score is seen to belong to GROUP 2. There is also a high overlap between GROUP 1 and GROUP 2. GROUP 3 is seen to have most individuals with positive PC scores in comparison to other groups. In general, the graph shows a contracting trend among all the groups with the highest variation seen among GROUP 2 and GROUP 4.

PC2 vs PC3

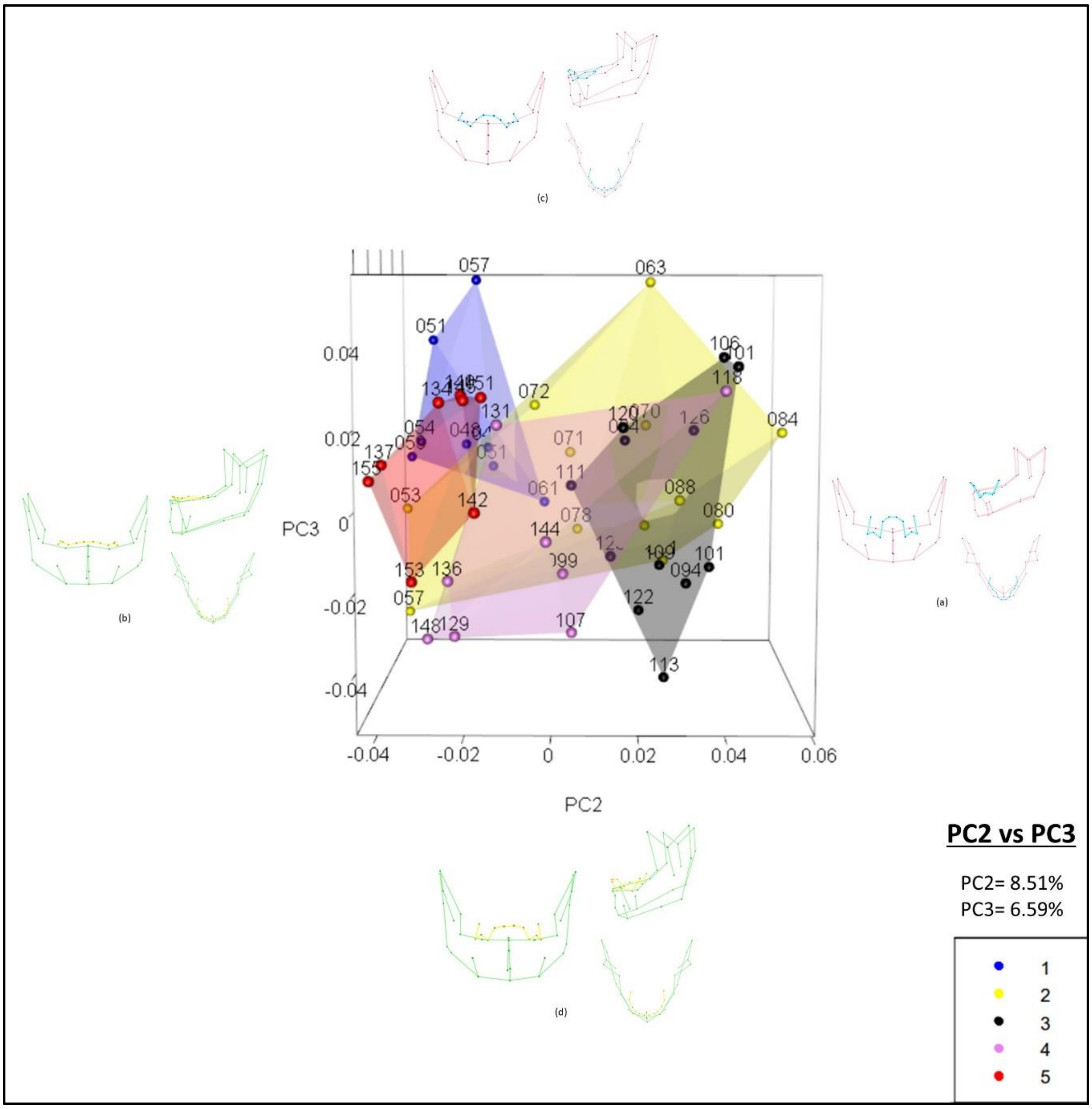

Figure 4.3: PC2 vs. PC3 of complete configuration. (a): maximum shape variation on PC2; (b): minimum shape variation on PC2; (c) maximum shape variation on PC3; (d): minimum shape variation on PC3

Figure 4.3 represents the distribution of sample groups in relation to PC2 and PC3. The 'x' axis represents the PC2, and the 'y' axis represents the PC3. The individuals in the graph have been named according to their respective ages and have been represented by the colour of the group they belong. The sum of the co-variance of PC2 and PC3 is 15.1% in which PC2 is 8.59% and PC3 is 6.59% (see annexe A.11). The shape variation along the PC2 and PC3 can also be seen where the 3-

dimensional model of the mandible and dental arcade from the frontal, lateral and occlusal view has been shown. In all the figures, the 3-three-dimensional constructed model of the mandible and dental arcade from the frontal, lateral and occlusal view based on landmark configuration has been shown. Like Figure 4.2 the mandibular configuration in green colour with yellow colour dental configuration (figure b;c) indicates minimum shape variation and the mandibular configuration in red colour with cyan blue coloured dental configuration (figure a;d) represents the maximum configuration respectively. Figure (a) represents the maximum shape variation of PC2 while (b) represents the minimum. Similarly, (c) is the maximum shape variation on PC3 and (d) is the minimum. As mentioned before, the shape variation of PC2 is not as clear as that along the PC1. For PC3, the maximum shape variation (c) is seen to have only the central incisor erupted while for the minimum (d) first molar, lateral incisor and central incisor are erupted. The first molar and lateral incisor are in the stage of alveolar eruption for figure (c) and both the premolar and canine are unerupted. In figure (d), the first premolar is in the stage of alveolar eruption and the rest of the dentition is unerupted. There are also differences in the mandibular morphology of maximum and minimum shape configuration. The mandibular notch has a smaller angle in Figure (d). the distance between the posterior alveolar process and anterior ramus (that is landmarks 11 and 10) is seen to be bigger in the minimum configuration. Figure (c) has a prominent curvature in the ramus flexure while it is more straight in the configuration relative to minimum values. The conoid process is wider in the maximum configuration and the ramus body ascending down from the conoid process is steeper. The position of the mental foramen is also more backward and the alveolar process is seen to be longer.

It is seen that GROUP 1 and GROUP 5 have lower PC2 scores in comparison to GROUP 3 because they are positioned nearer to the right side of the graph. GROUP 2 is seen to have the greatest variation in terms of the ‘y’ axis. In the graph, all the groups interlap with one another. All the groups are distributed medially in the plot with more concentration of individuals along the positive value of the ‘x’ axis. On the ‘y’ axis GROUP 2 has the most positive tendency.

Relation of PCA and AGE

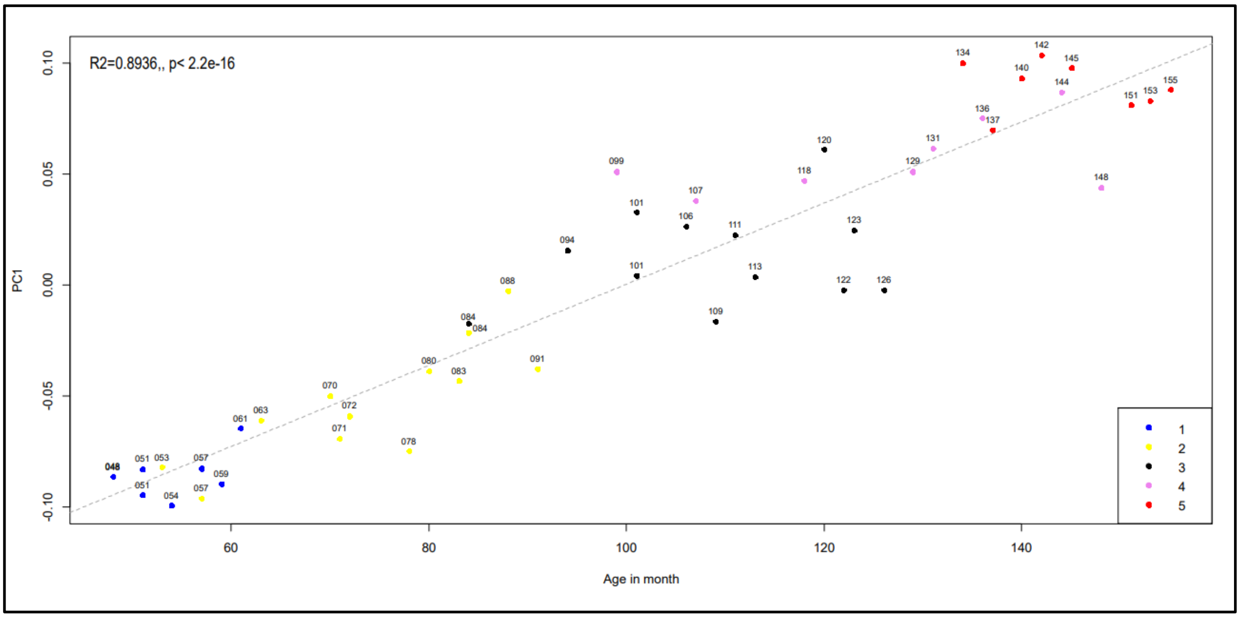

Figure 4.4:: Relation of PC1 of complete configuration with the age of the individuals

Figure 4.4 displays the relation of PC1 with the age of the individuals. The 'x' axis represents the age of the individuals in months while the 'y' axis represents the scores of PC1 where the age is the independent variable and the PC scores are the dependent variable. The r-square is 0.89 and the p value is inferior to 2.2e-16. Thus, the plot shows a high relation of the individuals on the PC1 with age. The line is sloped positively that is with the increase of age, the PC1 scores also increase.

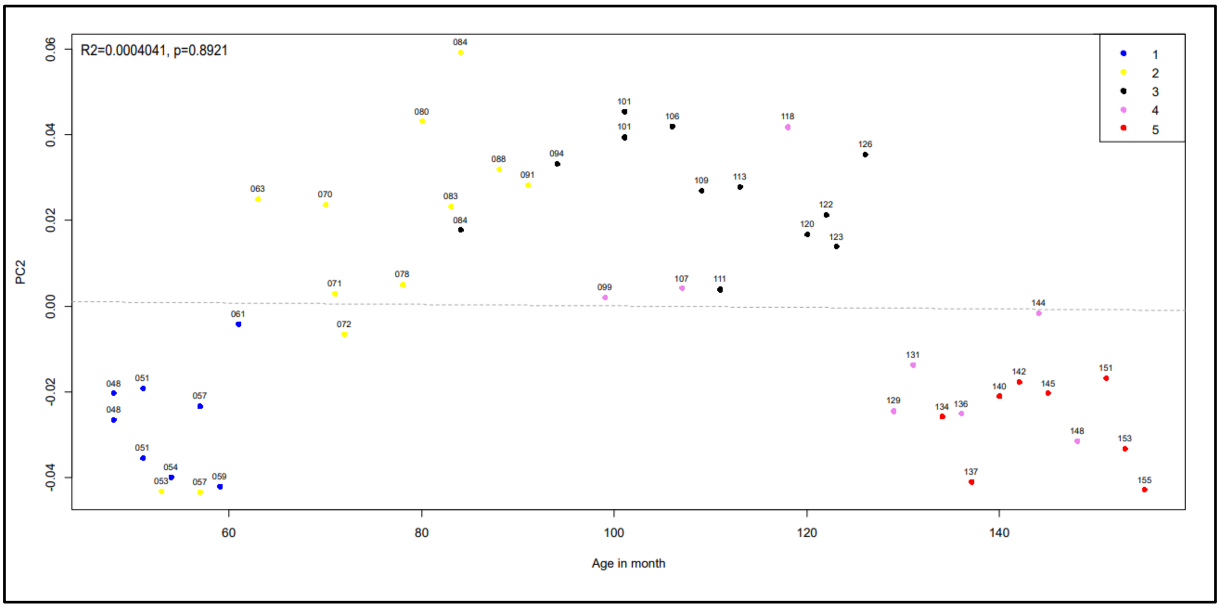

Figure 4.5: Relation of PC2 of complete configuration with age of the individuals

Figure 4.5 is a similar plot of the PC2 scores on the 'y' axis. The relation however is very weak in this case. The r-square is 0.0004041 which is very less than 1. The p-value is 0.8921 is more than 0.05. The line of regression is flat and closer to 0 than 1 as seen from the r-square value. In the plot GROUP 1 and GROUP 5 are seen to have lower scores on the axis and thus distributed lower than the line of intercept while the other groups are seen to have a higher score and are distributed above the line.

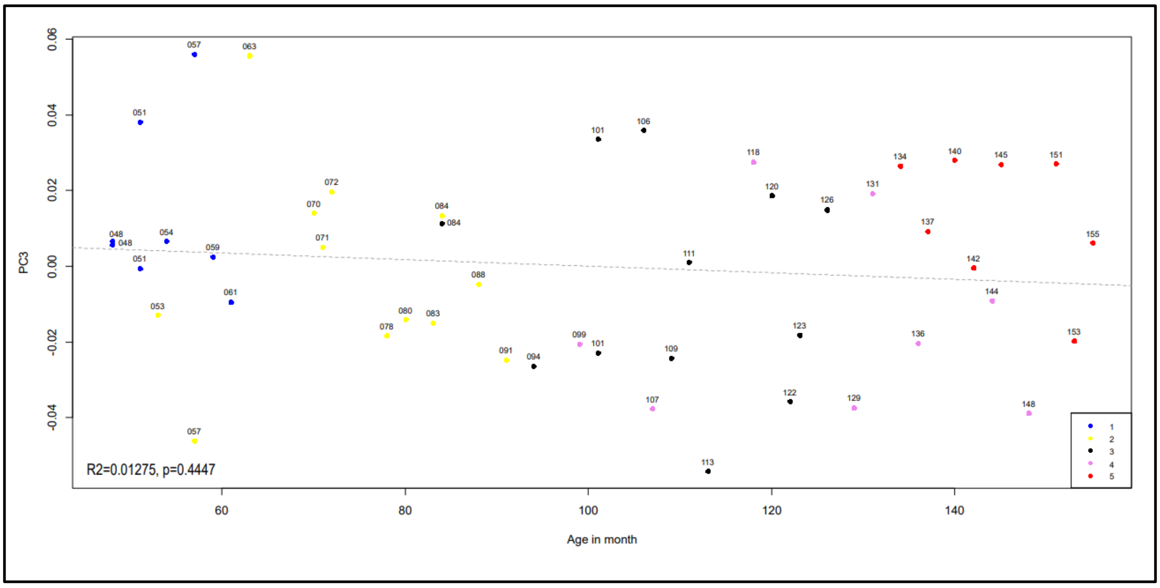

Figure 4.6: Relation of PC3 of complete configuration with age of the individuals

In the case of PC3, the r-square is 0.01275 which is more than the r-square of PC2 as seen in Figure 4.6 but less than PC1 Figure 4.4. The p-value of the plot is 0.4447 which is more than 0.05. The plot therefore displays a low correlation between the age and the pc scores as the r-square is closer to 0 than 1. The line of regression is seen to have a slight slope negatively which means that with the increase of age, the PC3 score decreases.

Allometric relation of PCA

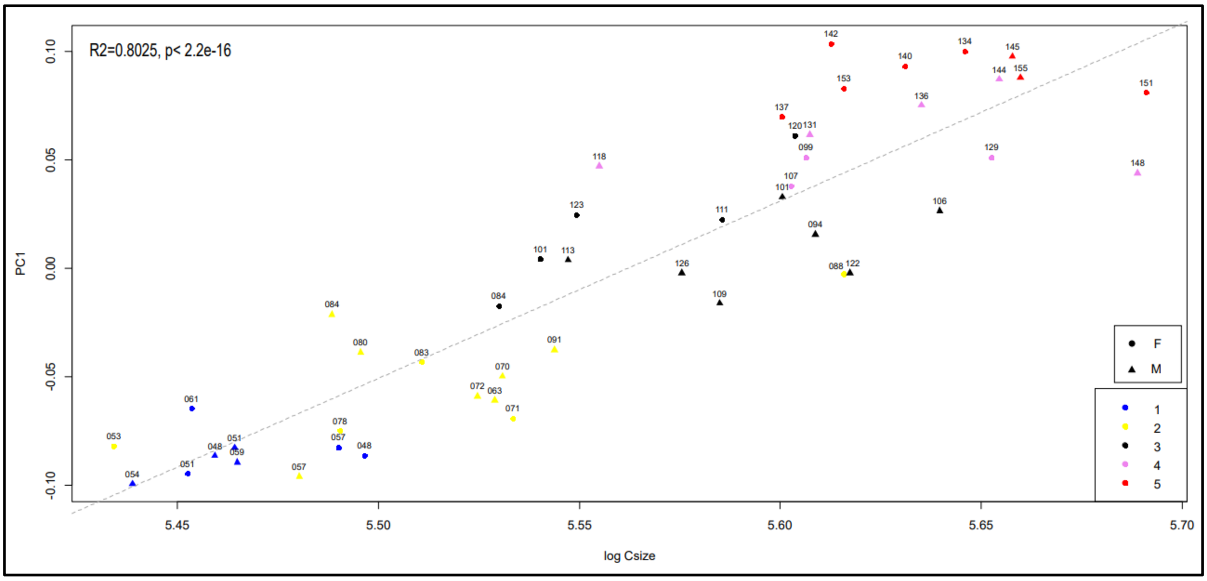

Figure 4.7: Allometry of PC1 of complete configuration

Figure 4.7 represents the allometric relation of PC1 and the logarithm of C-size where the ‘x’ axis represents the size, and the ‘y’ axis represents the PC scores. The r-square value of the plot is 0.8025 which is closer to 1 than 0 and the then-value is less than 2.2e-16. Therefore, the plot shows a strong allometric relation between the size and pc score. The plot also indicates male and female as round and triangle symbols respectively. However, no pattern of the effect of sex on the plot can be identified.

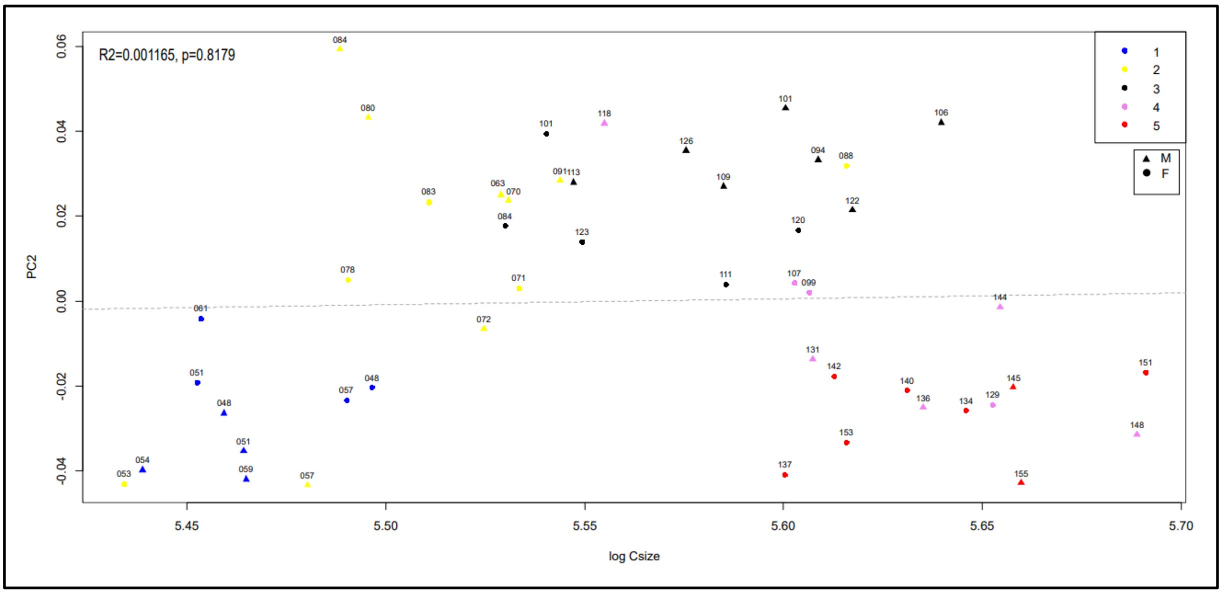

Figure 4.8: Allometry of PC2 of complete configuration

Figure 4.8 represents the allometric relation of PC2 with the 'y' axis representing the scores of PC2. The r-square value of the plot is 0.001165 which is closer to 0 than 1. The p-value is 0.8179 which is more than 0.05. Therefore, the plot shows a weak allometric relation between the size and pc score. The line of regression is flat and does not have close distribution of the individuals. The plot also indicates male and female as round and triangle symbols respectively. However, no pattern of the effect of sex on the plot can be identified.

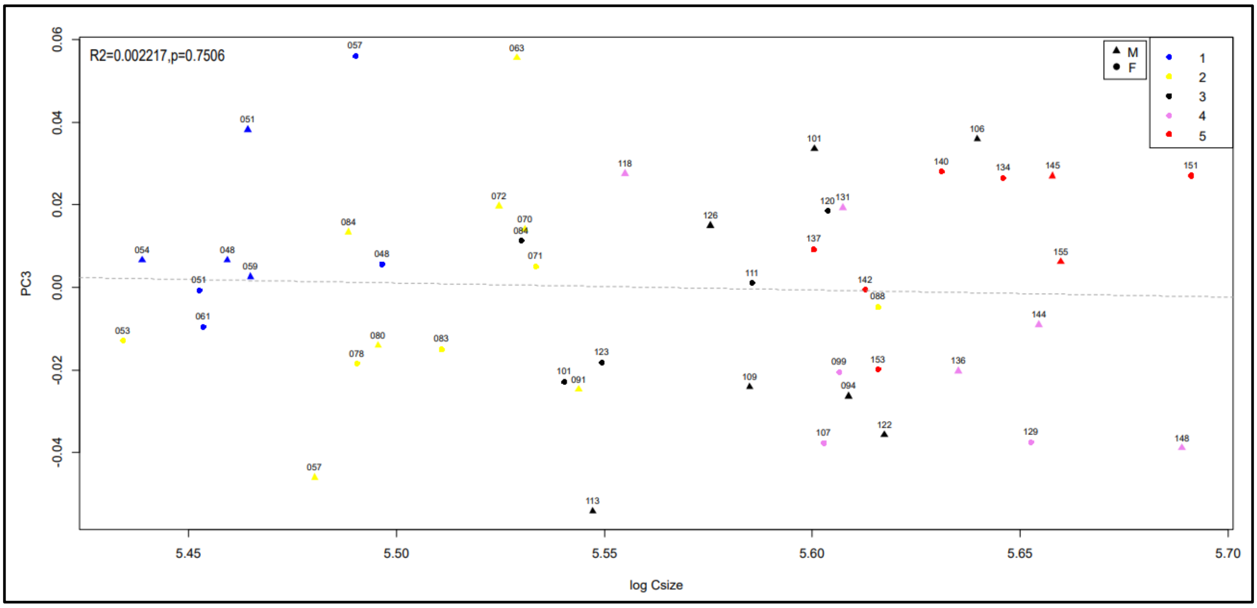

Figure 4.9: Allometry of PC3 of complete configuration

Lastly, Figure 4.9 represents the allometric relation of PC3 with the logarithm of C-size where the 'x' axis represents the size, and the 'y' axis represents the PC scores. The r-square value of the plot is 0.002217 which is closer to 0 than 1 and the p-value is 0.7506 which is more than 0.05. Therefore, the plot shows a weak allometric relation between the size and pc score. The line of regression is almost flat with a slight slope negatively meaning with the increase of size, the PC score decreases. The distribution of the individuals is dispersed around the plot and thus no visible pattern of concentration is seen near the line. The plot also indicates male and female as round and triangle symbols respectively. However, no pattern of the effect of sex on the plot can be identified.

4.3.3. Principal Component Analysis and Shape Variation of dental configuration.

In the following section, PCA analysis has been conducted on the landmark configuration of dentition. The data has been collected on 48 individuals, both male and female, distributed in 5 groups that have been previously mentioned in Chapter 3. Similar to the previous section, the results discuss the relation of the first and second principal components which have been described and then the second and third principal components and the maximum and minimum shape variation that occur on each PC. The relation of the PC1, PC2 and PC3 with the age of the individuals has been made. Lastly, the allometric relation of the principal components has also been discussed.

PC1 vs PC2

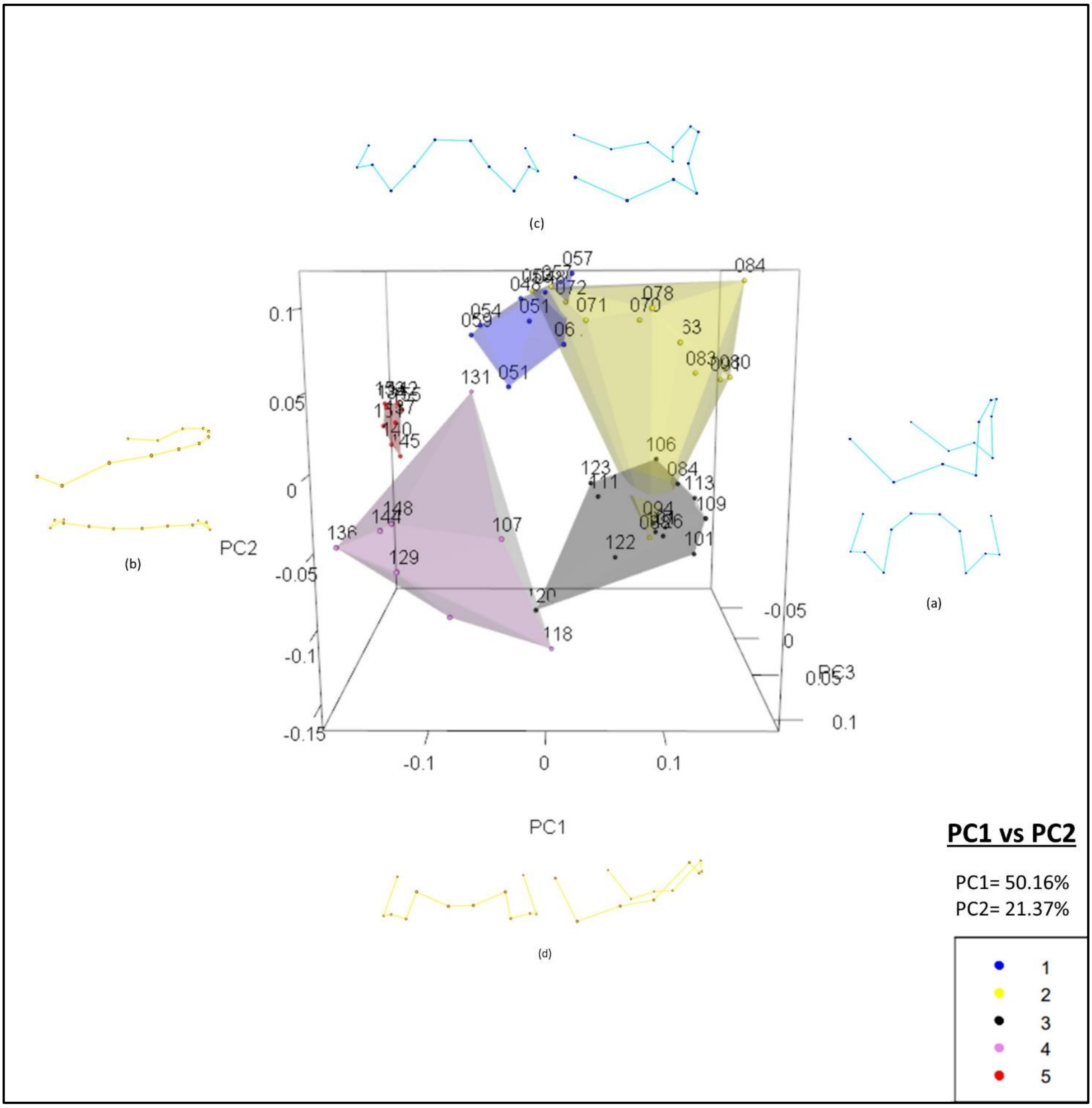

Figure 4.10: PC1 vs. PC2 of dental configuration. (a): maximum shape variation on PC1; (b): minimum shape variation on PC1; (c) maximum shape variation on PC2; (d): minimum shape variation on PC2

Figure 4.10 represents the distribution of sample groups in relation to PC1 and PC2. The 'x' axis represents the PC1, and the 'y' axis represents the PC2. The individuals in the graph have been named according to their respective ages and have been represented by the colour of the group they belong. The sum of the co-variance of PC1 and PC2 is 71.53% in which PC1 is 50.16% and PC2 is 21.37% (see annexe A.12). The shape variation along the PC1 and PC2 can also be seen. Figure (a) represents the maximum shape variation of PC1 while (b) represents the minimum. Similarly, (c) is the maximum shape variation on PC2 and (d) is the minimum. In all the figures, the 3 dimensional constructed model of the dental arcade from the frontal and lateral view based on landmark configuration has been shown. The yellow colour dental configuration (figure b;c) indicates minimum shape variation, and the cyan blue colour dental configuration (figure a;d) represents the maximum configuration. It can be seen that in the case of the maximum variation along PC1, the central incisor and the first molar are erupted while the premolars and canine are positioned much below in a stage of eruption. The lateral incisor depicts a position of partial eruption. On the other hand, the (b) is very linear thus depicting a stage where all the teeth are either erupted. In terms of the PC2, the minimum and maximum shape variation indicates mixed dentition. In (c) the dental arcade shows a higher position of the central incisor indicating a possible stage of eruption while for canine and second premolar, it is seen to be low. The lateral incisor, first premolar and first molar have a position in between which can range from alveolar eruption to partial eruption. The dental arcade in (d) shows a stage of eruption for the lateral incisor while the rest of the dentition has a lower position. The second premolar possibly indicates the stage of eruption since it is positioned at the lowest in the vertical morphospace.

In Figure 4.10, along the ‘x’ axis, GROUP 5 and GROUP 4 are seen to be separated from GROUP 1, GROUP 2 and GROUP 3 the former is found on the left side of the graph while the latter is found on the right side. That is to say, the individuals from GROUP 4 and GROUP 5 are seen to have more negative PC1 scores than the individuals from the other groups. There is overlap among groups. The GROUP 1 and GROUP 2 and the GROUP 3 and GROUP 4 overlap one another along the ‘x’ axis. The GROUP 5 is seen not to have any intersection with the other groups. In terms of the distribution of individuals along the ‘x’ axis, an individual with the most negative PC1 score belongs to GROUP 4 while the individual with the highest PC1 score belongs to GROUP 2. Along the ‘y’ axis, the PC2 is represented. GROUP 3 and GROUP 4 can be seen to have a distribution over negative PC2 scores while the other groups have a higher position among which

GROUP 1 has the highest position. Therefore, the individual with the highest PC2 score belongs to GROUP 1 while the individual with the lowest or most negative PC2 score belongs to GROUP 4.

PC2 vs PC3

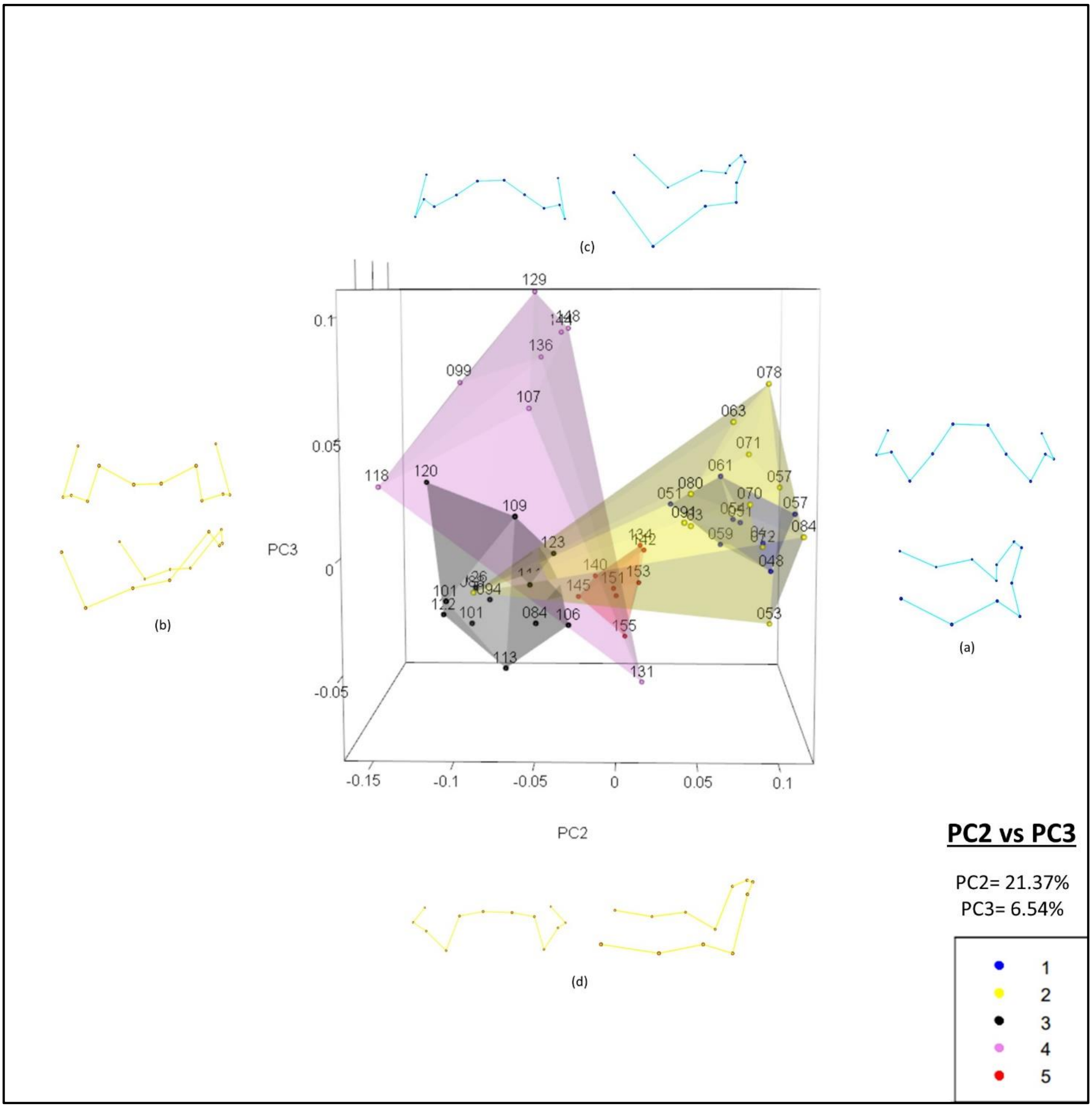

Figure 4.11: PC2 vs. PC3 of dental configuration. (a): maximum shape variation on PC2; (b): minimum shape variation on PC2; (c) maximum shape variation on PC3; (d): minimum shape variation on PC3

Figure 4.11 represents the distribution of sample groups in relation to PC1 and PC2. The 'x' axis represents the PC2, and the 'y' axis represents the PC3. The individuals in the graph have been named according to their respective ages and have been represented by the colour of the group they belong. The sum of the co-variance of PC2 and PC3 is 27.91% in which PC2 is 21.37% and PC3 is 6.54% (see annexe A.12). The shape variation along the PC1 and PC2 can also be seen. Figure (a) represents the maximum shape variation of PC1 while (b) represents the minimum. Similarly, (c) is the maximum shape variation on PC2 and (d) is the minimum. In all the figures, the 3 dimensional constructed model of the dental arcade from the frontal and lateral view based on landmark configuration has been shown. As seen previously, the yellow colour dental configuration (figure b;c) indicates minimum shape variation, and the cyan blue colour dental configuration (figure a;d) represents the maximum configuration respectively. In terms of the PC2, as discussed previously, the minimum dental arcade, that is (a) shows a higher position of central incisor indicating the possible stage of eruption while for canine and second premolar, it is seen to be low. The lateral incisor, first premolar and first molar have a position in between which can range from alveolar eruption to partial eruption. The maximum dental arcade in (b) shows a stage of eruption for the lateral incisor while the rest of the dentition has a lower position. The second premolar possibly indicates the stage of eruption since it is positioned at the lowest in the vertical morphospace. The shape variation along the PC3 shows a higher position of the incisors in comparison to the rest of the dentition. In (c), the central incisors are at the highest position while the canines are positioned lower thus indicating stages of eruption and uneruption respectively. The (d) indicates that both the incisors are erupting as seen from the higher position in the morphospace while the rest of the dentition is in the stage of uneruption.

The distribution of groups along the PC2 axis is as discussed before. Along the PC3 axis, it can be seen that all the groups have a central distribution in the graph. The GROUP 1 distribute within -0.05 to 0.05 with most of the individuals having a PC3 score near 0. The GROUP 2 ranges from -0.05 to more than 0.05. GROUP 3 has a more negative distribution ranging from -0.05 to more than 0 but less than 0.05. GROUP 4 has the biggest range in terms of PC3 having the individuals with the highest and lowest scores. Lastly, GROUP 5 is seen to range within -0.05 to 0 PC3 scores.

Relation of PCA and AGE

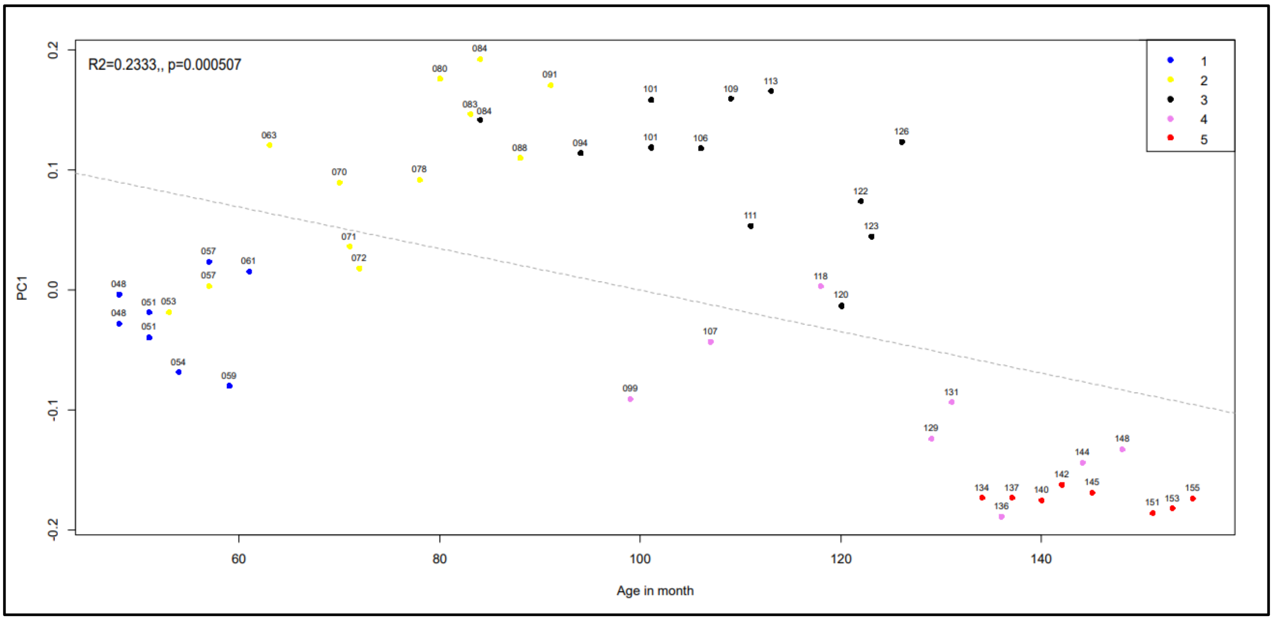

Figure 4.12: Relation of PC1 of dental configuration with age of the individuals

Figure 4.12 displays the relation of PC1 with the age of the individuals. The ‘x’ axis represents the age of the individuals in months while the ‘y’ axis represents the scores of PC1. The age is the independent variable, and the PC scores are the dependent variables. The r-square is 0.2333 which is closer to 0 than 1 and the p value is 0.000507. The plot therefore indicates the PC1 has a significant relation with age but the plot requires more variables for identifying the relation. The line of regression slopes negatively, which means that with the increase in age, the PC value decreases.

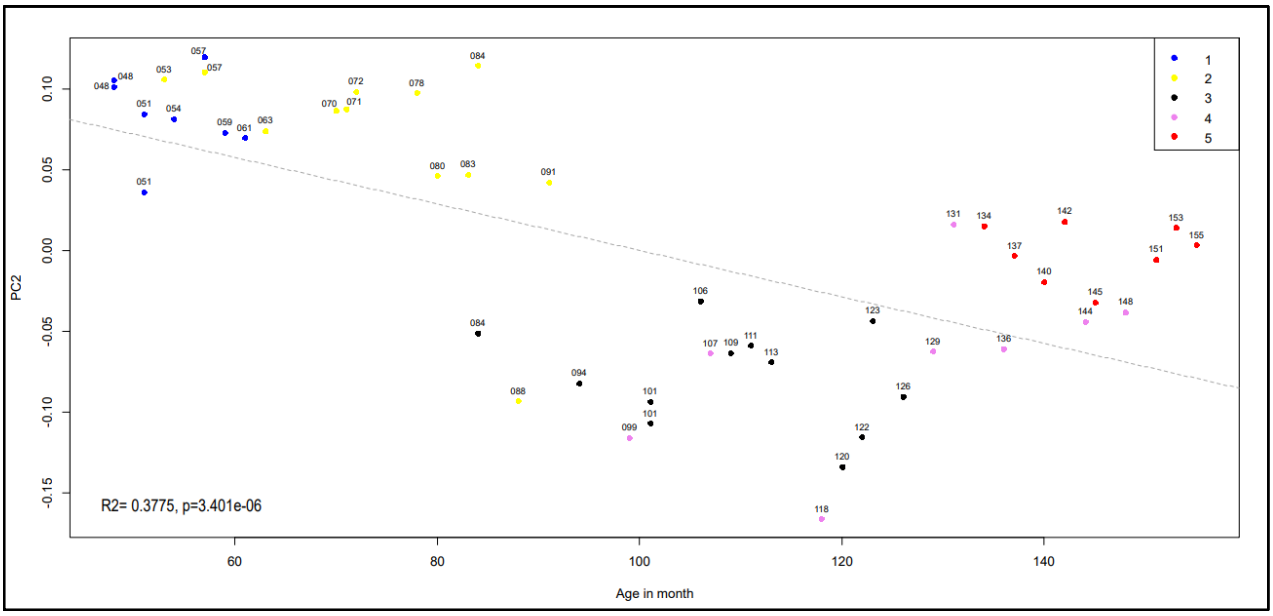

Figure 4.13: Relation of PC2 of dental configuration with age of the individuals

Figure 4.13 displays the relation of age with PC2 where again the 'x' axis represents the age of the individuals in months while the 'y' axis represents the scores of PC2. The relation, however, is very weak in this case. The r-square is 0.3775 which is less than 1 but more than the r-square seen previously in the plot of PC1 (Figure 4.12). The p-value is 3.401e-06 which is lesser than 0.05. Thus, the graph indicates that PC2 has a significant relation with age but the plot requires more variables for identifying the relation.

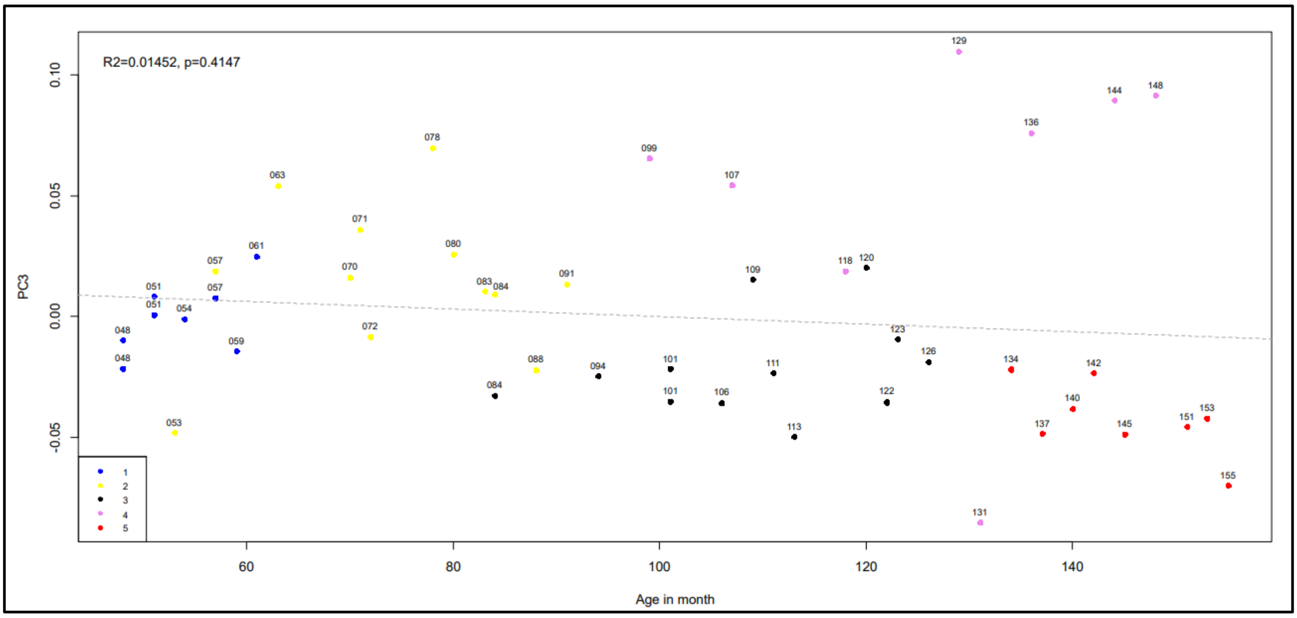

Figure 4.14: Relation of PC3 of dental configuration with age of the individuals

In the case of PC3, the r-square is 0.01452, which is very low from 1 as seen in Figure 4.14. The p-value is 0.4147, which is more than 0.05. The plot therefore displays a weak relation with age. The line of regression is seen to be almost flat with a slight negative tendency. The distribution of the individuals from GROUP 4 is seen to be farthest from the line.

Allometric relation of PCA

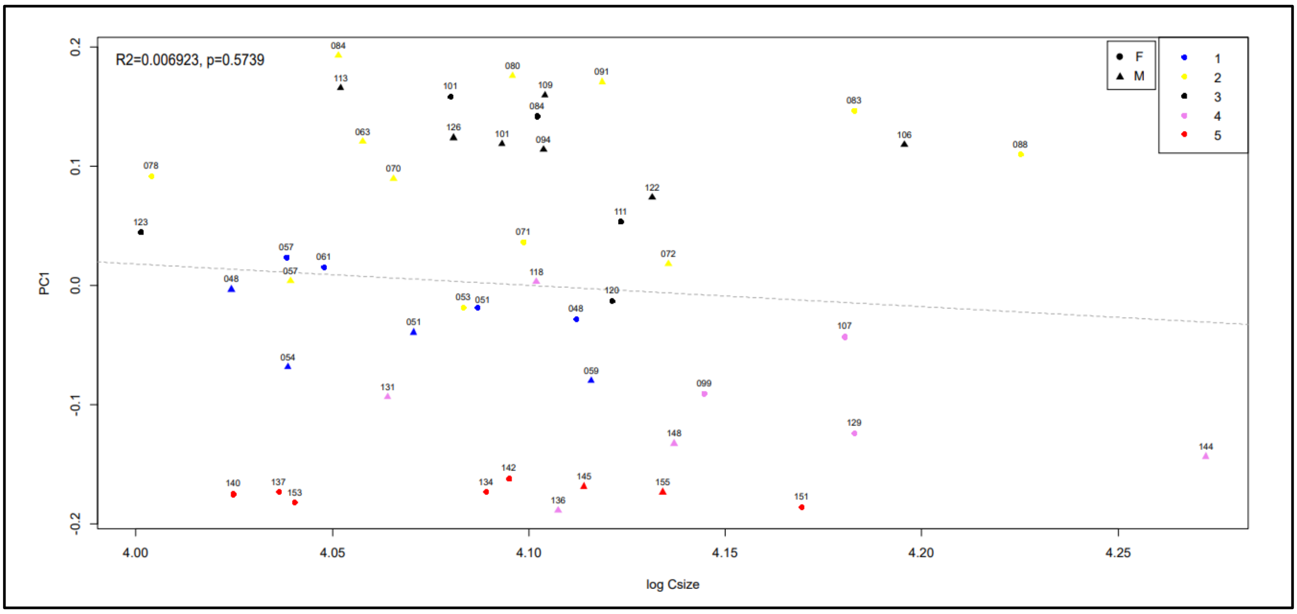

Figure 4.15: Allometry of PC1 of dental configuration

Figure 4.15 represents the allometric relation of PC1 and the logarithm of C-size where the 'x' axis represents the size, and the 'y' axis represents the PC scores. The r-square value of the plot is 0.006923 and the p-value is 0.5739. The plot therefore represents a weak allometric relation between the pc scores and age as evident from the low r-square which is closer to 0 than 1 and the p-value which is more than 0.05. The line of regression is sloped negatively, that is with the increase of size, the pc score decreases. The plot also indicates male and female as round and triangle symbols respectively. No pattern of the effect of sex on the plot can be identified. The individuals are seen to be dispersed in the plot and not concentrated near the line with a higher concentration within 4.00 and 4.15 values of x axis. Only 2 individual from GROUP 2, 1 from GROUP 3 and GRUP 4 is seen to distribute on the x axis more than 4.20, the highest being the male individual from GROUP 4 of age 144 months.

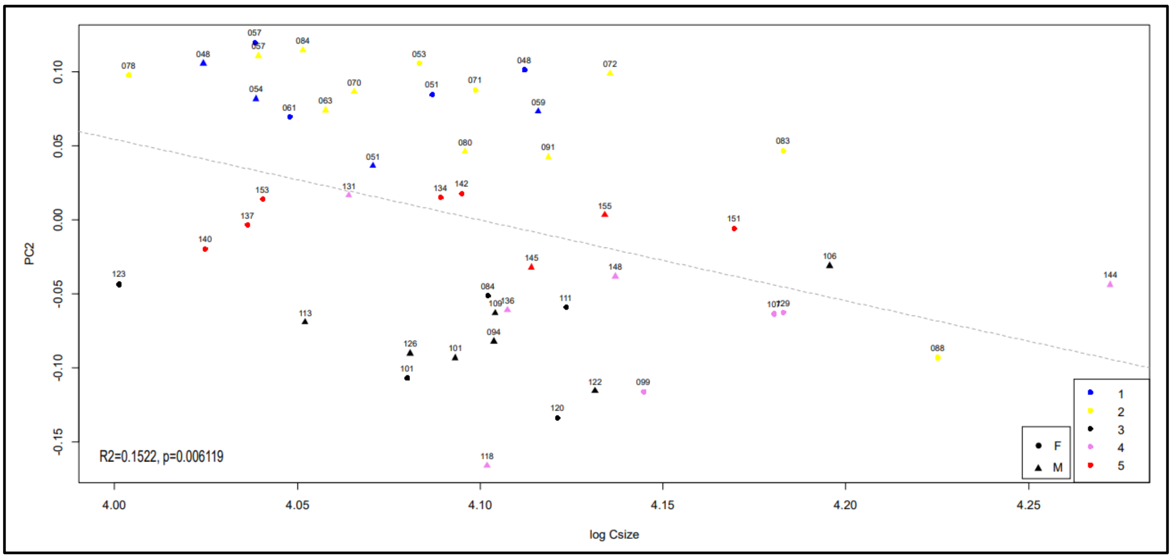

Figure 4.16: Allometry of PC2 of dental configuration

Figure 4.16 represents the allometric relation of PC2 and the logarithm of C-size where the ‘x’ axis represents the size, and the ‘y’ axis represents the PC scores. The r-square value of the plot is 0.1522 which is closer to 0 than 1 however it is more than the r-square value as seen in the previous graph. The p-value is 0.006119 less than 0.05. Thus there is a significant allometric elation with the PC2 but the plot requires more variables for identifying the relation. The line of regression is sloped negatively, that is with the increase of size, the pc score decreases. The plot also indicates male and female as round and triangle symbols respectively.

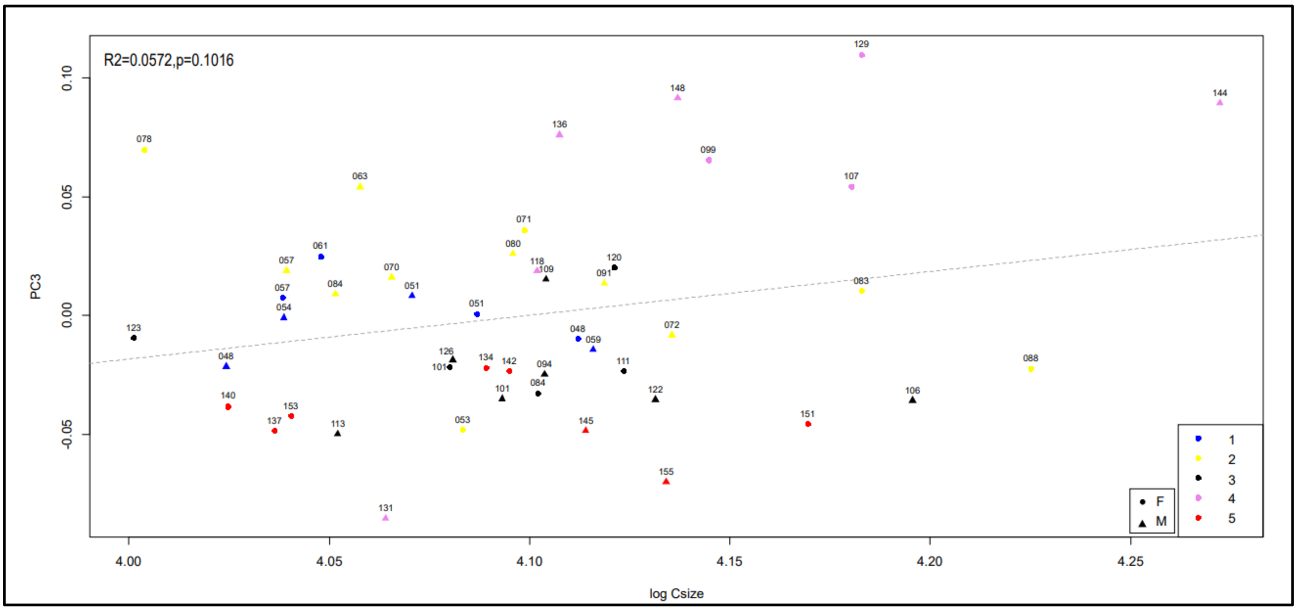

Figure 4.17: Allometry of PC3 of dental configuration

Lastly, Figure 4.17 represents the allometric relation between the PC3 and the logarithm of C-size where the ‘x’ axis represents the size, and the ‘y’ axis represents the PC scores. The r-square value of the plot is 0.0572 which is closer to 0 than 1. The p-value is 0.1016 more than 0.05. Therefore, there is a weak allometric relation between the PC3 and the size of the individuals. The line of regression is sloped positively and most of the individuals expect GROUP 4 to be close to the line but with a generally dispersed tendency. The plot also indicates male and female as round and triangle symbols respectively but no pattern of relation with the allometry can be seen.

4.3.4. Principal Component Analysis and Shape Variation of mandibular configuration.

In the following section, PCA analysis has been conducted on the landmark configuration of the mandible and the maximum and minimum shape variation of the principal components have been discussed alongside. The data has been collected on 48 individuals, male and female, distributed in 5 groups that have been previously mentioned in Chapter 3. Following the discussion of the PCA with shape variation, the relation of the principal components with the age of the individuals has been made. Lastly, the allometric relation has also been discussed.

PC1 vs PC2

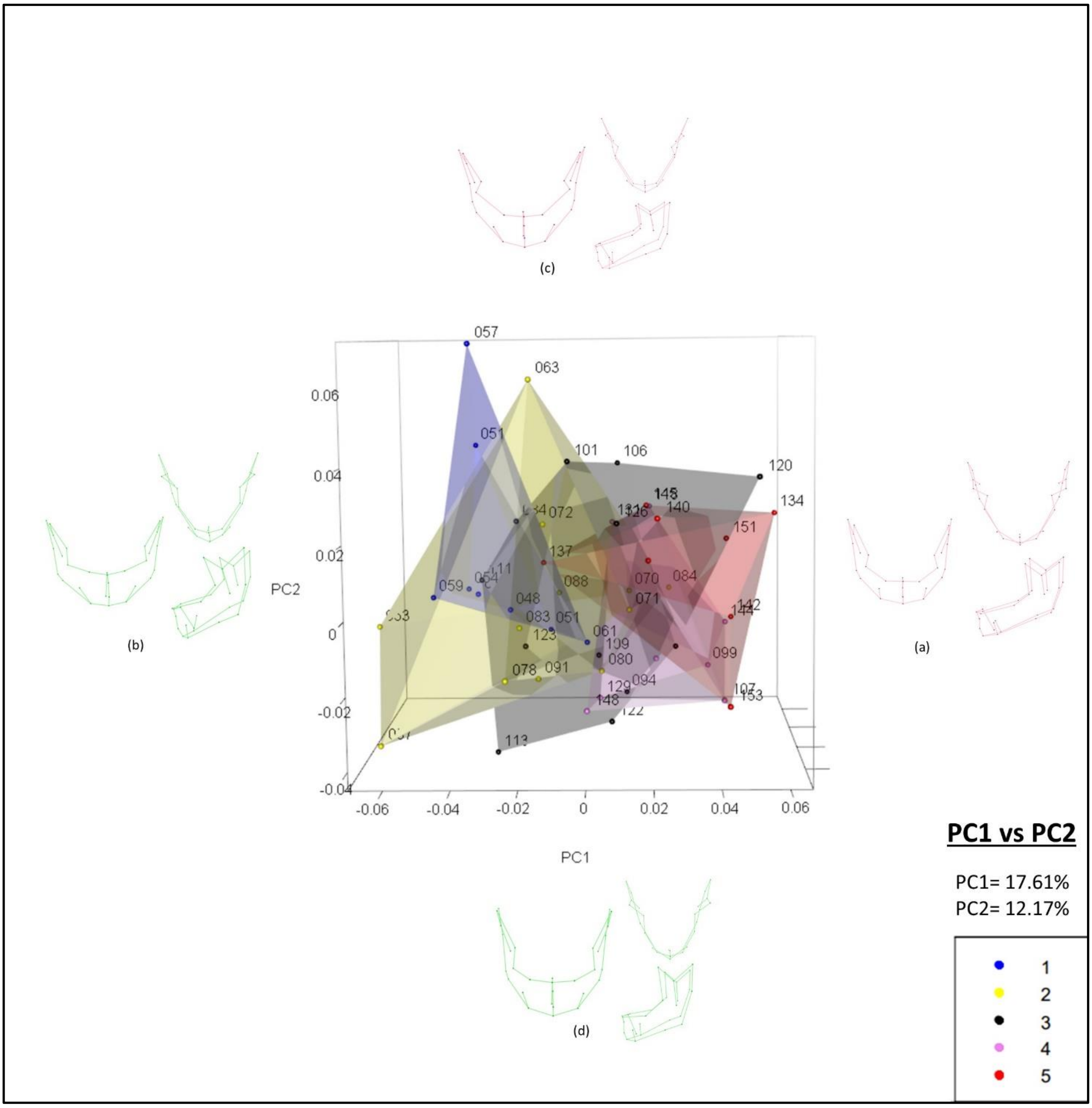

Figure 4.18: PC1 vs PC2 of mandibular configuration. (a): maximum shape variation on PC1; (b): minimum shape variation on PC1; (c) maximum shape variation on PC2; (d): minimum shape variation on PC2

Figure 4.18 represents the distribution of sample groups in relation to PC1 and PC2. The ‘x’ axis represents the PC1, and the ‘y’ axis represents the PC2. The individuals in the graph have been named according to their respective ages and have been represented by the colour of the group they belong. The sum of the co-variance of PC1 and PC2 is 29.78% in which PC1 is 17.61% and PC2 is 12.17% (see annexe A.13). In the figure, (a), (b), (c) and (d) represent the maximum and minimum shape variation along the PC1 and PC2 respectively. In all the figures, the 3-three-dimensional constructed model of the mandible and dental arcade from the frontal, lateral and occlusal view based on landmark configuration has been shown. The mandibular configuration in green colour (figure b;c) indicates minimum shape variation and the mandibular configuration in red colour (figure a;d) represents the maximum configuration. For PC1, it can be seen that the minimum configuration has a more divergent mandibular body in comparison to the maximum configuration. The symphysis also has a more important development in the maximum in Figure (a) in comparison to the minimum configuration (b). The mental foramen can be seen to be positioned posteriorly in terms of the beginning of the inferior basal border while it is almost parallel in Figure (b). The preangular notch is prominent in figure (b) while not so important in figure (a). Also, the ramus for the maximum configuration as seen in figure (a) is seen to be more elongated but with a smaller angle in the mandibular notch with a condyle that is shorter than the conoid process. For the minimum configuration, it is seen that the ramus is more divergent, short and has a smaller angle in the anterior end of the ramus process in conjunction with the superior alveolar process.

For PC2, the difference between the maximum and minimum shape variation as seen in figures (c) and (d) is morphologically less evident. For example, there is no difference in the general morphology of the mandibular body, mandibular symphysis or the gonion. Here is a probability of size difference which cannot be measured due to the performance of procrustes analysis. In terms of morphological differences, it can be seen that the superior alveolar process is longer in figure (c) than in figure (d). The condyle is seen to be more flexed and divergent in figure (c) than in figure (d).

In Figure 4.18, along the ‘x’ axis, it can be seen that all the groups intersect with each other expect GROUP 1 and GROUP 5. GROUP 1 has individuals with more negative PC1 scores in comparison to GROUP 5 where the individuals have more positive PC1 scores and thus are distributed more towards the right side of the ‘x’ axis while the former is distributed more to the left side. GROUP 3 has a central distribution in the ‘x’ axis with a distribution on the PC1 from -0-02 to

0.04. The GROUP 4 falls within the scores 0 to 0.04. It has no intersection with GROUP 1. The distribution along the PC2 also shows overlap and intersection of the groups. The general distribution of the individuals is on the higher part of the graph which shows that the individuals have a general positive score along PC2. The individual with the lowest score belongs to GROUP1 while the individual with the highest score can be seen to belong to GROUP 3.

PC2 vs PC3

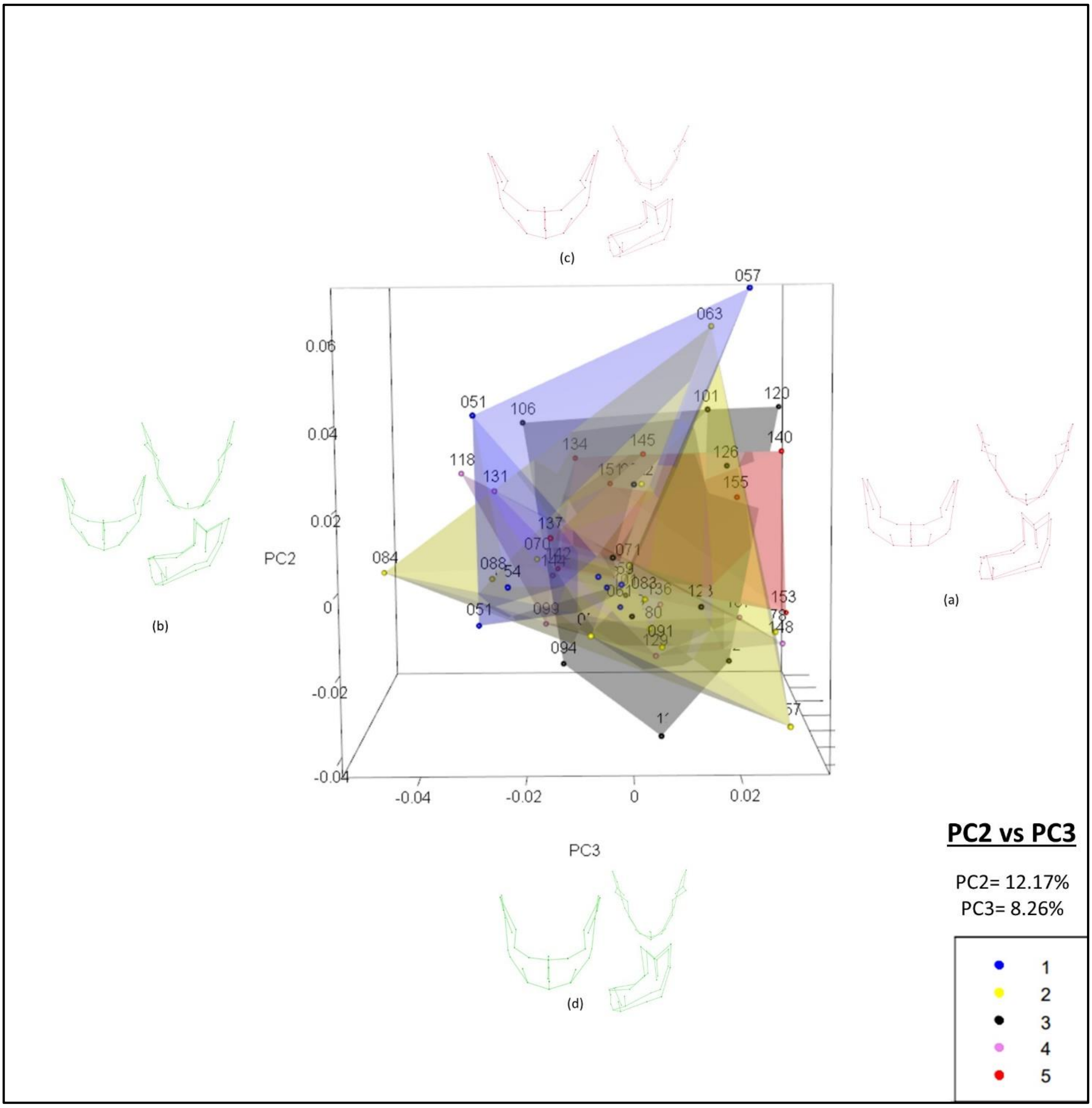

Figure 4.19: PC2 vs PC3 of mandibular configuration. (a): maximum shape variation on PC2; (b): minimum shape variation on PC2; (c) maximum shape variation on PC3; (d): minimum shape variation on PC3

Figure 4.19 represents the distribution of sample groups in relation to PC2 and PC3. The 'x' axis represents the PC2, and the 'y' axis represents the PC3. The individuals in the graph have been named according to their respective ages and have been represented by the colour of the group they belong. The sum of the co-variance of PC2 and PC3 is 50.43% in which PC1 is 12.17% and PC2 is 8.26% (see annexe A.13). It can be seen that all the groups are highly clustered with all the groups intersecting and overlapping with one another. No tendency of pattern can be deciphered from the distribution. The individuals in the groups can be seen to have a general tendency of positive scores along both PC2 and PC3.

In Figure 4.19, (a), (b), (c) and (d) represent the maximum and minimum shape variation along the PC2 and PC3 respectively. In all the figures, the mandible is shown from the frontal, lateral and occlusal view based on landmark configuration. The mandibular configuration in green colour (figure b;c) indicates minimum shape variation and the mandibular configuration in red colour (figure a;d) represents the maximum configuration. As discussed previously, there is a minimum morphological difference between the minimum and maximum configuration of PC2 which is evident from the similar morphological features of the mandibular body, the mandibular symphysis and the gonion. There is a small difference seen along the superior alveolar process which is longer in figure (a) which is the maximum configuration while it is shorter in figure (b), which is the minimum. The condyle is seen to be more flexed and divergent in figure (a) than in figure (b). In term of the shape variation along the PC3, figure (c) and (d) represents the maximum and minimum configuration. It is seen that the minimum configuration does not have an important development of the mandibular symphysis which is present in the other. The pre-angular notch is however more prominent in the maximum than the minimum configuration. In the morphology of the ramus, the condyle is more divergent and the angle at the mandibular notch is more in figure (c) than the figure (d).

Relation of PCA and AGE

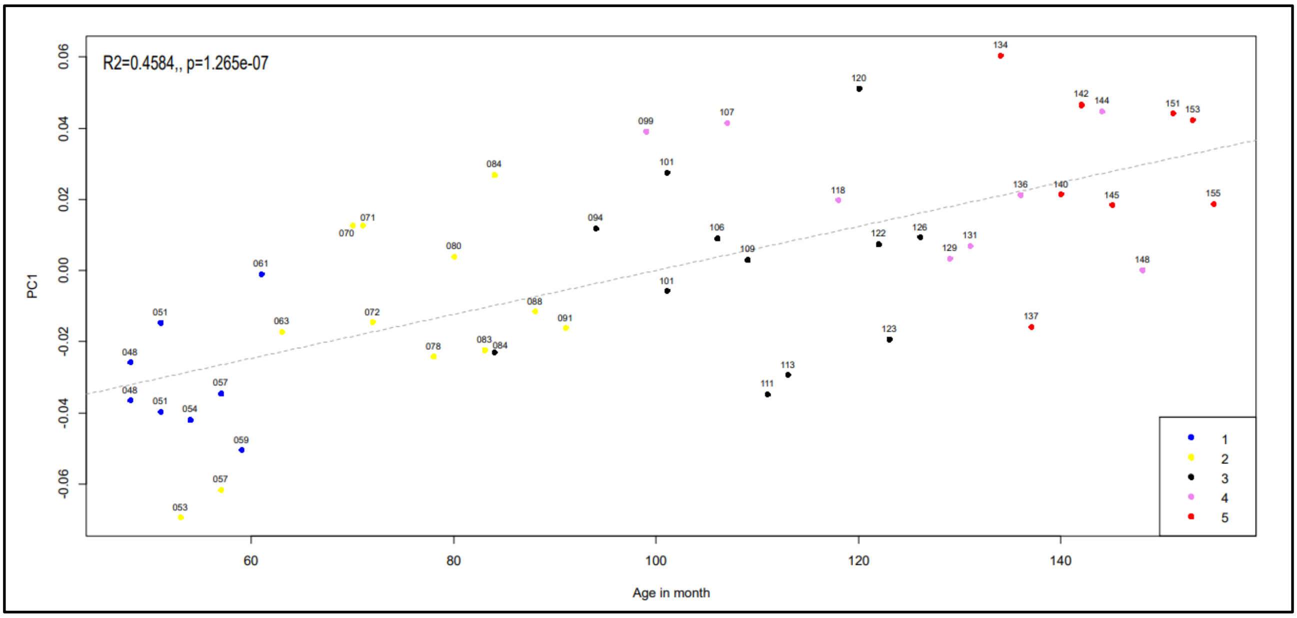

Figure 4.20: Relation of PC1 of mandibular configuration with age of the individuals

The relation of the PC scores with age in terms of the morphological variation of the mandible is represented in Figure 4.20, Figure 4.21and Figure 4.22. Figure 4.20 represents the PC1 and its relationship with the age of the individuals. The 'x' axis represents the age while the 'y' axis represents the pc scores. The r-square value of the plot is 0.4584 and the p value is 1.265e-0 which is less than 0.005. The r-square is almost equidistant from 0 and 1 thus indicating a medium variance. The plot therefore has a strong correlation between age and the pc score but can also be seen not to be very weak. The line of regression has a positive slope which shows that the pc score increases with the increase of age.

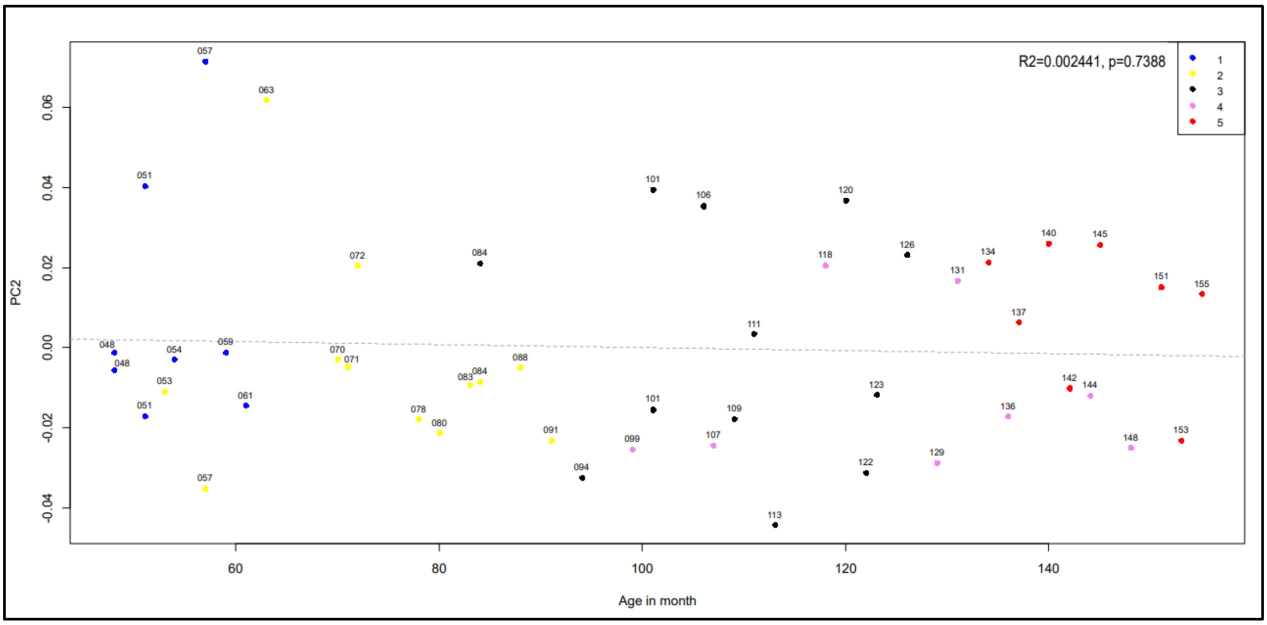

Figure 4.21: Relation of PC2 of mandibular configuration with age of the individuals

Figure 4.21 represents age on the 'x' axis and PC2 on the 'y' axis. The r-square value is 0.002441, which is lower than the previous plot Figure 4.20. The p-value is 0.7388 which is more than 0.05. The plot therefore shows a weak relationship between the PC2 scores and age. The line of regression is flat as indicated by the r-square value being closer to 0. GROUP 1 and GROUP 2 have a distribution of individuals closer to the line than the other groups.

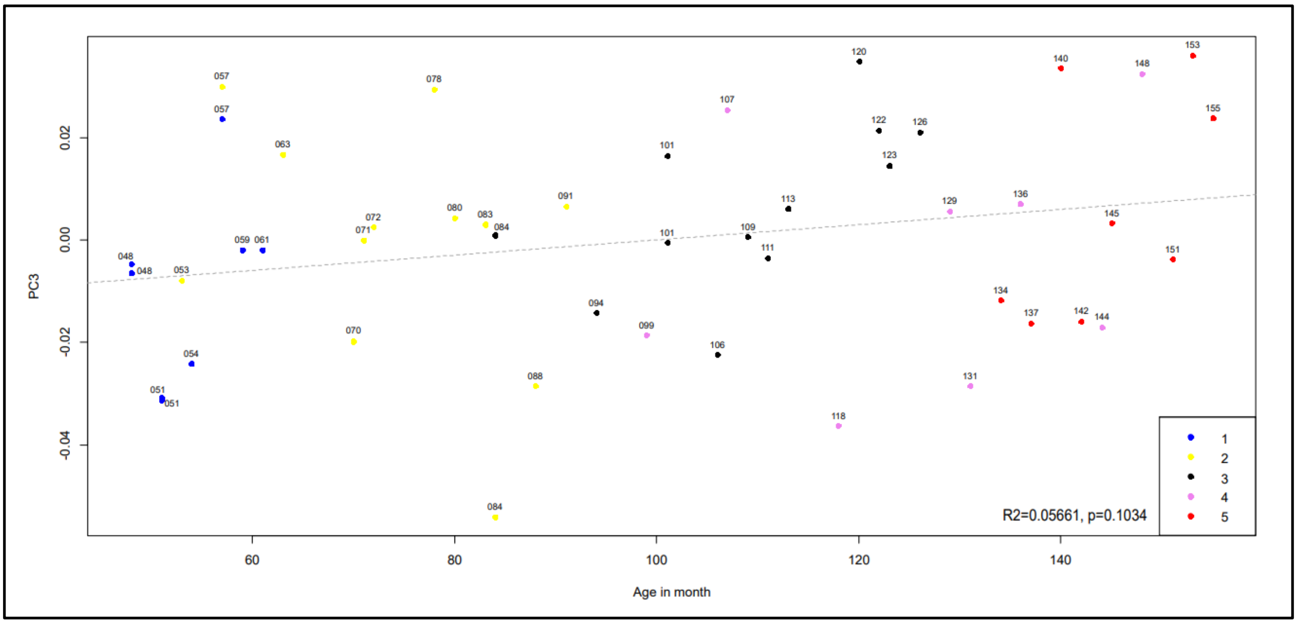

Figure 4.22: Relation of PC3 of mandibular configuration with age of the individuals

Figure 4.22 represents the plot of PC 3 scores in terms of the age of the individuals where the 'x' axis is age, and the 'y' axis is PC scores. The r-square value and the p-value are 0.05661 and

0.1034 respectively. It shows more degree of relation among the variables in comparison to Figure 4.20 of PC 2 scores however it is still very less than 1 and thus the relation is weak. The line of regression is seen to have a positive slope with individuals from GROUP 3 having a distribution closest to the line.

Allometric relation of PCA

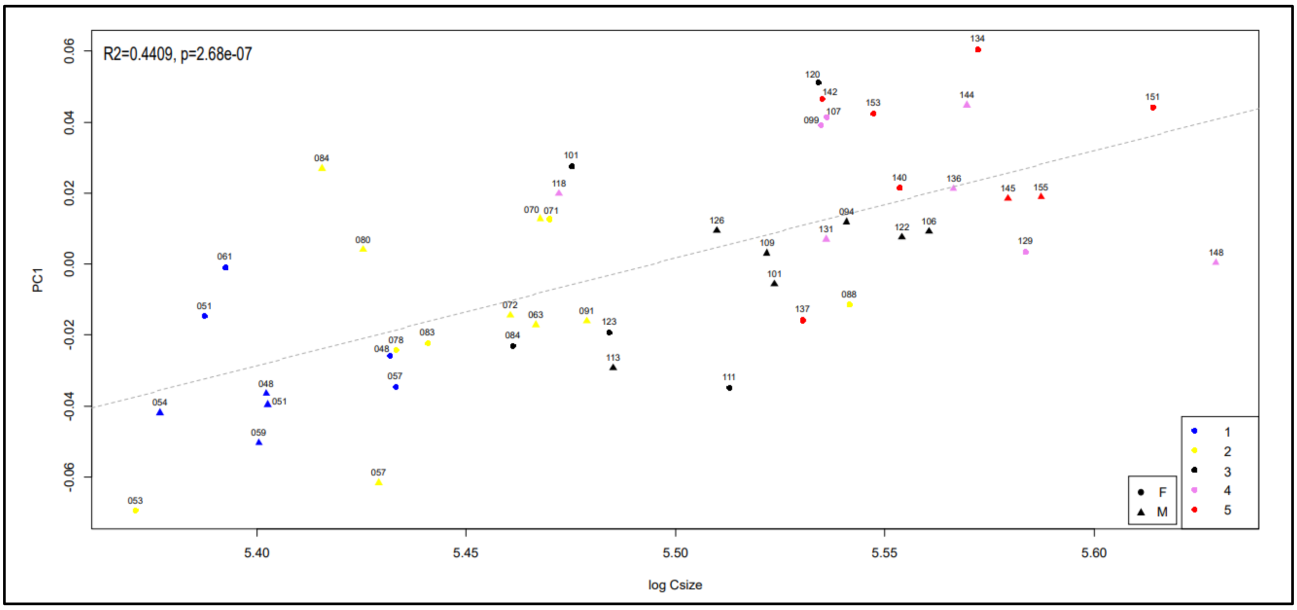

Figure 4.23: Allometry of PC1 of mandibular configuration

Figure 4.23 represents the allometric relation of PC1 and the logarithm of C-size where the 'x' axis represents the size, and the 'y' axis represents the PC scores. The r-square value of the plot is 0.4409 which is closer to 0 than 1. The p-value is 2.68e-07 which is more than 0.05. Therefore, the plot shows a strong allometric relation between the size and pc score. The line of regression is sloped positively and the individuals are seen to be distributed near the line with few individuals dispersed far. The plot also indicates male and female as round and triangle symbols respectively. However, no pattern of the effect of sex on the plot can be identified.

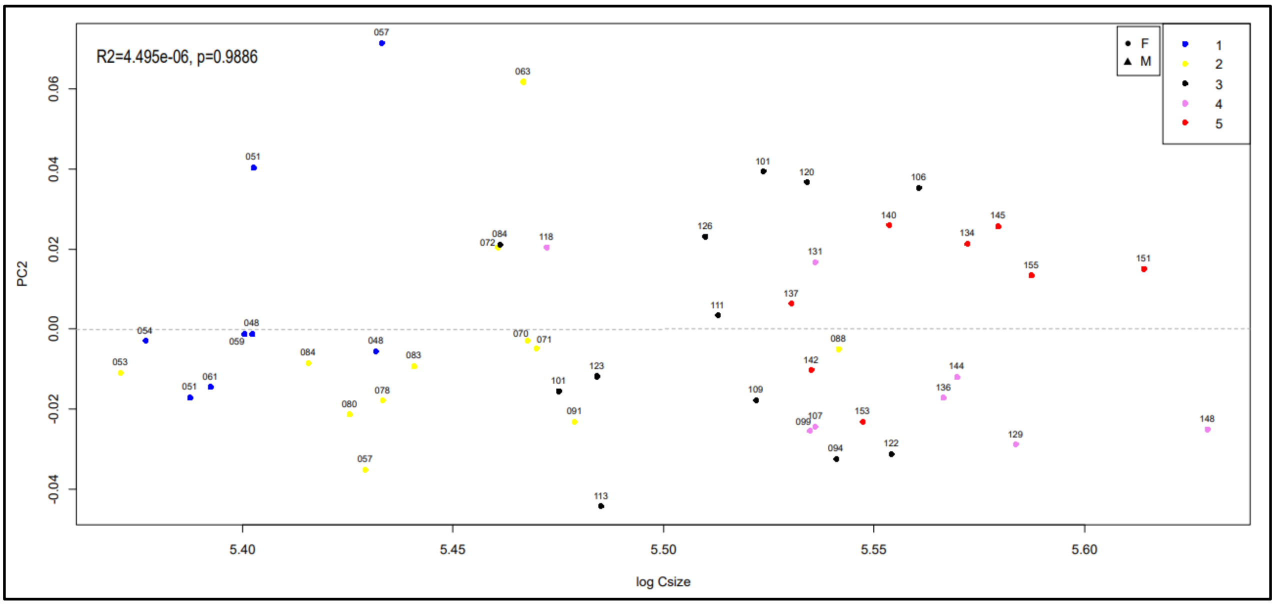

Figure 4.24: Allometry of PC2 of mandibular configuration

Figure 4.24 represents the allometric relation of PC2 and the logarithm of C-size where the ‘x’ axis represents the size, and the ‘y’ axis represents the PC scores. The r-square value of the plot is 4.495e-06 which is very than 1. The p-value is 0.9886 which is more than 0.05. Therefore, the plot shows a weak allometric relation between the size and pc score. The line of regression is flat, with few individuals distributed close to the line while most of the individuals have a dispersed distribution. The plot also indicates male and female as round and triangle symbols respectively. However, no pattern of the effect of sex on the plot can be identified.

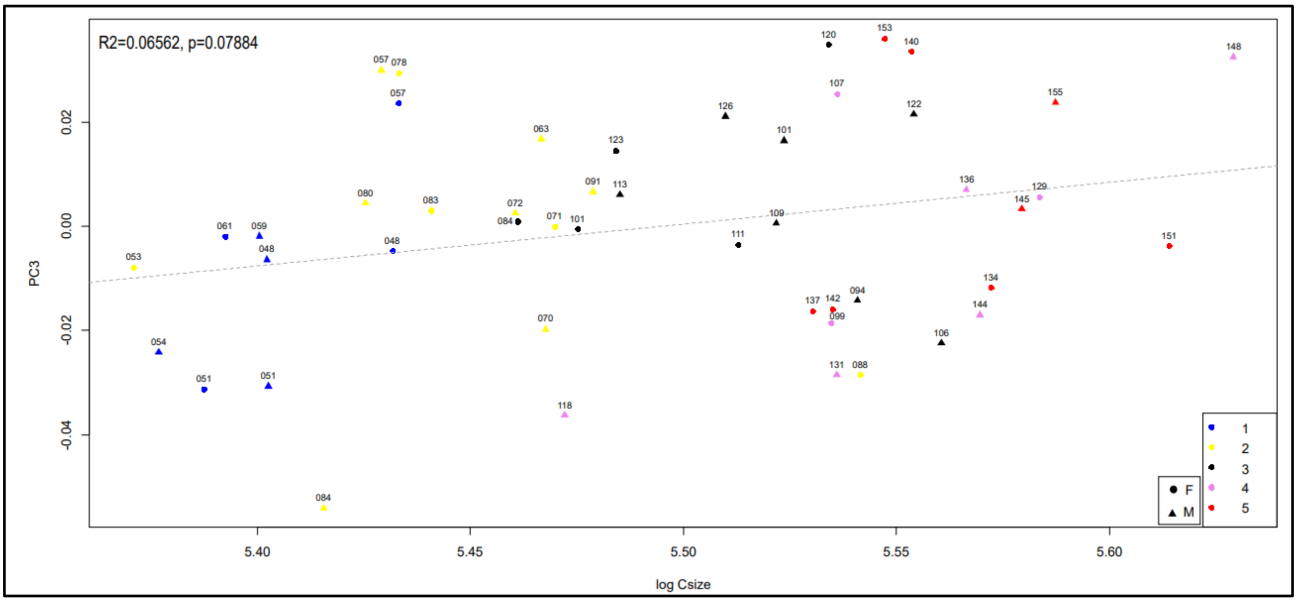

Figure 4.25: Allometry of P3 of mandibular configuration

Figure 4.25 represents the allometric relation of PC3 and the logarithm of C-size where the 'x' axis represents the size, and the 'y' axis represents the PC scores. The r-square value of the plot is 0.06562 which is closer to 0 than 1. The p-value is 0.07884 which is slightly less than 0.05. Therefore, the plot shows a weak allometric relation between the size and pc score. The line of regression is sloped positively, and the individuals are dispersed in the plot so no pattern if concentration near the line is visible. The plot also indicates male and female as round and triangle symbols respectively. However, no pattern of the effect of sex on the plot can be identified.

4.3.5. CVA Analysis

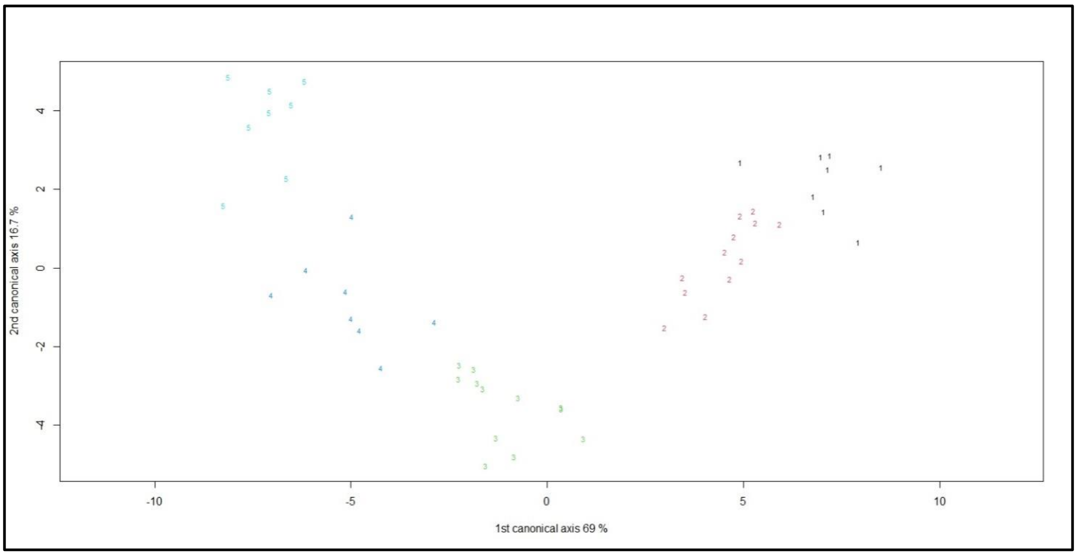

Figure 4.26: CVA analysis of GPA coordinates

Figure 4.26 represents the distribution of the groups in terms of their GPA coordinates in the CVA analysis. The 'x' axis is represented by the 1st canonical axis and the 'y' is represented by the 2nd canonical axis. The first two canonical variables explain a cumulative total of 85.7% of the variation (69% and 16.7%, respectively). In the plot, GROUP 1 has the highest value on the x axis while GROUP 5 has the highest score on the 'y' axis whereas it has the lowest score on the 'x' axis and GROUP 3 has the lowest score on the 'y' axis.

In order to visualise the accuracy of group assignments of each observation based on the probabilities calculated for each canonical variates, the typical probabilities of the canonical variant analysis have been shown in table (4.1.). The rows numbered 1, 2, 3, 4 and 5 indicate the GROUPS created for the analysis and the columns indicated as 1,2,3,4,5 and None are the levels of the CVA analysis. It is seen that 75% or 6 individuals in GROUP 1 have been accurately assigned to level 1 of the CVA. The rest 25% or 2 individuals belong to the level none. Thus, the group shows a strong probability of accuracy in terms of individual grouping. For GROUP 2 83.3% or 10 individuals have been accurately assigned while the rest 16.6% or 2 individuals are not suitable to be assigned to GROUP 2 and have been assigned to the level None. Therefore, the overall accuracy of the group seems to be very strong since the percentage of correct classification is close to 1. GROUP 3 has an accuracy of classification of 91.6% or 11 individuals as it has been classified to level 3. The rest 8.3% or 1 individual is seen to be not suitable for the group and has been classified under the level None. For both GROUP 4 and GROUP 5, the accuracy of classification is 100% (both have 8 individual in their assigned levels). The overall percentage of classification accuracy is 89.6 %. Therefore, the typical probability of the CVA analysis on the GPA coordinates provides a high level of accuracy in grouping of individuals which have been performed for the study.

Table 4.1: CVA analysis of GPA coordinates

Cross-validated classification results in frequencies						
	1	2	3	4	5	None
1	6	0	0	0	0	2
2	0	10	0	0	0	2
3	0	0	11	0	0	1
4	0	0	0	8	0	0
5	0	0	0	0	8	0
Cross-validated classification results in %						
	1	2	3	4	5	None
1	75.0000	0.0000	0.0000	0.0000	0.0000	25.0000
2	0.0000	83.3333	0.0000	0.0000	0.0000	16.6667
3	0.0000	0.0000	91.6667	0.0000	0.0000	8.3333
4	0.0000	0.0000	0.0000	100.0000	0.0000	0
5	0.0000	0.0000	0.0000	0.0000	100.0000	0

CHAPTER 5

DISCUSSION

5.1. Introduction

Chapter 5 entails the observation made based on the analysis conducted in Chapter 4 and the inferences that can be deduced from the research work done. The main objective of the present study is to assess the impact of dental eruption on the ontogenetic growth of the mandible. In chapter 2.2.4, it can be seen that even after decades of research conducted in estimating the exact age of stages of dental formation, the methodologies have failed to provide a precise age of individuals in a formation stage. The discrepancy within each stage of dental formation can be seen to extend from 6 months or 1 year to 4 to 5 years or more (Adserias-Garriga, 2019; Alqahtani et al., 2014; González-Colmenares et al., 2007; Kermani et al., 2019; Liversidge et al., 2010; Maber et al., 2006). The reason behind such discrepancy can be due to the high variability of human ontogeny within inter as well as intra-population (Cameron, 2002). Nevertheless, it is important to note that dentition is a crucial tool for age estimation in anthropological and forensic sciences since it is the most convenient method of estimating the age of skeletal remains. Multiple variations of methodologies have been formulated over the years to assess either the age of attainment of a dental stage (Anderson et al., 1976; Garn et al., 1959; Moorrees et al. 1963) or predict the age for a particular stage (Demisch & Wartmann 1956; Gleiser & Hunt Jr 1955; Haataja 1965; Trodden 1982) or dentition using maturity scores (Demirjian et al. 1973; Nolla 1952). This shows an important demand for age estimation methodologies in the world of science and medicine.

Following the topic of the implication of traditional methods of age estimation, it can be highlighted that it has been difficult to define methodologies for the classification of individuals into groups. The current study identifies the use of dental development as a sole indicator of maturity assessment does not bring high accuracy. However, instead, the implication of dental eruption along with mandibular deformation provides a better proxy for estimating the age of an individual. In the following section, a discussion of how the complete configuration that includes mandible morphology and dental eruption position can be a better proxy because of the strong ontogeny of the mandible is shown.

5.2. Limitations of the traditional methods of age estimation

5.2.1. Discrepancies of traditional methods

In Chapter 2.2.3 and Chapter 2.2.4, various methods for age estimation have been traditionally used in the field of biological and medical science Demirjian et al. (1973); AlQahtani et al. (2010); Anderson et al. (1976); Fanning & Brown (1971); Fass (1969); Garn et al. (1959); Garn & Lewis (1957); Gleiser & Hunt Jr (1955); Gustafson (1950); Hayes & Mantel (1958); Hurme (1948); Kent et al. (1978); Miles (1963); Moorrees et al. (1963); Nolla (1952); Schour & Hoffman (1939). For forensic and paleoanthropological studies, the methods that are primarily based on longitudinal radiographic data are not useful since they require evaluation of a living individual through its lifetime (Smith, 1991). Instead, the methodologies that can be applied to cross-sectional data are preferred. Besides this, there are also other issues with the traditional methodologies. First, the studies conducted in the early 20th century are seen to have a very constricted sample size. Although the initial studies like Peirce (1884) Legros & Magitot (1880), and Logan & Kronfeld (1933) led to the foundation of the studies on dental formation stages, they conducted studies on very few individuals belonging to one chosen population, which can be considered invalid in present-day statistical analogy. In the 1960s, which earlier in Chapter 2 has been mentioned as the second phase of age estimation studies, it is seen that there was a big development in the research methodology adopted by scholars. In the works of Moorrees et al. (1963), Gustafson (1950), Gleiser & Hunt Jr (1955) and others, it was used a big sample of individuals from different ethnic and geographic populations. There is also observation of more stages of dental formation as seen in the work of Moorrees et al. (1963). The studies repeatedly identify the variability of dental formation among males and females. One of the most popular methods of age estimation that was created during this phase is Moorrees et al. (1963) which has been used in the most recent work on dental age estimation as seen in the London Atlas of Human Tooth Development and Eruption (AlQahtani et al., 2010). The methodology of Moorrees et al. (1963) is based on dental formation as he mentions it is more consistent than the timing of dental eruption. He mentions how dental maturation helps in understanding the age of skeletal remains, but it has limitations in comparison to other maturity indicators like wrist due to the restriction of knowing somatic maturation from the former. However, as discussed in Chapter 2.1.1., there is some level of association between somatic maturation and dental maturation. Moorrees et al. (1963) in their study used a longitudinal sample and as pointed out by himself as well as Smith (1991), the lack of knowledge of the exact time between the two stages of attainment disrupts the level of accuracy of the methodology. This issue is not only present for the methodologies based on the age of attainment but also it is seen for methodologies based on maturity scores. Like for example, one of the most popular methodologies based on maturity scores was formed by Demirjian et al. (1973). In his work, each stage of dentition has been given a score and the cumulative dental score when plotted in a centile chart against known age if the individual provides the dental age. Although this methodology has a higher rate of accuracy, still there is significant variation in tooth development among individuals, even within the same age group (Figure 5.1).

Relationships of calcification, eruption and emergence

STAGE	BOYS		GIRLS	
	Mean age	S.D.	Mean age	S.D.
	months		*months*	
Crown completed	41.5	5.6	39.3	4.2
Minimal root formation	45.0	4.9	42.3	3.7
Alveolar emergence	64.6	8.0	61.2	7.7
1/4 of root completed	69.1	8.1	64.4	4.5
1/3 of root completed	74.1	9.2	69.0	5.3
Clinical emergence (Hurme, '48)	74.5	9.6	71.3	9.6
1/2 of root completed	76.8	8.8	74.2	5.4

Figure 5.1: Mean age of dental eruption Demirjian et al. (1973)

From the end of the 20th century to the early 21st century, it is seen that scholars have applied and modified these methodologies for age estimation to understand the variability of dental ontogeny in different populations. This is seen in the works of Tompkins (1996), Halcrow et al. (2007) and others.

In terms of the methodology of age prediction, as seen in the work of Gleiser & Hunt Jr (1955), the same issue arises. It is important to note that the work of Gleiser & Hunt Jr (1955) is based on both dental formation and eruption, comparing the relationship between dental calcification with the process of eruption. He points out that the tooth, which in this work is the first molar only, goes through a rapid process of calcification before its alveolar emergence. The cleft between two roots is formed and the timing of calcification of the cleft coincides with the timing of reabsorption of alveolar bone. The work of Gleiser & Hunt Jr (1955), therefore, helps in comparing dental eruption with tooth formation, something that was not highlighted in the previous works. It showed similarity with the work of Hurme (1948) but contradicted the studies of Massler et al. (1941), which was widely renowned. However, despite these positive aspects of his study, it is important to note that, he had a very limited sample size comprising of 25 male and female. Also, while he points out the changes in the dentition during the eruption, it fails to provide detailed information about the changes in the alveolar process. His study only mentions the work of Logan & Kronfeld (1933) on the recession of gingival bone. Besides, the standard deviation (SD) in between stages of dental eruption is quite significant as seen in Figure 5.2.

Relationships of calcification, eruption and emergence

STAGE	BOYS		GIRLS	
	Mean age	S.D.	Mean age	S.D.
	months		*months*	
Crown completed	41.5	5.6	39.3	4.2
Minimal root formation	45.0	4.9	42.3	3.7
Alveolar emergence	64.6	8.0	61.2	7.7
1/4 of root completed	69.1	8.1	64.4	4.5
1/3 of root completed	74.1	9.2	69.0	5.3
Clinical emergence (Hurme, '48)	74.5	9.6	71.3	9.6
1/2 of root completed	76.8	8.8	74.2	5.4

Figure 5.2: Stages of dental eruption Gleiser & Hunt Jr (1955)

While each of these methodologies has its profoundness and limitation in terms of estimation accuracy, applicability, and statistical significance, in this research, the focus has been made on the dental formation stages that they produced. In Chapter 4, the stages of dental formation as formulated in the studies of Logan & Kronfeld (1933), Gleiser & Hunt Jr (1955), Demirjian & Levesque (1980) and AlQahtani et al. (2010) have been observed against the current research sample.

5.2.2. Problem of Adaptability

In chapter 4.2.1., the standards of dental formation of Logan & Kronfeld (1933) have been applied to the current study sample. It is seen that the methodology is inadequate to establish a stage of dental formation for the whole sample group. The standard comprises two stages: crown completion and root completion. In the study, he provides the range of time taken by each dentition to finish the said stages which are seen to span over years. He does not provide information regarding any associated ontogenetic changes that take place simultaneously during this time. Therefore, the data from the study of Logan & Kronfeld (1933) are not sufficient to draw a conclusive analysis of the stages of dental development of the entire sample of the current study.

In the implementation of the standard of dental formation of Gleiser & Hunt Jr (1955) in chapter 4.2.2., only the first molar of the sample individuals has been observed since the methodology provides information only regarding the first molar. The research shows a correlation between dental formation and eruption stages. The individuals from GROUP 2, GROUP 3 and GROUP 4 show that while the first molar is going through one-fourth root formation to terminal divergence of the root canal, it undergoes eruption simultaneously. However, the age of completion of the first molar eruption of females is seen to be late by approximately 20 months according to the methodology of Gleiser & Hunt Jr (1955) in comparison to the observation made in this current research. The individual in the GROUP 3 is observed to have completed the eruption of the first molar. The average age of females in this group is 107.8 months. However, according to Gleiser & Hunt Jr (1955), the average age of occlusal lesion or decay of the first molar of females is 79.8 months (table 5.1). It is important to note that the study of Gleiser & Hunt Jr (1955) was conducted on children who as mentioned by him were ‘all ethnically White’ and ‘residents of Greater Boston’.

The sample that has been analysed in the current study is not based on any definitive ethnicity. Thus, intra-population variability can be one cause of the difference in the average time of emergence of the first molar, however, more analysis is required in this aspect.

The implication of dental formation stages of Demirjian et al. (1973), and Demirjian & Levesque (1980) showed the stages of dental formation for all the individuals in the current study sample (chapter 4.2.3.). The eight stages of Demirjian et al. (1973) are well described in the work which further helps in current observations (see annexe A.6). In terms of the age of emergence of the tooth, Demirjian & Levesque (1980) indicate the average age of clinical emergence of every detention for both males and females. The problem with the methodology is however that it requires the presence of complete dentition in the mandibular arcade to estimate the age. The method provides a score based on the stages of each dentition, then the cumulative score is calculated which is then plotted against chronological age (see annexe A.6). Thus, such a method can be seen to be difficult to apply in fragmented skeletal remains, where the complete dental arcade is often unavailable.

Lastly, the standard of the London Atlas of Human Tooth Development and Eruption is one of the most recent works available on dental formation and age estimation. It is a comprehensive resource that visually and textually presents the sequence and timing of tooth development and eruption in humans (AlQahtani et al., 2010). The standard has been created based on the work of Moorrees et al. (1963) and applied to a large sample population. In chapter 4.3.4., the stages of dental development based on the London Atlas of Human Tooth Development and Eruption (AlQahtani et al., 2010) are seen to provide more information regarding the dental formation of each group classification of the sample studied. In terms of age of emergence, the Atlas has provided medium age of alveolar eruption, clinical emergence and complete emergence for both males and females. The positive aspect of London Atlas AlQahtani et al. (2010) is that it comprises a very detailed description of the stages of dental formation of the current study sample. The stage of dental formation provides additional information regarding the sample data and can be utilised in future studies to check intra-population variability and test the universality of the London Atlas (AlQahtani et al., 2010).

5.2.3. Variability of Clinical age of emergence

The clinical age of emergence is described as the point at which a tooth becomes visible and is fully or partially exposed in the oral cavity (Hurme 1948). In literature, there have been long debates regarding the age of clinical emergence since eruption is a long ongoing process and no definitive description is yet available. In the study, it is seen that Gleiser & Hunt Jr. (1955) (annexe A.4) in his work, mention that it is based on the unpublished work of Hurme, which establishes the median age of clinical emergence of the first molar of male and female individuals as 74.5 and 73.1 months respectively (Gleiser & Hunt Jr 1955) (Table 5.1). While the study of Demirjian & Levesque (1980) (annexe A.6) and London Atlas AlQahtani et al. (2010) (annexe A.8) shows similar results with a slight variation of 1-3 months, the observation made in this study indicates the age of clinical emergence approximately 6 months earlier than the rest. It should be noted that, in the studies of Gleiser & Hunt Jr (1955) and Lonon Atlas, alveolar emergence and clinical emergence are indicated differently. However, accruing to the standard of Liversidge et al. (2004), which has been followed for the classification in this study, the scholar mentions no difference between clinical and alveolar emergence due to a lack of proper definition. This can suggest a bigger discrepancy among the results. The other explanation for the difference can be due to population variability. Therefore, further assessment regarding the age of emergence of the first mandibular molar is required. In general, the age of emergence shown by Demirjian & Levesque (1980) is seen to be more advanced in comparison to the London Atlas AlQahtani et al. (2010) and the current study. However, the current study does not have sufficient individuals with alveolar emergence or clinical emergence stage (see annexe A.1). Thus, analysis of more individuals is required to make any definitive conclusions.

Table 5.1: Clinical emergence of Tooth according to standards of Gleiser & Hunt Jr (1955), Demirjian & Levesque (1980), London Atlas AlQahtani et al. (2010) and observation of Current Study

Tooth	Gleiser & Hunt Jr (1955)		Demirjian & Levesque (1980)		London Atlas AlQahtani et al. (2010)		Current Study
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE & FEMALE
M1 (first molar)	74.5	73.1	75.6	73.2	75.6	75.6	67.2
P2 (second premolar)			139.2	134.4	133.2	121.2	138.6
P1 (first premolar)			128.3	123.5	123.6	115.2	116.7
C (canine)			126	115.2	124.8	110.4	114.4
I2 (lateral incisor)			88.8	85.2	87.6	81.6	82.5
I1 (central incisor)			76.8	72	75.6	74.4	72.3

5.3. Dental eruption and mandibular morphology as a proxy for age estimation

5.3.1. Practicality of complete configuration (dental and mandibular configuration)

Dental age is an important indicator for human age estimation (Demirjian et al., 1973; Moorrees et al., 1963; Smith, 1991). As discussed in chapter 2.1.2, it indicates biological maturity like height, stature, weight etc. whereas chronological age is dependent on birth and calendar years. Since they share a correlation, dental age is used to estimate chronological age. The dental eruption is seen to have a strong correlation with dental maturity (Garn et al., 1965). However, as discussed in the previous section of this chapter (5.2.3.), the stages of dental eruption, which is used to estimate dental age, have high variability caused due to changes in the sample population, or simply due to person-to-person observation which makes it difficult to create any strong standardisation of the system. The current study has tried to tackle this research question by analysing the accuracy of dental eruption and mandibular formation together as a proxy for age estimation.

In Chapter 4.3, the principal components 1 and 2 of the GPA coordinates of both the complete configuration comprising of the mandible and dental landmark placed on the individuals (Chapter 4.3.2) and dental configuration only (Chapter 4.3.2) shows the morphological variation that occurs along the PCs. In Figure 4.2, the plot of the complete configuration shows that GROUP 1 and GROUP 5 are the most separated on each extremity of the PC1. GROUP 1 signifies all unerupted tooth and is positioned on the negative scores of the PC while GROUP 5 signifies all unerupted tooth and are positioned on the positive scores. Moreover, the age range of the individual from GROUP 1 falls between 48 months to 61 months while GROUP 5 ranges from 134 months to 155 months which suggest that the age of the individuals have an impact on the distribution of the individuals on PC1 which can be confirmed based on the strong relation indicated in Figure 4.3. Therefore, it can be said that the individuals on the PC1 are separated based on morphological changes that occur along age. In Figure 5.3, the variation of maximum and minimum morphology of the PC1 is displayed.

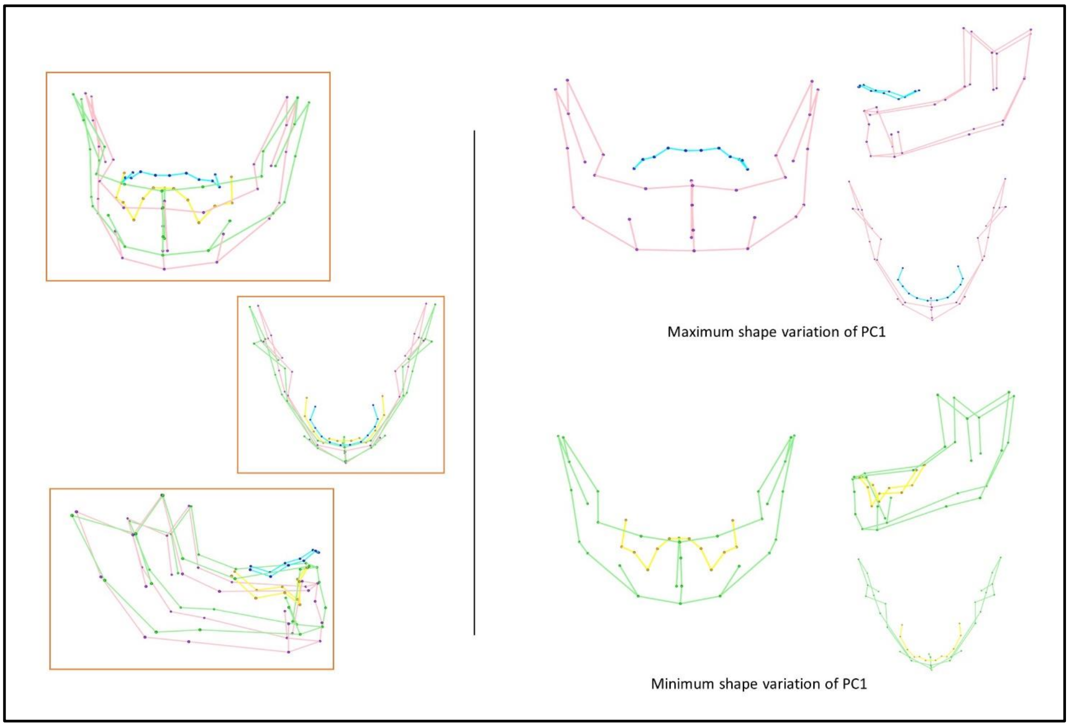

Figure 5.3: Shape variation of the complete configuration on PC1

The variation from minimum to maximum indicates advancement in the stages of eruption of dentition with some teeth like incisors showing complete eruption. In the mandibular morphology, a development in the mandibular symphysis is noticed. The mandibular symphysis is the area where the two halves of the mandible (lower jaw) fuse during development (Rogol et al., 2002). When the mandibular central incisors erupt, they can exert pressure on the mandibular symphysis, potentially contributing to its growth and development (Rogol et al., 2002). Some scholars like Sarrafpour et al. (2013) also suggest that the development is also related to tensile masticatory activities which gradually become complicated with an increase of age during growth causing modification in the mandibular symphysis. There is also an elongation of the ramus process which possibly suggests the development of the condyle to adapt to the temporal articulation of better support for masticatory activities (Nickel et al., 1988). Therefore, the PC1 suggest strong allometric changes which is proven by Figure 4.7. where the PC1 shows a strong allometric relation with the logarithm of the size of the individuals.

In the plot of PC1 and PC2 of the GPA coordinates of the complete configuration (Figure 4.2), GROUP 2 also shows the highest range of variability along PC2. However, the variability on PCs cannot be associated with age since the PC has a very weak relation with age (Figure 4.5). On comparing the maximum and minimum morphological variation of the PC2 (Figure 5.4), it is seen that the eruption of teeth has been most effective in the distribution of the individuals along the PC2.

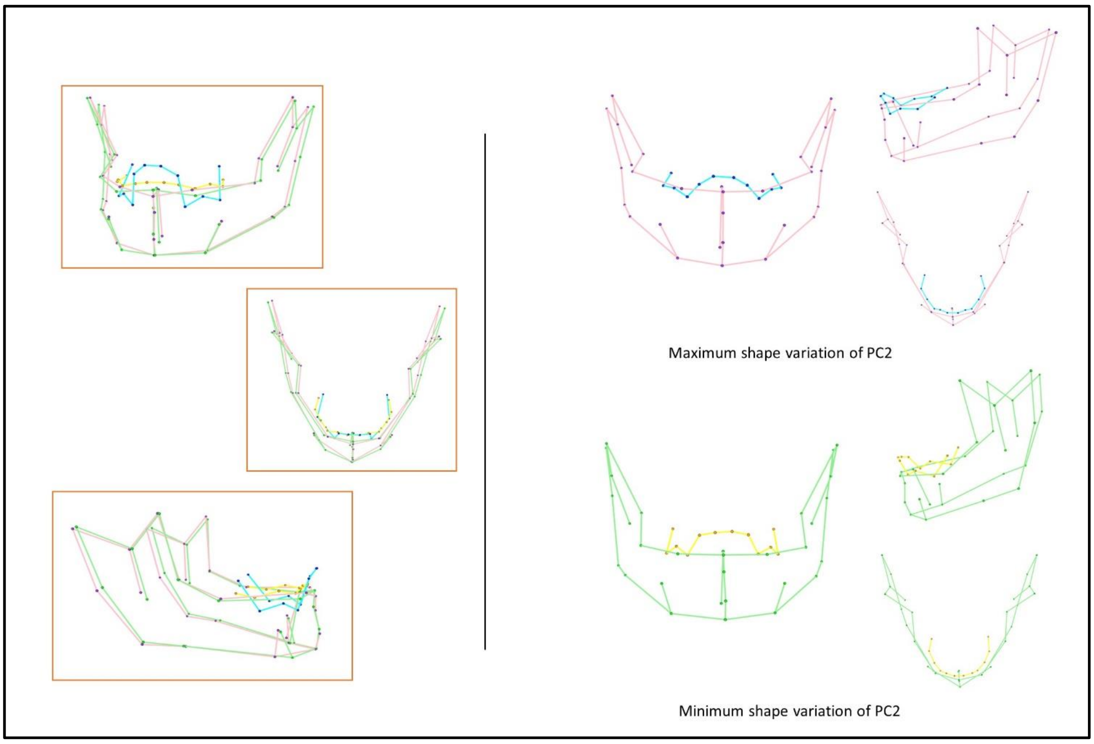

Figure 5.4: Shape variation of the complete configuration on PC2

In the configuration of the minimum shape variation (Figure 5.4) the dentition is on the same level. Such dental position corresponds to either the stage of all unerupted teeth or the stage of all erupted teeth which correspond with the group classification 1 and 5 respectively. That is why, GROUP1 and GROUP 5 are seen to have lower values on the PC2. The maximum variation indicates a stage of mixed dentition where the incisors and first molar are erupted. Therefore, it can be seen that along the PC2, the eruption of the first molar and incisor affects the distribution of the individuals. GROUP 2, therefore, has the highest variation since it represents individuals undergoing the process of eruption of the first molar and incisor passing through the stages of alveolar eruption and partial eruption. The mandibular morphology does not undergo much modification in comparison to the shape variation seen for PC1. This is probably signifying that during the eruption of the first molar and incisor, the mandible does not change morphologically. It is after the eruption of the incisors and first molar, that the mandibular symphysis becomes more prominent. The process does not show any allometric significance as seen in Figure 4.8.

The CVA analysis is conducted in Chapter 4.3.4. further validates the accuracy of the complete configuration as effective in terms of group classification. The groups formed in this current study are based on the observation made on dental eruption as seen in chapter 3.3. It is seen that the complete configuration shows a high percentage of accuracy (Table 4.1) of group assignments when the CVA analysis is conducted. This indicates each of the group correspond to a certain morphology of mandible and dentition which is significantly distinguishable from the other thus aiding in a high accuracy of group classification.

5.3.2. Limitation of the dental eruption as a sole indicator for age estimation

In comparison, in chapter 4.3.2., the plot of PC1 and PC2 of the GPA coordinates of the dental configuration does not show as much separation among GROUP 1 and GROUP 5 as the previous plot which is based on complete configuration (Figure 4.2). The PC1 does not have a strong relation with age as evident in Figure 4.13. Also, the variation in the distribution of GROUP 2 is not as important as in the case of the complete configuration. Based on the shape variation (Figure 5.3, Figure 5.4), the PC1 does represent mixed dentition to be affecting the distribution of the individuals (since minimum variation in dental position is indicated as minimum shape variation and vice versa is indicated as the maximum shape variation). However, the distribution of the individuals is not as separated as in the previous graph, and it is difficult to identify the position of the dental arcade without the presence of the mandible. It can be said that the inclusion of mandibular morphology along with the dental eruption provides a higher correlation with age and more accuracy for age estimation in comparison to just dental eruption.

5.3.3. consideration of the sexual variation

Before the pubertal growth spurt (post-natal to 7-8 years of age), the mandible undergoes a gradual increase in size. No difference between males and females can be noticed in the skeletal morphology of children during this period (White et al., 2011). In the plot of the allometric relation of the PCs of complete configuration (Figure 4.7, Figure 4.8, Figure 4.9.); dental configuration (Figure 4.15, Figure 4.16, Figure 4.17) and mandibular configuration (Figure 4.23, Figure 4.24, Figure 4.25.), where the sex of the individuals has been indicated using distinctive symbols, no pattern of distribution in relation to the age can be seen. This is because the sample collection of the individuals for this study comprises mainly males and females of pre-pubertal age as described in the data collection in chapter 3.2. With the onset of pubertal growth, the morphological difference changes individually between males and females. In terms of the mandible, the male develops a more pronounced mental eminence and robust jaw while in females, the jaw is more gracile, and the chin is more rounded (White et al., 2011). Sexual variation has also been identified in dental development byDemirjian et al. (1973; AlQahtani et al. (2010); Anderson et al. (1976); Fanning & Brown (1971); Fass (1969); Garn et al. (1959); Gates (1966); Gleiser & Hunt Jr (1955); Gustafson (1950); Hayes & Mantel (1958); Kent et al. (1978); Moorrees et al. (1963); Nolla (1952); Trodden (1982). Although there is high variability, each of the studies has identified a different range of age for males and females for dental eruption. The current observations however indicate that eruption is a highly variable process and the influence of sex of an individual is not noticed in the allomeric growth of either the mandible or the dentition. This observation contradicts the work of Demirjian et al. (1973); AlQahtani et al. (2010); Gleiser & Hunt Jr (1955) who have identified variation in the age of dental eruption between males and females (Chapter 4.2). The current study sample is not sufficient for the determination of variation based on sex. Thus, a bigger sample collection can help in the identification of any pattern of sexual variation if present.

5.4. Indication of pubertal growth spurt and its relation with dental age

In the growth pattern of modern humans, sexual dimorphism is not prominent from post-natal until age 11 to 18 just before the pubertal growth spurt (Cameron, 2002). Pubertal growth spurt refers to a rapid and intense increase in height and skeletal growth that occurs during puberty in humans. Puberty is a period of significant physical and psychological development that typically occurs during adolescence, marked by the maturation of sexual and reproductive characteristics (Rogol et al., 2002). The hormonal regulation of the growth spurt and the alterations in body composition depend on the release of gonadotropins, leptin, sex steroids, and growth hormones (Rogol et al., 2002).

The impact is also noticed in the mandibular alterations. The individuals in the sample age range of 10 to 13 years (120 to 156 months) which according to Cameron (2002), should indicate signs of pubertal growth. The mandible is a period of minimal velocity in somatic growth immediately before the onset of the adolescent growth spurt also known as prepubertal minimum (Franchi et al., 2001). This results in the upward-forward direction of condylar growth determining an overall "shrinkage" of the mandibular configuration along the measurement of total mandibular length. Closure of the gonial angle is associated with an upward-forward direction of growth at the condyle and with an upward-backwards direction of growth at the symphysis, thus confirming the tendency to the anterior morphogenetic rotation of the mandible (Rogol et al., 2002). In the analysis (4.3.3), which has been performed solely on the mandibular configuration, it is seen that the PC1 that has been conducted on the GPA coordinates of the mandibular configuration has a strong relation with age. It also shows a strong allometric relation with size. Thus, the mandible grows allometrically among children of the age of 4 years to 14 years.

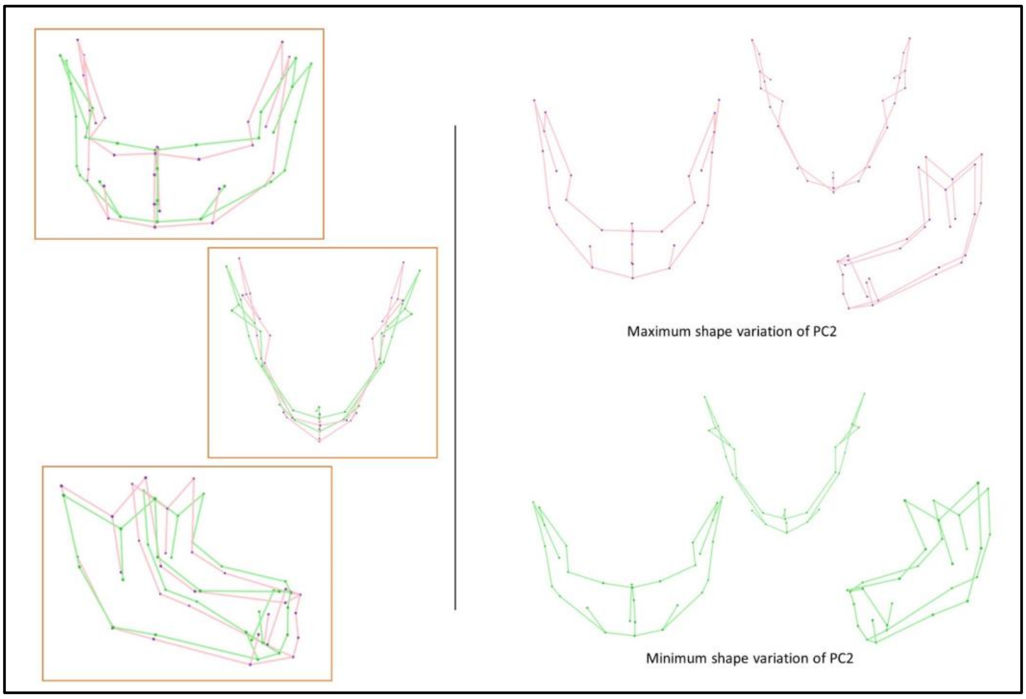

Figure 5.5: Shape variation of the mandibular configuration on PC2

The shape variation along the PC1 as seen in Figure 5.5 indicates the development of frontal symphysis; a more pronounced angle, that is premandibular notch, between the body of the mandible and the ascending ramus (vertical portion) and an increase in the length of the condyle process as it becomes more developed. Both the PC1 of the complete configuration and mandibular configuration indicated a strong relation with age (Figure 4.4 and Figure 4.20 respectively) showing that the individuals who belong to the higher score on the mentioned PCs are associated with a morphology like the maximum shape configuration. In the case of the PC1 of complete configuration, these individuals associate with GROUP 5 (Figure 4.2). The plot of PC1 of mandibular configuration (Figure 4.18) although has a high level of overlapping among all the groups, the individual from GROUP 3, GRPPUP 4 and GROUP 5 can be associated with the maximum shape variation configuration in comparison to the GROUP 1 and GROUP 2. Thus, it shows that some individuals in the sample do represent morphological changes which could be associated with pubertal spurt.

The ontogenetic growth of the mandible also has a close relation with maturity indicators like maturation of the cervical vertebra and dental age (Franchi et al., 2001). More factors like masticatory adaptations, facial aesthetics, secondary ossification, and sexual dimorphism impact the deformation of the mandible Wong et al. (2010). Since all the changes take place simultaneously, it is difficult to point to one exact cause for morphological adaptations during mandibular ontogeny. Many scholars estimate the impact of the dental eruption on the morphological changes of the mandible during this time (Banu et al., 2018; Fränkel et al., 1987; Krarup et al., 2005c; Liu & Buschang, 2011; Sampson & Richards, 1985; Vucic et al., 2019). As seen in section 5.3.1, after the completion of the eruption of the incisors and first molar, the mandibular symphysis joins (Endo et al., 2007; Fukase, 2007; Ikai et al., 1997). This leads to an important development of the mental eminence. The dental eruption process entails absorption of alveolar bone which causes somatic alteration in the mandibular corpus (Sarrafpour et al., 2013). Not to mention, in between the deciduous dentition and permanent dentition, there is an increase in the number and size of the tooth which requires definitive alteration among the mandibular corpuses. The current study has indicated that the morphological changes of the mandible that take place before and during the onset of pubertal growth are related to dental eruption.

CHAPTER 6

CONCLUSION

The morphometric analysis has led to successful testing of the reliability of using mandibular morphology and dental eruption as a proxy for age estimation in modern humans. In comparison to using only dental eruption, the combined configuration has a stronger relation to age as well as allometric growth of individuals. The studies have successfully shown that the use of morphological deformation of the mandible beside dental eruption can act as a proxy for age estimation. The success of this combined approach highlights its potential to enhance the accuracy and reliability of age estimation methods in diverse contexts. The research has also laid the foundation for a methodology whose universality is not constrained by ethnicity, rendering it a versatile tool applicable across different population groups. The fact that the chronological age is with stages of dental eruption, shows that the changes in the growth rate will equally change the age of attainment of a dental stage. The sample studied is also based on individuals from no predefined ethnicity which shows the success of the study as a proxy for any sample population.

An essential aspect of this study is the new classification that has been established. The new group classification that has been made in the current study is based on a sequence of dentition and their corresponding stage of dental eruption. For example, one group identifies the partial eruption stage of the incisor and first molar and the unerupted stage of premolar and canine. This is not contingent upon human growth rates. That is to say, these stages are seen to be dependent on the amount of the tooth visible from the alveolar margin to the occlusal level. Thus, it does not rely on the rate at which the tooth will attain a certain stage of eruption. In comparison, the methodologies of age prediction are seen to be relying on the mean age of a subject in a stage (Smith, 1991). The methodologies shown in the works of Demisch & Wartmann (1956); Gleiser & Hunt Jr (1955); Haataja (1965); and Trodden (1982) are shown to be specifically useful for the age estimation of skeletal remain by Smith (1991). However, these methodologies indicate a known rate of growth seen from dental formation which is then applied for age estimation. Therefore, such methodologies may not provide accurate results among other species due to the changes in thew growth rate.

The scope of this research is far-reaching, and it can be suggested that a larger sample size can further help to assess the limitations faced in the current study. High variability in the pattern of mixed dentition (during the phases of alveolar eruption to complete eruption of incisor, molar, canine and premolars until before all the dentitions are completely erupted) is noticeable among the individuals. A bigger sample collection can further help in the identification of patterns in the mixed dentition stages which can shed new light on the effects of dental growth on the morphological variation of the mandible. This holds the scope for a comprehensive assessment of the impact of mandibular ontogeny that occurs at different stages of dental eruption. A larger sample can also help in identifying any pattern of sex variance in the alteration of mandibular morphology during dental eruption. In the works of Demirjian et al. (1973); Gleiser & Hunt Jr (1955); and Nolla (1952), a variation in the age of attainment of stages of dental development among boys and girls has been shown which has not been seen in the current study. This can be due to the smaller sample size or the presence of a mixed population where each ethnicity differs in terms of growth rate. An expansion of the sample collection of individuals could thus provide a more comprehensive understanding of the interplay between these factors and their contributions to age prediction.

One of the notable advantages of this approach is its cost efficiency. The methodology of the current study can be applied successfully using open-resource software and low-resolution images. Software like R studio and 3D Slicer are free resources and compatible with various currently widespread computer systems. The CT scans utilised in this research are also sourced from NMDID which provides free access to their resources on request. It is important to note that the availability of such open-access sources of medical data and CT scans allows extensive studies from an open science perspective. This is a pragmatic option, particularly in resource-constrained settings. While not imperative, the availability of good-quality imaging can significantly enhance segmentation accuracy, thus contributing to better results.

In summary, this thesis has demonstrated the potential of mandibular morphological changes and dental eruption stages as a reliable and versatile age estimation method. Taking into consideration the transferability of this new methodology of the current study, in future, after enhancing the modern human samples, it might be interesting to test the methodology on fossil hominins. This would allow us to achieve an alternative way to assess the growth stage in sub-adult fossil humans. Since it is based on landmark configuration on almost all the permanent dentition that undergoes eruption in the pre-pubertal age of modern humans, this methodology can also be adapted for the application of fragmentary fossils.

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ANNEX

A.1. Sample collection and associated group and observed eruption stage.

U= Unerupted
AE=Alveolar eruption
PE= Partial Eruption
E= Complete/Occlusal eruption
R= Right side
L=Left side

Group	File_no	ID	AGE (MONTHS)	SEX	M1	P2	P1	C	I2	I1
unerupted (01	114841	12017	48	Female	U	U	U	U	U	U
	164851	6183	51	Female	U	U	U	U	U	U
	116077	8509	57	Female	U	U	U	U	U	U
	114998	861	61	Female	U	U	U	U	U	U
	106974	2620	48	Male	U	U	U	U	U	U
	112307	11123	51	Male	U	U	U	U	U	U
	149738	760	54	Male	U	U	U	U	U	U
	110562	2892	59	Male	U	U	U	U	U	U
Molar and Incisor partial eruption (02)	149066	13702	53	Female	AE	U	U	U	U	U
	144737	11178	71	Female	AE	U	U	U	U	U
	101285	10935	78	Female	PE	U	U	U	U	U
	108330	13394	83	Female	E	U	U	U	AE	E
	173453	12269	88	Female	E	U	U	U	E	E
	135745	3096	57	Male	AE	U	U	U	U	U
	175526	14899	63	Male	E	U	U	U	U	U(L)/AE(R)
	191675	4611	70	Male	AE	U	U	U	U	U
	104676	8127	72	Male	AE	U	U	U	PE	PE
	167431	14264	80	Male	E	U	U	U	U	E
	157644	1441	84	Male	PE	U	U	U	U	E
	122960	9438	91	Male	E	U	U	U	U	E
Molar and Incisor complete eruption (03)	145492	11176	84	Female	E	U	U	U	E	E
	142005	151	101	Female	E	U	U	U	E	E
	127657	9415	111	Female	E	U	U	U	E	E
	177700	12742	120	Female	E	U	U	U	E	E
	103712	9582	94	Male	E	U	U	U	E	E
	165123	135	101	Male	E	U	U	U	E	E
	179579	8392	106	Male	E	U	U	U	E	E
	169985	9229	109	Male	E	U	U	U	E	E
	107776	6808	113	Male	E	U	U	U	E	E
	129915	10208	122	Male	E	U	U	U	E	E
	159165	13253	123	Female	E	U	U	U	E	E
	174130	10516	126	Male	E	U	U	U	E	E
premolar, canine partial eruption (04)	126034	3932	99	Female	E	U	U	E	E	E
	177365	4949	107	Female	E	U	PE(L)/E(R)	PE	E	E
	160103	12270	129	Female	E	U	E	E	E	E
	118089	6434	118	Male	E	U	U	R(AE)/L (U)	E	E
	150492	5501	131	Male	E	PE(L)/E(R)	E	PE	E	E

	136645	7365	136	Male	E	U	E	E	E	E
	114745	12176	144	Male	E	U	E	E	E	E
	133875	6256	148	Male	E	U	E	E	E	E
complete eruption (05)	194332	1493	134	Female	E	E	E	E	E	E
	121316	5620	137	Female	E	E	E	E	E	E
	112356	4807	140	Female	E	E	E	E	E	E
	179973	8906	142	Female	E	E	E	E	E	E
	155748	1456	151	Female	E	E	E	E	E	E
	118172	4563	153	Female	E	E	E	E	E	E
	111920	11143	145	Male	E	E	E	E	E	E
	141547	6428	155	Male	E	E	E	E	E	E

A.2. Stage of dental formation according to Kronfeld (1933)

Tooth	First evidence of calcification	Crown completed	Root completed
Deciduous dentition			
di1	5 mo in utero	4 mo	1½–2 yr
di2	5 mo in utero	5 mo	1½–2 yr
dc	6 mo in utero	9 mo	2½–3 yr
dm1	5 mo in utero	6 mo	2–2 ½ yr
dm2	6 mo in utero	10–12 mo	3 yr
Permanent dentition			
Upper jaw			
I1	3–4 mo	4–5 yr	10 yr
I2	1 yr	4–5 yr	11 yr
C	4–5 mo	6–7 yr	13–15 yr
P1	1½–1¾ yr	5–6 yr	12–13 yr
P2	2–2¼ yr	6–7 yr	12–14 yr
M1	at birth	2½–3 yr	9–10 yr
M2	2½–3 yr	7–8 yr	14–16 yr
M3	7–9 yr	12–16 yr	18–25 yr
Lower jaw			
I1	3–4 mo	4–5 yr	9 yr
I2	3–4 mo	4–5 yr	10 yr
C	4–5 mo	6–7 yr	12–14 yr
P1	1¾–2 yr	5–6 yr	12–13 yr
P2	2¼–2½ yr	6–7 yr	13–14 yr
M1	at birth	2½–3 yr	9–10 yr
M2	2½–3 yr	7–8 yr	14–15 yr
M3	8–10 yr	12–16 yr	18–25 yr

A.3. Application of stages of dental formation of Kronfeld (1933) on current sample

Group	ID	AGE (MONTHS)	SEX	M1	P4	P3	C	I2	I1
1	12017	48	Female					Crown completed	Crown completed
	2620	48	Male					Crown completed	Crown completed
	6183	51	Female					Crown completed	Crown completed
	11123	51	Male					Crown completed	Crown completed

	760	54	Male					Crown completed	Crown completed
	8509	57	Female					Crown completed	Crown completed
	2892	59	Male					Crown completed	Crown completed
	861	61	Female			Crown completed			
2	13702	53	Female					Crown completed	Crown completed
	3096	57	Male					Crown completed	Crown completed
	14899	63	Male			Crown completed			
	4611	70	Male			Crown completed			
	11178	71	Female			Crown completed			
	8127	72	Male		Crown completed	Crown completed	Crown completed		
	10935	78	Female		Crown completed		Crown completed		
	14264	80	Male		Crown completed		Crown completed		
	13394	83	Female		Crown completed		Crown completed		
	1441	84	Male		Crown completed		Crown completed		
	12269	88	Female		Crown completed		Crown completed		
	9438	91	Male						
3	11176	84	Female		Crown completed		Crown completed		
	9582	94	Male						
	151	101	Female						
	135	101	Male						
	8392	106	Male						
	9229	109	Male	Root completed				Root completed	
	9415	111	Female	Root completed					
	6808	113	Male	Root completed					
	12742	120	Female	Root completed					Root completed
	10208	122	Male						
	13253	123	Female						
	10516	126	Male						
4	3932	99	Female						
	4949	107	Female	Root completed					
	6434	118	Male	Root completed					
	12270	129	Female						
	5501	131	Male						
	7365	136	Male						

	12176	144	Male			Root completed	Root completed		
	6256	148	Male			Root completed	Root completed		
5	1493	134	Female						
	5620	137	Female						
	4807	140	Female						
	8906	142	Female						
	11143	145	Male			Root completed	Root completed		
	1456	151	Female			Root completed	Root completed		
	4563	153	Female			Root completed	Root completed		
	6428	155	Male			Root completed	Root completed		

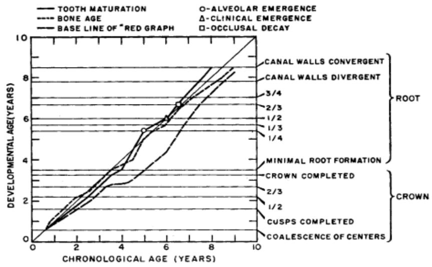
A.4. Stage of dental formation according to Gleiser (1955)

Chronolgy of calcification of the permanent mandibular first molar

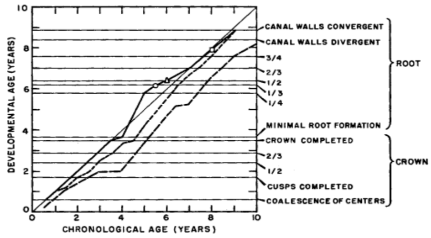
STAGE OF CALCIFICATION	BOYS			GIRLS		
	N	Mean age	S.D.	N	Mean age	S.D.
		months			*months*	
Coalescence of at least 2 centers	22	7.0	1.4	22	7.0	1.4
Outline of cusps completed	20	20.7	3.0	18	19.5	2.6
Half of crown completed	17	28.4	4.5	20	25.9	2.8
2/3 of crown completed	14	35.1	4.0	18	32.3	3.0
Crown completed	17	41.5	5.6	21	39.3	4.2
Minimal root formation	16	45.0	4.9	19	42.3	3.7
1/4 of root completed	23	69.1	8.1	24	64.4	4.5
1/3 of root completed	17	74.1	9.2	20	69.0	5.3
1/2 of root completed	21	76.8	8.8	22	74.2	5.4
2/3 of root completed	22	84.3	8.4	18	80.7	5.9
3/4 of root completed	20	90.0	8.1	17	84.7	5.9
Root canal terminally divergent	22	100.4	7.7	16	94.1	8.1
Root canal terminally convergent	13	106.6	7.4	12	102.5	6.0

Relationships of calcification, eruption and emergence

STAGE	BOYS		GIRLS	
	Mean age	S.D.	Mean age	S.D.
	months		*months*	
Crown completed	41.5	5.6	39.3	4.2
Minimal root formation	45.0	4.9	42.3	3.7
Alveolar emergence	64.6	8.0	61.2	7.7
1/4 of root completed	69.1	8.1	64.4	4.5
1/3 of root completed	74.1	9.2	69.0	5.3
Clinical emergence (Hurme, '48)	74.5	9.6	71.3	9.6
1/2 of root completed	76.8	8.8	74.2	5.4



INDIVIDUAL GRAPH OF FEMALE OSSEOUS AND DENTAL MATURATION



INDIVIDUAL GRAPH OF MALE OSSEOUS AND DENTAL MATURATION

A.5. Application of stages of dental formation of Gleiser (1955) on current sample

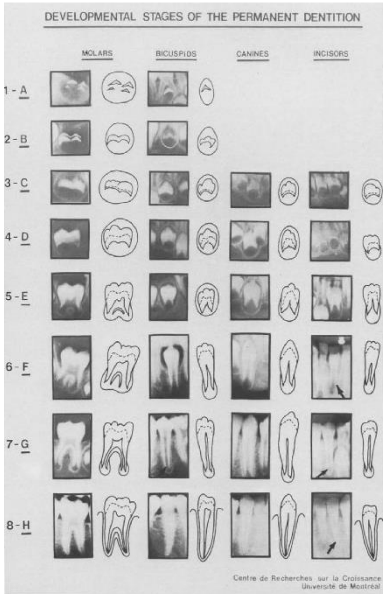
Group	ID	AGE (MONTHS)	SEX	Formation stage (M1)	Eruption stage (M1)
	12017	48	Female	Minimal root formation	
	6183	51	Female	Minimal root formation	
	8509	57	Female	Minimal root formation	
	861	61	Female	Root 1/4	
	2620	48	Male	Minimal root formation	
	11123	51	Male	Minimal root formation	
	760	54	Male	Minimal root formation	
1	2892	59	Male	Minimal root formation	
	13702	53	Female	Minimal root formation	
	11178	71	Female	root 1/3	Alveolar emergence
	10935	78	Female	root 1/2	Clinical emergence
	13394	83	Female	root 1/2	Clinical emergence
	12269	88	Female	root 2/3	Occlusal decay
	3096	57	Male	Minimal root formation	
	14899	63	Male	Root 1/4	Alveolar emergence
	4611	70	Male	Root 1/3	Alveolar emergence
	8127	72	Male	Root 1/2	Clinical emergence
	14264	80	Male	Root 2/3	Occlusal decay
	1441	84	Male	Root 3/4	
2	9438	91	Male	Root canal terminally divergent	
	11176	84	Female	root 2/3	Clinical emergence
	151	101	Female	Root canal terminally divergent	Occlusal decay
	9415	111	Female		
	12742	120	Female		
	13253	123	Female		
	9582	94	Male	Root canal terminally divergent	
	135	101	Male		
	8392	106	Male		
	9229	109	Male		
	6808	113	Male		
	10208	122	Male		
3	10516	126	Male		
	3932	99	Female	Root canal terminally divergent	Occlusal decay
	4949	107	Female	Root canal terminally convergent	
	12270	129	Female		
	6434	118	Male		
	5501	131	Male		
	7365	136	Male		
	12176	144	Male		
4	6256	148	Male		
	1493	134	Female		
5	5620	137	Female		

	4807	140	Female		
	8906	142	Female		
	1456	151	Female		
	4563	153	Female		
	11143	145	Male		
	6428	155	Male		

A.6. Stage of dental formation according to Demirjian (1976

		A (1)	B (2)	C (3)	D (4)	E (5)	F (6)	G (7)	H (8)	EMERG.
M_2	GIRLS	3.5	4.0	4.6	5.9	7.9	9.9	11.5	14.9	11.3
	*diff.	*0.0*	*0.0*	*0.3*	*0.4*	*0.6*	*0.5*	*0.5*	*0.4*	*0.3*
	BOYS	3.5	4.0	4.9	6.3	8.5	10.4	12.0	15.3	11.6
M_1	GIRLS	–	–	–	–	3.7	5.2	6.3	9.5	6.1
	diff.	–	–	–	–	*0.4*	*0.2*	*0.4*	*0.7*	*0.2*
	BOYS	–	–	–	–	4.1	5.4	6.7	10.2	6.3
PM_2	GIRLS	3.8	4.1	4.7	5.6	7.1	9.3	11.1	13.6	11.2
	diff.	*0.0*	*0.0*	*0.0*	*0.3*	*0.5*	*0.3*	*0.5*	*0.6*	*0.4*
	BOYS	3.8	4.1	4.7	5.9	7.6	9.6	11.6	14.2	11.6
PM_1	GIRLS	–	–	3.5	4.2	6.0	8.6	10.1	12.7	10.3
	diff.	–	–	*0.1*	*0.3*	*0.5*	*0.5*	*0.7*	*0.7*	*0.4*
	BOYS	–	–	3.6	4.5	6.5	9.1	10.8	13.4	10.7
C	GIRLS	–	–	–	2.9	4.9	7.6	9.6	12.2	9.6
	diff.	–	–	–	*0.4*	*0.5*	*0.9*	*1.0*	*1.2*	*0.9*
	BOYS	–	–	–	3.3	5.4	8.5	10.6	13.4	10.5
I_2	GIRLS	–	–	–	–	3.7	6.1	7.3	9.2	7.1
	diff.	–	–	–	–	*0.7*	*0.4*	*0.4*	*0.4*	*0.3*
	BOYS	–	–	–	–	4.4	6.5	7.7	9.6	7.4
I_1	GIRLS	–	–	–	–	3.5	5.3	6.5	8.1	6.0
	diff.	–	–	–	–	*0.4*	*0.4*	*0.3*	*0.4*	*0.4*
	BOYS	–	–	–	–	3.9	5.7	6.8	8.5	6.4

*The figures in italics represent the differences between the sexes (boys minus girls).



Stage	Uniradicular teeth	Molar
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 point	
B	Fusion of the calcified points forms one or several cusps which unite to give a regularly outlined occlusal surf	
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 uniradicu- lar 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 trapezoid form.	
	c. Beginning of root formation is seen in the form of a spicule	
E	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.	a. Initial formation of the radicular bifurcation is seen in the form of either a calcified point or a semi-lunar shape.
	b. The root length is less than The crown height	b. The root length is less than The crown height

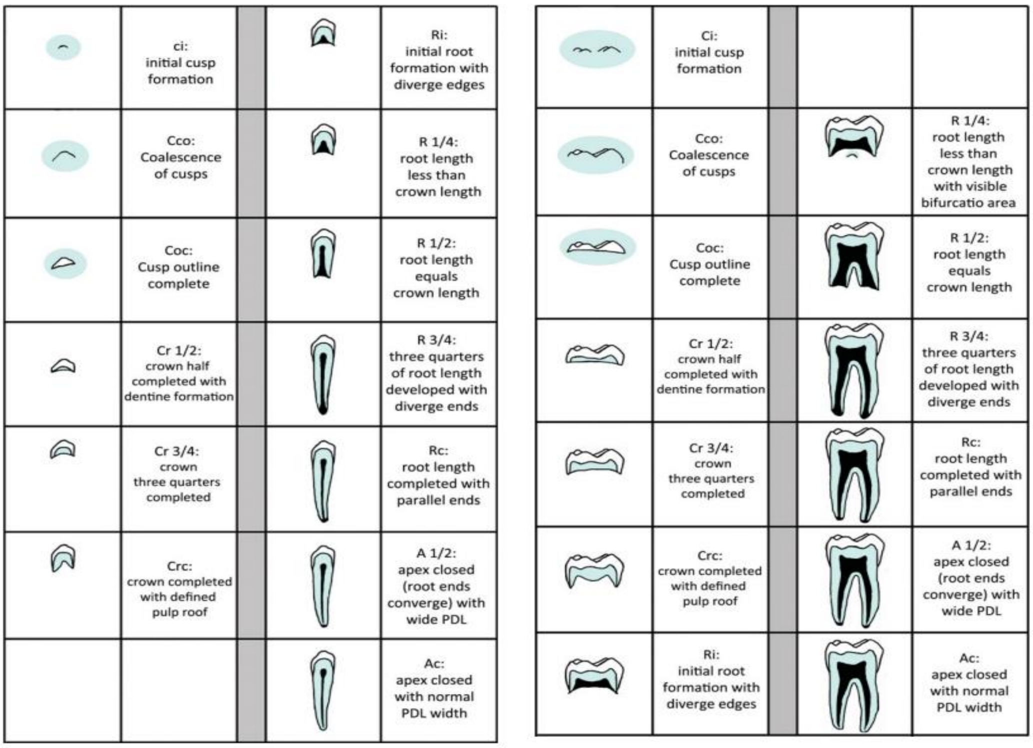
F	a. The walls of the pulp chamber now form a more or less isosceles triangle. The apex ends in a funnel shape.	a. The calcified region of the bifurcation has developed fur- ther down from its semi-lunar stage to give the roots a more definite and distinct outline with funnel shaped endings.
	b. The root length is equal to or greater than the crown height.	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 in molars)	
H	a. The apical end of the root canal is completely closed. (Distal root in molars	
	b. The periodontal membrane has a uniform width around the root and the apex	

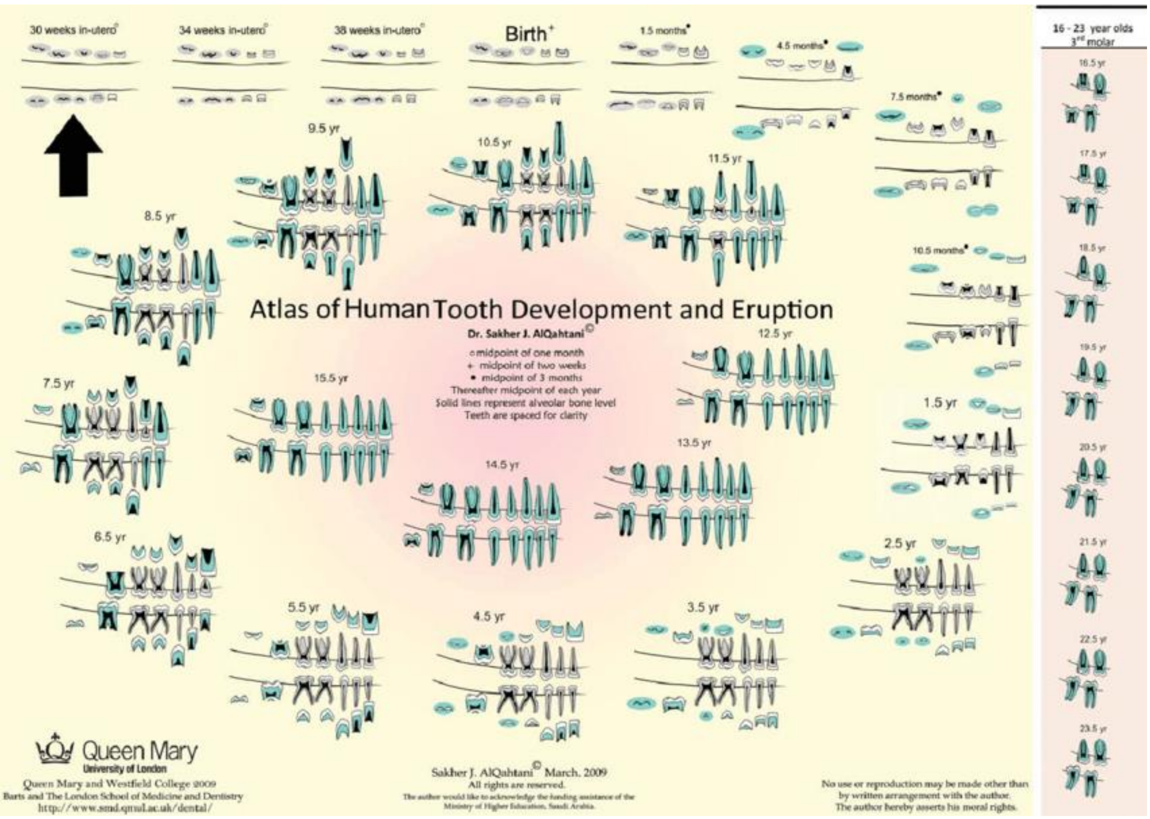
A.7. Application of stages of dental formation of Demirjian (1976) on current sample

Group	ID	AGE (MONTHS)	SEX	M1	P4	P3	C	I2	I1
1	12017	48	Female	E	A	C	D	E	E
1	2620	48	Male	D	A	C	D	D	E
1	6183	51	Female	E	B	D	D	E	E
1	11123	51	Male	E	B	C	D	D	E
1	760	54	Male	E	B	D	D	E	E
1	8509	57	Female	E	C	D	D	E	E
1	2892	59	Male	E	C	D	D	E	E
1	861	61	Female	F	C	D	E	E	E
2	13702	53	Female	E	B	D	D	E	E
2	3096	57	Male	E	C	D	D	E	E
2	14899	63	Male	E	C	D	D	E	E
2	4611	70	Male	F	C	D	E	E	F
2	11178	71	Female	F	D	D	E	E	F
2	8127	72	Male	F	D	D	E	E	F
2	10935	78	Female	G	D	E	E	F	G
2	14264	80	Male	F	D	E	E	F	F
2	13394	83	Female	G	D	E	E	F	G
2	1441	84	Male	G	D	E	E	F	G
2	12269	88	Female	G	E	E	E	G	G
2	9438	91	Male	G	D	E	E	F	G
3	11176	84	Female	G	D	E	E	F	G
3	9582	94	Male	G	E	E	E	G	G
3	151	101	Female	G	E	E	F	G	H
3	135	101	Male	G	E	E	E	G	G
3	8392	106	Male	G	E	E	F	G	G
3	9229	109	Male	G	E	E	F	G	H
3	9415	111	Female	G	E	F	F	H	H
3	6808	113	Male	G	E	F	F	G	H
3	12742	120	Female	H	F	F	G	H	H
3	10208	122	Male	G	F	F	F	H	H
3	13253	123	Female	H	F	G	G	H	H
3	10516	126	Male	H	F	F	F	H	H

4	3932	99	Female	G	E	E	F	G	H
4	4949	107	Female	G	E	F	F	G	H
4	6434	118	Male	G	F	F	F	H	H
4	12270	129	Female	H	F	G	G	H	H
4	5501	131	Male	H	F	G	G	H	H
4	7365	136	Male	H	F	G	G	H	H
4	12176	144	Male	H	G	G	G	H	H
4	6256	148	Male	H	G	G	G	H	H
5	1493	134	Female	H	G	G	G	H	H
5	5620	137	Female	H	G	G	G	H	H
5	4807	140	Female	H	G	G	G	H	H
5	8906	142	Female	H	G	G	G	H	H
5	11143	145	Male	H	G	G	G	H	H
5	1456	151	Female	H	G	G	H	H	H
5	4563	153	Female	H	G	H	H	H	H
5	6428	155	Male	H	G	G	G	H	H

A.8. Stage of dental formation according to London Atlas of Human Tooth development and Eruption (2010)





A.9. Application of stages of dental formation of London Atlas of Human Tooth development and Eruption (2010) on current sample

Group	ID	AGE (MONTHS)	SEX	M1	P4	P3	C	I2	I1
	12017	48	Female	Cr1/2		Ci	Coc	Cr1/2	Cr3/4
	2620	48	Male	Cr1/2		Ci	Coc	Cr1/2	Cr3/4
	6183	51	Female	Cr1/2		Ci	Coc	Cr1/2	Cr3/4
	11123	51	Male	Cr1/2		Ci	Coc	Cr1/2	Cr3/4
	760	54	Male	R1/4	Coc	Cr1/2	Cr3/4	Ri	Ri
	8509	57	Female	R1/4	Coc	Cr1/2	Cr3/4	Ri	Ri
	2892	59	Male	R1/4	Coc	Cr1/2	Cr3/4	Ri	Ri
1	861	61	Female	R1/4	Coc	Cr1/2	Cr3/4	Ri	Ri
	13702	53	Female	Cr1/2		Ci	Coc	Cr1/2	Cr3/4
	3096	57	Male	R1/4	Coc	Cr1/2	Cr3/4	Ri	Ri
	14899	63	Male	R1/4	Coc	Cr1/2	Cr3/4	Ri	Ri
	4611	70	Male	R1/4	Cr3/4	Cr3/4	Crc	R1/4	R1/4
	11178	71	Female	R1/4	Cr3/4	Cr3/4	Crc	R1/4	R1/4
	8127	72	Male	R1/4	Cr3/4	Cr3/4	Crc	R1/4	R1/4
	10935	78	Female	R1/2	Crc	Crc	Ri	R1/4	R1/2
	14264	80	Male	R1/2	Crc	Crc	Ri	R1/4	R1/2
	13394	83	Female	R1/2	Crc	Crc	Ri	R1/4	R1/2
	1441	84	Male	R1/2	Crc	Crc	Ri	R1/4	R1/2
	12269	88	Female	R3/4	Crc	Ri	R1/4	R3/4	Rc
2	9438	91	Male	R3/4	Crc	Ri	R1/4	R3/4	Rc

	11176	84	Female	R1/2	Crc	Crc	Ri	R1/4	R1/2
	9582	94	Male	R3/4	Crc	Ri	R1/4	R3/4	Rc
	151	101	Female	R3/4	Ri	R1/4	R1/4	A1/2	Ac
	135	101	Male	R3/4	Ri	R1/4	R1/4	A1/2	Ac
	8392	106	Male	R3/4	Ri	R1/4	R1/4	A1/2	Ac
	9229	109	Male	R3/4	Ri	R1/4	R1/4	A1/2	Ac
	9415	111	Female	R3/4	Ri	R1/4	R1/4	A1/2	Ac
	6808	113	Male	R3/4	Ri	R1/4	R1/4	A1/2	Ac
	12742	120	Female	A1/2	R1/4	R1/2	R1/2	R1/2	Ac
	10208	122	Male	A1/2	R1/4	R1/2	R1/2	R1/2	Ac
	13253	123	Female	A1/2	R1/4	R1/2	R1/2	R1/2	Ac
3	10516	126	Male	Ac	R1/2	R1/2	R3/4	Ac	Ac
	3932	99	Female	R3/4	Crc	Ri	R1/4	R3/4	Rc
	4949	107	Female	R3/4	Ri	R1/4	R1/4	A1/2	Ac
	6434	118	Male	A1/2	R1/4	R1/2	R1/2	R1/2	Ac
	12270	129	Female	Ac	R1/2	R1/2	R3/4	Ac	Ac
	5501	131	Male	Ac	R1/2	R1/2	R3/4	Ac	Ac
	7365	136	Male	Ac	R1/2	R1/2	R3/4	Ac	Ac
	12176	144	Male	Ac	R3/4	R3/4	R3/4	Ac	Ac
4	6256	148	Male	Ac	R3/4	R3/4	R3/4	Ac	Ac
	1493	134	Female	Ac	R1/2	R1/2	R3/4	Ac	Ac
	5620	137	Female	Ac	R3/4	R3/4	R3/4	Ac	Ac
	4807	140	Female	Ac	R3/4	R3/4	R3/4	Ac	Ac
	8906	142	Female	Ac	R3/4	R3/4	R3/4	Ac	Ac
	11143	145	Male	Ac	R3/4	R3/4	R3/4	Ac	Ac
	1456	151	Female	Ac	Rc	Rc	A1/2	Ac	Ac
	4563	153	Female	Ac	Rc	Rc	A1/2	Ac	Ac
5	6428	155	Male	Ac	Rc	Rc	A1/2	Ac	Ac

A.10. Individuals selected for Error Test

Group	File_no	ID	AGE (MONTH)	SEX	M1	P4	P3	C	I2	I1
unerupted (01) 8 ind	164851	6183	51	Female	U	U	U	U	U	U
	112307	11123	51	Male	U	U	U	U	U	U
Molar and Inscisor partial eruption (02) 12 ind	144737	11178	71	Female	AE	U	U	U	U	U
	157644	1441	84	Male	PE	U	U	U	U	E
Molar and Inscisor complete eruption (03) 12 ind	145492	11176	84	Female	E	U	U	U	E	E
	169985	9229	109	Male	E	U	U	U	E	E
premolar, canine partial eruption (04) 8 ind	160103	12270	129	Female	E	U	E	E	E	E
	136645	7365	136	Male	E	U	E	E	E	E
complete eruption (05) 8ind	118172	4563	153	Female	E	E	E	E	E	E
	141547	6428	155	Male	E	E	E	E	E	E

A.11. Percent of variance of the PCA analysis of the complete configuration

eigenvalues	% Variance	Cumulative %
0.004277	41.92588	41.92588
0.000869	8.517778	50.44366
0.000673	6.596302	57.03996
0.000507	4.967182	62.00714
0.000433	4.240774	66.24791
0.000409	4.008722	70.25664
0.000315	3.08961	73.34625
0.000271	2.659976	76.00622
0.000234	2.295649	78.30187
0.000222	2.172634	80.4745
0.000187	1.834532	82.30904
0.000164	1.611379	83.92042
0.00015	1.468296	85.38871
0.000139	1.360186	86.7489
0.000122	1.195222	87.94412
0.000119	1.163292	89.10741
0.000101	0.992338	90.09975
9.11E-05	0.892702	90.99245
9E-05	0.881895	91.87435
7.62E-05	0.746471	92.62082
7.29E-05	0.714904	93.33572
6.27E-05	0.614542	93.95026
5.48E-05	0.537127	94.48739
5.34E-05	0.523762	95.01115
5.03E-05	0.49278	95.50393
4.7E-05	0.461165	95.9651
4.49E-05	0.440082	96.40518
4.09E-05	0.401347	96.80653
3.45E-05	0.338625	97.14515
3.33E-05	0.326357	97.47151
3.02E-05	0.296462	97.76797
2.71E-05	0.265415	98.03339
2.42E-05	0.237511	98.2709
2.25E-05	0.220816	98.49171
1.93E-05	0.189424	98.68114
1.76E-05	0.172505	98.85364
1.66E-05	0.162941	99.01658
1.55E-05	0.152079	99.16866
1.45E-05	0.142403	99.31107
1.35E-05	0.1328	99.44387
1.21E-05	0.118861	99.56273
1.16E-05	0.113405	99.67613

1.06E-05	0.10347	99.7796
8.31E-06	0.081459	99.86106
5.56E-06	0.054501	99.91556
4.66E-06	0.045689	99.96125
3.95E-06	0.038748	100

A.12. Percent of variance of the PCA analysis of the mandibular configuration

eigenvalues	**% Variance**	**Cumulative %**
0.000954	17.61524	17.61524
0.00066	12.17833	29.79356
0.000448	8.26319	38.05675
0.000413	7.630396	45.68715
0.000335	6.19132	51.87847
0.00031	5.719418	57.59789
0.000259	4.778884	62.37677
0.000253	4.679899	67.05667
0.000212	3.911936	70.96861
0.000184	3.402514	74.37112
0.000142	2.621105	76.99222
0.000134	2.472456	79.46468
0.000109	2.019791	81.48447
0.000101	1.864227	83.3487
9.81E-05	1.811317	85.16002
8.56E-05	1.580146	86.74016
7.89E-05	1.455879	88.19604
6.94E-05	1.282264	89.4783
6.02E-05	1.111623	90.58993
5.28E-05	0.974322	91.56425
4.72E-05	0.872062	92.43631
4.27E-05	0.788362	93.22467
3.81E-05	0.703651	93.92832
3.42E-05	0.631105	94.55943
3.32E-05	0.613803	95.17323
3.04E-05	0.562028	95.73526
2.79E-05	0.514697	96.24996
2.55E-05	0.470521	96.72048
2.26E-05	0.417242	97.13772
1.87E-05	0.344616	97.48234
1.71E-05	0.316318	97.79866
1.59E-05	0.292916	98.09157
1.52E-05	0.281436	98.37301
1.38E-05	0.254813	98.62782
1.16E-05	0.213796	98.84162
1.03E-05	0.189404	99.03102
8.84E-06	0.1633	99.19432

8.11E-06	0.149739	99.34406
7.48E-06	0.138137	99.4822
5.55E-06	0.102547	99.58474
5.21E-06	0.096199	99.68094
4.33E-06	0.079872	99.76081
3.76E-06	0.069373	99.83019
3.09E-06	0.057004	99.88719
2.27E-06	0.041928	99.92912
2.05E-06	0.037784	99.9669
1.79E-06	0.033098	100

4.13. Percent of variance of the PCA analysis of the dental configuration

eigenvalues	**% Variance**	**Cumulative %**
0.014728	50.16558	50.16558
0.006275	21.37343	71.53902
0.001922	6.545825	78.08484
0.001324	4.508604	82.59344
0.001015	3.458202	86.05165
0.000792	2.697938	88.74958
0.000607	2.067522	90.81711
0.000505	1.719823	92.53693
0.000417	1.419969	93.9569
0.000332	1.129901	95.0868
0.000259	0.882488	95.96929
0.000194	0.659589	96.62888
0.000177	0.603273	97.23215
0.000123	0.419038	97.65119
0.000112	0.382262	98.03345
8.83E-05	0.300725	98.33417
8.49E-05	0.289023	98.6232
7.64E-05	0.260384	98.88358
6.94E-05	0.2365	99.12008
5.56E-05	0.189275	99.30936
4.08E-05	0.139069	99.44842
3.67E-05	0.125007	99.57343
3.35E-05	0.114063	99.68749
2.46E-05	0.083741	99.77124
2.17E-05	0.073922	99.84516
1.7E-05	0.057866	99.90302
1.2E-05	0.04101	99.94403
1.02E-05	0.034898	99.97893
6.19E-06	0.021068	100