PRE AND POST NATAL FACIAL DEVELOPMENT IN SOUTH AFRICANS OF AFRICAN DESCENT

Abduljalil Adetola Adebesin

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science (Med) in Anatomy.

Johannesburg, 2012

DECLARATION

I, ABDULJALIL ADETOLA ADEBESIN, declare this thesis to be my own work. It is being submitted in fulfilment of the requirements for the degree of Master of Science (Med) in Anatomy, at the University of the Witwatersrand. It has not been submitted before for any degree or examination at this or any other University.

_____

_____ day of _____ 2012.

DEDICATION

To My Family,

Tawakkaltu and Jamila

for all their support and encouragement

and to the memory of my late mother Mrs Mmordiyyah Mojisola Adebesin

ABSTRACT

Knowledge of development of the facial complex is the key to grasping the anatomical finished product and what can go wrong. Only a full understanding of the growth of the numerous components of the craniofacial structure can lead to successful intervention in the complex interactions that exist during development (Sperber, 1989). The form of the apparently involved structural make–up of the head differs from one population to another (Standring *et al.*, 2005). At the moment there are no published data on facial dimensions of fetal and postnatal South Africans of African descent, as well as the facial asymmetry associated with development thereof.

This research was undertaken to generate facial morphometric data for the African South African populations using a cross section sample. It is envisaged that findings from this study will aid in the management of facial complex dysgenesis and will be useful for other purposes in obstetrics, perinatology and forensic medicine.

In this study anthropometric points were used to define measurements that were taken on skulls to assess changes in facial dimensions with crown rump length and dental eruption schedules used as a proxy for age. The general outcome is an ontogenetic change that is harmonious amongst the facial bony components over time. Both males and females showed similar rate of growth, although, male growth curve was generally ahead of the female. There is no generalised directional asymmetry between the two sides of the face. Similarly, statistically significant fluctuating asymmetry level was found to be raised in the maxillary length and anterior nasal spine-zygion measurements. The prenatal sample also shows a higher level of fluctuating asymmetry than the postnatal sample, in conflict with the high levels of odontometric fluctuating asymmetry observed in the samples used in the study by Kieser and Groenveld (1988). These changes in fluctuating asymmetry after postnatal period

may be attributed to a decreasing stability in the population with later growth and development.

ACKNOWLEDGEMENTS

All praise is due to **Almighty God**, the knowledgeable and the wise, for making the completion of this thesis a fulfilled dream. I am grateful to my supervisor, **Mr Jason Hemingway**, for the opportunity, your guidance and support during the study. The initial setback and disappointment due to resignation and withdrawal of my first supervisor was a blessing in disguise. To my co-supervisor, **Dr Okobi Ekpo**, I am grateful for your positive comments and guidance.

I wish to thank **Dr Mubarak Bidmos** immensely for his assistance and guidance; from the first day to the end of the project. You are indeed a dependable friend and brother.

To my wife, **Mrs Tawakkaltu Adebesin**, thank you for your consistent support, encouragement and for being a source of inspiration. And to my lovely daughter, **Jamila**, thank you for being part of my life.

Last but not the least; I thank all the technical staff of the School of Anatomical Sciences, for assisting with retrieval of the fetal samples. Specifically, I am grateful to **Mr Brendon Billings,** for assisting with the retrieval of skeletal sample in the Dart Collection and arranging the radiography. I thank **Sr Mary-Ann Costello** of the Central Animal Services, for taking the radiographs and **Libby W. Cowgill** for the access to some of her radiographic material.

Thank you to the **University of Limpopo** for providing financial assistance and giving me time-off for the project.

I share the success of this project with everybody that assisted me in one way or another.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

Development is a complex process that is linked to a variety of concepts, when viewed from a morphological perspective it is generally regarded as a change in form during the course of time (Trenouth, 1985). The facial skeleton grows by displacement and remodelling. The active surface resorption and deposition of bone has been considered as the most important process in facial growth (Enlow, 1968; Bjork, 1969). At the same time, Moss and Salentijn (1969) argued that growth of articular edges of bones is central to facial growth. These edges grow into the space created by the movement of individual bones away from the articular junctions by the action of soft tissue. The observed displacement and remodelling of the facial bones in all directions has been described as a primary event that is followed by a secondary bony growth (Beals, 1986).

The face has been described as a part of the identity of a human being, often used as a form of expression and physical appearance (Oladipo *et al.*, 2010). Variable features determine facial appearance (Ari *et al.*, 2005) and the attractiveness of the face is determined by each culture. The facial appearance is determined by the genome (Hartsfield *et al.*, 2012) and greatly influenced by the environment.

Studies on the development of the face are useful tools to outline the dimensions along which populations are classified and assign individuals to groups on the basis of shared features. These shared features are a consequence of the genetic make-up of the population group; the facial dimensions of one group may not necessarily be similar to the other (Farkas *et al.*, 2005). A series of norms have to be developed for each population group, which will not satisfy the so called divine proportion.

The African communities of South Africa arrived about 3,000 years ago from West Africa (Nurse *et al.*, 1985). The Khoikhoisan were the earliest group to arrive followed closely by the Bantu speaking groups. Newman (1995) claimed that the Bantu speakers displaced the earlier settlers further South and this was followed by the fissions and fusions that resulted in the formation of subgroups; the Sotho-Nguni tribes being the majority groups.

The admixture of these subgroups increasingly dilutes the morphological differences amongst the population, including, but not limited to the facial features. The geographical spread also exposed the subgroups to different environmental conditions and the ability to buffer against environmental noises differed greatly.

1.1 Rationale and Motivation

There are limited available data on the development of South Africans of African descent as may be required for standardisation of treatment and other uses. The field of orthodontics and aesthetic clinical dentistry is growing and many South Africans of African descent are interested in such services being offered by these disciplines. Also, the increasing availability and use of sophisticated ultrasound machines means that some facial dysgenesis can be diagnosed in utero. This can only be possible if standardised data is available for South Africans of African descent. Thus, the current study aspires to contribute to the establishment of such a database.

1.2 Study Objectives

The main objective of this study is to obtain the morphometric information of the craniofacial components of the South Africans of African descent using standardised methods, and to determine the degree of asymmetry of the components. Thus the research aims at the following:

- To determine the dimensions of the fetal and postnatal maxilla and mandible in relation to crown-rump length (CRL) and dental development.

- To determine if there are any sexual differences in development of the facial dimensions.
- To compare the asymmetry of the facial dimensions.
- To compare the data obtained from the current study with previously published data of similar population for any differences.

1.3 Scope and Limitation

The research is limited to the linear measurements of the external bony contours of the craniofacial skeleton. The cranium is not disarticulated and so, no sagittal dimension was studied. The research did not focus on the cranial shapes but rather the trend of the dimensions over time.

1.4 Ethical Consideration

The School of Anatomical Sciences allowed the use of all the cadaveric and skeletal materials and all procedures complied with the provisions of the Human Tissue Act, No. 65, of 1983.

1.5 Literature Review

In the literature review chapter, an attempt was made to describe the available scholarly studies done on the development of the craniofacial complex from intra-uterine life to postnatal period. Sequences of bone formation were addressed. The role of the genetics, coupled with environmental factors on the stability of bony structures was discussed. Studies that compared population differences were explored and the conflicting findings on the growth pattern analysed. The history of the present day population of South Africa was discussed briefly, and studies available worldwide on the development of the face summarised.

1.6 Materials and Methods

The materials and methods chapter describes the materials that were used for the research and provides full descriptions of the methods employed in the project.

The research employed the use of 27 cadaveric fetal skulls and 44 juvenile skeletal skulls. The cadaveric materials were part of the collection in the School of Anatomical Sciences and the skeletal materials were obtained from the Raymond A Dart Collection. The fetuses were dissected to expose all the anthropometric points that were used for the measurements of the facial dimensions. Similar measurements were taken in both cadaveric and skeletal materials; however, the CRL and stage of teeth formation were employed as a proxy for age in the prenatal and postnatal groups respectively.

The methods utilised in this project included sampling techniques, as elaborated below, as well as data collection and measuring techniques. Measurements that enable accurate readings were incorporated; the test for precision and accuracy of measurement was done with Lin's concordance correlation coefficient of reproducibility (Lin, 1989). The regression statistics were performed with the SMATR statistic package, developed by Warton *et al*., (2006). The principal components analysis (PCA) was used to analyse patterns of variation between the two samples. The arithmetic mean, median and variance were calculated with Microsoft Excel 2010 and further analyses of fluctuating and directional asymmetry were performed with PAST statistical package Version 2.11 (Falster *et al.*, 2006) using a rescaled asymmetry value (d*) to eliminate individual size differences.

1.7 Results

In this chapter, the results of the study were presented in table and graphic formats. In each of the sets of results presented, the results for the prenatal groups are followed by those for the postnatal group.

The results presented included the regression analysis and loading of the PCA. The arithmetic mean to determine asymmetry was presented including the student *t*-test for significant shift from zero and Levene's test for homogeneity of variance.

1.8 The Discussion and Conclusion

The study was discussed on the basis of existing knowledge in the field of development of the craniofacial complex. The limitations of the study design were discussed and compared with earlier studies. The directions of growth were comparable with previous studies. Only the nasion-zygomaxillare and maxillary length measure of the postnatal group showed relative deviation that was not statistically significant. The maxillary length, anterior nasal spine-zygion, mandibular ramus and coronoid-condyle measurements showed significant high level of fluctuating asymmetry (FA) in comparison to other measurements. Furthermore, there was presence of increased level of FA in post natal group compared to prenatal group that is pronounced more in the mandibular ramus than other measured parameters. There is no significant sexual dimorphism observed within the sample and in addition, the rates of growth are similar. There are no available data for other similar population groups in the literature for direct comparison.

CHAPTER 2: LITERATURE REVIEW

2.1 Facial Skeleton

Every society places undue emphasis on the 'normal' dento-facial appearance of individuals (Shaw, 1975). The face is often used as a measure of an individual's beauty and aspersions are shone on individuals with faces that are not within the realm of the 'societal norm'. The need to address inconveniences that may be associated with real or perceived facial disharmony have led to the multidisciplinary team approach in the management of facial discrepancies (Bull *et al.*, 1990).

Most of the facial skeleton is made up of four paired bones and an unpaired mandible. The paired facial bones are the nasal, the zygomatic, the palatine and the maxilla (Moore, 1992). Majority of muscles that originate from these bones are inserted onto the facial skin, with the exception of the muscles of mastication. The face generally looks smaller at birth than in childhood, this is because of the relatively huge size of the neurocranium at birth and subsequent development of the sinuses while the rate of growth of neurocranium stabilised (Sperber, 1989).

The face is generally divided into three parts for ease of description. The boundaries of these divisions correspond to a horizontal line passing through the pupillary fissure and the rima oris (Sperber, 1989). These divisions, although arbitrary, broadly correlate with the embryonic frontonasal, maxillary and mandibular prominences of the first brachial arch (Sperber, 1989).

The upper third of the face is part of the neurocranium, with the frontal bone as the dominant structure. The lower two-third is the masticatory apparatus that is made up of maxilla and mandible (Standring *et al.*, 2005).

2.2 Prenatal Facial Growth

The formation of the head region is governed by specific genes that signal the mechanisms and morphogenetic processes for development (Waddington, 1942; Standring *et al.*, 2005). The early facial structures develop between the seventh and ninth week of gestation from the five prominences that surround the stomadeum (Moore and Persaud, 1993). These are made up of a single frontonasal prominence as well as paired maxillary and mandibular prominences. These prominences are induced by the migrating neural crest cells that originate from the crest of the neural fold before the completion of neurulation. These neural crest cells have the potential to form connective and skeletal tissues. Diewert (1985) noted that the transition of the embryo to the fetus in the eighth week of gestation is marked by a rapid growth and extensive changes in the shape of the face. Most of the structures within the craniofacial region are evident by the thirteenth week (Houpt, 1970). The early visibility of the developing craniofacial structures, with a human facial appearance prompted Zalel *et al*., (2006) to suggest that micrognathia can be detected in a ten week old fetus.

An analysis of the upper face in the second and third trimester revealed that the fastest rate of growth is observed between the sixteenth and twentieth week of gestation followed by a plateau (Burdi, 1969). The part of the face that is noted to be of a recent development is growing faster than the part that developed much earlier (Ford, 1956).

2.2.1 The Growth Centres in the Face

Facial bones develop from differentiation of mesenchymes that originate from neural crest cells, the precursor of ectomesenchyme and mesoderm. They do not grow by cell divisions but rather by the ossification of the laid down organic matrix. The calcification of the amorphous and crystalline apatite deposit happens directly or indirectly. The direct ossification results in the formation of a membranous bone while indirect ossification results in the formation of endochondral bone. The indirect ossification goes through a hyaline

cartilage prototype model of the future bone (chondrification) before undergoing intracartilaginous ossification. Bones are therefore laid down in two ways, ossification of cartilage and intramembranous ossification.

The initial ossification point is visible as the primary growth centre. The time of appearance of the primary centres differs from one bone to another, but typically appears between the seventh and twelfth prenatal life. Secondary centres appear at or after birth and may be in response to functional activities around the bone. The resultant effect of ossification is the expansion of bone which is not only an increase in size or weight but the sum of all measurable changes in the region (Tanner, 1981).

Facial growth is achieved by direct continuous accretion on the surface of the laid down bony contour accompanied by a sequential adjustment in the structure, i.e. remodelling growth. For intramembranous bones, activities revolve around the periosteum and endosteum wherein bones are laid down and resorbed to maintain shape. However for endochondral bones, activities involve intrinsic cartilage growth and endochondral bone apposition (Enlow, 1968).

In addition to cartilaginous growth, periosteal and endosteal deposition of bone, there are three major sites of growth of the facial bones namely: sutures, condyles and synchrondroses. These factors explain why the bony contour laid down eventually becomes disproportionate and the earlier configuration lost, i.e. reshaping and resizing. The complex remodelling thereafter involves all regional areas of the bone leading to overall growth.

The condylar process of the mandible is a secondary growth centre that developed from a separate cartilage. It eventually merges with the intramembranously ossifying ramus and increases in size due to muscular activities. Together with the bony processes of the mandible, it contributes to the overall form of the mandible. The growth of the maxilla is

dominated by growth at the suture edges, interstitial growth of the interposed synchrondroses, the bony processes and expansible effect of the sinus.

2.2.1.1 The Nasomaxillary Complex

The nasomaxillary segment develops from the frontonasal and paired maxillary prominences. It plays a significant role in the developing face. The traditional assumption that the upper face grows forward and downwards has been queried by Enlow (1968) who described the assumption as an oversimplification of events. Sperber (1989) argued that a thrust and pull effect created by the developing nasal septum separates to some degree the sutures around the face. This allows osseous accretion at the articular edges of the sutures and anterior surface (Moss and Salentijn, 1969). The effect of the developing temporal lobes of the cerebrum also plays a role. The temporal lobes force the anterior cranial base and the nasomaxillary segment downwards as they increase in size; this mechanism results in flattening of the anterior cranial base and displacement of the facial complex (Trenouth, 1985) and the resulting displacement causes a slight downward movement of the nasion.

The nasal septum is a dominant structure in the anterior cranial base and maintains a constant proportion. It has been shown to increase by six fold within this constant proportion by the 36^{th} week of gestation. The position of the alveolar process of the maxilla relative to the nasal septum determines the prognathism observed in an individual. According to Ford (1956), it is fixed at the commencement of the fetal life.

2.2.1.2 The Mandible

The mandible develops from paired mandibular prominences of the first brachial arch. The Mackle's cartilage, found medial to the mesenchyme that forms the mandible, serves as a template for the developing mandible. The ossification of the mesenchyme begins in the sixth week (Malas *et al.*, 2006). With the presence of the cartilage, the mandible grows ahead of the maxilla during the eighth and ninth week. By the 11^{th} week, the Meckel's cartilage

becomes smaller and less important. The developing temporomandibular joint and the secondary cartilage of the condyle now set the growth rate of the mandible (Ford, 1956). The effects of the masseter and temporalis muscles also manifest on the developing ramus (Diewert, 1985). From the 12th week of fetal life, the growth of the mandible lags behind that of the maxilla (Bareggi *et al.*, 1995) and any distortions in the rate or timing of these changes (developmental process) will result in disharmony between the mandible and maxilla. The mandibular shape changes due to the development of the ramus, the condyle and the enlargement of the coronoid process (Trenouth, 1985). The two halves of the mandible eventually fuse between the first and second post natal years arresting the growth activities at the symphysis. The fusion of the joint results in a single distinctive adult V-shaped bony structure (Standring *et al.*, 2005).

2.2.1.3 Pattern of Prenatal Growth

Changes in the proportional relationships between the different parts of craniofacial bone in time determine the pattern of growth observed. There are different bony components developing simultaneously with added soft tissue influences. The pattern of facial growth is the result of the interplay between the growth processes of the different parts. The different parts of the skull follow different growth patterns. Overall, the face grows downwards and forwards away from the cranial base, but there is no general consensus on the directional growth of the face amongst researchers. While some of them, including Scammon and Calkins (1929) and Birch (1968) are of the opinion that the face grew faster in height than in length others such as Inoue (1961) maintained that the rate of growth observed is greater for the length than the height. Houpt (1970) observed that the components of the craniofacial bones have a similar growth rate, but Johnson (1974) opined that an isometric growth pattern is the norm.

2.3 Postnatal Growth

The face grows continuously beginning in the post somite period and continues throughout postnatal life, with a constant geometrical shape change (Inoue, 1961; Houpt, 1970). The shape of the face is predicted on the functions and the remodelling of the bone as it simultaneously increases in size (Enlow, 1968). Bone however does not develop in similar manner as soft tissue. The remodelling is often a response to the biomechanical force exacted by the adjacent soft tissue (Smartt *et al.*, 2005). The hardness of bone and the influence of the soft tissues require a distinctive specialised adaptation. The increase in size does not happen only by surface accretion and enlargement of existing contours, but by a measured epigenetic control, otherwise a disproportionate face will result (Waddington, 1942; Jefferson, 1996). The change in form noticed in the postnatal lower third of the face is more pronounced than the upper two thirds due to the active remodelling taking place.

Bjork (1969) did not notice any major remodelling of the anterior surfaces of the jaws of the Swedish children he followed in his ground-breaking research on the growth of the jaw. The effect of basal cranial growth on the face reduces after the fourth year, and the skull sutures cease to be a growth centre but serve as sites of fibrous union of skull base (Sperber, 1989). The lack of major remodelling lends support to the theory that condylar cartilage reacts in response to the muscular displacement of the mandible (McNamara, 1972; Petrovic and Strutzman, 1972). The bony deposition achieved at the posterior surface of the condyle results in the forward and downward movements of the mandible (Enlow, 1968). The trabeculae that are related to the mandibular canal are stationary and are not subjected to remodelling changes (Captier *et al.*, 2006). This phenomenon limits any noted changes in the symmetry of the mandible to the functional units (Captier *et al.*, 2006; Malas *et al.*, 2006).

All the work done so far on the craniofacial disorders have pointed to the fact that not all facial asymmetry is as a result of developmental disorders (Captier *et al.*, 2006). Other

notable factors that can manifest as facial asymmetry include muscular matrix (Captier *et al.*, 2006), as well as disturbances with the sutural growth and fusion (Captier *et al.*, 2003). A posterior plagiocephaly will inevitably displace the ipsilateral temporomandibular joint (St John *et al.*, 2002). The activity of the masseter muscle is known to contribute to the prominence of the gonion. However, if the masseter fails to develop, this will have an effect on the development of the gonion as a prominent structure (Kane *et al.*, 1997).

2.4 Developmental Instability and Fluctuating Asymmetry

It is generally agreed that external tensions are an essential factor responsible for the morphology of bone. This tension, within the threshold, stimulates osteogenesis in the area of stress. This extrinsic force leads to the formation of bony markings. The shape of the bone that results thereof is augmented by the intrinsic genetic factors that are operating within the connective tissue membrane of the bone (Enlow, 1968). In paired bony components, the phenotypic similarities are governed by the shared genetic influences (Waddington, 1942), the shared stresses, and functional matrices. Thus, the ability of a fetus to develop a regular facial phenotype under a given environmental and genetic condition is a sign of developmental stability (Møller and Manning, 2003). Under given adverse environmental condition, instability in the form of developmental noise may result i.e.; when the introduced disturbance cannot be corrected. When such perturbations are corrected, then a developmental stability prevails. Fluctuating asymmetry (FA) is used as a measure of the level of developmental instability.

FA is a small, random deviation from perfect symmetry in a given population of organisms. It quantifies the level of developmental noise, that reflects population's average state of gene changes and interaction (Jones, 1987; Graham *et al.*, 2010). The ability to defy such developmental accidents by an organism is referred to as 'buffering against developmental

instability'. In addition, the ability to buffer well in one character may confer similar ability in other characters.

It is generally agreed that a more perfectly symmetrical organism handled its developmental stress better i.e. has more developmental stability (Van Valen, 1962). FA may therefore be attributed to a measure of developmental processes that occur in disharmony with controlling regulatory systems in place (Brown and Brown, 1998; Kegley and Hemingway, 2007). It is the asymmetry that results from the inability of organisms to develop in precisely determined paths (Van Valen, 1962). Instability during morphogenesis could result in directional, anti or fluctuating asymmetry. FA is therefore a measure of developmental instability of a given population rather than individuals within the population.

Palmer and Strobeck (1992), and Parsons (1992) proposed that three factors should be considered when using FA as a measure of developmental instability:

1. Without intrinsic or extrinsic perturbations, all individuals in a sample should consistently be symmetrical;
2. All biological systems cannot consistently achieve bilateral symmetry, even in an ideal condition;
3. All biological systems often make correction for imperfections during development.

There is a disproportionately high degree of dental asymmetry among South African of African descent children compared to other groupings (Kieser and Groeneveld, 1988). This developmental instability was attributed to inequalities within communities living in the same locality as a result of the then political order in South Africa. Although the presence of a high level of disease burden on the African South African population has been documented (Wyndham and Irwig, 1979), the primary key assumption that developmental instability is

not due to any genetic predisposition needs to be substantiated at all times (Palmer and Strobeck, 1992).

2.5 Facial Morphometrics

There is a continuous osseous accretion on the surfaces of the bone. These are accompanied early in life by growth at the sutures, the secondary endochondrium of the mandibular condyle and interstitial growth of the interposed cartilage (synchrondroses) of the cranial base. The resultant effect is a shift of the relative position of bony landmarks. Apposition and remodelling therefore adjust bone to new positions after displacement (Sperber, 1989). The soft tissues exert pressure on the bone and continue to induce formation of various markings. The sutural growth also stressed the adjacent soft tissues thereby augmenting the markings. These activities are more pronounced and relatively larger during fetal than postnatal life (Trenouth, 1984).

Facial development can therefore be measured using defined anatomical landmarks. The slope of the regression line of a measurement between such landmarks in time represents the absolute growth rate which may vary (Ford, 1956).

2.6 Stability of Anthropometric Points

There is a differential growth pattern observed within and between the different bone components. The remodelling changes are due to the differences in the extent and rate of deposition and resorption of bone. This is governed by the local tension factors under epigenetic influence and ensures a predetermined geometric shape at a given time. Inoue (1961), Levihn (1967) and Sardi *et al*. (2007) supported the hypothesis that the geometrical shape of the face remains constant due to the stability of the anthropometric lines. However, Trenouth (1984) argued that undetected changes in angular measurements that are not picked up in linear measurements may summate to a potential change in overall shape.

Synchrondroses and sutural growth are proportional such that the suture retains its comparative position; adjusting bone to their new position after displacement.

Different patterns of prenatal growth have been suggested by various authors, some have claimed a linear, curvilinear or sigmoid pattern. Burdi (1969) outlined that the various patterns mentioned are encountered during development and suggested that the study design and limitations of various studies were responsible for the different views.

The high positive correlations between CRL and increasing height-length parameters attest to a curvilinear growth (Levihn, 1967), whereas a positive linear relationship is found between anterior and posterior parts of the cranial base (Ford, 1956). Houpt (1970) observed craniofacial growth to be relatively constant.

2.7 Sexual Variation

Studies by Scammon and Calkins (1929) have suggested that there are no major differences between the sexes in the facial development until puberty. De Villiers (1968) reported that the degree of sexual dimorphism is higher in the mandible than the cranium of adult South Africans. In addition, it is suggested that the adult variation, independent of population group, is greater within than between sexes (Standring *et al.*, 2005). Malas *et al.* (2006) did not find any sexual differences in the mandibular dimensions of a fetal sample of Turkish origin. However, adult males grow at a faster and longer rate than females. Males tend to have significant larger features than females. Both sexes present a small chin in utero and childhood while male chins are generally larger in adulthood (Sperber, 1989). The current study will look for any sexual dimorphism within the current sample.

2.8 Studies Conducted on Facial Growth

There is a glut of scholarly work on the development of the craniofacial complex but the design and research approaches are not necessarily comparable. The earliest of these studies by Scammon and Calkins (1929) analysed growth patterns in 369 formalin fixed un-dissected

human fetuses. Anthropometric measurements were carried out on the prenatal cranium and it was concluded that most dimensions of the fetal head correlate with the crown rump heel (Scammon and Calkins, 1929). Ford (1956) exposed the anthropometric points and sagittally sectioned the head to obtain similar results as Scammon and Calkins (1929). The results tend to explain how age related morphological changes of the craniofacial complex are a result of their differential growth rates. Later studies used a lateral radiographic method (Meastre, 1959; Inoue, 1961; Levihn, 1967), which in some cases were complemented with photography (Burdi, 1965; Miotti *et al.*, 1983), histological sections (Diewert, 1985; Johnston, 1962) and direct measurement (Plavcan and German, 1995). Other researchers such as, Ortiz and Brodie (1949) and Miotti *et al*., (1983) considered not only fetal materials but extended to early postnatal life.

2.9 Population Specificity

Standring *et al*. (2005) accumulated evidence to demonstrate differences in craniofacial dimensions within and between population groups. The magnitude and nature of these differences have been acknowledged in metrical analyses. The existence of interwoven ontogenetic mechanisms in the facial profile of population groups have been documented and Vioarsdóttir *et al.* (2002) attributes the differences in the facial profiles to:

- an early development of major aspects of population-specific morphogenesis;
- dissimilarity in the direction of the ontogenetic path;
- ontogenetic scaling.

The international anthropometric study of facial morphology in various cultural groups by Farkas *et al*. (2005) gave valuable insight into the major differences in facial characteristics. Data for a given population group shows variation from the normal distribution values of other groups. Altemus (1959; 1960) had previously reported that, differences exist in the head size of European American and African American children and observed that the head

sizes and dental protrusion of African American children are greater in absolute terms when compared to children of European descent. However, the upper facial height of the African American sample was found to be lower than that of the European American sample. In addition, Flynn *et al.* (1989) observed that the length of the mandibular body in African American children was significantly greater than European American children amongst the inhabitants of Pittsburgh. de Freitas (2010) recently reported a greater maxillomandibular discrepancy and a more horizontal craniofacial growth pattern amongst Black Brazilian teenagers compared to their White counterparts.

The work of Naido and Miles (1997) found differences in craniofacial measures of adult South Africans and Americans of African descent. In 1968, De Villiers showed that the different tribes that constitute the so called 'South African Blacks' or better referred to as African South Africans (South African census categorisation) are a single homogeneous group based on the analysis of the dimensions of skull (de Villiers, 1968), implying that the African South African population has similar metrical values. A recent analysis by Franklin *et al.* (2005), using geometric morphometry on same material used by De Villiers (1968) however found that differences existed among the African South African population. Similarly Sibisi and Hlongwa (2007) found significant differences in upper face height, lower face height and gonial angle of Tswana specimens when compared to Sotho-Tswana specimens of Botswana normative values from the work of Barter *et al.* (1995). It has been reported that the Tswana of Botswana and South Africa can be regarded as cousins both having migrated from the West of Africa over 3000 years ago (Nurse *et al.*, 1985).

2.9.1 The Southern Africans of African descent Population

The current study utilises the terms 'South Africans of African descent' or 'African South Africans' to denote peoples belonging to the Bantu speaking tribes, namely the Nguni (Hlubi,

Mpondo, Swazi, Xhosa and Zulu), Sotho-Tswana (comprising the Sotho and Tswana) and Shangaan / Tsonga tribes.

The Bantu speaking African tribes of Southern Africa started their migration about 2,000 years ago from the Western part of Africa and some of them stayed behind in the present East and Central Africa and adapted to the environment (Nurse *et al.*, 1985). The earliest group to occupy Southern Africa were the Khoesan, generally referred to as the indigenous communities. They are represented by the Khoekhoe and San groups in present day South Africa. By the fifth century AD, the newly arriving Bantu speaking (Sotho-Nguni) groups had started to displace the Khoesan (Newman, 1995). Hammond-Tooke (2000) noted that the numerous fissions and fusions resulted in considerable number of subgroups; the Nguni groups settled in the east/south coast and the Sotho groups settled in the hinterland. Ribot *et al*., (2010) reported that skeletal remains from KwaZulu-Natal, older than 400 AD, tended to be morphologically closer to individuals of Khoesan ancestry, whereas remains from around 1000 AD share more in common with modern-day Sotho-Tswana and Nguni groups. This suggests that population replacement and or gene flow occurred significantly between these periods in this region.

The traditional distribution of the various groups was subsequently blurred out due to intertribal marriages. Jenkins *et al*. (1970) observed gene flows from the Khoesan to the Bantu speaking people that arrived later, the level of the genetic affinity is noted to correlate with the geographic closeness of each of the group to the Khoesan settlements areas. Franklin *et al.* (2005) found that the geographically proximal group i.e. the Xhosa, shares more morphological features with the Khoesan than any of the other bantu-speaking tribes.

2.10 Summary

This chapter reviewed available published studies on the development of the face. Events in literature relating to facial development starting from the somite period, at birth and

postnatally were traced. The effects of epigenesis and stress on the developing face were also reviewed. The differences within and between populations groups were explored; including the differences within and between the African South African populations. This literature review provides a basis for the current study to accomplish its broad objective which included the determination of the dimensions of the fetal and postnatal maxilla and mandible, sexual differences in their growth curves and associated asymmetry in their development.

CHAPTER 3: MATERIALS AND METHODS

3.1 The Materials

Pre and post-natal skulls were used for the project. The skulls were made of two components namely the cadaveric and the skeletal specimens. The cadaveric specimens were the formalin fixed fetuses which represented the prenatal group while the postnatal group were represented by the skeletal skulls.

3.1.1 The Cadaveric Specimens

A total of 27 dissected fetuses were used. Twelve of these fetuses were females while the rest (15) were male. The CRL ranged between 24.3cm and 40.7cm. Each of the fetuses was observed for any physical anomalies so that only specimens without gross anomalies were included in the sample. The fetal cadavers were part of the collections housed at the School of Anatomical Sciences of the University of Witwatersrand and stored in fixative-filled tanks. There is a paucity of authentic information on the collections but it is known that the collections were gathered over a long period of time as donations from the community (Dayal *et al.*, 2009). Some of the specimens were unclaimed, aborted fetal bodies from the surrounding Gauteng Provincial health facilities (Hutchinson *et al.*, 2011)

3.1.1.1 Desirability of Aborted Fetal Cadaver

There are no major physical differences between fetuses that were aborted by intervention in health facilities and those that were aborted naturally. According to Trenouth (1985), there is a high correlation in the ultrasonic growth curves between *in utero* and *ex utero* fetuses. In other words, the growth curves for aborted fetuses correlate with those that did not abort and continue growth. Therefore the aborted fetus can be considered as a representative of a normal growth.

Fixation and storage in formalin have been demonstrated to have little effect on the physical condition of cadavers. Plavcan and German (1995) observed no gross differences in the shape or condition of bones from post-fixed cadavers on a visual inspection when compared with unfixed cadavers. The result of the regression line for CRL and bi-parietal measures of non-fixed (recently aborted) fetuses compared favourably well with formalin-fixed fetuses (Scammon and Calkins, 1929; Plavcan and German, 1995).

3.1.2 The Skeletal Specimens

A total of 52 human skulls (23 female, 26 male and three unspecified) were used. All the skulls were obtained from the Raymond A Dart Collection of Human Skeletons. The skulls used in this study did not exhibit any obvious abnormalities except for post-preparation damages to different parts ((Dayal *et al.*, 2009). Tooth eruption stage has been considered suitable for postnatal studies (Smith, 1991) and the eruption stages of specimens used in this study were found to range from the presence of only the lower deciduous incisors to the first permanent molar (mixed dentition).

It was possible to take all desired measurements on only 24 skulls out of the 52 skulls. The condition of one of the maxillae was such that only four measurements could be taken. Five mandibles were missing and a few measurements could not be taken in some of the mandibles included in the study.

3.1.2.1 The Raymond A Dart Collection of Human Skeletons - 'the Dart Collection'

The Raymond A Dart Collection of Human Skeletons housed at the School of Anatomical Sciences of University of Witwatersrand is the largest collection of Human skeletons in South Africa (Dayal *et al.*, 2009). The collection was initiated by the late eminent physical anthropologist, Professor Raymond Arthur Dart (1893-1998) who was appointed in 1923 as the head of the then Department of Anatomy (Tobias, 1987; 1991). Close to 2,000 skeletons of cadaver-derived origin were collected.

The Raymond A Dart Collection has become world renowned, hosting researchers from different parts of the world. The addition of skeletons to the collection has become institutionalised within the School of Anatomical Sciences, with the collection now sitting above 2,500 cadaver-derived skeletons (Dayal *et al.*, 2009). The emphasis on the collection today is to add only skeletons of South African groups that were not well represented and that are of research interest as anatomic variant subjects. Unfortunately, there are very few juvenile skeletons, about 1% (Dayal *et al.*, 2009) within the Dart Collection and even fewer elsewhere in South Africa.

There is availability of the demographic information for each of the specimens i.e. age, population affinity and sex. The degree of completeness of skeletons is also listed on each box. However there are few cases of strange bones in the boxes (Dayal *et al.*, 2009).

3.1.3 Measuring Instruments

The measuring instruments used in this study include a sliding digital calliper with accuracy nearest to 0.01mm and a spreading calliper with accuracy nearest to 0.1mm.

3.2 Method

A similar set of anthropometrical points was measured in both groups of specimens and the data subjected to the same analysis to determine the development of facial bones. However, different criteria were used as the index of time for the two groups. The CRL and stage of teeth formation were employed as a proxy for age for the prenatal and postnatal groups respectively.

3.2.1 Sampling

The initial study design was to make use of 50 skeletal and 30 cadaveric skulls, but only 44 and 27 skulls respectively were available for the study.

There is a catalogue of skeletal material in the Dart Collection, the correctness of some of the data are doubtful (Dayal *et al.*, 2009) especially considering that a few of the boxes that have

skulls assigned ages below six years were found to have erupted the second maxillary molar. Consequently skeletal materials were included based on the observable deciduous tooth eruption stage rather than the ages indicated on the boxes.

Similarly, all the cadaveric specimens were tagged but no accurate biographic data was available. Therefore, the cadaveric materials were selected on basis of the physical appearance of the skin colour and hair curling patterns; all cadaveric materials that could not be readily assigned as African South African were excluded. Furthermore only specimens that demonstrated no physical deformity were included in the study.

3.2.2 The Anthropometric Points

Six bilateral anthropometric points and four median anthropometric points were chosen for the facial bone measurements. These anatomical landmarks were used as a measure of development of the facial bone. The anatomical landmarks were visible on the external surfaces of the facial bones as shown in Figure 3.1 (page 25) and Figure 3.2 (page 26). The points were chosen because they are easily recognisable at various stages of development and measuring across them does not involve cutting of bone. In addition, the chosen landmarks enhance the reproducibility of the measurements that were taken. The landmarks as defined in Table 3.1 (page 24) were based on the description of Knußmann (1988), Bareggi *et al*., (1995), Proffit (2000) and Captier *et al*., (2006).

Table 3.1: Definition of the anthropometric points

Anthropometric Point	Abbreviation	Description
Condylion	**Co**	Most superior ossified point of the condylar process
Coronoid point	**Cr**	Most superior ossified point of the coronoid process
Gonion	**Go**	Midpoint of the contour connecting the ramus and body of the mandible
Gnathion	**Gn**	Centre of the inferior point on the mandibular Symphysis
Anterior nasal spine	**Ans**	The most anterior point of the nasal cavity floor i.e. the tip of the anterior nasal spine
Zygion	**Zy**	Most lateral aspect of the zygoma
Zygomaxillare	**Zm**	Junction point, most lateral and inferior, between the zygomatic bone and the zygomatic process of the maxillary bone.
Pterygomaxillary point	**Pt**	Point on the vertical fissure between the pterygoid plate and back of maxilla at same level with anterior nasal spine.
Nasion	**Na**	Central point of the intersection between the nasal and frontal bone
Prosthion	**Pr**	The most anterior and inferior point of the maxillary alveolus on the median plane.
Vertex	**V**	The highest point on the median sagittal plane when the cranium is aligned to the Frankfort Horizontal
Breech	**B**	The midpoint on the medial plane between the apices of the buttocks i.e. the rump

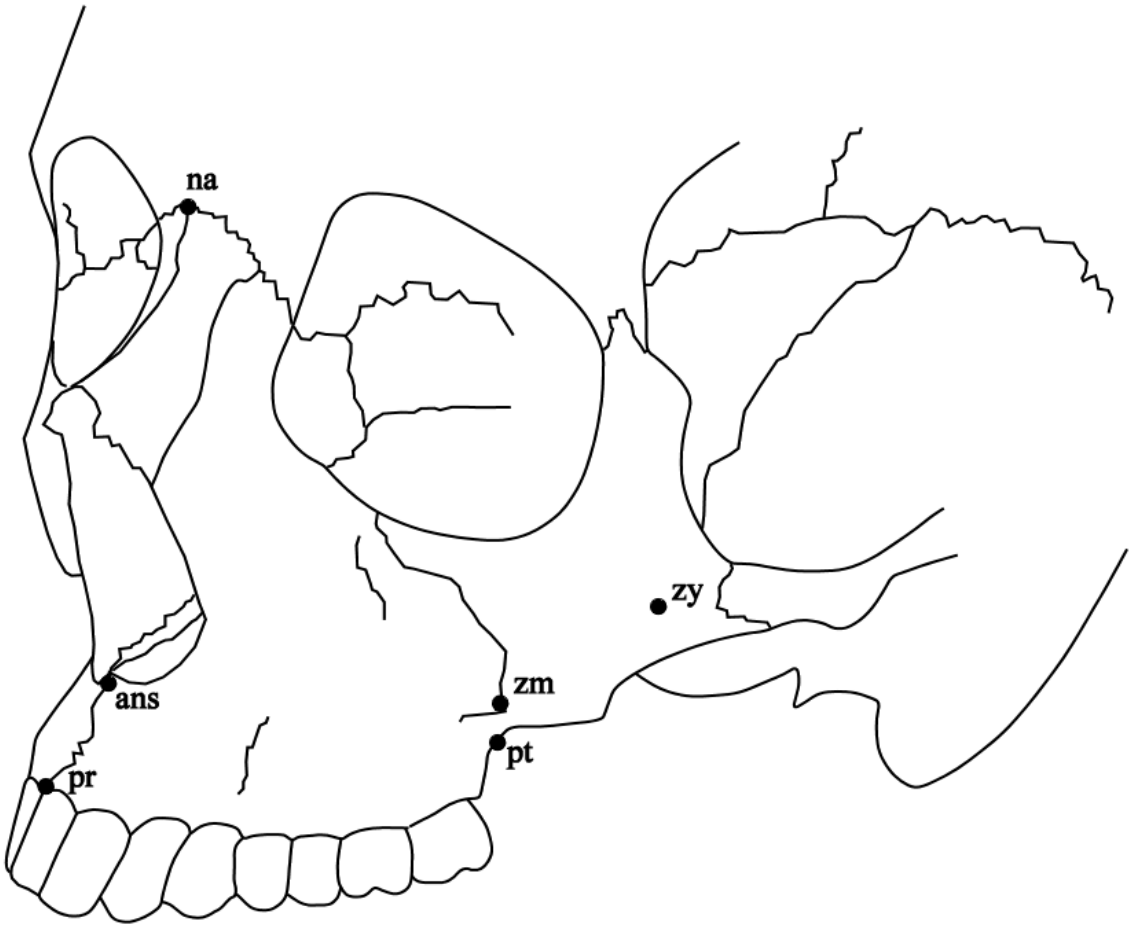


Figure 3.1: **Skull showing the anthropometric points. Adapted from Williams and Slice (2010), landmarks names and definitions are provided in Table 3.1 page 24.**

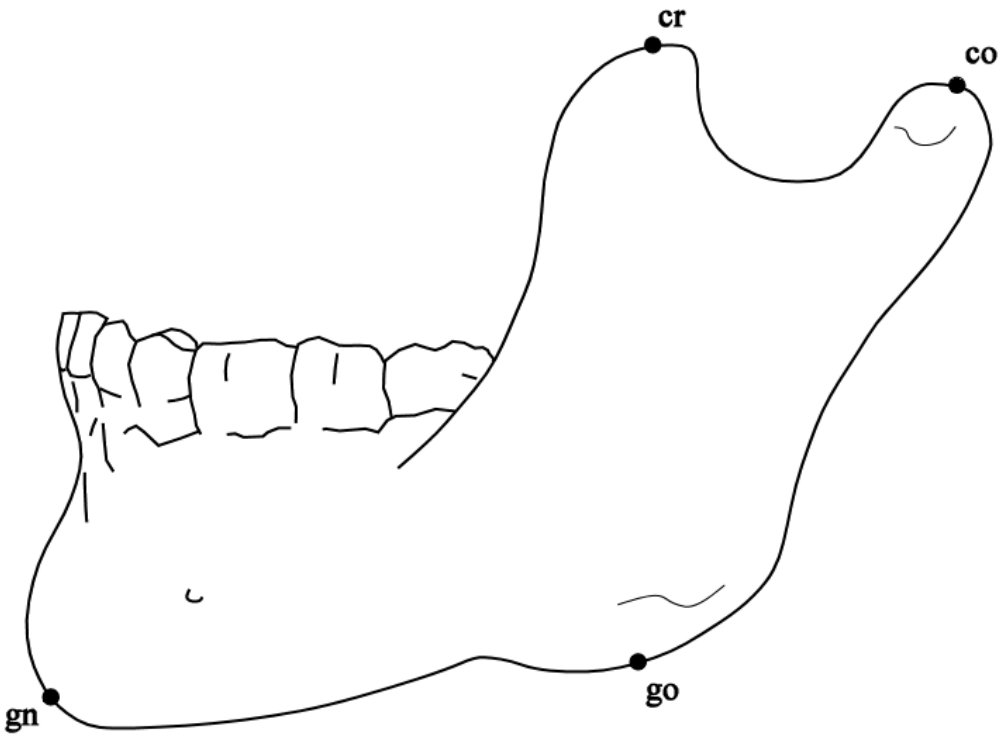


Figure 3.2: Mandible showing the anthropometric points. Adapted from Sperber (1989) landmarks names and definitions are provided in Table 3.1 page 24.

3.2.3 Anthropometric Measurements

Fifteen defined anthropometric measurements presented in Table 3.2 (page 28) were taken on the facial bones and seven of these measurements were bilateral. The measurements were developed on the basis of identified anthropometric points. The current study did not involve complete gross dissection and sectioning of bone structure. All measurable surfaces of the nasomaxillary complex and the mandible were included.

Each anthropometric point was marked except Vertex and Zygion that intrinsically rely on the maximal measurements; one end of the anthropometric distance to be measured was designated as point A and the other point designated as B. One end of the tips of the calliper was placed on point A and the other end was drawn out and placed on point B. The reading on the calliper was then recorded as the distance measured.

Table 3.2: Definition of measurements

Measurement	Abbreviation	Description
Facial width	**bi-zy**	The distance between the right and left zygion.
bi-zygomaxillare distance	**bi-zm**	The distance between the right and left zygomaxillare.
Nasal septal height	**na-ans**	The distance between nasion and the anterior nasal spine.
Nasion-zygomaxillare distance	**na-zm**	The distance between the nasion and zygomaxillare on the same side.
Inter-maxillary alveolar height	**ans-pr**	The distance between anterior nasal spine (ans) and prosthion (p) i.e. distance represented by the suture between the right and left maxillary alveolar processes.
ans-zy distance	**ans-zy**	The distance between the anterior nasal spine (ans) and the zygion (zy) on the same side.
na-zy distance	**na-zy**	The distance between the nasion (na) and the zygion (zy) on the same side.
Maxillary length	**ans-pt**	The distance between the anterior nasal spine (ans) and the pterygomaxillary point (pt).
bi-condylion distance	**bi-co**	The distance between the right and left condylion.
bi-coronoid distance	**bi-cr**	The distance between the right and left coronoid point.
Mandibular width	**bi-go**	The distance between the right and left gonion
Mandibular ramus distance	**go-co**	The distance between the gonion and condylion point on same side
Mandibular body	**go-gn**	The distance between the gonion and gnathion
Symphyseal height	**sh**	The distance from the gonion to the highest level of the alveolus on the median plane; i.e. the height of the mandible at the Symphysis.
co-cr distance	**co-cr**	The distance between the condylion and coronoid point on the same side.
Crown rump length	**crl**	The distance between the vertex and breech

3.2.4 Assignment of Age

CRL and tooth eruption stage have been widely used as a measure of development. A strong relationship has been demonstrated between chronological age, CRL and tooth eruption stage (Moore and Persaud, 1999; Standring *et al.*, 2005) . Unfortunately there are no published data regarding the relationship between CRL, tooth eruption stage and age among South African populations. Hence, the current study utilised CRL and tooth eruption stage as a proxy for the ages of the skulls in order to provide a set of normal values for South African population.

3.2.4.1 The Determination of CRL

Each fetal specimen was retrieved from the storage tank and placed on the dissection table. The breech, parietal tuber and calcaneal tuberosity were identified with a marker. Thereafter, the CRL measurements were taken from the vertex to the breech with a spreading calliper. Similarly the foot length and bi-parietal diameter were taken and recorded. Some of the cadavers have missing feet or partially dissected skulls and so foot length and bi-parietal measurements were not taken from such cadavers. The inability to collect all the parameters necessary to determine all the fetal ages has necessitated the assignment of ages based on the Streeter's Table of 1920 (Moore and Persaud, 1993). Ford (1956) had argued that when there are discrepancies in the use of foot length, bi-parietal diameter and CRL in the establishment of fetal age, the CRL is the most reliable parameter to determine fetal ages. Therefore only the CRL was considered as a parameter for fetal age as shown in Table 3.3 (page 30).

Table 3.3: Assigned CRL ages

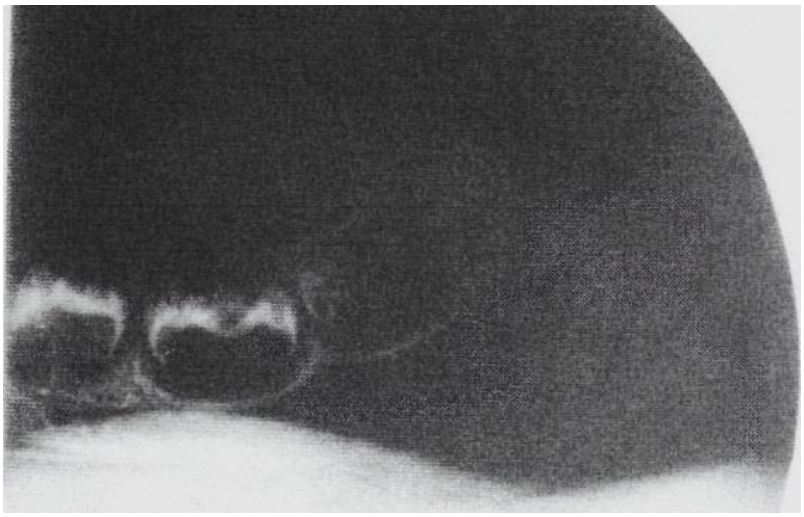
Crl (cm)	Age (wk.)
24.3	24-26
25.6	26-28
25.8	26-28
26	26-28
26.4	26-28
26.6	26-28
27	28-30
27.3	28-30
28	30-32
28.7	30-32
29.8	30-32
30.5	32-36
31.4	32-36
31.8	32-36
32.1	32-36
32.8	32-36
33.2	32-36
33.5	32-36
34.1	36-38
34.5	36-38
35	36-38
35.3	36-38
35.6	36-38
36	36-38
36.1	38-40
39.8	38-40
40.7	38-40

3.2.4.2 Determination of the Tooth Eruption Stage

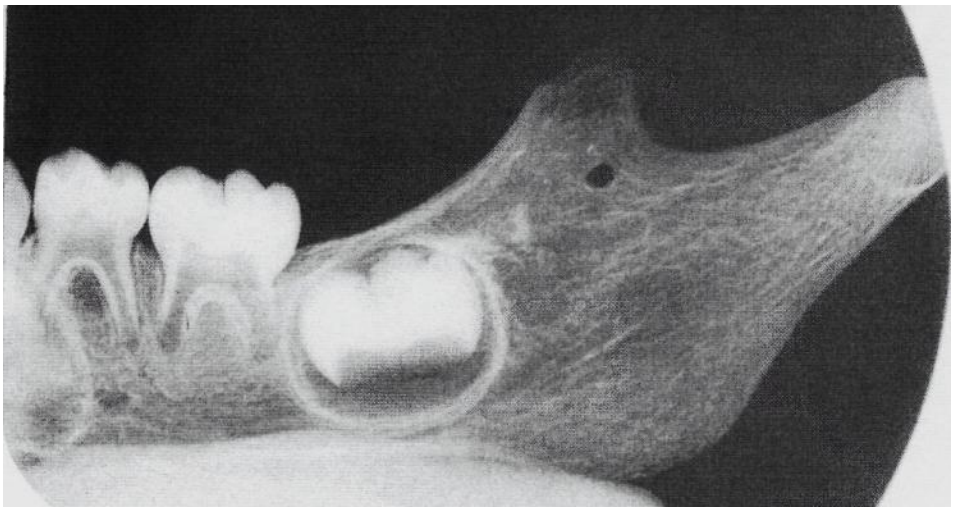
Dental age has been noted to compare with skeletal development; however the coefficient of variation exhibited by the stages of the tooth formation is lower than the general skeletal development (Lewis and Garn, 1960). In addition, its development is less affected by endocrinopathies and other environmental impacts (Smith, 1988) with the morphology being a highly heritable trait. Dental development has consistently tied to chronological age than other skeletal indicators and given first consideration in forensic age asseessment (Klepinger, 2006).

In the past there were inconsistencies in the determination of the dental age. Some researchers relied on the formation of the teeth while others used eruption of teeth. Tooth eruption is an instantaneous event of emergence of the tooth through the gingiva while formation of a tooth is a continuous development (Smith, 1988). However, the time of eruption may be missed while busy documenting eruption stages in a longitudinal study. It is therefore better to rely on tooth development rather than eruption. Determination of the stage of tooth formation is based on the modification of the criteria espoused by Liversidge and Molleson (2004) on the formation of roots, crowns and eruption of deciduous tooth.

Radiographs of the skeletal skulls were taken at the Central Animal Services of the University of Witwatersrand. The projections were oblique lateral; such that each radiograph showed the teeth and the bony components distinctively and un-obscured as shown in Figures 3.3 A and B (page 32). The mandibular radiographs were taken except for the cases of four missing mandibles where the maxillae were taken. No adjustments were made for the usual late formation of maxillary teeth compared to mandibular teeth as there is a high degree of overlap in the estimates.



A: Stage 4



B: Stage 7

Figure 3.3: Radiographs of the mandibles showing tooth eruption at stages 4 and 7

From the adaptation of Liversidge and Molleson (2004) criteria, the tooth eruption stage for the current study is divided into Stages 1-10 as presented in Table 3.4 (page 34), Stage 1 represents the beginning of the mineralisation of the deciduous canine while Stage 10 represents the emergence of the first permanent molar from the alveolar processes. The ages were assigned with reference to the eruption guideline published in the pocket notes of the Department of Orthodontics of the University of the Witwatersrand (Witwatersrand, n.d). The assignment of ages is merely to establish trends between the groups as the published guideline relates more to South Africans of European descent (SAED).

Table 3.4: Crown and root formation stages, adapted from Liversidge and Molleson (2004)

Stage of development Score	Description	Age assigned (in months)
1	Mineralisation of the deciduous canine crown and occlusal surface of the cusp tips of molar visible.	3 +/- 3
2	The dentine of the deciduous molar is visible below the enamel	6 +/- 3
3	The roof of the pulp chamber of the canine is fully formed and the formation of roots of the deciduous molars begin as dentine spicules	15 +/- 3
4	Root length is equal to the crown height	13 +/- 3
5	The root walls are thicker than the root canal	24 +/- 3
6	The apex of the roots are open and the cusps of the permanent first molar is visible as an outline of occlusal surface	36 +/- 3
7	The apex of the deciduous are closed, the dentine of the permanent molar is visible under the enamel	48 +/- 3
8	The formation of roots of the molars first permanent molar begins as dentine spicules	60 +/- 3
9	The root length is longer than the crown height	72 +/- 3
10	The apex of the first permanent molar are open	80 +/- 3

3.2.5 Dissection of the Fetuses

The primary aim of the dissection was to expose the anatomical landmarks, to free and remove the mandible from the skull. Each cadaver was laid in supine position on the dissection table. The anthropometric points needed were noted and marked. Guide -lines were made between the landmarks to denote each anthropometric distance and incisions were made along these lines.

To expose nasion, an approximately 5-cm incision was made from the nasion above and below in the median plane. The skin along with subcutaneous tissue was then reflected. Similarly the incision from the nasion was continued along the border of the nose to the philtrum and extended to the tragus of the auricle and the skin reflected to expose the zygion, the anterior nasal spine, the prosthion and the zygomaxillare.

In order to remove the mandible, incisions were made along the contour of the lower border of the mandible from gonion to gonion via gnathion; the skin along with subcutaneous tissue was reflected inferiorly. All the muscles attached to the mandible were cut without striping the fibres from the bone, starting from the right side to the left. The developing submandibular gland was displaced medially and the parotid gland removed to expose the temporomandibular joint. The exposed temporomandibular joint was opened by a horizontal cut through the capsule. The lateral pterygoid muscle was then cut from the capsule and condyle; followed by the temporalis muscle from the coronoid process.

Finally, the mucosa was cut with a scalpel passing from the midline to the ramus posteriorly; the temporalis muscle was then pushed through the zygomatic arch and the mandible removed. The removal of the mandible exposed the pterygomaxillary fissure.

Some of the cadavers used in this study had previously been used for other studies. The skins had been incised, reflected and dissected to expose the mandible. The additional dissection

done on these cadavers was merely to remove the mandible and expose further landmarks that were not properly exposed.

3.2.6 Data Collection

The available biographic data for each of the specimens used was recorded in a separate worksheet (in order to avoid bias towards predetermined values) before the commencement of measurement of anthropometric distances. The data for male and female groups were recorded separately in the data sheet.

3.2.6.1 Cadaveric Skull Measurements

After each dissection, the anthropometric points were noted with a marker and each of the measurements was taken as described in section 3.2.3 above, using the sliding calliper for CRL and bi-parietal measurements, and the digital sliding calliper for the remaining measurements.

3.2.6.2 Skeletal Skull Measurements

Each of the skulls was placed in a specially prepared skull holder to stabilise them during measurement. All the anthropometric points were identified and marked, and the measurements outlined in section 3.2.3 above were taken with a digital calliper.

3.2.7 Test of Reliability

The reliability of any anthropometric analysis is as good as the degree of precision of measurement and the error of the statistical analysis. Cameron (1984) advised that a reliability test must not be an ‘afterthought’ to give some measure of validity to the results. Since the reliability is also dependent on the accuracy and clarity of the measurement definitions. Anthropologists are therefore encouraged to incorporate measures that will result in taking accurate measurements and use appropriate statistics. As a result of the need for a repeatable and an unbiased measurement, Lin’s concordance correlation coefficient of reproducibility (Lin, 1989) was employed in this study.

Ten skeletal and cadaveric skulls were randomly selected from the samples. They were part of the skulls that have been measured previously for the study. The measurements were repeated approximately eight days after the first measurements. The measurements i.e. retest were recorded in a separate worksheet. The test and retest values were subjected to Lin's formula to calculate the correlation coefficient, Pc, using Microsoft Excel 2010:

$$Pc = 1 - [\frac{1}{n}\Sigma(x_1 - x_2)^2 / (sdx_1^2 + sdx_2^2) + (mx_1 - mx_2)^2],$$

where n = sample size, x_1 = first (test) measurement, x_2 = second (retest) measurement, sdx_1 = standard deviation of the first set of measurements, sdx_2 = standard deviation of the second set of measurements, mx_1 = mean of the first set of measurements and mx_2 = mean of the second set of measurements.

The acceptable range for coefficient of reproducibility is 0.90-1.00 (Cameron, 1984).

Unlike measurement errors, equation errors cannot be estimated from a repeated measurement, therefore appropriate statistical software have to be employed. It must be noted that component independent lines change across functional groups, populations and environment. It is suggested that equation error is often large in allometry when compared with measurement error and that there is no single correct method to find the line-of-best-fit in a regression (Warton *et al.*, 2006). The method chosen for estimation of the line is a function of the direction in which the errors are measured to the line-of–best-fit.

Standardised major axis, SMA (or reduced major axis, RMA), is noted to summarise relation between two variables. It corrects errors on both axes unlike the least squares method of regression that only reduces error on the dependent variables (y-axis). The current study does not aim to predict the facial dimensions from the index of time, i.e. the CRL and the tooth development stage, but to summarise the relationship. As such, single dimension will be used to describe a two-dimensional data. In effect, SMA is a data or dimension reduction (Warton

et al., 2006). Therefore SMATR version 2.0 (Falster *et al.*, 2006; Warton *et al.*, 2006), a statistical package that employs SMA, was used for the regression analysis of the data obtained in this study. The natural logarithms of the values were used in performing regression analysis.

The rescaled asymmetry (d*) as suggested by Harris and Nweeia (1980) for analyses of asymmetry attributable to random side difference was used in this study. It eliminates the effect of individual measurement difference among the paired measurements, i.e. paired values are viewed as independent variables within a group.

When data are re-used in a multiple fashion to arrive at an answer, it is suggested that the data be adjusted for multiple comparison. Consequently, multiple comparisons using sequential Bonferroni correction was used in order to use Brown and Forsythe test (1974) to determine absolute FA amongst variables.

3.2.8 Regression Analysis

Correlation has been described as an association or relationship between variables as shown by their measurements or scores (Allan, 1982). In non-biological matters, and a few biological circumstances, the variable which causes the observed phenomenon in the relationship is the independent variable, while the phenomenon observed is the dependent variable. A plot of the scores usually creates a scattergram which could take the form of a positive correlation, a no correlation or a negative correlation.

A positive correlation is obtained when the magnitude of an increase on one axis corresponds to similar magnitude on the other axis and vice versa. The plot is thus regarded as a (linear) positive correlation that can be represented by the general formula for a straight line

$$y = bx + c$$

where b represents the slope of the line, and c the intercept on the y axis.

In practice variables do not lie in a perfect straight line, more so with biological matter but closely approximate a straight line. The best line that fits the values of the subject will be the average line i.e. regression line.

The correlation coefficient (r) is a descriptive tool that measures the relative co-variance and its magnitude for the two paired variables. It returns a numerical value showing the degree of association between the variables. These values extend from +1, 0 to -1, representing positive correlation, no correlation and negative correlation respectively. Experimental variables will always give a fractional value because the regression line is always not perfect. The closer the fractional value to +1 or -1, the greater the association.

The correlation coefficient is often a means of commenting on the population from which a sample was taken. However we do not want results occurring by chance due to error, therefore a probability level will have to be set. The correlation coefficient is then regarded as being statistically significant, if its probability of being zero falls within the usual set level of less than 0.05.

The approximation of the degree of correlation that existed between the two sets of measurement is known as Pearson's correlation coefficient (r), represented by the general formula

$$r = \frac{\sum xy}{\sqrt{\sum x^2 \sum y^2}}$$

where *x* represents the variable usually plotted on the horizontal axis, and *y* represents that of the vertical axis. Comparison of the value of slope estimates is an indicator of the relationship of rates of growth amongst the variables considered. However, because of the deviations of cases from the regression line, the line is then estimated by minimising the sum of the residuals from the line i.e. corrected by least square regression. The line that minimises the

squared deviations is called the least squares line or least squares regression. It is prudent to consider how much variation in the y-values is accounted for by the variation in the x-values. This will be the coefficient of determination (r^2), which is the square of coefficient of correlation.

If the dependent variables i.e. y-values are normally distributed, one can draw lines parallel to the regression line to determine the values of y that fall within probabilities of 68%, 95% and 99% of the regression line. The positions of the lines are determined by the calculated standard error of the estimate.

The regression analysis was computed using the SMATR software. The confidence interval was set at 95% and the critical p-value at 0.05. The data was loaded and the requested output from the program included slope, intercept and the correlation coefficient (r^2) for each of the following analysis:

- Regression for cadaveric and skeletal male, female, and combined data and;
- Comparison of growth curves amongst cadaveric and skeletal male and female data;

The slope, r^2 and the p-values generated by the input was tabulated. The comparison of slope included the test and p-values.

3.2.9 Principal Components Analysis (PCA)

When there is a data set with high dimensionality and the pattern of the variation needs to be discerned, PCA is one of the techniques of choice. It identifies patterns in data, especially when such data cannot be presented graphically. It is based on applied linear algebra and widely used in face recognition and image compression (Smith, 2002). It essentially reduces a complex dataset to reveal underlying hidden simplified structures, and can function as a roadmap to identify further analytical options. (Shlens, 2005)

PCA identifies those measurements that can best answer the dynamics in question, determines if more than necessary measurements were taken, and takes care of the noise problems and contaminants. It then identifies the pattern, and establishes similarities and differences by reducing the number of dimensions without losing information.

Principal component eigenvectors are always perpendicular and independent of one another often revealing important information about the dataset. Since the current study relied on CRL and tooth development score as proxies for age in the fetal and skeletal samples respectively, direct comparisons between the samples would be problematic. The eigenvector with the highest eigenvalue is the first Principal component (PC1) of the dataset; lower eigenvalues may not be considered especially when the first few values account considerably for the studied phenomenon. In developmental studies the PC1 is often aligned with the size and size related shape changes and thus its loading can provide evidence as to the measurements substantially involved with growth and development. This allowed for a direct comparison between the two samples.

Principal components were calculated from the mean-adjusted covariance matrix using PAST and provided the opportunity to interrogate the facial growth pattern and development between the two samples.

3.2.10 Asymmetrical Analysis

All bilaterally symmetrical structures are expected to develop as mirror image of one another given that the genetic influence on both sides is the same. Nonetheless, biological elements are influenced differently by the environment. The individual level of buffer will determine the resulting phenotypic outcome of the genetic expression. Directional asymmetry (DA) is a preferential development on either side of midline; i.e. one side of the body is consistently larger than the opposite side. The trait shows a variation of left and right that is normally distributed about the mean. However the mean of the distribution deviates significantly from

zero when the data is plotted on histogram. On the other hand, FA is a variable attributed to random side difference and is thus normally distributed about a mean of zero.

Analysis of FA was done using a rescaled asymmetry value (d*); the side difference measure was divided by the mean of the right and left measure. The absolute values were used to avoid positive values cancelling out negative values, it is represented by:

$$\text{d*} = L - R \Big/ \frac{L+R}{2}$$

where L and R represent the left and right measures (Harris and Nweeia, 1980).

The arithmetic mean, median and variance of the paired measures were calculated.

3.2.10.1 Directional Asymmetry

The data for paired measurements including maxillary and mandibular length, mandibular ramus, nasion-anterior nasal spine, nasion-zygion and nasion-zygomaxillare were entered into Excel and the arithmetic mean computed. Mean values of zero denotes that the antimeres are developing in equal length, a positive value indicates a shift in development to the right and a negative value denotes a shift to the left. The arithmetic mean that differs from zero was tested using Student's *t*-test to confirm whether the mean is significantly different from zero. The data were also presented graphically.

3.2.10.2 Fluctuating Asymmetry

The same data that was used for DA was also used to compute the median and the variance. The d* values were entered into PAST version 4.1 software (Hammer *et al.*, 2001) to establish homogeneity of variance using Levene's test based on the mean and median (Levene, 1960). The variance of the measurements about the mean of zero determines the degree of FA. The results obtained using the medians were adjusted for multiple comparisons via sequential Bonferroni correction. Brown & Forsythe (1974) showed that the median

performed better with skewed distributions. Correlation and regression analysis between absolute FA and development was analysed with the SMATR (Warton *et al.*, 2006).

3.3 Summary

This chapter looked at the materials used in the current study. The anthropometric points that were employed to generate the data were listed and defined. Measures taken to avoid errors and establish the validity of the research design was elucidated. The data were analysed with Microsoft Excel, PAST and SMATR software. The processes involved in the computation were discussed. Regression models were computed to compare rates within the prenatal and postnatal samples, and facial growth pattern and development between groups assessed with the PC1 of the PCA, the DA and FA computed and the generated data will be presented under results.

CHAPTER 4: RESULTS

4.1 Test of Reliability

Anthropologists are encouraged to incorporate measures to achieve repeatability and non-bias in their research design (Cameron, 1984). This study relied on the concordance correlation coefficient of reproducibility as presented by Lin (1989). The 23 variables measured were considered and the P*c* values for the prenatal and postnatal groups fell between 0.98 and 1.00 as shown in Table 4.1 page 45. This is the range of value advocated by most researchers (Cameron, 1984).

Table 4.1: Test of reliability (Lin, 1989)

Measurement	Prenatal P*c*	Postnatal P*c*
crl	1.00	-
bi-zy	0.99	0.99
bi-zm	0.99	0.99
na-ans	0.99	0.99
ans-pr	0.99	0.99
L ans-zy	0.99	0.99
R ans-zy	0.99	0.99
L na-zy	0.99	0.99
R na-zy	0.99	0.99
L na-zm	1.00	0.99
R na-zm	0.99	0.99
L ans-pt	0.99	0.99
R ans-pt	0.99	0.99
bi-co	0.99	0.99
bi-cr	0.99	0.99
bi-go	0.99	0.99
Sh	0.99	0.98
Lgo-gn	0.99	0.99
R go-gn	1.00	0.99
L go-co	1.00	0.96
R go-co	0.99	0.99
L co-cr	0.99	0.96
R co-cr	0.99	0.96

4.2 Facial dimensions

4.2.1 Prenatal facial dimensions

The average facial dimension in the third trimester ranges between 0.84 and 5.57 cm, the facial width has the highest average dimension while the average value of the nasal septal height is 0.84 cm as shown in Table 4.2 page 47. The mean value for mandibular width and bi-coronoid distance are 4.23 cm and 5.13 cm respectively while the maxillary length is 2.94 cm. The average height of the middle-third of the face is 2.20 cm while the total lower two-third facial height is 3.43 cm.

Table 4.2: Prenatal facial dimensions

Measurement	n	Minimum value	Maximum value
bi-zy	27	4.600	7.180
bi-zm	27	3.320	6.040
na-ans	27	0.830	2.020
ans-pr	27	0.480	1.250
ans-zy	27	2.620	4.110
na-zy	27	2.695	4.375
na-zm	27	2.525	4.585
ans-pt	27	2.125	3.675
bi-co	27	3.930	6.430
bi-cr	27	3.540	6.440
bi-go	27	2.980	5.370
sh	27	0.850	1.790
go-gn	27	2.530	4.540
go-co	27	1.205	2.355
co-cr	27	0.645	1.600
na-pr	27	1.520	2.900
na-gn	27	2.550	4.640

4.2.2 Postnatal facial dimensions

The average facial dimension of the postnatal group ranges between 1.27 and 7.34 cm, the facial width has the highest average dimension while the average value of the nasal septal height is 1.27 cm as shown in Table 4.3 page 49. The mean value for mandibular width and bi-coronoid distance are 5.59 cm and 6.71 cm respectively while the maxillary length is 4.04 cm. The average height of the middle-third of the face is 2.20 cm while the total lower two-third facial height is 5.95cm.

Table 4.3: Postnatal facial dimensions

Measurement	n	Minimum value	Maximum value
bi-zy	50	5.459	9.533
bi-zm	51	4.635	8.326
na-ans	52	1.730	4.081
ans-pr	52	0.772	1.962
ans-zy	51	3.219	5.407
na-zy	51	3.220	5.905
na-zm	51	3.155	6.154
ans-pt	52	2.804	4.900
bi-co	41	4.707	8.948
bi-cr	38	4.726	7.885
bi-go	41	4.324	7.464
sh	42	0.858	2.833
go-gn	46	3.497	7.366
go-co	45	1.238	4.902
co-cr	40	1.737	4.934
na-pr	52	2.558	5.528
na-gn	43	3.844	8.186

4.3 Regression Analysis

4.3.1 Prenatal Regression

The SMA regression analysis showed association between all the measured facial dimension and CRL as shown in Table 4.4 page 51. Due to multiple testing, the probabilities were adjusted using the sequential Bonferroni technique, which did not alter any of the outcomes. Mandibular width dimension showed the strongest association ($r^2 = 0.82$), while the nasal septal height showed the least ($r^2 = 0.14$). With the exception of nasion-anterior nasal spine (na-ans), there was a statistically significant association between all the measurements and CRL. The variable with the highest slope is condyle-coronoid process measurement and the facial width dimension registered the lowest slope. The condyle-coronoid process dimension is growing almost at one and a half times that of facial width. The mandibular width appears to be growing faster ahead of the facial width. Similarly the body of mandible appears to be growing slightly ahead of the maxilla. When tested for isometric growth ($\alpha = 0.05$), only symphyseal height and condyle-coronoid process dimension show statistically significant positive allometry. All the measurements show a negative intercepts which indicates that development started much earlier than the late fetal period considered in the current study.

Table 4.4: Prenatal regression analysis

Measurement	n	r^2	p	Slope	Slope: Lower confidence level (-0.95)	Slope: Upper confidence level (0.95)	Intercept	Intercept Lower confidence level (-0.95)	Intercept Upper confidence level (0.95)
bi-zy	27	0.775	0.000*	0.885	0.729	1.074	-1.333	-1.928	-0.738
bi-zm	27	0.456	0.000*	1.039	0.770	1.401	-2.000	-3.086	-0.913
na-ans	27	0.140	0.060	1.383	0.952	2.009	-4.470	-6.288	-2.651
ans-pr	27	0.445	0.000*	1.510	1.117	2.043	-5.385	-6.979	-3.791
ans-zy	27	0.561	0.000*	0.917	0.700	1.202	-1.974	-2.835	-1.112
na-zy	27	0.682	0.000*	0.959	0.762	1.207	-2.058	-2.824	-1.291
na-zm	27	0.550	0.000*	1.058	0.809	1.390	-2.409	-3.415	-1.403
ans-pt	27	0.632	0.000*	0.942	0.736	1.206	-2.167	-2.977	-1.358
bi-co	27	0.794	0.000*	0.974	0.809	1.173	-1.722	-2.348	-1.096
bi-cr	27	0.814	0.000*	1.151	0.964	1.373	-2.381	-3.084	-1.677
bi-go	27	0.822	0.000*	1.146	0.964	1.362	-2.505	-3.190	-1.820
sh	27	0.549	0.000*	1.300	0.989	1.709	-4.275	-5.512	-3.038
go-gn	27	0.760	0.000*	1.042	0.853	1.273	-2.331	-3.055	-1.608
go-co	27	0.237	0.010*†	1.165	0.819	1.657	-3.610	-5.052	-2.167
co-cr	27	0.565	0.000*	1.487	1.137	1.945	-5.005	-6.394	-3.616

n= sample number

p= probability

r^2= coefficient of determination

† = the probability of no relationship (a = 0) increases to 0.020 after sequential Bonferroni correction, which remains significant at $\alpha = 0.05$.

* = statistically significant values

4.3.2 Prenatal sample loadings of PCA

Figure 4.1 page 53 shows the first principal component (PC1) loading which accounts for over 80% of the variation in growth and development observed. The other components individually accounted for less than 10% of the variation, and are probably not related to growth and development, as such they were not analysed further. Spearman's rank correlation identified that PC1, alone, was significantly related with CRL (r_s = 0.914, P = 2.79x10^{-11}), however the remaining components failed to come close to showing significance (P values all greater than 0.5). This suggests that growth related change in the face was largely accounted for by the first component, and reporting further on the other components would reflect non-allometric changes and are thus out of the scope of this project.

The sample was grouped into three (early, mid and late 3rd trimester) groups for display purposes only, as shown in Figure 4.2 page 54 and Figure 4.3 page 55. The facial width, bi-zygomaxillare, nasion-zygion and nasion-zygomaxillare display high loading values while nasal septum and alveolar process display low values for the maxilla. While the mandibular body, width, bi-coronoid, and bi-condylar measurements display high loading values and the ramus, symphyseal and coronoid-condylar measurements have low loading values. These loadings suggest that the various measurements contribute differently to the PC1.

Figure 4.2 page 54 shows the variables extending along PC1 and across the principal component 2 demonstrating the association between growth and PC1, while Figure 4.3 page 55 shows a linear positive correlation between changes in the size and size related shape changes and PC1.

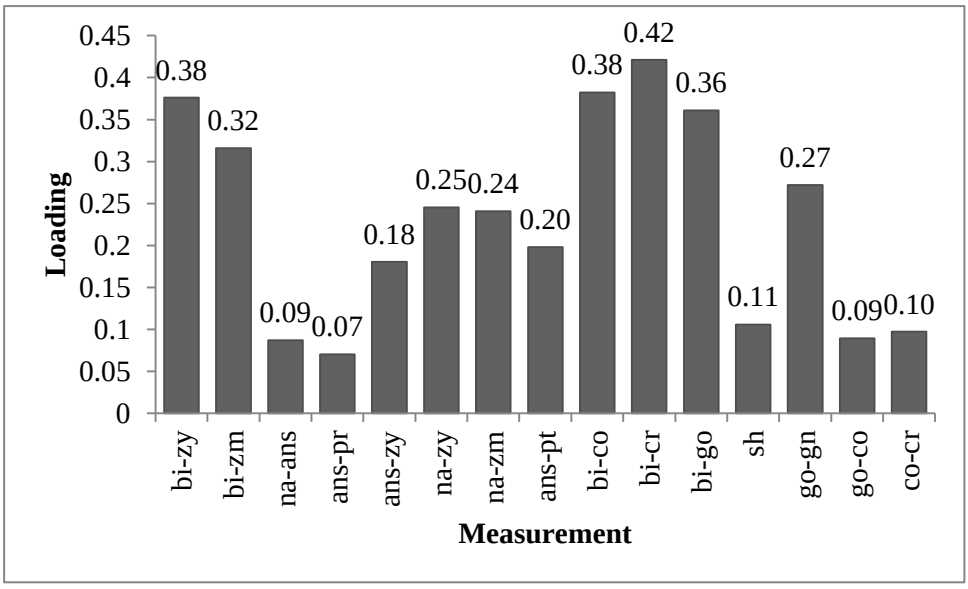


Figure 4.1: First principal component loading

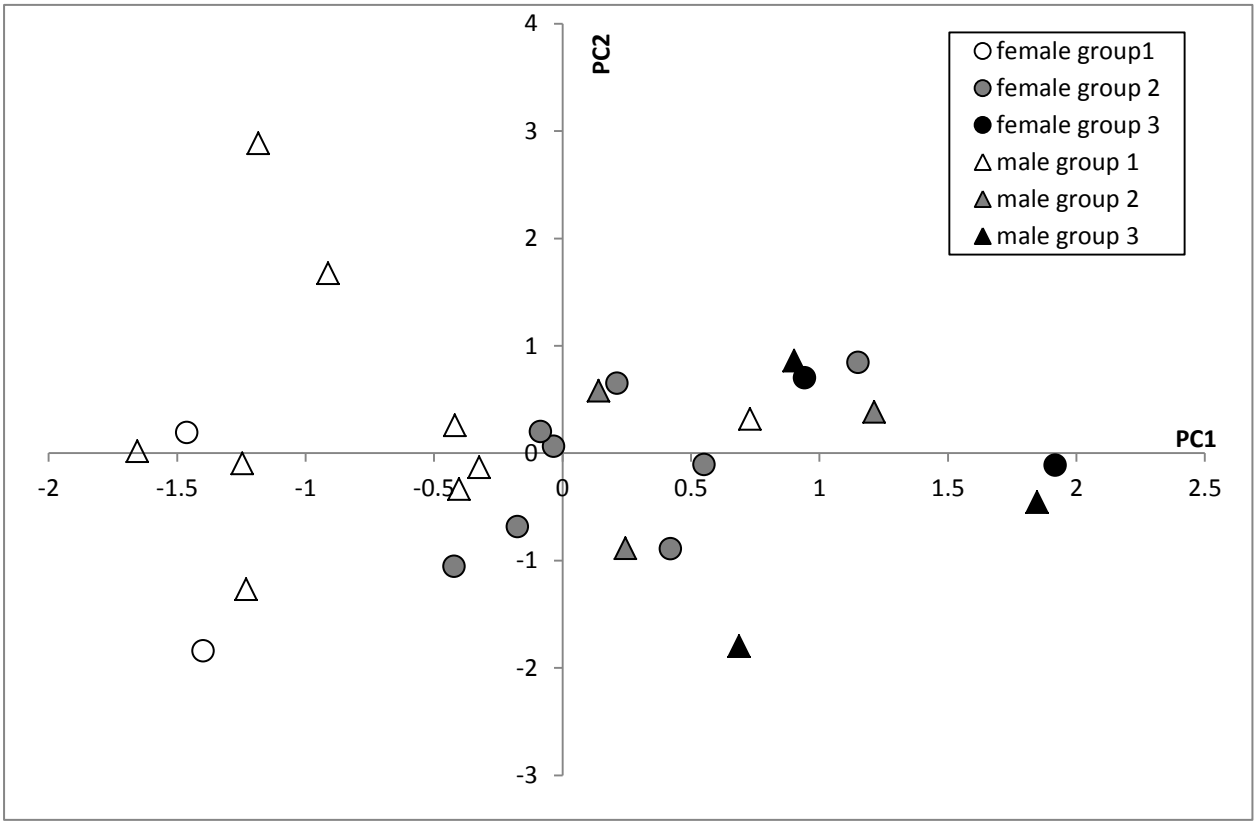


Figure 4.2: Plot of the scores associated with PC1 and PC2 from the PCA of prenatal dataset

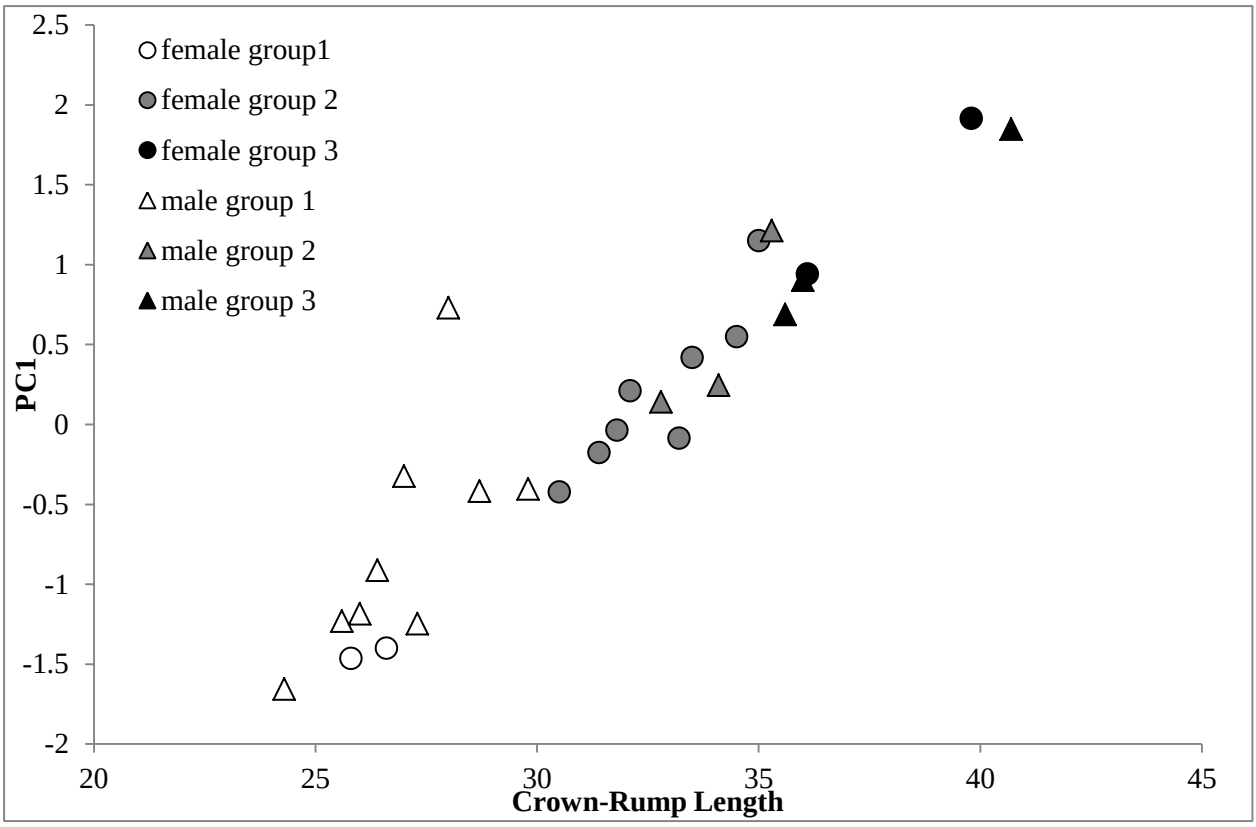


Figure 4.3: Association between PC1 and CRL explaining how PC1 represents size and size-related shape shange

4.3.3 Postnatal Regression Analysis

The SMA regression analysis shows association between all measured facial dimension and the stage of tooth development as shown in Table 4.5 pages 57. The nasion-zygion dimension shows the strongest association (r^2 = 0.77) while mandibular symphyseal dimension (r^2 = 0.33) shows the least. The variable with the highest slope was the condyle-coronoid process dimension (0.66) while the anterior nasal spine-zygion dimension had the least slope of 0.29. The mandibular width is growing at one and a half times the facial width, whereas the ramus grows one and half times faster than the body of the mandible. The nasal septum is growing at a similar rate as the dimension of the maxillary alveolar process. When tested for isometry (α = 0.05), all variables demonstrate statistically significant positive allometry. Only maxillary alveolar process, symphyseal height, mandibular ramus and condylar-coronoid process measurements show negative allometry.

Table 4.5: Postnatal regression

Measurement	n	r^2	p	Slope	Slope: Lower confidence level (-0.95)	Slope: Upper confidence level (0.95)	Intercept	Intercept: Lower confidence level (-0.95)	Intercept: Upper confidence level (0.95)
bi-zy	50	0.702	0.000*	0.290	0.248	0.340	1.497	1.417	1.577
bi-zm	51	0.731	0.000*	0.355	0.306	0.411	1.218	1.127	1.310
na-ans	52	0.704	0.000*	0.456	0.391	0.532	0.234	0.112	0.357
ans-pr	52	0.300	0.000*	0.470	0.372	0.595	-0.569	-0.765	-0.374
ans-zy	51	0.739	0.000*	0.275	0.238	0.319	0.949	0.880	1.020
na-zy	51	0.766	0.000*	0.326	0.284	0.374	0.971	0.893	1.050
na-zm	51	0.739	0.000*	0.352	0.304	0.407	0.927	0.838	1.017
ans-pt	52	0.701	0.000*	0.294	0.2512	0.343	0.800	0.720	0.879
bi-co	41	0.700	0.000*	0.369	0.309	0.440	1.246	1.128	1.363
bi-cr	38	0.746	0.000*	0.344	0.291	0.408	1.229	1.122	1.336
bi-go	41	0.556	0.000*	0.368	0.2927	0.456	1.066	0.924	1.220
Sh	42	0.325	0.000*	0.542	0.418	0.702	-0.290	-0.539	-0.041
go-gn	46	0.702	0.000*	0.443	0.376	0.523	0.842	0.7190	0.973
go-co	45	0.598	0.000*	0.645	0.531	0.783	-0.071	-0.297	0.155
co-cr	40	0.502	0.000*	0.662	0.526	0.834	-0.117	-0.397	0.163

n= sample number

p= probability

r^2= coefficient of determination

* = statistically significant values

4.3.4 Postnatal sample loading of PCA

Figure 4.4 page 59 shows the PC1 loading, the sample was arranged into three groups based on the dental development (low, mid and high scores) as shown in Figures 4.5 page 60 and 4.6 page 61. Spearman's rank correlation between the components and tooth development scores found that only the first principal components reflected allometic change in the face (r_s = 0.889, P = 7.42x10^{-16}), whereas the remaining components failed to indicate any form of association (P values all greater than 0.19). Thus, as with the preceding prenatal sample, presentation and discussion of the first components was considered sufficient for the scope of the project.

All the maxillary parameters display high loading values, except for the maxillary alveolar process. As for the mandible, only the symphysis displays a low loading. These different component loadings suggest that the various measurements contribute differently to the PC1. Figure 4.5 page 60 shows the variables in relation to PC1 and PC2 while Figure 4.6 page 61 shows correlation between the size and size related shape changes and PC1.

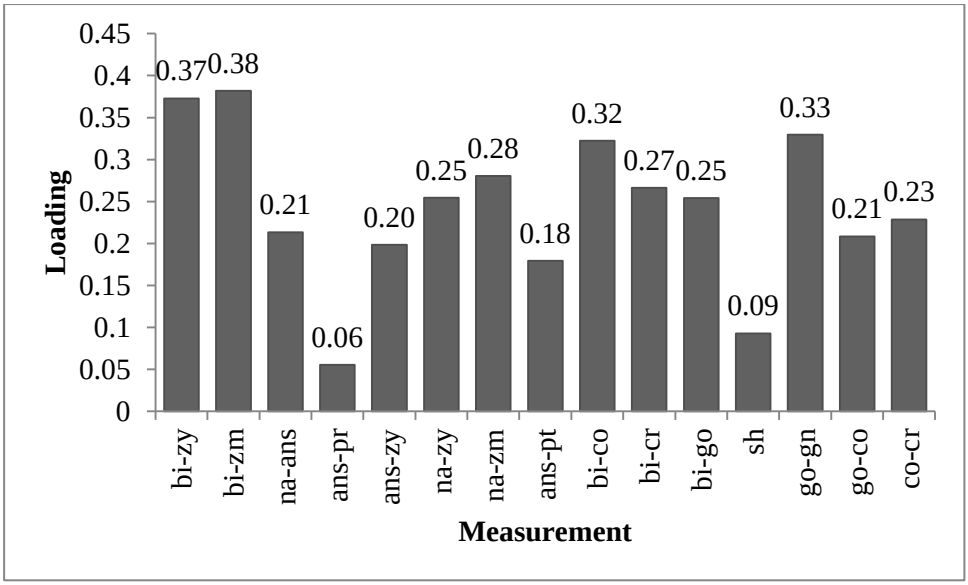


Figure 4.4: Postnatal sample First principal components loading

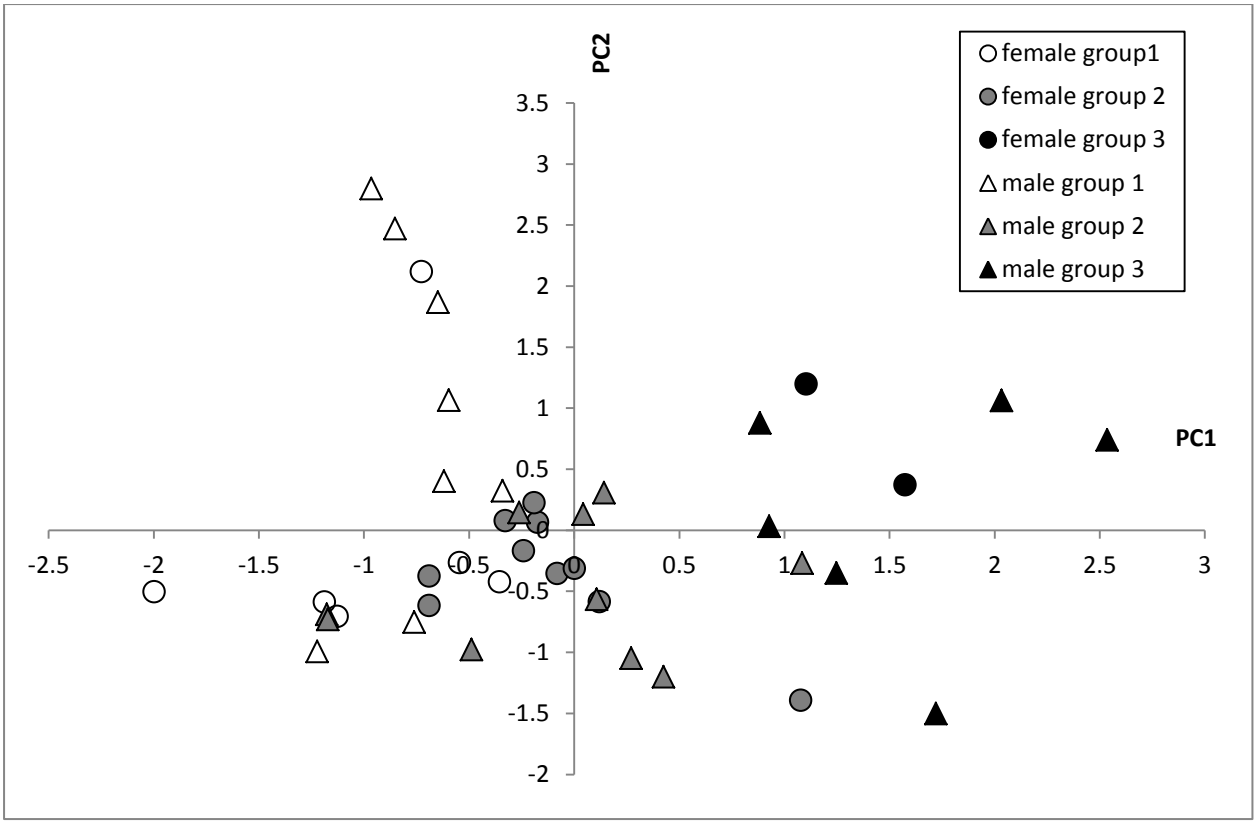


Figure 4.5: Plot of the scores associated with PC1 and PC2 from the PCA of postnatal dataset

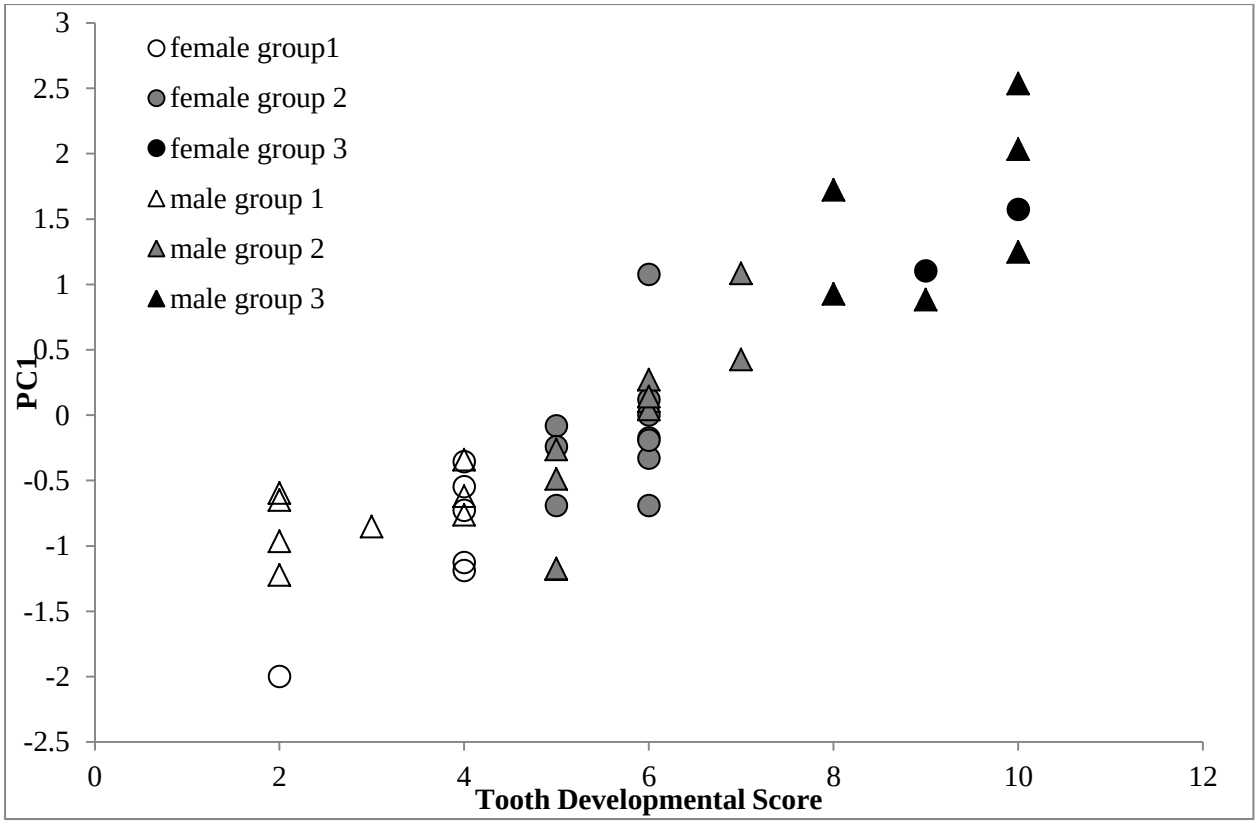


Figure 4.6: Association between PC1 and CRL explaining how PC1 represents size and size-related shape shange in postnatal sample

4.4 Asymmetry Analysis Results

The data obtained for paired variables were rescaled to obtain the d* values which were then subjected to analysis of mean, median and variance.

4.4.1 Directional Asymmetry for Prenatal Group

The arithmetic mean value from the measurements using the one sample *t* test for mean = 0 as detailed in Table 4.6 page 63 shows two variables (mandibular ramus and maxillary length) with a negative value i.e. right mandibular ramus longer than the left and the right maxillary length longer than the left. Four measurements (mandibular length, anterior nasal spine –zygion, nasion-zygion and nasion-zygomaxillare measurements) show a positive mean value i.e. the left side measurements are longer than the right. The arithmetic mean suggests a shift of the maxillary length to the left side of the body with lower border of the mandibular ramus not at the same horizontal plane while the mandibular length, anterior nasal spine-zygion, nasion-zygion, nasion-zygomaxillare shift to the right. However when the deviation from mean of zero was tested for significance using the student *t*-test, none of the variable show any statistical significant shift from symmetry as illustrated in the frequency distribution (histogram) in Figure 4.7 page 64.

Table 4.6: Prenatal directional asymmetry

Measure	Mean	t (p)
ans-zy	0.008	0.915 (0.369)
na-zy	0.017	1.467 (0.154)
na-zm	0.014	1.105 (0.279)
ans-pt	-0.001	0.132 (0.896)
go-gn	0.016	1.340 (0.191)
go-co	-0.006	0.284 (0.779)
co-cr	0.010	0.434 (0.668)

Values in parenthesis = probability

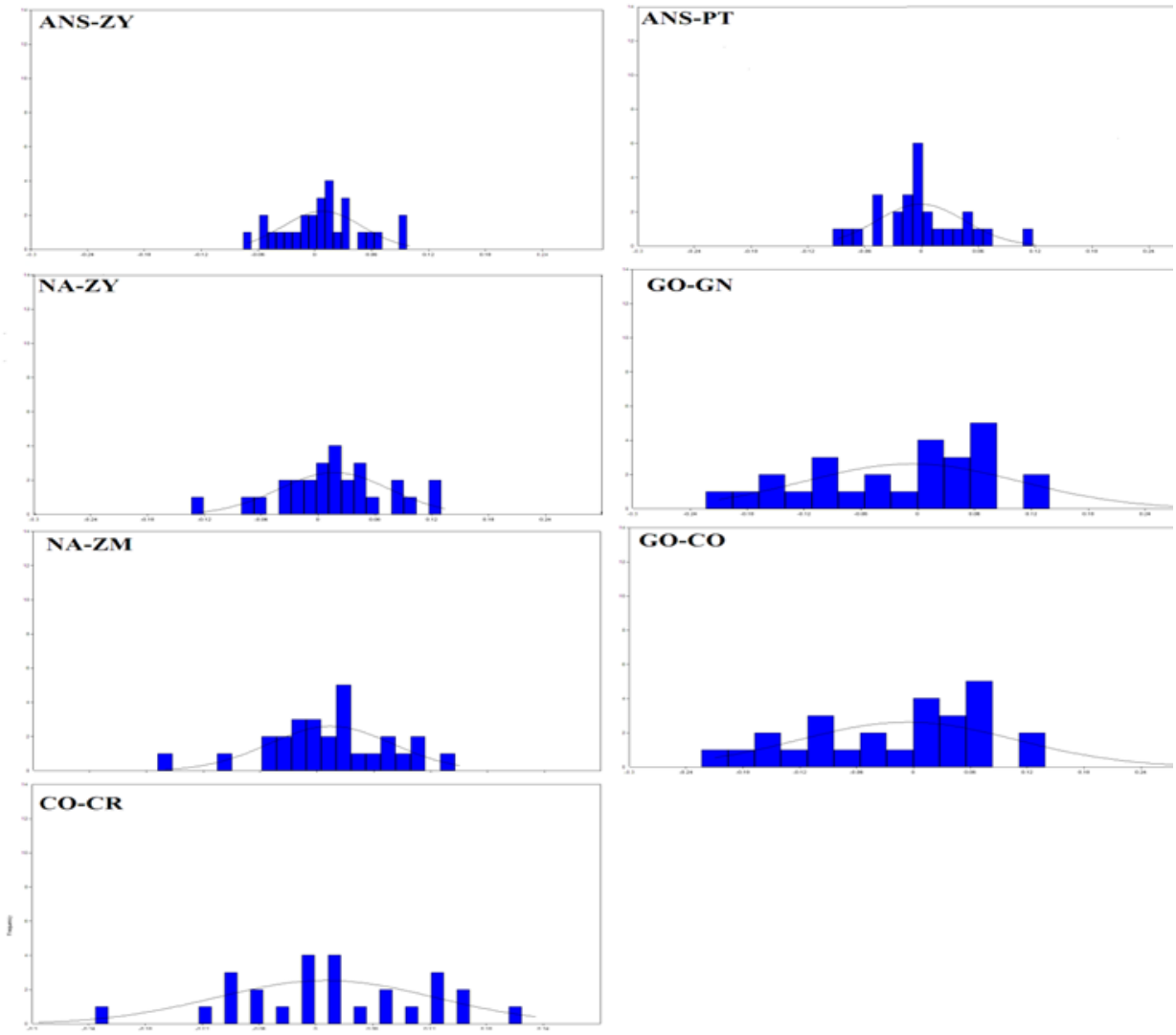


Figure 4.7: Frequency distribution of the prenatal group measurements

4.4.2 Directional Asymmetry for Postnatal Group

Table 4.7 page 66 shows the analysis measurements for a DA using the one sample t test for mean = 0. The arithmetic mean of three of the paired variables namely nasion-zygion, nasion-zygomaxillare, and maxillary length show deviation to the left, while the arithmetic mean of the remaining variables (anterior nasal spine-zygion, condyle-coronoid process, mandibular length and ramus) show a deviation to the right. When the arithmetic mean values were subjected to student's *t*-test for significant deviation from zero, only nasion-zygomaxillare length showed a shift to the left direction i.e. the right side is significantly larger than the left side as shown in the frequency distribution in Figure 4.8 page 67. When the significant value of 0.043 was adjusted for multiple testing using the sequential Bonferroni technique, it was not significant which implies that it may likely be a type I error of multiple testing.

Table 4.7: Postnatal directional asymmetry

Measurement	Mean	t (p)
ans-zy	0.006	0.974 (0.335)
na-zy	-0.008	-1.530 (0.133)
na-zm	-0.008	-2078 (0.301)
ans-pt	-0.009	-2.881 (0.006)
go-gn	0.011	1.290 (0.204)
go-co	0.001	0.052 (0.959)
co-cr	0.009	1.058 (0.297)

Values in parenthesis = probability

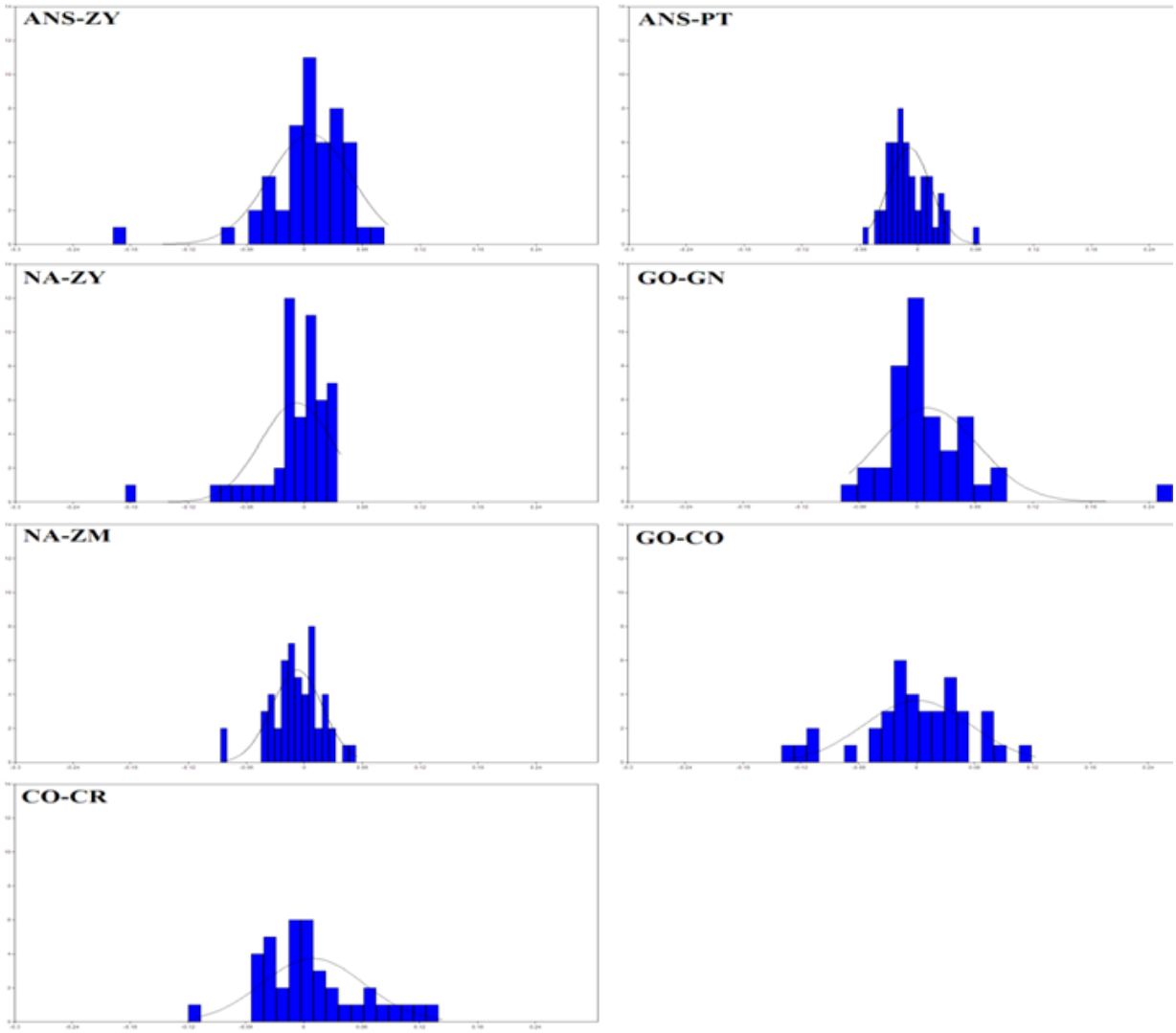


Figure 4.8: Frequency distribution of the postnatal group measurements

4.4.3 Fluctuating Asymmetry

The computed values of the variance is presented as signed variance and the arithmetic median values were rescaled (d*) to obtain absolute values. The signed values are values considered in the same direction while unsigned values indicate the true directional differences in sample size. Levene's (1960) test for homogeneity of variance using the mean (W_0) and median (W_{50}) were performed on the unsigned data. The use of the median was proposed by Brown–Forsythe (1974) as it is more robust for non-normal samples; their names are commonly associated with this form of the Levene's test. The median values (W_{50}) were further adjusting for multiple comparisons using sequential Bonferroni correction ($W_{50}C$) as proposed by Holm's (1979).

4.4.3.1 Fluctuating Asymmetry for Prenatal Group

Table 4.8 page 69 shows the signed variance and unsigned median values. The condyle-coronoid measurement, ramus and body of the mandible show statistically significant level of FA when compared with the maxillary length, the anterior nasal spine-zygion and nasion-zygomaxillare measurements using the Levene's test. However, only the maxillary length and the anterior nasal spine-zygion measurements show statistically significant level of FA when subjected to Brown and Forsythe test as shown in Table 4.9 page 70.

Table 4.8: Prenatal fluctuating asymmetry

Measurement	ans-zy	na-zy	na-zm	ans-pt	go-gn	go-co	co-cr
Signed variance	0.002	0.004	0.004	0.002	0.004	0.014	0.014
Unsigned Median	0.030	0.037	0.030	0.028	0.044	0.072	0.075

Table 4.9: Comparison of fluctuating asymmetry in prenatal group

Measurements		W_0	W_{50}	$W_{50}C$
ans-zy	na-zy	0.205	0.213	0.213
ans-zy	na-zm	0.096	0.102	0.102
ans-zy	ans-pt	0.784	0.785	0.785
ans-zy	go-gn	0.052	0.072	0.072
ans-zy	go-co	0.000*	0.001*	0.021*
ans-zy	co-cr	0.001*	0.001*	0.028*
na-zy	na-zm	0.656	0.663	0.663
na-zy	ans-pt	0.332	0.339	0.339
na-zy	go-gn	0.607	0.658	0.658
na-zy	go-co	0.004*	0.011*	0.193
na-zy	co-cr	0.015*	0.015*	0.244
na-zm	ans-pt	0.169	0.176	0.176
na-zm	go-go	0.987	0.975	0.975
na-zm	go-co	0.012*	0.027*	0.380
na-zm	co-cr	0.035*	0.035*	0.426
ans-pt	go-gn	0.112	0.141	0.141
ans-pt	go-co	0.000*	0.002*	0.034*
ans-pt	co-cr	0.000*	0.003*	0.045*
go-gn	go-co	0.008*	0.021*	0.315
go-gn	co-cr	0.028*	0.028*	0.362
go-co	co-cr	0.828	0.921	0.921

Definitions for the abbreviated measurements are provided in Table 3.1 page 24. W_0 and W_{50} refer to the probabilities obtained using the Levene's (1960) and Brown and Forsythe (1974) tests respectively. $W_{50}C$ is the Brown and Forsythe (1974) probability after sequential Bonferroni correction.

4.4.3.2 Fluctuating Asymmetry for Postnatal Group

Table 4.10 page 72 shows the signed variance and unsigned median values. The ramus of the mandible and the condyle-coronoid measurements show statistically significant level of FA when compared with the maxillary length and the nasion-zygomaxillare distance using the Levene's test as shown in Table 4.11 page 73. However when the median values were adjusted for multiple comparisons, only maxillary length and the anterior nasal spine-zygomaxillare shows significantly level of FA as shown in Table 4.11 page 73. The nasion-zygion measurement level of FA was only statistically significant with Levene's test and not with the Brown and Forsythe test.

Table 4.10: Postnatal fluctuating asymmetry

Measure	ans-zy	na-zy	na-zm	ans-pt	go-gn	go-co	co-cr
Signed variance	0.007	-0.001	-0.006	-0.012	0.001	-0.001	0.002
Unsigned median	0.026	0.016	0.017	0.019	0.024	0.035	0.031

Table 4.11: Comparison of fluctuating asymmetry in postnatal group

Measurements		W_0	W_{50}	$W_{50}C$
go-co	co-cr	0.768	0.674	1.348
go-co	go-gn	0.278	0.225	1.797
go-co	ans-zy	0.061	0.061	0.856
go-co	na-zy	0.009*	0.008*	0.142
go-co	na-zm	0.000*	0.000*	0.002*
go-co	ans-pt	0.000*	0.000*	0.000*
co-cr	go-gn	0.413	0.414	1.656
co-cr	ans-zy	0.115	0.165	1.487
co-cr	na-zy	0.020*	0.033	0. 457
co-cr	na-zm	0.000*	0.001	0.021*
co-cr	ans-pt	0.000*	0.000	0.002*
go-gn	ans-zy	0.565	0.713	0.713
go-gn	na-zy	0.222	0.301	2.104
go-gn	na-zm	0.034*	0.071	0.851
go-gn	ans-pt	0.008*	0.021	0.315
ans-zy	na-zy	0.459	0.419	1.256
ans-zy	na-zm	0.078	0.079	0.864
ans-zy	ans-pt	0.017*	0.016	0.257
na-zy	na-zm	0.330	0.401	2.005
na-zy	ans-pt	0.101	0.133	1.331
na-zm	ans-pt	0.377	0.358	2.148

Definitions for the abbreviated measurements are provided in Table 3.1 page 24. W_0 and W_{50} refer to the probabilities obtained using the Levene's (1960) and Brown and Forsythe (1974) tests respectively. $W_{50}C$ is the Brown and Forsythe (1974) probability after sequential Bonferroni correction.

4.4.4 Comparison of fluctuating asymmetry across samples

Figure 4:9 page 75 shows the differences in FA between the two samples. The mandibular measurements show a higher level of FA in the prenatal sample compared to the postnatal sample. The condyle-coronoid measurement shows the highest level of FA, about four fold levels, and the nasion-zygion measurement (two fold) the lowest, while nasion-zygion measurement did not demonstrate any differences between the two samples.

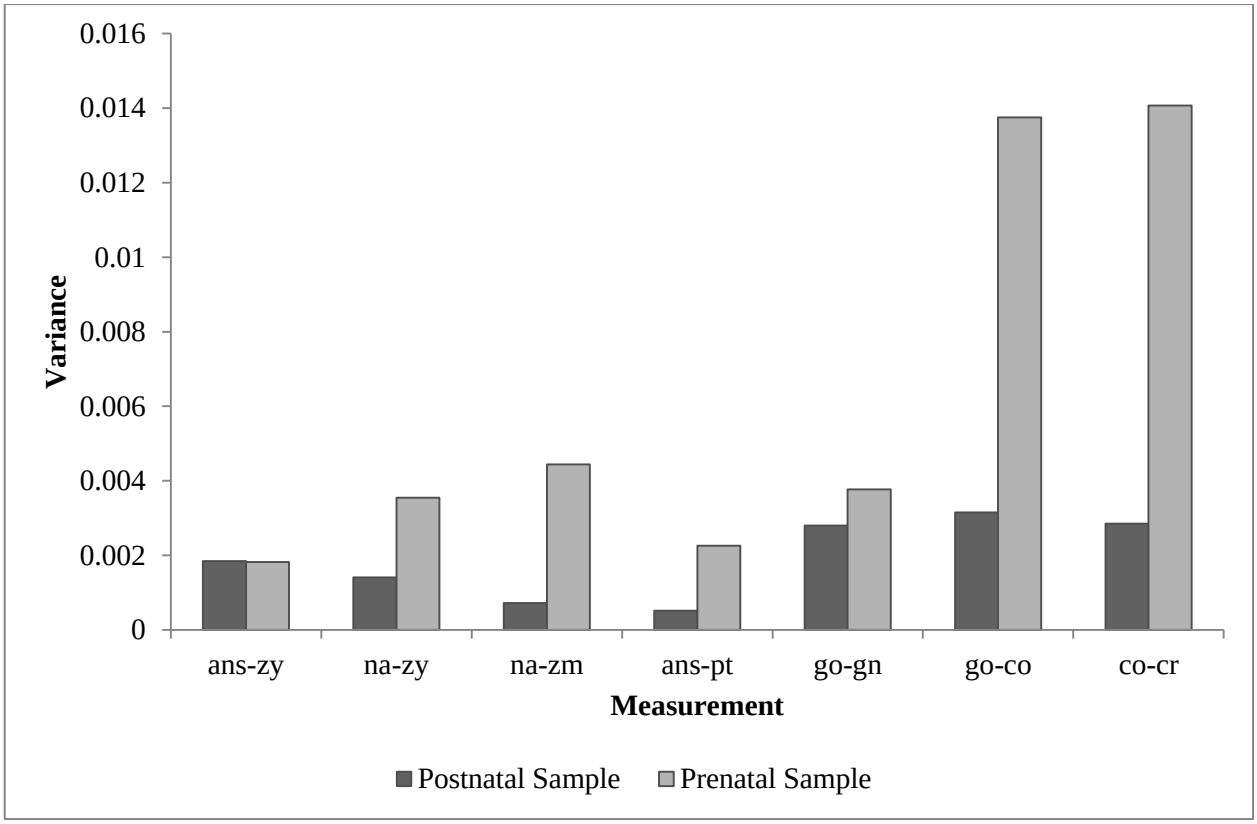


Figure 4.9: Comparison of FA between samples

4.4.5 Correlation between Absolute Asymmetry and developmental stages

The unsigned arithmetic means were subjected to Pearson's correlation coefficient analysis in order to assess whether there is a relationship between the degree of asymmetry and developmental stage. None of the parameters considered in prenatal group show any statistically significant asymmetric changes with development as shown in Table 4.12 page 77. Within the postnatal group, the mandibular ramus and nasion-zygion measurement show a statistically significant asymmetry with development as shown in Table 4.13 page 78. However, when p values were corrected for multiple comparisons, using the sequential Bonferroni method, there were no significance differences between the asymmetry of the various developmental stages. The lack of significance suggests that these values were possibly type I errors.

Table 4.12: Correlation between absolute asymmetry and CRL in the pre-natal sample

Measurement	Test mean	p
ans-zy	-0.027	0.894
na-zy	0.141	0.483
na-zm	0.225	0.258
ans-pt	-0.124	0.539
go-gn	0.081	0.689
go-co	0.155	0.440
co-ro	-0.314	0.110

Table 4.13: Correlation between absolute asymmetry and tooth development stage in the post-natal sample

Variable	Test mean	p	pC
ans-zy	-0.250	0.074	0.370
na-zy	0.290	0.037*	0.222
na-zm	-0.078	0.584	1.000
ans-pt	0.003	0.982	0.982
go-gn	-0.238	0.090	0.360
go-co	-0.295	0.033*	0.231
co-ro	-0.074	0.601	1.000

pC is corrected for by multiple comparisons – showing no significance between any of the asymmetry and developmental stage

4.5 Analysis of Sexual dimorphism during development

The obtained data was grouped according to sex and analysed for differences in growth curves among the sexes.

4.5.1 Prenatal group sexual dimorphism and growth rate

There is a strong correlation between the male and female measurements except for the female nasal septal and mandibular height that show no correlation. There is no difference in the growth rate of the male and female facial measurements demonstrated by the lack of statistically significant difference in the common slope and elevation among the sexes as presented in Table 4.14 page 80.

Table 4.14: Sexual dimorphism and growth rate in Prenatal group

Measurement	Common slope	p	Shift in elevation	p
bi-zy	0.919	0.335	1.915	0.166
bi-zm	1.588	0.204	3.042	0.081
na-ans	-	-	-	-
ans-pr	2.153	0.13	2.055	0.152
ans-zy	2.153	0.13	2.055	0.152
na-zy	1.785	0.167	1.171	0.279
na-zm	3.001	0.079	2.327	0.127
ans-pt	0.148	0.703	0.546	0.460
bi-co	0.04	0.846	1.499	0.221
bi-cr	0.372	0.535	1.006	0.316
bi-go	0.466	0.499	2.068	0.150
Sh	0.825	0.371	0.391	0.532
go-gn	0.423	0.478	0.647	0.421
go-co	-	-	-	-
co-cr	0.181	0.698	0.049	0.825

4.5.2 Postnatal Group Sexual Dimorphism and growth rate

There is a strong correlation between the rate of growth of male and female measurements, except for the female maxillary alveolar process and symphyseal height that show no correlation. Although all the male measurements show elevated shifts, none of these elevated shift in growth translates to a statistically significant faster growth rate except the facial width as presented in Table 4.15 page 82. However, the significance is marginal and when corrected for multiple comparisons, suggests no significance.

Table 4.15: Sexual dimorphism and growth rate in Postnatal group

Measurement	common slope	p	shift in elevation	p
bi-zy	1.560	0.190	4.139	0.042†
bi-zm	0.969	0.317	1.598	0.206
na-ans	1.469	0.231	2.418	0.120
ans-pr	-	-	-	-
ans-zy	0.293	0.574	2.212	0.137
na-zy	0.816	0.370	0.508	0.476
na-zm	0.928	0.337	0.657	0.418
ans-pt	1.967	0.168	0.407	0.523
bi-co	0.942	0.301	0.411	0.521
bi-cr	0.061	0.808	0.003	0.953
bi-go	0.028	0.847	0.579	0.447
sh	-	-	-	-
go-gn	3.692	0.052	2.192	0.139
go-co	1.681	0.207	2.645	0.104
co-cr	0.032	0.861	0.231	0.631

† Not significant when corrected for multiple tests, there is a high likelihood of a type I error

CHAPTER 5: DISCUSSION

5.1 The Research Design

Previous studies used various methods in the assessment of the correlation between the facial dimension and development. It is difficult to make comparisons between these various works because the authors used different anatomical reference points thereby making a direct comparison problematic. Scammon and Callkins (1929) used the largest sample size of 369 specimens, but studied the external features which cannot be compared readily with the radiographic sample of 242 fetuses used by Inoue (1961). The difference in the range of gestational ages of the samples also presents a challenge. For example, Johnston (1962) considered a sample (n=30) with age ranging between 12 and 22 weeks compared with 12 to 27 weeks of the sample (n= 24) used by Burdi (1965). These shortcomings are not unrelated to the difficulties in sourcing the fetuses. In the current study, 27 fetuses could be secured and utilised which made it impossible to analyse the subpopulation within the South Africans of African descent as would have been desirable. The use of CRL as the sole criterion for the ages of the fetal sample became necessary due to missing feet of some of the cadavers; coupled with incomplete cranium that could have been used in the measurement of bi-parietal diameter and inability to determine the weight of the fetuses accurately. The use of tooth eruption sequence as a proxy for postnatal group age is motivated by lack of any sexual differences in the timing of the dental development in childhood until the sixth year (Demirjian and Levesque, 1980).

A substantial number of previous studies including those that were conducted on craniofacial form such as Bareggi *et al* (1995) and Plavcan and German (1995) relied on linear measurements (like in the current study) which are considered good for the estimation of size rather than shape. Shape may be better analysed when angular measurements are integrated.

Data from linear measurements alone will be insufficient and inappropriate when analysing the facial shape; because it is unlike geometric morphometry that considers changes in-between multiple anatomical points simultaneously. Similarly, the inclusion of angular measurements may also not fully describe the facial form. Bareggi *et al.* (1995) pointed out that what is apparent in a direct observation of the craniofacial bone shape contradicts the reported stability in the angular measurements. The attempt to demonstrate changes between anatomical points using coordinate reference grid (histomorphograms) by Trenouth (1984) has its own weaknesses. According to Bareggi *et al.* (1995), there are drawbacks to changes observed in shape studies and these include, but not limited to the following:

- The limited choice of reference points (which must also be distinguishable in young embryos) useful for descriptive purposes;
- The intermediate points not adequately describable by measurement of two anthropometric points, as well as failure of the angular measurements to give exhaustive descriptions and;
- The choice of appropriate statistical formulae to fit the data generated.

Researchers on development of the skull used specially prepared fetal skulls, such as skulls macerated under running water and bleached (Mandarim-de-Lacerda and Alves, 1992; Bareggi *et al.*, 1995) as well as a sagittal section of formalin preserved fetuses (Johnston, 1974). In order to maximise the use of the available cadaveric and skeletal materials for research in the School of Anatomical Sciences, the reference points used in the current study were restricted to external surfaces of the dissected skull. Since the current study is not intended to assess shape changes, the external linear dimensions are appropriate to monitor growth and asymmetry.

5.2 Methods and techniques

5.2.1 Measurements

The parameters that were measured were designed to assess the length, width and height of the face. Although no sagittal or coronal sections were used for any of the measurements in the current study, the use of bi-zygion as a parameter for the facial width is consistent with previous research (Ford, 1956; Houpt, 1970; Plavcan and German, 1995), as well as the bi-gonion measurement for mandibular width (Houpt, 1970; Mandarim-de-Lacerda and Alves, 1992; Malas *et al.*, 2006). The current study also used the gonion-gnathion measurement as a parameter for the length of the mandible as was described by Houpt (1970) and Mandarim-De-Lacerda and Alves (1992). Other studies however used the gnathion-coronoid parameter (Kieser and Groeneveld, 1986; Plavcan and German, 1995) as total length of the mandible.

Unlike the nasion-prosthion used in the current study, the nasion-gnathion parameter has been used previously to represent the facial height (Houpt, 1970), while nasion-anterior nasal spine has also been used as maxillary height (Ford, 1956; Houpt, 1970). The facial height has previously been divided into three components namely nasion-anterior nasal spine (Burdi, 1969), anterior nasal spine-prosthion and symphyseal height measurements. This division was to minimise error variance and achieve consistency with pre and post natal samples. Plavcan and German (1995) used the anterior nasal spine to posterior nasal spine measurement in their study to represent the length of maxilla. Because the samples used in the current study did not have sagittal sections, pterygomaxillary fissure was substituted for the posterior nasal spine for maxillary length. Only studies by Mandarim-De-Lacerda and Alveus (1992) and Plavcan and German (1995) among the literature consulted studied the rate of growth of the condyle-coronoid parameter as was the case with the current study.

The assessment of bi-zygomaxillare, nasion-zygomaxillare, nasion-zygion and nasion-zygomaxillare are novel measures regarding studies assessing the relative rate of growth

among the craniofacial bones as far as literature search is concerned. These additional parameters added value to the overall assessment of relative rate of growth. In addition, the zygomaxillare as a reference point is easier to measure than the zygion which is fraught with a higher degree of measurement error, which is particularly important regarding the assessment of asymmetry of the maxilla. The lateral and vertical growth rate on both sides of the maxilla is expected to be similar, since both are governed by similar genetic influence.

5.2.2 Repeatability of Measurements

It has been recommended that Physical Anthropologist should ensure that accurate readings are obtained and the level of reproducibility of the measured variables should be documented in a manner that can be understood by other researchers (Cameron, 1984) Various simple test of reliability such as Pearson's (Malas *et al.*, 2006), Lin's correlation coefficient (Lin, 1989), the paired *t*-test (Captier *et al.*, 2006), interclass correlation and technical error of measurement (Cameron, 1984) have been used in the past. The current study used the concordance correlation coefficient of Lin (1989).

It was chosen for its ability not to merely correlate but to elicit the differences between the mean and standard deviation of the test and retest, thereby ensuring accuracy and precision of measurements. Three of the prenatal measurements (Lgo-co, Lna-zn and Rgo-gn) exhibit P*c* values of 1.00 and the remaining measurements exhibit P*c* values of 0.99 as shown in Table 4.1 page 45. As for the postnatal sample, P*c* value for symphyseal height is 0.98 and the right and left condyle-coronoid is 0.96 while the remaining parameters exhibited P*c* values of 0.99.these marginal differences in the P*c* values may be related to post-mortem changes that affected precision of anatomical points. Nonetheless, the P*c* values obtained demonstrated that measurements taken have same degree of accuracy each time the measurement is taken on each of the sample. These P*c* values fall within advocated range (Lin, 1989; Cameron,

1984), It is therefore reasonable to argue that the current study will compare favourably well with any other similar work.

5.2.3 Statistical methods

The first consideration in the studies of ontogenetic allometry is the statistical model to fit the data generated. The use of linear and non-linear models have been suggested and debated amongst researchers (Mandarim-de-Lacerda and Alves, 1992). It is generally agreed that for any model that fits the data adequately, the resulting regression coefficients (Mandarim-de-Lacerda and Alves, 1992) or correlation (Johnston, 1974) for dependent variables must be easily comparable. In the current study, the bivariate regression equation used data that were transformed using the natural logarithm prior to model fitting to examine the relationship between x and y. Logarithm is known to allow multiplicative properties of the data generated and ease slope comparison among variables. The method of least-squares was favoured by earlier researchers, such as, Ford (1956), Burdi (1965) and Johnston (1974). The realisation that comparison of relative changes of linear measurement against each other using the least-squares gives distinctly different slopes has made SMA the new technique of choice (Plavcan and German, 1995). Although with increasing correlation, the differences between RMA and least-squares slope decreases (Bareggi *et al.*, 1995). The current study needs to account for the error variance in both the dependent and independent variables and the objective involved comparison as opposed to prediction, hence the use of SMA as applied by Mandarim-De-Lacerda and Alves (1992) and Plavcan and German (1995), although Smith (2009) has criticised the overuse of SMA against ordinary least square by some investigators.

As far as a linear model is concerned it is immaterial whether the independent variable is the CRL or stage of tooth eruption. It assumes that the rate of growth of the y variable relative to the x variable as measured by the coefficient b is constant. More so, that the relative

developmental rates between measurements were of concern in the current study and not their actual temporal rates.

The SMATR package used for the regression analysis is adequate to generate reliable output. The slopes are therefore useful tools that determine specific growth rates of each dimension measured. A slope of one indicates a geometrical similarity with increase in dimension amongst the bony components and illustrates an isometric growth. The PCA provided the opportunity to compare the growth between the two samples, bearing in mind that CRL and tooth development scores were used as proxies for the ages.

The asymmetry technique used in the current study analysed each paired parameter as an independent variable unlike in the study by Captier *et al*. (2006) that considered absolute differences. The use of d^* ensures that the effect of individual size differences is eliminated.

5.3 Results

The major finding of the current study is that the facial growth from late trimester to mixed dentition stage of development was characterised by decreasing level of FA. The asymmetry observed in the sample was due to increasing random differences rather than preferential development of one side of the face over the other. In addition, PC1 accounted for over 80% of the variation in both samples showing a greater significance in the first components than the others. All the variables have positive loadings with PC1 and show a continuous trend in facial form. Furthermore, there is a strong association between facial dimensions and the observed development.

Majority of the previous studies on the quantitative growth trends in the prenatal period have correlated facial dimensions with CRL (Burdi, 1969; Bareggi *et al.*, 1995), with length of the spinal column C_1 - L_5 (Trenouth, 1985), with birth weight (Mandarim-de-Lacerda and Alves, 1992), and with head length (Johnston, 1974). Studies on postnatal growth trends have however referenced postcranial skeleton against individuals with known ages at death (Sardi

et al., 2007) or used tooth development as reference (Klepinger, 2006; Smith, 1988; Demirjian and Levesque, 1980), as was the case in the current study.

All the studies agreed that there is a correlation between facial dimensions and time irrespective of the choice of the index of time. In the current study, the mandibular width shows the strongest correlation with the CRL but the association progressively reduced with eruption of teeth; probably because of the level of accuracy of the proxy used. As the temporal lobes reach maturity, the lateral expansion of the skull diminishes so is the bi-gonion distance i.e. the mandibular width (Table 4.2 page 47).

The slope of the regression is a measure of rate of growth (Ford, 1956). The higher the value of the slope, the higher the rate of growth; this demonstrates a positive allometry. The observation made by Ford (1956), 'that the region that developed earlier grew slower than that which developed thereafter' was confirmed with the slope for the ramus being higher than the body of the mandible (Table 4.4 page 51). It should be emphasised that the ossification of the mandible begins at the region of the bifurcation of the inferior alveolar nerve, the position of the definitive mental foramen, and proceeds forward and backward. The secondary condylar cartilage develops subsequently and acts as a growth centre to sustain the mandibular growth in a forward and downward direction. As the condylar cartilage increasingly contributes to the anteroposterior growth of the mandible in the molar region, the growth around the canine region diminishes in tandem.

5.3.1 Development of the Face

The face is regarded as being relatively underdeveloped at birth (Sperber, 1989). The current study demonstrated a progressive increase in the dimensions of the lower two-third of the face from prenatal to postnatal period as shown in the PCA loading for fetal and skeletal samples growth pattern (Figure 4.1 page 53 and Figure 4.4 page 59). The precocious development of the brain fuels the rapidly expanding calvarium ahead of the other

components of the skull until after two years (Sperber, 1989). The slowdown in the growth rate of neural cranial components and the unhindered growth of the facial skeleton change the comparatively underdeveloped face. The facial complex then begins an intensive change throughout childhood. The bi-coronoid distance as a good measure of growth of the neural cranial component; maintain average similar growth ratio with the mandibular width from the third trimester to mixed dentition stage.

These changes according to Standring *et al*. (2005) are partly in response to the development of alveolar processes needed for eruption of the deciduous teeth, as well as the activities of the muscles of mastication including a push effect of the developing tongue and formation of sinuses.

These changes are reflected in the increasing magnitude in the elongation of the maxilla and the mandible as represented by the mandibular and maxillary length measurements. The mandibular length increased from average of 2.94 cm in the prenatal group to 3.67 cm in the postnatal group while the maxillary length increased by double fold (Table 4.2 page 47 and Table 4.3 page 49).The upper third is the first to achieve its growth potentials and ceases growth while the lower two third responds to the base of skull and grows till adolescence.

5.3.2 Facial Dimensions

The current study established facial dimensions from the 26^{th} to 40^{th} week of gestation and from birth to the sixth year of childhood. Most of the variables show a strong correlation with overall growth, notable exceptions are the prenatal mandibular ramus and the nasal septum (Table 4.4 page 51). The exhibited low correlation may be related to the initial rapid increase of the nasal septum (Ford, 1956) and the influence of the condylar cartilage, a secondary growth centre, on the mandibular ramus. Similarly the facial width showed a stronger correlation with growth than bi-zygomaxillare (Table 4.6 page 63), this may be due to continuous shifting position of the zygomaticomaxillary suture (Enlow, 1968). The

correlation coefficients for mandibular dimensions are higher than those for maxilla in both sample and therefore may be better predictors of age. The average height of the lower two thirds of the face of the fetuses in the current study is one and half times smaller than the facial width, while the body of the mandible is on average slightly longer than the maxillary length and the mandibular width lesser than the facial width by a small margin (Table 4.2 page 47). The average facial height and width of the fetuses in the current sample are generally lower, as expected, than the Asian values obtained by Malas *et al*. (2006), the standard deviation from the mean been similar for the two samples. Within the postnatal group, the differences between facial width and lower two third dimensions, as well as body of mandible and maxillary length narrowed down, while the relationship between the facial and mandibular width on average remain the same (Table 4.3 page 49). These reported changes in the postnatal sample parameters of the current study compared favourably well with the Miotti *et al.,* (1983) sample.

5.3.3 Sexual Dimorphism

Only few of the studies on pre and postnatal facial growth have analysed data on whether there existed a significant difference between the linear growth models for males and females (Houpt, 1970; Malas *et al.,* 2006). None of these studies have reported any significant differences in growth rate among the sexes. The current study demonstrates existence of a strong correlation between each of the facial measurements and relative developmental stage within each of the sexes. However, some parameters did not demonstrate correlation within the sexes such as nasal septum and mandibular height within the prenatal sample (Table 4.14 page 80) and maxillary alveolar process and symphysis menti in the postnatal sample (Table 4.15 page 82). Nonetheless only the post natal facial width shows a significant higher growth rate in male than female, but loses statistical significance after Bonferroni correction (Table 4.15 page 82). De Villiers (1968) considered the shape of the chin and zygomatic arch as

parameters to differentiate adult South Africans sexually; while Kieser and Groeneveld (1986) used the maximum length of the mandible. The rate of growth of the samples used in the current study did not support these two findings and Robinson (2007) cautioned against the use of the mandibular ramus for sex differentiation because of its doubtful accuracy and lack of consensus among researchers. The current study supported the argument by Scammon and Calkins (1929) that there are no major differences in the facial development until puberty given that this sample extends till first permanent molar eruption stage. Standring *et al.* (2005) also suggested that the variation observed in a population is greater within than between sexes; this assertion cannot however be tested with the small sample size used in the current study. The current study observed a generalised elevation in the slopes of the male parameters over the female similar to the findings of Houpt (1970) and Malas *et al*. (2006). This is suggestive of male parameters growing ahead of female; except that all the noted shifts were not statistically significant.

This may however be the beginning of the noticeable differences between adult male and female physical features. Male generally has a propensity to be larger than the female in a number of features such as a large and rugged skull (Standring *et al.*, 2005; Robinson, 2007), these features are secondary sexual characteristics brought about by changes in hormonal levels during and after puberty. The sample used in the current study is too early for such hormonal changes and no differences were observed between the sexes.

5.3.4 Facial Asymmetry

The facial bones demonstrated similar dimensions on both sides for the pre and postnatal samples. None of the parameters returned absolute zero for mean as it is expected of a biological growth, the randomness in development is a biological phenomenon irrespective of whether the organism possesses the most ideally balanced and structured genome for the environment that it occupies (Lens *et al.*, 2002).

It is only in the post natal group that the maxillary length and nasion zygion show relative asymmetry with a directional shift to the left. The presence of relative facial asymmetry in only the postnatal group attests to diminishing buffering ability or increased environmental stressors. Population levels of bilateral asymmetry have been shown to relate positively to a wide range of parasitic and genetic stresses (Palmer and Strobeck, 1992) couple with the inability of individuals to buffer their development against small, random perturbations of cellular processes. With the FA present in same region of the face, decreasing in level from prenatal to postnatal period, it is suggestive of an environmental influence. Previously, environmental stress was found to increase significantly the number of developmentally-less-stable persons in a community, without preference for either sex (Kieser and Groeneveld, 1988). As recent as 2010, Özener and Fink (2010) noticed a remarkable difference in the level of FA among high School Students living in the slum and affluent areas of Ankara in Turkey. The higher level of FA observed in the prenatal group may be as a result of contortion in the uterus (Kellner and Alford, 2003),couple with the reflex activities of the temporomandibular joint (Sperber, 1989) or alternatively, correction of abnormal growth deviation during fetal period (Kellner and Alford, 2003) that are beyond developmental haemostasis (Waddington, 1942; 1957) provided by the mother.

5.3.5 Rate of Growth

In the fetal maxilla, the nasal septum shows the highest positive allometry, this rate reduces drastically in the postnatal sample as suggested by Ford (1956). However, the coronoid-condyle measurement shows highest rate of growth that is isometry contrary to the findings of Mandarim-De-Lacerda and Alves (1992) whose fetal samples did not demonstrate any mandibular dimension with isometric growth. The coronoid-condyle measurement continued similar rate of growth in the postnatal sample, although all the parameters demonstrate isometric growth in postnatal sample.

The samples assessed are in the period where growth is associated with essentially re-proportioning of body components. Most of the parameters show similar trend in the rate of growth in relation to height, length and width of the face between the prenatal and postnatal samples. However the facial parameters regarding length, height and width grow differently. Facial height is growing faster than length and the length faster than the width.

5.4 Secular trends on Facial Complex Growth

Changes in external features of individuals over time in a given population have been attributed to environmental factors. Zong *et al.* (2011) reported a rapid positive secular trend in the height of Chinese children aged between six and seven years over a period of three decades. The height of the children increased by a mean of 5.00 and 5.30 cm over the specified period among boys and girls respectively (Zong *et al.*, 2011). The South African population of African descent could also be affected by environmental influences. Gene flow between population and genetic drift determine the physiological responses and ability of individual members of the population to withstand differences in the local climatic conditions, such as altitude and the availability of suitable infrastructures. Balanced diet and a healthy environment also have greater influence. Pawson *et al*., (2001) demonstrated that the adverse effects of weather and a high altitude on skeletal development can be ameliorated with improved infrastructure and diet in childhood. Inadequate nutrition during pregnancy and early childhood diseases will affect not only growth but the eventual adult size.

The differences in the level of FA among the groups could be stress related. The low level of postnatal FA is not preserved as reported in the odontometric study by Kieser and Groenveld (1988), and generally increases with age. However, this study is not directly comparable because of different parameters and variables considered. Nevertheless, decrease in buffering ability of the population caused by migration to urban centres and urbanisation (Kieser and Groeneveld, 1988) in a politically unstable environment of the then South Africa could

explain the changes. In addition, the associated consumption of new and inadequate diet types also leads to dietary and parasitic stress (Wyndham and Irwig, 1979). This obviously contributes to the increased number of developmentally-less-stable individuals in later generations.

CHAPTER 6: CONCLUSION

The development of the face in South African of African descent was studied using a sample of 27 fetuses (12 female and 15 male) and 44 skeletal skulls (17 female and 27 male). The samples were part of the collections at the School of Anatomical Sciences of the University of the Witwatersrand. The fetuses CRL ranged between 24.3cm and 40.7cm while the skeletal skull sample was a postnatal group up to the period of mixed dentition. The growth was studied using changes in the facial dimensions. 15 defined anatomical landmarks were used for the study; seven of the anthropometrical points were bilateral and were used to determine the symmetry of the maxilla and mandible. The degree of accuracy of the repeated measurements was satisfactory based on the range of the P_c values (0.96 to 1.00) of concordance correlation coefficient (Cameron, 1984; Lin, 1989).

The study showed a progressive trend in the ontogeny of the dimension of the face from the last trimester to the mixed dentition stage. The trend of these dimensions are similar to findings of previous studies (Malas *et al.*, 2006; Miotti *et al.*, 1983). The facial dimensions correlate with CRL and the sequence of eruption. The nasal septum grew almost two times the facial width throughout the period considered. The maxillary and mandibular length grew initially at similar rate before an increase of about one and a half in the rate of mandibular growth. The growth curves for the male group were generally higher than the female; however, there was no statistically significant sexual dimorphism in growth of any of the measurements.

The facial dimensions of the current sample were found not to show directional asymmetry. However the condyle-coronoid region of the mandible, maxillary length and the zygion-zygomaxillare region of the maxilla displayed statistically significant greater fluctuating asymmetry than the rest of the face. In addition, there is decrease in level of fluctuating

asymmetry from prenatal group to postnatal group. The level observed in this current study may probably be lesser than the general high level of odontometric asymmetry documented by Kieser and Goeneveld (1988).

It can be argued that the level of FA of the postnatal sample compared to fetal sample used in the current study is due to correction of the contortion *intra uterine,* as well as correction of ontogenetic pathway (Kellner and Alford, 2003). The reversal of the low level of FA achieved in postnatal stage in a teenage group as reported in odontometric analysis need investigation. Research with a larger sample, using a current sample of the South Africans of African descent will shed light on the environmental effects on the population.

6.1 Recommendation

It is recommended that a follow up study be conducted on more recent Skeletal Collections containing samples of Southern Africans of African descent to establish if there is any secular trend or differences in the level of fluctuation asymmetry. In addition, a comparative analysis of different population groups, sexes and subpopulation will be desirable. Since there is no sufficient collection in existence at the moment within the Southern African region, it will be recommended that all Departments or Schools of Anatomy within the Southern African region can commence a robust fetal cadaver collection programme, to study trends, detailed fluctuating asymmetry analyses and any differences in facial morphometry within the region.

REFERENCES

Allan, J. C. (1982) *Learning about statistics* Johannesburg, Macmillan South Africa (Pty) Publishers.

Altemus, L. A. (1959) Frequency of the incidence of malocclusion in American Negro Children aged twelve to sixteen. *Angle orthod,* 53, 189 – 200.

Altemus, L. A. (1960) A comparison of cephalofacial relationships. *Angle orthod,* 30, 223 – 240.

Ari, I., Kafa, I. M., Basar, Z. & Kurt, M. (2005) The Localization and Anthropometry of Mental Foramen on Late Byzantine Mandibles *Coll Antropol* 29, 233-236.

Bareggi, R., Sandrucci, M. A., Baldini, G., Grill, V., Zweyer, M. & Narducci, P. (1995) Mandibular growth rates in human foetal development. *Archs Oral Biol,* 40, 119-125.

Barter, M. A., Evans, W. G., Smit, G. L. & P.G, B. (1995) Cephalometric analysis of a Sotho-Tswana group. *J. Dent Assoc SA,* 50, 539-544.

Beals, S. (1986) Development and growth of the normal face. *BNI Ouarterly,* 2, 09-16.

Birch, R. H. (1968) Foetal retrognathia and the cranial base. *Angle Orthodont,* 38, 231-235.

Bjork, A. (1969) Prediction of mandibular growth rotation. *Am J Orthod,* 55, 589-599.

Brown, C. R. & Brown, M. B. (1998) Intense natural selection on body size and wing and tail asymmetry in cliff swallows during severe weather. *Evolution* 52, 1461–1475.

Brown, M. B. & Forsythe, A. B. (1974) Robust Tests for the Equality of Variances. *J Am Stat Asso,* 69 364-367.

Bull, M. J., Givan, D. C. & Sadove, A. M. (1990) Improved outcome in Pierre Robin syndrome sequence: effect of multidisciplinary evaluation and management. *Pediatrics,* 86, 294 – 301.

Burdi, A. R. (1965) Sagittal growth of the nasomaxillary complex during the second trimester of human prenetal development. *J Dent Res,* 44, 112-125.

Burdi, A. R. (1969) Cephalometric growth analyses of the human upper face region during the last two trimester of gestation. *Am J Anat,* 125, 113-122.

Cameron, N. (1984) *The measurement of human growth* London, Croom Helm.

Captier, G., Leboucq, N., Bigorre, M., Canovas, F., Bonnel, A., Bonnafe, A. & Montoya, P. (2003) Plagiocephaly: morphometry of skull base asymmetry. *Surg Radiol Anat,* 25, 226-233.

Captier, G., Lethuilier, J., Oussaid, M., Canovas, F. & Bonnel, F. (2006) Neural symmetry and functional asymmetry of the mandible. *Surg Radiol Anat,* 28, 379-386.

Dayal, M. R., Kegley, A. D. T., Strkalj, G., Bidmos, M. A. & Kuykendall, K. L. (2009) The history and composition of the Raymond A. Dart Collection of Human Skeletons at the University of the Witwatersrand, Johannesburg, South Africa. *Am J Phys Anthropol,* 140, 320–335.

De Freitas, L. M. A., De Freitas, K. M. S., Pinzan, A., Janson, G. & De Freitas, M. R. (2010) A comparison of skeletal, dentoalveolar and soft tissue characteristics in white and black brazilian subjects. *J Appl Oral Sci,* 18, 135-42.

De Villiers, H. (1968) Sexual dimorphism of the skull of the South African Bantu-speaking Negro. *S Afr J Sci,* 64, 118-124.

Demirjian, A. & Levesque, G. Y. (1980) Sexual Differences in Dental Development and Prediction of Emergence. *J Dent Res* 59 1110-1122.

Diewert, V. M. (1985) Development of human craniofacial morphology during the late embryonic and early foetal periods. *Am J Orthod,* 88, 64-75.

Enlow, D. H. (1968) *The human face: an account of the postnatal growth and development in cranionfacial skeleton.* New York, Harper & Row.

Falster, D. S., Warton, D. I. & Wright, I. J. (2006) *SMATR: Standardised major axis tests and routines.* Version 2.0. Accessed on 13/07/2011. Available at http://www.bio.mq.edu.au/ecology/SMATR

Farkas, L. G., Katic, M. J., Forrest, C. R., Alt, K. W., Bagic, I. & Baltadjiev, G. (2005) International anthropometric study of facial morphology in various ethnic groups/races. *J Craniofac Surg* 16, 615-46.

Flynn, T. R., Ambrogio, R. I. & Ziecner, S. J. (1989) Cephalometric norms for orthognatic surgery in black American adults. *J Oral Maxillofac Surg,* 47, 30-38.

Ford, H. E. R. (1956) The growth of the foetal skull. *Am J Anat,* 90, 63-72.

Franklin, D., Freedman, L. & Milne, N. (2005) Three-dimensional technology for linear morphological studies: a re-examination of cranial variation in four southern African indigenous populations *HOMO,* 56, 17-34.

Graham, J. H., Raz, S., Hel-O, H. & Nevo, E. (2010) Fluctuating Asymmetry: Methods, Theory, and Applications. *Symmetry* 2, 466-540.

Hammer, Ø., Harper, D. a. T. & Ryan, P. D. (2001) *PAST: Paleontological Statistics Software Package for Education and Data Analysis.* Version 4.1. Accessed on 15/09/2011. Available at http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Hammond-Tooke, W. D. (2000) 'Ethnicity' and 'Ethnic Group' in Iron Age South Africa. *S Afr J Sci,* 96, 421–422.

Harris, E. F. & Nweeia, M. (1980) Dental asymmetry as a measure of environmental stressing in the Tucana Indians of Columbia. *Am J Phys Anthropol,* 53, 133-142.

Hartsfield, J. K., Jr, Morford, L. A. & Otero, L. M. (2012) *Orthodontics.* IN Bourzgui, F. (Ed.) *Genetic factors affecting facial growth.* InTech.

Holm, S. (1979) A simple sequentially rejective multiple test procedure. *Scandinavian J Stat,* 6, 65-70.

Houpt, M. I. (1970) Growth of the craniofacial complex of the human foetus. *Am J Orthod,* 58, 373-383.

Hutchinson, E. F., L'abbe, E. N. & Oettle, A. C. (2011) *An assessment of growth and sex from mandibles of cadaver foetuses and newborns*. *39th Annual Conference of the Anatomical Society of Southern Africa.* Johannesburg.

Inoue, N. (1961) A study of the developmental changes of dentofacial complex during foetal period by means of roentgenographic cephalometrics. *Tokyo Med Dent Uni Bull,* 8, 205-227.

Jefferson, Y. (1996) Skeletal types: key to unraveling the mystery of facial beauty and its biologic significance. *J Gen Orthod* 7, 7-25.

Jenkins, T., Zoutendyk, A. & Steinberg, A. G. (1970) Gammaglobulin groups (Gm and Inv) of various southernAfrican populations *Am J Phys Anthrop* 32, 197–218.

Johnston, L. E. (1962) *Sagittal growth changes in the foetal face; 12 to 22 weeks*. Mimeographed Material

Johnston, L. E. (1974) A cephalometric investigation of the sagittal growth of the second trimester foetal face. *Anat Re,* 178, 623-630.

Jones, J. S. (1987) An asymmetrical view of fitness. *Nature,* 325, 298-299.

Kane, A. A., Lo, L., Christensen, G. E., Vannier, M. W. & Marsh, J. L. (1997) Relationship between Bone and Muscle of Mastication in Hemifacial Microsomia. *Plast Reconstr Surg,* 99, 990 – 997.

Kegley, A. D. T. & Hemingway, J. (2007) Assessing fluctuating odontometric asymmetry among fossil hominin taxa through alternative measures of central tendency: Effect of outliers and directional components on reported results. *HOMO—J of Comparative Human Biol* 58, 33–52.

Kellner, J. R. & Alford, R. A. (2003) The Ontogeny of Fluctuating Asymmetry. *Am Nat,* 161, 931-947.

Kieser, J. A. & Groeneveld, H. T. (1986) Multivariate Sexing of the Human Visceocranium. *The Journal of Forensic Odonto-Stomatology,* 4, 41-46.

Kieser, J. A. & Groeneveld, H. T. (1988) Fluctuating Odontometric Asymmetry in an Urban South African Black Population. *J Dent Res* 67, 1200-1205

Klepinger, L. L. (2006) *Fundamentals of Forensic Anthropology.* New Jersey, John Wiley & Sons.

Knußmann, R. (Ed.) (1988) *Wesen und Methoden der Anthropologie Fischer*, Stuttgart.

Lens, L., Van Dongen, S., Kark, S. & Matthysen, E. (2002) Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? *Biol. Rev,* 77, 27-38.

Levene, H. (1960) *Robust Tests for Equality of Variances*. IN Olkin, I. (Ed.) *Probability and Statistics* Palo Alto, Califonia, Stanford University Press.

Levihn, W. C. (1967) A cephalometric roentgenographic cross-sectional study of the craniofacial complex in foetus from 12 weeks to birth *Am J orthod* 53, 822–848

Lewis, A. B. & Garn, S. M. (1960) The relationship between tooth formation and other maturational factors *Angle Orthod,* 30, 70-77.

Lin, L. I. (1989) A Concordance Correlation Coefficient to Evaluate Reproducibility *Biometrics,* 45, 225–268.

Liversidge, H. M. & Molleson, T. (2004) Variation in Crown and Root Formation and Eruption of Human Deciduous Teeth. *Am. J. Phys Anthropol,* 123, 172–180.

Malas, M. A., Üngor, B., Tagil, S. M. & Sulak, O. (2006) Determination of dimensions and angles of mandible in the foetal period *Surg Radiol Anat,* 28, 364–371.

Mandarim-De-Lacerda, C. A. & Alves, M. U. (1992) Human mandibular prenatal growth: bivariante and multivariante growth allometry comparing different mandibular dimensions *Anat Embryol* 186, 537–541.

Mcnamara, J. A. (1972) *Neuromuscular and skeletal adaptations to altered orofacial function* The University of Michigan, Ann Arbor.

Meastre, J. C. (1959) A cephalometric appraisal of cranial and facial relationships at various stages of human feotal development. *Am J Orthodont,* 45, 473.

Miotti, F., Miotti, A. & Drusini, A. (1983) Sagittal dimensions of the anterior cranialbase, maxilla and mandibular body in newborn male infants. *Euro J Orthod,* 5, 245-248.

Møller, A. P. & Manning, J. (2003) Growth and developmental instability. *The Veterinary Journal* 166 19–27.

Moore, K. L. (1992) *Clinically oriented Anatomy.* 3rd ed. Baltimore, Williams and Wilkins.

Moore, K. L. & Persaud, T. V. N. (1993) *The developing human* 5th ed. Philadelphia, WB Saunders Company

Moore, K. L. & Persaud, T. V. N. (1999) *Clinically Oriented Anatomy.* 5th ed. Philadelphia, WB Saunders Company.

Moss, M. L. & Salentijn, L. (1969) The primary role of functional matrices in facial growth. *Am J Orthod,* 55, 566-577.

Naidoo, L. C. & Miles, L. P. (1997) An evaluation of the mean cephalometric values for orthognathic surgery for black South African adults. Part 1: Hard tissue. *J Dent Assoc S Afr,* Jul 52, 495-502.

Newman, J. L. (1995) *The Peopling of Africa: A Geographic Interpretation.* London, Yale University Press.

Nurse, G. T., Weiner, J. S. & Jenkins, T. (1985) *The peoples of southern Africa and their affinities. Research Monographs on Population Biology*. Oxford, Clarendon Press.

Oladipo, G. S., Esomonu, C. & Osogba, I. G. (2010) Craniofacial Dimensions of Ijaw Children and Adolescents in Nigeria. *Biomedicine International* 1, 25-29.

Ortiz, M. H. & Brodie, A. G. (1949) On the growth of the human head from birth to the third month of life *Anat Rec* 103, 311 – 33.

Özener, B. & Fink, B. (2010) Facial symmetry in young girls and boys from a slum and a control area of Ankara, Turkey. *Evol Hum Behav*, 31, 436-441.

Palmer, R. A. & Strobeck, C. (1992) Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. *Acta Zool. Fennica*, 191, 57-72.

Parsons, P. A. (1992) Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity*, 68, 361-4.

Pawson, I. G., Huicho, L., Muro, M. & Pacheco, A. (2001) Growth of children in two economically diverse Peruvian high-altitude communities. *Am J Hum Biol* 13, 323-40.

Petrovic, A. & Strutzman, J. (1972) Le muscle pterygoidien externe et la croissance du condyle mandibulaire. Recherches experimentales chez le jeune rat. *Orthodontie francaise*, 43, 271-285.

Plavcan, J. M. & German, R. Z. (1995) Quantitative Evaluation of Craniofacial Growth in the Third Trimester Human *Cleft Palate Caniofac J*, 32, 393 – 404.

Proffit, W. R. (2000) *Contemporary Orthodontics* 3rd ed. St Louis, Mosby year book.

Ribot, I., Morris, A. G., Sealy, J. & Maggs, T. (2010) Population history and economic change in the last 2000 years in KwaZulu-Natal, RSA. *Southern African Humanities*, 22, 89-112.

Robinson, M. (2007) *Assessment of the Reliability of Metrical Methods of Sex Determination of South African Whites*. MSc Dissetation, University of the Witwatersrand

Sardi, M. L., Ventrice, F. & Rozzi, F. R. (2007) Allometries Throughout the Late Prenatal and Early Postnatal Human Craniofacial Ontogeny. *Anat Rec*, 290, 1112–1120.

Scammon, R. E. & Calkins, L. A. (1929) The development and growth of the external dimensions of the human body in the foetal period *Minneapolis*

Shaw, W. C. (1975) The influence of children dentofacial appearance on the social attractiveness as judged by peers and lay adults *Am J orthod* 79 399 – 415.

Shlens, J. (2005) *A Tutorial on Principal Component Analysis*. Accessed on 2011/09/21. Available at www.snl.salk.edu/~shlens/pca.pdf.

Sibisi, L. N. & Hlongwa, P. (2007) *Cephalometric values in a sample of South African blacks at Medunsa oral health centre. University of Limpopo Academic Day.* Medunsa.

Smartt, J. M., Low, D. W. & Bartlett, S. P. (2005) The pediatric Mandible: I. A Primer on Growth and development *Plast Reconstr Surg* 116, 14e – 23e

Smith, B. H. (1988) *Standards of Human Tooth Formation and Dental Age Asessment*. IN Kelly, M. A. & Larsen, C. P. (Eds.) *Advances in Dental Anthropology.* Kansas City, Mo, Wiley-Liss, Inc.

Smith, L. I. (2002) *A tutorial on Principal Components Analysis*. Accessed on 2011/09/21. Available at http://www.cs.otago.ac.nz/cosc453/student_tutorials/pricipal_components.pdf.

Smith, R. J. (2009) Use and Misuse of the Reduced Major Axis for Line-Fittin. *Am J Phys Anthropol*, 140, 476–486.

Sperber, G. H. (1989) *Craniofacial embryology* 4th. Oxford, Wright.

St John, D., Mulliken, J. B., Kaban, L. B. & Padwa, B. L. (2002) Anthropometric Analysis of Mandibular Asymmetry in Infants With Deformational Posterior Plagiocephaly. *J Oral Maxillofac Surg* 60, 837 – 877.

Standring, S., Berkovitz, B. K. B., Hackney, C. M., Ruskell, G. L., Collins, P. & Wigley, C. (2005) *Head and Neck* IN Standring, S., Ellis, H., Healy, J. C., Johnson, D., Williams, A., Collins, P., Wigley, C. & Berkovitz, B. K. B. (Eds.) *Gray's Anatomy.* 39th ed. Edinburgh, Elsevier Churchill Livingstone.

Tanner, J. M. (1981) The control of growth. *British Medical Bulletin*, 37, 207-208.

Tobias, P. V. (1987) Memories of the Robert James Terry (1871-1966) and the genesis of the Terry and Dart collections of human skeletons *Adler Museum Bulletin* 13 31–34.

Tobias, P. V. (1991) On the scientific, medical, dental and educational value of collections of human skeletons. *International Journal of Anthropology*, 6, 277–280.

Trenouth, M. J. (1984) Shape changes during human foetal craniofacial growth *J Anat* 139, 639–651.

Trenouth, M. J. (1985) The relationship between differences in regional growth rates and changes in shape during human foetal craniofacial growth *Archs oral Biol* 30, 31–34.

Van Valen, L. V. (1962) A study of flutuating asymmetry. *Evolution,* 16 125-142.

Vioarsdóttir, U. S., O'higgins, P. & Stringer, C. (2002) A geometric morphometric study of regional differences in the ontogeny of the modern human facial skeleton. *J. Anat.,* 201, 211–229.

Waddington, C. H. (1942) Canalization of development and the inheritance of acquired characters. *Nature* 150, 357-565.

Waddington, C. H. (1957) *The Strategy of the Genes.* London, UK George Allen Unwin.

Warton, D. I., Wright, I. J., Falster, D. S. & Westoby, W. (2006) Bivariate line-fitting methods for allometry. *Biol. Rev,* 81, 259–291.

Williams, S. E. & Slice, D. E. (2010) Regional Shape Change in Adult Facial Bone Curvature With Age. *Am J Phys Anthrop,* 143, 437–447.

Witwatersrand, U. O. T. (n.d) *Pocket notes*. Johannesburg.

Wyndham, C. H. & Irwig, L. M. (1979) A comparison of mortality rates of various population groups in the Republic of South Africa. *SA Med J,* 55, 796-802.

Zalel, Y., Gindes, L. & Achiron, R. (2006) The foetal mandible: an in utero sonographic evaluation between 11 and 31 weeks' gestation. *Prenat Diagn* 26, 163–167.

Zong, X., Li, H. & Zhu, Z. (2011) Secular trends in height and weight for healthy Han children aged 0–7 years in China, 1975–2005. *Am J Hum Biol* 23, 209-15.

Appendix1: Caveric specimen data

ID	6312	7137	3210	5935	7067	7103	3958	6289	6963	7047	6575	6326
Sex	M	F	F	F	F	M	F	M	M	M	M	F
Foot length	4.67	6.86	5.6		4.36	9.44	8.7	5.45	5.3	6.36	6.65	7.4
bi-parietal	6.2	7.1	7.6	9.2	7.1	11.8		7.3	7	8	7.8	10.2
CRL	24.3	30.5	25.8	31.8	26.6	40.7	39.8	25.6	27.3	28.7	27	36.1
bi-zy	4.71	5	4.6	5.74	4.86	7	7.18	4.7	4.64	5.69	5.73	6.06
bi-zm	3.9	4.23	4.13	4.84	3.32	5.94	6.04	3.84	4.07	4.67	4.53	5.29
na-ans	1.15	1.17	1.18	1.17	1.04	1.41	1.57	1.25	1.33	1.37	1.32	1.55
ans-pr	0.54	0.81	0.7	0.81	0.48	1	1.01	0.55	0.96	0.88	0.85	0.95
Lans-zy	2.9	3.04	2.67	2.9	2.79	4.17	3.98	3.86	2.75	3.08	2.6	3.44
Rans-zy	2.99	3.04	2.8	3.06	2.7	4.05	3.92	3.76	2.76	3.07	2.64	3.43
Lna-zy	2.96	3.3	2.74	3.6	2.86	4.48	4.3	2.95	2.86	3.35	3.45	3.93
Rna-zy	3.16	3.18	2.8	3.59	2.7	4.27	4.38	2.83	2.75	3.31	3.39	3.9
Lna-zm	3.08	3.03	2.86	3.6	2.71	3.72	4.38	2.51	2.89	3.33	3.38	4.65
Rna-zm	2.95	2.97	2.89	3.34	2.43	3.83	4.15	2.54	2.82	3.35	3.28	4.52
Lans-pt	2.25	2.89	2.48	3.26	2.68	3.65	3.59	2.62	2.49	2.82	2.94	3.33
Rans-pt	2	2.89	2.33	3.1	2.49	3.7	3.64	2.62	2.42	2.94	2.99	3.16
bi-co	3.93	5.15	4.05	5.04	4.32	6.43	6.32	4.29	4.48	4.45	4.93	5.87
bi-cr	3.54	4.7	3.97	4.9	4.2	6.2	6.44	4.06	3.94	4.52	4.39	5.19
bi-go	2.98	4.05	3.02	4.22	3.53	5.37	5.02	3.5	3.32	3.95	3.98	5.13
Sh	0.99	1.29	0.94	1.1	1.03	1.63	1.79	1.12	1.14	1.14	1.55	1.29
Lgo-gn	2.49	3.76	2.83	3.18	2.8	4.63	4.29	2.86	3.3	3.23	3.6	4.12
Rgo-gn	2.57	3.33	3.03	3.52	2.74	4.45	4.1	2.85	3.17	3.32	3.57	3.92
Lgo-co	1.36	1.79	1.47	1.25	1.37	1.49	2.43	1.22	1.24	1.4	1.66	1.19
Rgo-co	1.21	1.98	1.38	1.45	1.45	1.56	2.28	1.19	1.19	1.22	1.62	1.49
Lco-cr	0.75	1.03	1	1.1	0.77	1.39	1.45	1.16	1	1.15	0.62	1.16

Rco-cr	0.68	1.29	0.95	0.96	0.85	1.29	1.43	0.92	0.85	1.13	0.67	1.26

ID	5160	6607	6833	7108	4238	6510	6367	6746	6311	4533	4366	5939	7268	6605	6459
Sex	F	M	F	M	M	M	F	M	M	F	F	M	M	M	F
Foot length	6.72			5.73	6.15	7.43			5.05		6.17	7.3	6.25	7.8	8.14
bi-parietal	9.3	9.5	8.6	6.7	8.7	8.7	8.5	9.1	6.8	9.8	8.5	9.6	7.5	9	10
CRL	32.1	35.6	33.5	26.4	28	35.3	33.2	32.8	26	34.5	31.4	34.1	29.8	36	35
bi-zy	5.37	6.04	6.11	4.64	5.88	6.15	5.65	5.4	4.6	6.2	5.3	5.64	5.29	6.3	5.9
bi-zm	5.09	4.29	4.89	4.82	5.43	5.7	4.89	5.06	5.64	5.23	4.71	4.74	4.5	5.79	5.72
na-ans	2.02	1.49	1.16	1.29	1.95	1.6	1	1.45	1.07	1.49	0.83	1.38	1.2	1.49	1.65
ans-pr	0.75	1.06	0.9	0.78	0.89	0.89	0.66	0.73	0.7	0.98	0.88	0.84	0.9	0.96	1.25
Lans-zy	3.48	3.94	3.56	2.85	3.47	3.67	3.72	3.67	2.8	3.64	3.05	3.08	2.92	3.53	3.35
Rans-zy	3.27	4	3.84	2.84	3.3	3.62	3.4	3.33	2.63	3.59	3.01	3.24	3.04	3.57	3.25
Lna-zy	3.88	4.31	3.62	3.29	3.81	3.64	3.46	3.44	2.64	3.89	3.46	3.6	3.44	3.5	3.92
Rna-zy	3.9	3.96	4.14	3.23	3.94	3.62	3.12	3.32	2.75	3.58	3.07	3.16	3.4	3.77	3.95
Lna-zm	3.9	3.95	3.1	3.57	3.56	3.8	3.48	3.9	3.1	3.62	3.24	3.16	3.43	3.72	4.07
Rna-zm	3.37	3.97	3.26	3.62	3.62	4.5	3.6	3.85	3.03	3.34	2.94	3.07	3.09	4.12	4.26
Lans-pt	3	2.9	3.18	2.73	3.44	3.08	2.95	3.1	2.46	3.08	2.46	2.94	2.51	3.2	3.3
Rans-pt	2.99	3.05	3.43	2.73	3.53	3.05	2.85	3.12	2.46	3.29	2.59	3.23	2.58	3.2	3.24
bi-co	5.31	5.7	5.39	4.5	5.83	6.1	4.93	5.07	4.02	5.29	5.27	5.54	5.03	5.45	5.79
bi-cr	4.69	5.43	4.95	3.57	5.36	6.18	4.58	5	3.89	5.35	5.35	5.43	4.76	5.65	5.66
bi-go	4.62	4.84	4.57	3.84	4.56	5.05	4.28	4.4	3.29	4.27	4.25	4.65	4.1	4.83	5.07
Sh	1.23	1.38	1.28	0.85	1.2	1.24	1.22	1.2	0.92	1.27	1.15	1.17	1	1.48	1.74
Lgo-gn	3.8	4.07	3.93	3.14	3.95	4.14	3.5	3.68	2.88	4.07	3.14	3.68	3.19	3.67	4.28
Rgo-gn	3.59	3.67	3.58	3.29	3.74	4.04	3.95	3.7	2.59	3.9	3.28	3.44	3.07	3.73	4.4
Lgo-co	1.29	1.56	1.7	1.38	1.73	1.26	1.43	1.33	1.2	1.63	1.56	1.45	1.42	1.57	2.1
Rgo-co	1.2	1.82	1.89	1.27	1.67	1.5	1.02	1.47	1.37	1.7	1.51	1.49	1.39	1.55	1.95
Lco-cr	1.25	1.12	1.42	0.99	1.26	0.99	1.17	1	1.13	1.33	1.04	1.24	1.15	1.46	1.6
Rco-cr	1.3	1.12	1.5	0.85	1.17	1.36	1.15	1.07	1	1.3	1.04	1.35	1	1.46	1.6

Appendix 2: Skeletal specimen data

ID	712	722	808	809	810	854	1235	1320	1327	1439	3014	3019	3119	3020	3190	4092	659	1128	1145
Group	Hlubi	Zulu	N/A	Shangaan	N/A	Sotho	Sotho	Swazi	Zulu	Sotho	Xhosa	N/A	N/A	Swazi	N/A	N/A	N/A	N/A	N/A
Sex	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	N/A	N/A	N/A
T_score	6	8	2	2	5	9	6	10	7	10	6	9	10	4	5	5	4	10	6
bi-zy	7.70	9.23	6.36	6.62	7.18	7.77	7.20	8.81	8.45	8.79	7.32	8.33	8.42	6.80	6.57	6.54		8.74	8.16
bi-zm	6.64	8.33	4.79	5.19	5.71	6.57	6.24	7.65	7.26	7.91	6.08	7.56	7.61	5.68	5.12	5.16	5.92	7.62	7.14
na-ans	3.07	3.72	2.17	2.32	2.79	3.33	2.77	3.69	3.22	3.89	2.97	3.49	3.53	2.51	2.06	2.05	2.25	3.28	2.95
ans-pr	1.61	1.50	1.03	1.07	1.21	1.82	1.20	1.43	1.45	1.41	1.12	1.62	1.56	1.24	1.27	1.23	1.08	1.48	1.22
Lans-zy	4.25	5.09	3.66	3.46	3.46	4.25	3.97	4.94	4.79	5.20	4.23	4.55	4.63	3.88	3.53	3.60		5.03	4.54
Rans-zy	4.26	4.91	3.48	3.28	4.23	4.23	3.98	4.83	4.62	4.98	3.89	4.59	4.57	3.75	3.70	3.74	3.88	4.90	4.43
Lna-zy	4.97	4.83	3.71	3.78	4.21	5.01	4.57	5.66	5.37	5.69	4.80	5.35	5.35	4.01	3.74	3.74		5.48	5.19
Rna-zy	5.05	5.82	3.76	3.84	4.55	5.08	4.62	5.83	5.24	5.63	4.70	5.18	5.20	3.98	3.77	3.77	4.11	5.55	5.13
Lna-zm	5.00	6.15	3.67	4.17	4.15	4.98	4.48	5.80	5.43	5.54	4.69	5.56	5.52	4.03	3.79	3.80	3.84	5.47	5.05
Rna-zm	4.99	6.16	3.82	4.07	4.30	4.93	4.62	5.93	5.39	6.01	4.75	5.47	5.23	4.12	3.82	3.82	3.88	5.57	5.07
Lans-pt	3.77	4.47	3.27	3.27	3.53	3.92	3.63	4.42	3.78	4.45	3.43	3.82	3.82	3.31	3.27	3.18	3.23	4.54	3.70
Rans-pt	3.81	4.54	3.24	3.32	3.65	4.04	3.85	4.50	3.77	4.49	3.49	3.89	3.88	3.34	3.20	3.26	3.15	4.60	3.78
bi-co						7.59	6.88	8.88	7.44	7.55	6.90	7.59	7.64	5.83	5.42	5.50	5.94	7.65	
bi-cr						7.05	6.09	7.54	7.18	7.63	6.73	7.30	7.37	5.46	5.44	5.33	5.81	7.65	
bi-go		6.09		5.17		6.35	5.94	7.19	6.28	7.46	5.86	5.42	6.87	4.66	4.43	4.38	5.32	7.15	
Sh	2.11	2.61	1.68	1.65	1.54	2.48	1.94	2.20	2.11	2.28		2.63	2.71	1.71	1.68	1.71	1.62		
Lgo-gn	5.49	6.91		4.61	4.50	5.64	5.19	7.15	5.67	6.61	5.03	6.29	5.90	4.46	4.02	4.07	4.51	6.80	
Rgo-gn	4.21			4.38		5.67	5.22	7.43	5.40	6.62	5.03	6.19	6.04	4.11	4.07	4.09	4.31	6.97	
Lgo-co		4.21			2.53	3.61	2.79	3.79	3.48	4.87	2.61	3.59	3.80	2.48	2.11	2.12	2.15	3.32	
Rgo-co	2.62					3.67	2.69	3.51	3.59	4.94	2.57	3.64	3.90	2.47	2.12	2.12	2.06	3.35	
Lco-cr		4.62				3.86	2.77	4.10	3.52	3.03	3.24	3.93	2.59	2.88	2.56	2.58	1.68	3.96	
Rco-cr						3.93	2.61	4.19	3.44	2.81	3.13	3.87	2.58	2.87	2.48	2.43	1.89	4.01	

ID	169	476	535	608	614	652	656	657	713	740	806	868	1244	1247	1469	1572	3016
Group	Mpondo	Zulu	Zulu	Sotho	Sotho	N/A	N/A	Sotho	Zulu	Sotho	Sotho	Sotho	Ndebele	Sotho	Zulu	Shangaan	N/A
Sex	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
T_score	6	5	5	6	4	2	6	4	4	4	4	6	10	9	6	5	5
bi-zy		7.44	6.79	6.90	5.83	5.46	6.67	6.60	6.59	6.24	7.35	7.39	8.91	7.72	8.71	7.02	7.21
bi-zm		6.28	5.45	5.84	4.73	4.67	6.02	5.19	5.49	5.25	6.32	6.35	7.96	7.02	7.44	5.88	5.76
na-ans	2.75	2.39	2.35	2.57	2.15	1.73	2.52	2.23	2.61	2.29	3.10	3.06	3.85	3.24	3.40	2.46	2.37
ans-pr	1.47	1.30	0.98	1.11	1.28	0.83	1.96	1.02	1.90	1.23	1.50	1.46	1.43	1.30	1.60	1.14	1.30
Lans-zy		4.11	3.99	3.93	3.48	3.30	3.79	3.60	3.94	3.46	4.20	4.19	4.98	4.28	4.82	4.14	4.02
Rans-zy		4.07	3.98	3.91	3.47	3.13	3.67	3.77	3.79	3.45	4.10	4.07	4.68	4.42	4.84	3.98	4.16
Lna-zy		4.62	4.48	4.71	3.88	3.23	4.36	3.70	4.45	3.74	4.71	4.78	5.46	4.92	5.56	4.66	4.15
Rna-zy		4.47	4.65	4.56	3.87	3.21	4.27	3.91	4.35	4.02	4.68	4.74	5.55	4.91	5.51	4.55	4.20
Lna-zm		4.43	4.21	4.56	3.72	3.17	4.44	3.86	4.32	3.84	4.92	4.91	5.73	5.35	5.49	4.61	4.20
Rna-zm		4.42	4.28	4.43	3.77	3.14	4.45	4.00	4.48	4.00	4.88	4.89	5.76	5.19	5.38	4.51	4.26
Lans-pt	4.00	3.47	3.28	3.82	3.09	2.98	3.39	3.19	3.59	3.15	3.74	3.73	4.74	4.26	3.87	3.60	3.56
Rans-pt	4.12	3.51	3.29	3.91	3.21	2.94	3.28	3.17	3.72	3.07	3.75	3.72	4.75	4.33	3.83	3.52	3.64
bi-co	7.01	6.58	5.66	6.58		4.94	6.71	5.56	6.28	5.58	6.73	6.57	7.57	7.84	7.77	6.43	6.69
bi-cr	6.56	6.34	5.55	6.27		4.73	5.76	4.90	5.57	4.73	6.27		7.35	6.96	7.22		6.04
bi-go	4.32	5.26	5.26	5.22		4.32	5.59	4.95	5.28	4.95	6.02		6.41	7.03	4.71	5.13	5.25
Sh	0.86	1.90	1.91	1.87		1.29	1.96	1.65	1.21	1.68	2.19		2.83	2.18	2.23	1.17	1.75
Lgo-gn	5.24	4.89	4.38	5.33		3.45	4.73	3.74	4.21	3.78	4.90	4.75	7.26	5.68	5.28	4.73	4.69
Rgo-gn	5.67	4.97	4.40	5.34		3.54	4.63	3.93	4.14	3.80	4.88	4.61	7.47	5.83	5.20	4.68	4.54
Lgo-co	2.98	2.71	2.53	2.53		1.57	2.86	2.48	2.62	2.47	3.12	3.13	3.98	3.85	3.59		2.03
Rgo-co	2.85	2.61	2.33	2.58		1.40	2.66	2.42	2.75	2.42	3.04	3.02	3.92	3.68	3.45	2.85	2.26
Lco-cr	2.95	2.85	2.72	2.81		1.73	2.85	2.60		2.57	1.97		2.99	4.19	3.61	3.07	1.80
Rco-cr	3.09	2.94	2.47	2.63		1.75	2.52	2.26		2.56	1.96		3.12	4.00	3.64	3.24	1.79

ID	3017	3018	3021	3023	3148	3191	161	472	615	660	661	662	664	667	668	669
Group	Swazi	Zulu	N/A	N/A	Sotho	N/A	Xhosa	Sotho	Zulu	Zulu	Zulu	Swazi	Sotho	Zulu	Shangaan	Sotho
Sex	F	F	F	F	F	F	M	M	M	M	M	M	M	M	M	M
T_score	6	6	4	6	10	6	10	4	2	7	3	2	4	5	6	8
bi-zy	7.52	7.02	7.03	7.08	8.35	6.46	9.53	6.84	5.62	7.91	5.83	6.41	6.50	7.03	7.50	8.42
bi-zm	6.23	5.87	5.86	5.94	7.64	5.41	8.09	5.99	4.64	6.50	4.70	5.25	5.26	5.84	6.22	6.89
na-ans	2.56	2.47	2.73	2.49	3.68	2.61	4.08	2.36	1.93	3.15	1.95	2.15	2.41	2.51	2.88	2.95
ans-pr	1.00	0.89	0.80	1.01	1.24	0.96	1.45	1.16	0.88	1.41	0.77	1.16	1.16	1.34	1.24	1.31
Lans-zy	4.22	4.11	3.90	3.90	4.79	3.77	5.40	3.96	3.34	4.66	3.33	3.52	3.86	4.22	4.40	4.70
Rans-zy	4.24	4.04	3.98	4.25	4.72	3.82	5.42	4.04	3.19	4.60	3.32	3.63	3.71	4.05	4.41	4.72
Lna-zy	4.81	4.41	4.51	4.29	5.56	4.41	5.84	4.27	3.32	5.12	3.54	3.94	4.19	4.44	4.94	5.32
Rna-zy	4.90	4.45	4.53	4.69	5.47	4.40	5.97	4.33	3.32	5.40	3.54	3.99	4.14	4.37	4.85	5.16
Lna-zm	4.64	4.29	4.40	4.45	5.64	4.36	5.94	3.99	3.11	4.88	3.50	3.99	4.16	4.35	4.77	5.04
Rna-zm	4.73	4.40	4.49	4.40	5.70	4.34	6.05	4.03	3.24	5.33	3.60	3.95	4.12	4.18	4.80	4.94
Lans-pt	3.38	3.74	3.27	3.47	4.42	3.41	4.88	3.47	2.82	4.19	2.83	2.96	3.46	3.96	3.56	4.06
Rans-pt	3.52	3.81	3.36	3.50	4.56	3.43	4.92	3.43	2.79	4.31	2.82	3.05	3.53	3.85	3.65	3.81
bi-co	6.53	6.40	6.42	6.47	8.07	5.96	8.95					5.55	4.71	6.30	6.77	7.50
bi-cr	6.28	6.41	6.28	6.19	7.28	6.00	7.89					5.19		5.56	6.01	7.11
bi-go	5.57	5.42	4.65	5.30	6.81	4.60	7.24					4.45		4.90	5.60	6.33
Sh	1.69	1.80	1.62	2.09		1.78	2.66	1.50				1.91		1.94	1.89	2.02
Lgo-gn	4.87	4.64	4.64	4.72	6.64	4.49	6.87	4.73				3.91		4.44	4.76	5.70
Rgo-gn	4.92	4.63	4.44	4.72	6.15	4.23	6.84					4.15	4.40	4.26	4.87	5.56
Lgo-co	2.68	2.98	2.62	2.18	3.32	2.42	4.80	2.48				1.99	4.32	2.21	2.99	3.22
Rgo-co	2.74	2.98	2.92	2.27	3.73	2.58	4.86					1.86		2.54	3.09	3.11
Lco-cr	2.10	3.34	2.91	1.85	4.00	2.84	4.94	2.88				1.94		2.55	2.85	3.28
Rco-cr	1.89	3.29	3.01	1.92	4.04	2.86	4.93					2.02		2.64	2.87	3.44

Note: T-Score = Tooth development score