TRANSITION ANALYSIS OF AGE-RELATED CHANGES TO THE DISTAL RADIUS AND ULNA IN A MODERN SOUTH AFRICAN POPULATION

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ABSTRACT

A forensic anthropologist's primary role involves establishing a biological profile from unknown skeletal remains. Extensive research has been conducted on methodology to construct the biological profile from adult remains. However, the estimation of subadult biological parameters is lacking, mainly as a result of the paucity of known skeletal material for research. Numerous methods have been assessed to conduct subadult age estimation, with epiphyseal fusion being the preferred method for the adolescent age cohort. The application of epiphyseal fusion has been extensively researched on several populations; differences in the maturation rate of populations have been observed and demonstrate the need for population-specific standards. While some studies have been conducted on South Africans, the approach lacks the robust statistical component to make the method compliant with standards of best practice required of forensic methodology. The aim of the current study was to re-evaluate age estimation standards from epiphyseal fusion of the distal radius and ulna.

A sample of 782 hand-wrist radiographs of male and female black and white South Africans were collected from Mediclinic, Bloemfontein. The ages ranged between eight and 30 years. Degree of epiphyseal fusion for the radius and ulna was assessed and scored using a four-stage system. Differences in the rate of fusion between the radius and ulna, the sexes, as well as the population groups were assessed with a Kruskal-Wallis test. Transition analysis and Bayesian statistics were applied to obtain the maximum likelihood age estimate and the average age of transition among the stages, respectively. Significant differences ($p<0.05$) were noted between the fusion of the radius and ulna. Furthermore, there were no significant differences between males and females.

While significant differences were noted between black and white South Africans, the differences only amount to a few months and therefore do not justify separating the populations for the creation of standards, as group separation would affect the practical applicability of the method. Complete fusion was observed between the ages of 16 and 19 years in the pooled sample (95% CI). The results indicate an earlier age of complete fusion compared to previous South African studies, particularly for the males.

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ACRONYMS AND ABBREVIATIONS

CI	–	Confidence Interval
CT	–	Computed Tomography
GH	–	Growth Hormone
GP	–	Greulich and Pyle
ICC	–	Interclass Correlation Coefficient
IGF-1	–	Insulin Growth Factor-1
LODOX	–	Low Dose X-ray
MRI	–	Magnetic Resonance Imaging
SD	–	Standard Deviation
SES	–	Socio-Economic Status
TA	–	Transition Analysis
TW2	–	Tanner-Whitehouse

Chapter 1 INTRODUCTION

1.1 BACKGROUND

South African law stipulates that the age of majority, defined as the age at which a person is no longer considered a minor and recognised by law as an adult, is 18 years of age. Criminal capacity in South Africa, however, is set at age 14. Therefore, children 14 years or older are considered to have criminal capacity and can be tried and prosecuted for crimes committed (RSA, 2005). Recent statistics show that crimes committed by subadults are on the increase. Further, subadults appear to commit increasingly more violent crimes as a result of the influence of gang violence throughout much of South Africa; these crimes are inclusive of murder, assault, and rape (Fischer, 2017). In 2014, children between the ages of 10 and 17 years were responsible for committing 800 crimes throughout South Africa; and in the Gauteng province, subadults were responsible for 49 murders. The term subadult is used to refer to individuals who have not yet reached adulthood but have passed through the juvenile period (Du Plessis, 2006; Lamprecht, 2015).

Recent years have shown a major influx of foreign nationals and asylum seekers without valid identification documents from various neighbouring countries (Maromo, 2015). Individuals flee from countries such as Democratic Republic of Congo, Somalia, Burundi, Ethiopia and Zimbabwe due to political unrest, civil war and violence (Magqibelo and Londt, 2016). According to the United Nations High Commissioner for Refugees (UNHCR) approximately 65 000 refugees are currently seeking asylum in South Africa; with an estimated 50% being younger than 18 years, many of whom are unaccompanied and without documents of identification (UNCHR, 2015). Additionally, authorities frequently deal with individuals whose exact age is of utmost importance with regard to further legal proceedings (i.e. whether the person is older or younger than the age of majority). For instance, children under the age of 18 years but older than 14 years may receive reduced sentencing and are sent to juvenile institutions, thereby keeping them separate from older offenders (RSA, 1997). Whereas children between the ages of 10 and 14 years are presumed to lack criminal capacity unless proven otherwise (RSA, 2008). Problems arise when individuals cannot provide or do not possess proof of identification stating their date of birth. As a result, alternative methods for obtaining age for these individuals, such as assessing skeletal development, need to receive closer scrutiny (Schmeling *et al.*, 2004:a).

Estimation of age from skeletal indicators is a reflection of the physiological state and level of maturation of an individual (Uysal *et al.*, 2004). The process of age estimation involves the estimation of biological age of an individual in an attempt to correlate biological age with chronological age, via comparison to a standard level of normal controls (Ubelaker, 1987, 2005; Rikhasor *et al.*, 1999; Lewis and Flavel, 2006; Christensen *et al.*, 2014). While chronological age refers to the amount of time (years, months, days) that has passed since the birth of an individual, biological age refers to the physiological state of the individual as demonstrated by skeletal material and other bodily structures (Garvin *et al.*, 2012; Christensen *et al.*, 2014). Forensic anthropologists are often required to estimate biological age for legal, academic or clinical purposes (Schmeling *et al.*, 2006:a; Nemade *et al.*, 2010; Dembetembe and Morris, 2012; Christensen *et al.*, 2014). Numerous age estimation techniques currently exist and may be applied to both living and deceased individuals. This is inclusive of dental, osteometric and radiographic methods.

Radiographic images of the bones of the hand and wrist are commonly used as an indicator for skeletal maturity. Various age estimation standards have been created based on the skeletal development and maturity of the hand and wrist, such as the Greulich and Pyle (GP) skeletal age estimation standards in 1959 and the Tanner-Whitehouse (TW2) skeletal age estimation standards in 1975. However, the applicability of these methods to modern South Africans have been questioned, as various factors such as sex, ancestry, socio-economic status (SES) and secular trends have been found to influence skeletal maturation as well as the fusion times of different epiphyses (Introna and Campobasso, 2006; Baumann *et al.*, 2009; Dembetembe and Morris, 2012; Schmidt *et al.*, 2013).

Age estimation from the distal wrist among South Africans has received some attention in recent years. Dembetembe and Morris (2012) reported that the GP atlas method underestimates age in black South African males and females, as maturation occurs approximately two years later than the individuals utilised for the GP reference sample. Lakha (2015) reported reference standards on the epiphyseal fusion times of multiple epiphyses in a South African population between the ages of six and 24 years. However, both studies present with some limitations, ranging from a lack of population variation (Dembetembe and Morris, 2012), to limited statistical analyses (Lakha, 2015). Revised population-specific standards for the timing of epiphyseal plate fusion among South Africans is required as well as fairly robust statistical techniques to address complex variation within biological age. A revision of epiphyseal fusion methodology in South Africa can increase reliability, accuracy and consistency of skeletal

maturity estimation (Mora *et al.*, 2001). The current study will examine age related changes of the distal radius and ulna through transition analysis (TA) to better understand and quantify the levels of variation and patterns of aging in a South African sample. Furthermore, Bayesian statistics will be applied in order to recalibrate maximum likelihood age standards of epiphyseal plate fusion in the distal radius and ulna for a South African population.

1.2 AIM

The aim of this current study is to use a four stage classification system to evaluate age related changes to the epiphyseal surfaces of the distal radius and ulna of modern white and black South Africans with the use of TA and Bayesian statistics.

1.3 OBJECTIVES

The objectives of the study are as follows:

a) To compare skeletal maturation of the distal radius and ulna between black and white South Africans.
b) To compare skeletal maturation of the distal radius and ulna between males and females.
c) To determine the timing of epiphyseal plate fusion of the distal radius and ulna in black and white South African male and female subadults through the use of TA.

Chapter 2 LITERATURE REVIEW

2.1 AGE ESTIMATION

Age estimation is enabled by the analysis of the growth and development of skeletal material associated with chronological age (Ubelaker, 1987; Schmeling *et al.*, 2004:a; Uysal *et al.*, 2004; Baumann *et al.*, 2009). The process of age estimation aims to estimate biological age of an individual in an attempt to correlate biological age with chronological age. However, while closely related, biological age and chronological age are not synonymous and therefore biological age indicators are only estimates of the physiological status of a given person (Kemkes-Grottenthaler, 2002). Due to this, age estimation is a difficult task, resulting in age intervals given with a certain degree of accuracy (95% confidence interval (CI)) rather than a point estimate. Age estimation can be used for clinical, academic or medico-legal purposes (Schmeling *et al.*, 2006:a; Nemade *et al.*, 2010; Dembetembe and Morris, 2012; Christensen *et al.*, 2014).

Clinicians may use age estimation as a method to evaluate level of skeletal maturity and treat children with growth or metabolic disorders (Loder *et al.*, 1993; Uysal *et al.*, 2004). Age-at-death of unidentified skeletal remains is estimated in order to narrow the list of possible missing persons, and age estimation may also be applied during the study of past or historic populations to determine life expectancy, health status and disease patterns (Scheuer and Black, 2000; Christensen *et al.*, 2014; Davies *et al.*, 2016). Additionally, age estimation can be applied to living persons whose age is of legal relevance, or individuals, such as refugees or illegal immigrants, lacking legal documentation (Scheuer and Black, 2000; Schmeling *et al.*, 2006:a). These individuals may therefore benefit from the privileges of juvenile penal law, such as reduced sentencing and being sent to separate juvenile facilities (RSA, 1997). For this reason, the field of forensic anthropology has both ethical and socio-political implications (Baumann *et al.*, 2009).

Skeletal maturation is attained through the growth and development of bones, which continue throughout childhood until sexual maturity is reached. Thereafter, in adulthood, skeletal degeneration starts to occur due to numerous factors, including biomechanical loading and socio-economic influences (Scheuer, 2002; Ubelaker, 2005; Christensen *et al.*, 2014). Owing to the vast differences in the various stages of bone maturation throughout an

individual's life, skeletal age can be divided into two broad categories: subadult (inclusive of embryo, foetus, infant, child and adolescent) and adult (Christensen *et al.*, 2014).

Adult age estimates are dependent on morphological and degenerative indicators, whereas age estimation in children and adolescents involves measuring growth and development. The most common skeletal indicators used for adult age estimation include the degenerative changes to the pubic symphysis, auricular surface of the ilium and sternal rib changes (Rissech *et al.*, 2012; Christensen *et al.*, 2014). As a result of the heteroscedastic nature of the aging process, adult age indicators tend to produce wider age ranges than techniques applied to subadults. Wider age ranges are due to the fact that degeneration is influenced by many environmental factors, resulting in pronounced individual variation; referred to as the trajectory effect (Christensen *et al.*, 2014). The trajectory effect is resultant of changes that occur in skeletal indicators due to various biomechanical and physiological factors over time; small changes may result in large differences as time passes. Due to the fact that different individuals experience different biomechanical and physiological factors and that individuals are variably affected by these factors, high levels of variation are seen with an increase in age (Buikstra and Rhine, 2010). For instance, a woman who has given birth, resulting in stress to the pubic symphysis, may exhibit more degeneration to that area compared to a woman who did not experience that physical stress (Ubelaker and De La Paz, 2012). Consequently, the former will likely appear older although they are the same chronological age.

Subadult age methods typically produce narrower prediction intervals as the techniques involved rely on the predictable sequence of different growth and development processes that occur throughout childhood. These techniques may produce age estimates accurately within one to two years, with minimal bias, variance and an acceptable margin of error (Boldsen *et al.*, 2002; Introna and Campobasso, 2006; Christensen *et al.*, 2014). The age category of the individual in question will dictate which methods should be applied to obtain an age estimate, because different approaches or skeletal areas achieve greater accuracies at certain age intervals (Cardoso *et al.*, 2014; Christensen *et al.*, 2014). Table 2.1 reviews the methods of age estimation applied during different stages of development (Christensen *et al.*, 2014).

Table 2.1: Age estimation methods for different categories of development in subadults

Category	Time period	Method
Embryo	First 8 intra-uterine weeks	Diaphyseal growth and dental development
Foetus	8th intra-uterine week to birth	Diaphyseal growth and dental development
Infant	Birth to 1 year	Fusion of primary ossification centres, dental development and long bone diaphyseal lengths
Child	1 to 15 years	Fusion of primary ossification centres, epiphyseal plate fusion, dental development and long bone diaphyseal lengths
Adolescent	15 to 17 years	Epiphyseal plate fusion

With the assessment of skeletal material, the most relevant methods used for the estimation of age in subadults include union of primary ossification centres, long bone (diaphyseal) growth, epiphyseal plate fusion as well as dental development (Christensen *et al.*, 2014). Typically, dental development is the preferred method of age estimation in subadults; however, all permanent teeth (with the exception of the third molar) erupt between the ages of six and 12 years. Thus, dental development will only be a good indicator of age in pre-adolescents (under the age of 12 years) (Christensen *et al.*, 2014). Due to the fact that fusion of epiphyseal growth plates generally occurs between the second and third decades of life, epiphyseal union is a reliable and favoured method for estimating the age of older children, adolescents and young adults. As such, an integral knowledge of the growth, development and maturation of bones as well as the timing of skeletal changes is paramount to the age estimation of subadults (Noback, 1954; Cardoso *et al.*, 2014; Christensen *et al.*, 2014).

2.2 BONE GROWTH AND DEVELOPMENT

Bone growth occurs through a process called osteogenesis, which is defined as the "deposition of bone matrix on a pre-existing surface" (Christensen *et al.*, 2014). During embryological development pluripotent stem cells differentiate to form bone. Bone growth occurs through two different processes, namely intramembranous or mesenchymal ossification and endochondral ossification.

Intramembranous ossification is the direct formation of bone through ossification within a connective tissue membrane (i.e. the transformation within a highly vascular membrane) and forms the cranio-facial bones. An ossification centre is formed through the aggregation and ultimate differentiation of central mesenchymal cells into osteoblasts. Osteoblasts secrete osteoid (uncalcified bone matrix), which only begins to mineralise a few days later when binding to calcium salts. Within an individual ossification centre, osteoblasts may become trapped within the osteoid and differentiate into osteocytes. As osteoid deposition continues around the embryonic vasculature, ossification thereof results in a random network of finely woven trabeculae. Vascularisation condenses the outer surface mesenchymal cells in order to produce the periosteum. At the outer edges of the trabeculae, lamellar bone is deposited in structured layers in order to form osteons, typical of compact bone. Trabecular bone is thus found between two layers of compact bone, which is the defining characteristic of flat bones (Gilbert, 2000; Scheuer and Black, 2004; Allen and Burr, 2013). Mesenchymal bone models are formed during embryonic development, while direct ossification of the mesenchymal bone models occur during the foetal period (Ubelaker, 1987).

Majority of bones, including long bones, are formed through endochondral ossification. During this process, mesenchymal cells condense and differentiate into chondrocytes which then form the cartilage precursors or cartilage models during the foetal period. The cartilage models are subsequently replaced by bone (Scheuer and Black, 2004; White and Folkens, 2005; Christensen *et al.*, 2014; Moore *et al.*, 2014). Long bones, bones of the hands and feet, ribs, vertebrae, clavicles and scapulae develop from two or more ossification centres. The primary or first ossification centre generally appears before birth; however, some of these ossification centres may only appear during late childhood (such as the pisiform of the hand which ossifies between eight and ten years). Primary ossification centres develop in the shaft of long bones or in the body of irregular bones (Cardoso *et al.*, 2014). Scheuer and Black (2004) describes the primary ossification centre as "a temporal indication of the initial locus of ossification…". Primary ossification is initiated through penetration of periosteal blood vessels into the calcified cartilage bone model, which together with osteogenic (bone-forming) cells, form an osteogenic bud. In long bones, the diaphysis or shaft ossifies from the primary ossification centre, whereas epiphyses are formed by the ossification of secondary ossification centres as indicated in Figure 2.1 (Noback, 1954; Scheuer and Black, 2004; White and Folkens, 2005; Christensen *et al.*, 2014; Moore *et al.*, 2014). Primary and secondary ossification centres are

separated from one another by a cartilaginous layer, known as the epiphyseal or growth plate (Weise *et al.*, 2001; Scheuer and Black, 2004).

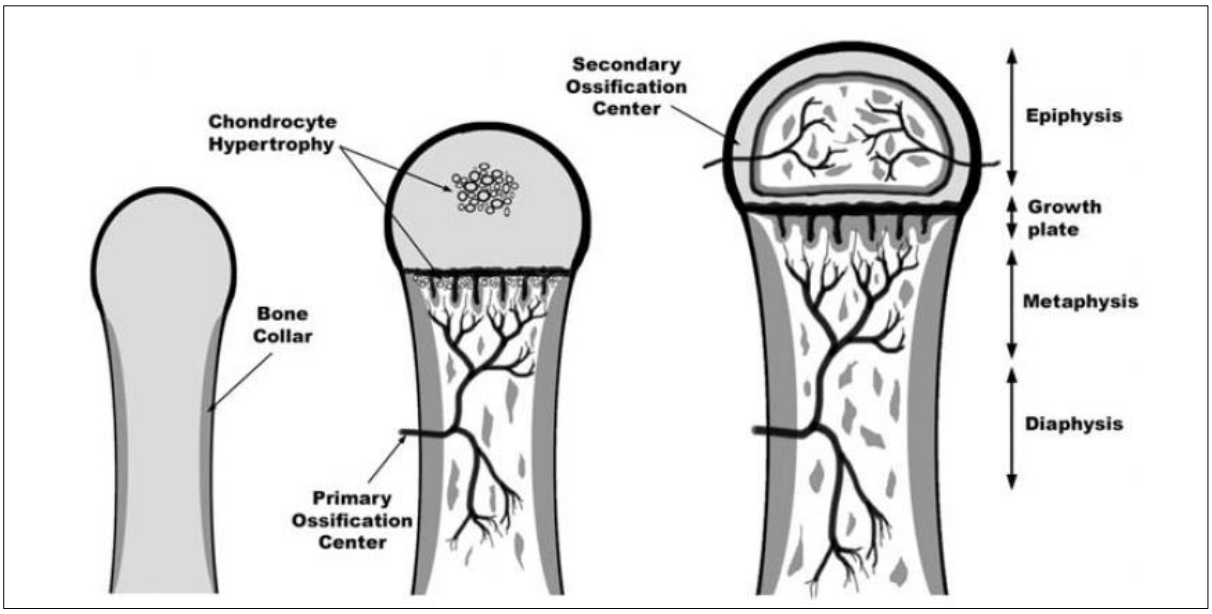

Figure 2.1: Formation of primary and secondary ossification centres (Gilsanz and Ratib, 2005:2)

Longitudinal bone growth occurs at the epiphyseal plate, through the process of endochondral bone formation (Nilsson *et al.*, 1994; Weise *et al.*, 2001). The metaphysis is the flared section of the diaphysis, closest to the epiphysis. To allow bone growth at these sites, the diaphysis and epiphysis do not fuse until the bone is fully grown or matured (i.e. has reached the adult state) (Scheuer and Black, 2004; Christensen *et al.*, 2014; Moore *et al.*, 2014).

2.3 EPIPHYSEAL PLATE FUSION

The process through which the diaphysis and epiphysis of a bone fuse is known as epiphyseal plate fusion and is generally identified by a dense epiphyseal scar after recent union (Emons *et al.*, 2009; Moore *et al.*, 2014). With time the epiphyseal scar will fade and eventually disappear completely. Epiphyseal plate fusion occurs when bone deposition exceeds the rate of cartilage proliferation, ceasing longitudinal bone growth (Scheuer and Black, 2004).

Throughout childhood, the epiphyseal growth plate gradually diminishes until maturity is reached and epiphyseal plate fusion is completed (Emons *et al.*, 2009, 2011). Epiphyseal plate fusion of secondary ossification centres commence at approximately 10 years of age and continue until early adulthood (Lewis and Flavel, 2006; Christensen *et al.*, 2014).

2.3.1 Regulation of epiphyseal plate fusion

The epiphyseal plate consists of four distinctive zones, namely (i) resting or reserve, (ii) proliferative, (iii) proliferative-hypertrophic transition and (iv) hypertrophic (Figure 2.2). The resting zone is located adjacent to the epiphysis and contains small, randomly distributed chondrocytes which rarely multiply. The proliferative zone contains mature, replicating chondrocytes which are arranged in columns running parallel to the bone shaft. The proliferative-hypertrophic transition zone represents the transitioning from the proliferative zone to the hypertrophic zone. Nearest to the metaphysis of the bone lies the hypertrophic zone, containing hypertrophic chondrocytes (chondrocytes that have replicated and enlarged) (Weise *et al.*, 2001; Serrat *et al.*, 2009). In this zone, the chondrocytes undergo preparations to be replaced by bone (Scheuer and Black, 2004).

Longitudinal bone growth (i.e. increase in length) is regulated by growth hormone (GH), insulin-like growth factor-1 (IGF-1), thyroid hormones, sex steroids and vitamin D. Growth hormone is secreted by cells of the adenohypophysis which stimulate the synthesis and secretion of IGF-1 in the liver and epiphyseal growth plates, subsequently activating slow-dividing pre-chondrocytes. In turn, IGF-1 is responsible for differentiation, proliferation and hypertrophy of chondrocytes, extra-cellular matrix formation and ultimately ossification of the epiphyseal growth plate after skeletal maturity is reached (Nilsson *et al.*, 1994; Shim, 2015).

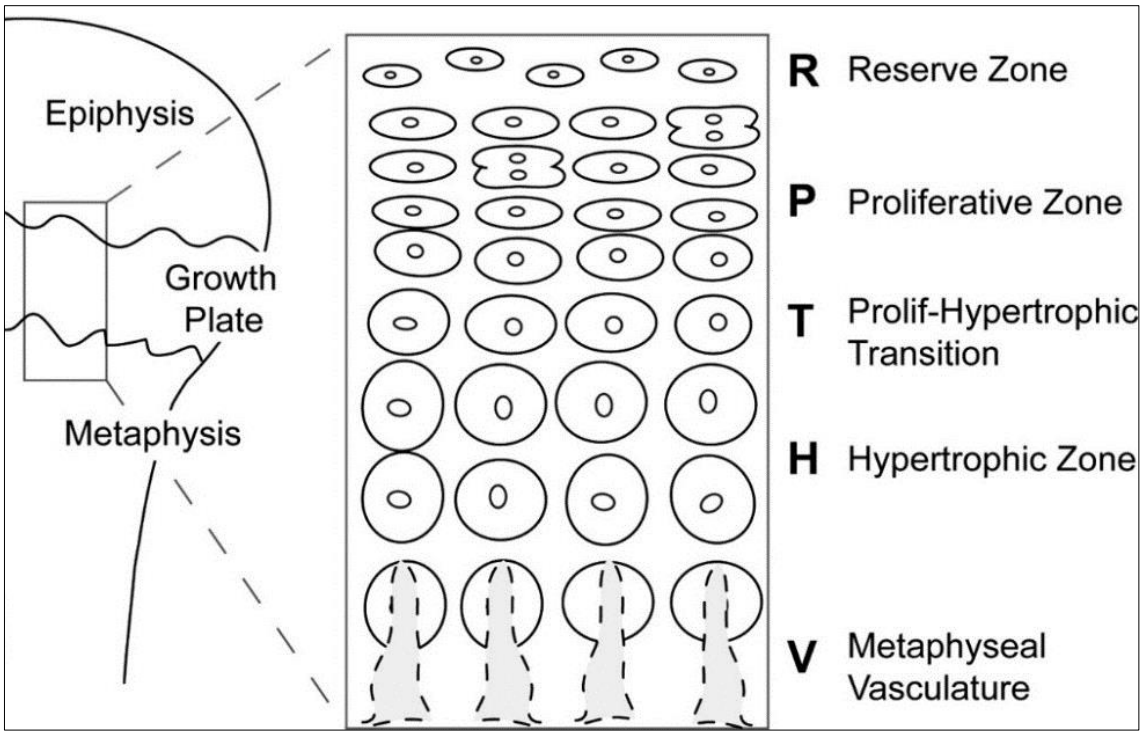

Figure 2.2: Zones of the epiphyseal plate (Serrat *et al.*, 2009:2017)

Thyroid hormones have been found to promote GH synthesis. An increase in GH stimulates secretion of IGF-1 in both the liver and the epiphyseal growth plates, thereby resulting in longitudinal growth. Furthermore, research indicates that thyroid hormones play a role in the formation of hypertrophic cells in the epiphyseal growth plate. Sex steroids (androgens and oestrogens) have long been known to play a significant role in the longitudinal growth of bones during the pubertal growth spurt. Androgens and oestrogens may influence skeletal maturation both directly and indirectly through the stimulation of GH secretion, thereby increasing concentrations of GH and in turn IGF-1. Initially, sex steroids promote growth by stimulating secretion of GH. However, near the end of the pubertal growth spurt steroids play a role in the fusion of the epiphyseal growth plate (Nilsson *et al.*, 1994; Shim, 2015).

The active form of vitamin D binds to the receptors found in the epiphyseal growth plate where it has been demonstrated to restrict IGF-1 induced clonal expansion and stimulate proliferation and maturation. Accordingly, vitamin D plays an imperative role in the maturation of epiphyseal plate chondrocytes (Nilsson *et al.*, 1994).

2.3.2 Persistent epiphyseal scar

At the location of the fused epiphyseal growth plate, an epiphyseal scar in the form of a thin, white line may be observed through the use of various medical imaging techniques. An epiphyseal scar generally obliterates shortly after completion of epiphyseal plate fusion, resulting in a lack of distinction between the metaphysis and diaphysis of the bone. Thus, indicating full maturation of the skeletal element (Davies *et al.*, 2014, 2016). However, some anomalies have been noted.

A persistent epiphyseal scar was first noted by Cope (1920), who stated that they can often be seen until much later in life, even though epiphyseal plate fusion has long since been completed. As the presence of an epiphyseal scar should indicate recent epiphyseal plate fusion, a persistent scar brings about difficulties in the estimation of skeletal age. Baumann *et al*. (2009) reported that the minimum age of epiphyseal scar obliteration in the radius was 18.7 and 16.2 years in males and females, respectively. The same study demonstrated that the maximum age at which an epiphyseal scar was still visible was 31.0 years in males and 30.8 years in females (Baumann *et al.*, 2009). A study by Davies *et al.* (2016) stated that the presence or absence of a persistent epiphyseal scar should not be used as an indicator for age estimation as they found no statistically significant correlations between the obliteration of an epiphyseal scar and chronological age. This statement is emphasised by the work of Stevenson

(1924) who stated that the visibility of an epiphyseal scar may overestimate true chronological age and should therefore not be taken into account when estimating age.

2.3.3 Epiphyseal plate fusion as an age estimation technique

The timing of epiphyseal plate fusion has been studied from as early as the 1920's (Stevenson, 1924). Epiphyseal plate fusion for age estimation involves the visual, qualitative assessment of epiphyses (Cardoso *et al.*, 2016). Skeletal development occurs in a specific chronological sequence at relatively predictable times throughout skeletal maturation. Epiphyseal plate fusion commences at the elbow, followed by the hip, ankle, knee, wrist and ultimately the shoulder joint (Stevenson, 1924; Greulich and Pyle, 1959). Therefore, it is a relatively good indicator of maturity and an accurate biological indicator of chronological age (Lewis and Flavel, 2006; Suri *et al.*, 2013; Christensen *et al.*, 2014). Figure 2.3 indicates the location of various post-cranial epiphyses as used for age estimation.

Epiphyseal plate fusion typically commences at the age of 10 years and ceases during early adulthood (around the age of 25 years). Thus, epiphyseal plate fusion is regularly implemented for age estimation in older children, adolescents and young adults. The process of initial epiphyseal plate fusion to complete fusion may take several months to years. As such, variation in the timing of epiphyseal plate fusion is observed due to numerous external influences such as socio-economic factors, population group, sex and level of urbanisation (Christensen *et al.*, 2014).

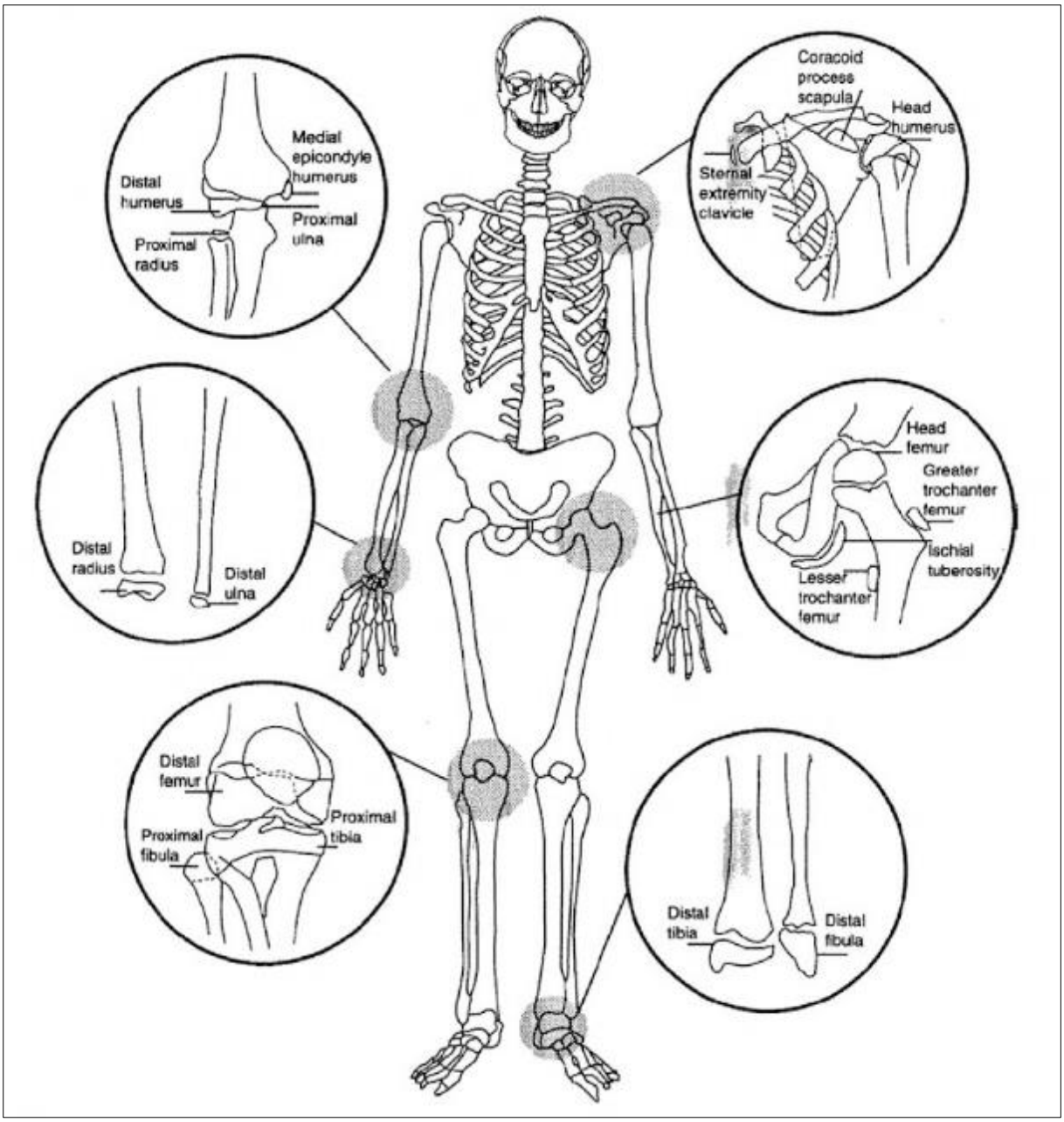

Figure 2.3: Location of epiphyses used for age estimation (Buikstra and Ubelaker, 1994:40)

Standards regarding the timing of epiphyseal plate fusion have been developed for the radius and ulna, in males and females, respectively. However, individual variation may influence the timing of epiphyseal plate fusion, which may lead to accelerated or delayed skeletal development (Noback, 1954; Schmeling *et al.*, 2000; Soegiharto *et al.*, 2008; Suri *et al.*, 2013; Christensen *et al.*, 2014). Ubelaker (1987) lists three factors that should be considered when estimating age through epiphyseal plate fusion:

i. Specific stage of union of all available epiphyses as not all epiphyses play an equal role with regard to accuracy in age estimation and it has been shown that epiphyseal plate fusion is a process that occurs over a period of time.
ii. Sex of the individual in combination with the range of variation as described for a specific population as differences in the timing of epiphyseal plate fusion between males and females as well as population groups have regularly been reported in literature. Furthermore, individual variation within the same population have been reported.
iii. Differences between gross examination and radiographic imaging should be considered.

The applicability of radiographic standards to the gross examination of dry bone have prompted some concerns as caution should be given when applying standards developed using radiographic methods to dry skeletal remains (Ubelaker, 1987; Lakha, 2015). Skeletal remains under assessment may display delayed growth and maturation when compared to healthy individuals undergoing radiographic imaging for the purpose of compiling standards. Delays may be produced by a difference in nutritional intake, environmental influences as well as health status. Epiphyseal scars have been reported to display more prominently and for a prolonged period of time on dry bone when compared to radiographic images. With this said, Lakha (2015) applied a four stage scoring method to dry bone specimens. By modifying the methodology to include both gross examination and radiographic imaging of skeletal remains and subsequent analysis of the epiphyseal surfaces, high levels of agreement and low standard errors were noted between her standards derived from radiographic imaging and the application thereof to skeletal remains (Lakha, 2015).

Current age estimation is based on internationally recognised standards published by Schaefer *et al.* (2009), Scheuer and Black (2000) and Greulich and Pyle (1959) (see Table 2.2). Schaefer *et al.* (2009) compiled their standards based on preceding studies using both dry bone and radiological methods. Various population groups were included in the compilation of their standards; including American, Indian, Portuguese and Bosnian.

Scheuer and Black (2000) published age estimation standards for the distal radius and ulna based on findings by Greulich and Pyle (1959), Paterson (1929), Flecker (1942) and Hansman (1962). Standards were compiled from European populations using radiographic images.

Greulich and Pyle (1959) published standards of age estimation for the hand and wrist based on the radiographs from 1000 white American children of North-European ancestry. This method allows the investigator to compare radiographs to a series of 'standard' radiographs as formulated by the authors, in an atlas method. Standard radiographs were based on the most commonly observed skeletal indicators for a specific age and sex (Dembetembe, 2010).

Table 2.2: International standards for the fusion times of the distal radius and ulna

Epiphysis	**Males**	**Females**
Radius		
Schaefer *et al.* (2009)	16-20	14-19
Scheuer and Black (2000)	16-20	14-17
Greulich and Pyle (1959)	17-18	15-16
Ulna		
Schaefer *et al.* (2009)	17-20	15-19
Scheuer and Black (2000)	17-20	15-17
Greulich and Pyle (1959)	17-18	16-17

* Values in years

Standards as reported in Table 2.2 include mean age ranges for the epiphyseal plate fusion of the distal radius and ulna (Johnston, 2008). The problem with employing point estimates is that mean values are influenced by factors such as outliers and skewed data, thereby influencing the interpretation and ultimately leading to imprecise conclusions. The application may produce reliability and accuracy issues. More sophisticated statistical models are required to effectively assess the complex variation associated with the aging process, and to use this known variation to provide a prediction of chronological age.

Some South African-specific research has been conducted on the estimation of age from the wrist. Dembetembe and Morris (2012) conducted a study on the applicability of the Greulich and Pyle (1959) radiographic standards of the hand and wrist on black South African males between the ages of 13 and 22 years. This study compared 131 pre-existing hand-wrist radiographs to the male standards as published by Greulich and Pyle (1959) in order to estimate skeletal age. Estimated age was thereafter compared to chronological age. Results indicated that black South African males exhibited a developmental delay of approximately 2.1 years compared to the Greulich and Pyle (1959) standards. In conclusion, the Greulich and Pyle (1959) standards were found not to be directly applicable to black South African males and

that population-specific age estimation standards were recommended for the South African population (Dembetembe and Morris, 2012).

More recently, Lakha (2015) proposed standards for epiphyseal plate fusion in a South African population between 6 and 24 years of age. The sample consisted of black, coloured, Indian as well as white males and females. In this study, a scoring system was used to classify the degree of epiphyseal plate fusion from one (non-fusion) to four (complete fusion). Full body low-dose digital X-ray (LODOX) images of 2151 individuals were assessed for the study. Epiphyseal plate fusion of the shoulder, elbow, wrist, iliac crest, hip, knee and ankle joints were studied and classified. A summary of age ranges for the fusion of the radius and ulna is depicted in Table 2.3 (highlighted in bold). While the study comprised a large sample of a wide variety of population groups, the statistical analysis was limited to the calculation of mean values (Lakha, 2015).

The current study focused on the epiphyseal plate fusion of the distal radius and ulna. Assessment of degree of epiphyseal plate fusion is often referred to as a method of age estimation for medico-legal purposes where age of majority is of concern. According to the literature, epiphyseal plate fusion and visibility of an epiphyseal scar of the distal radius and ulna generally occur between the ages of 16 and 20 years in males and between 14 and 19 years in females (Baumann *et al.*, 2009; Christensen *et al.*, 2014). However, different age ranges have been published for various population groups. Table 2.3 reports age ranges for various population groups using the epiphyseal plate scoring method (varying from two to five stages of fusion).

Table 2.3: Fusion times of the distal radius and ulna for various population groups

Author	Population	No stages	Age			
			Radius		Ulna	
			♂	♀	♂	♀
Abbie and Adey (1953)	Australian Aborigine	2	>16.0	18.0-19.0	>16.0	18.0-19.0
Banerjee and Agarwal (1998)	Indian	2	16.0-19.0	14.0-18.0	16.0-20.0	14.0-18.0
Baumann *et al.* (2009)	German	5	14.5-30.8	12.2-31.0	14.5-29.0	13.6-29.9
Flecker (1932)	Australian	2	18.4-23.0	16.5-20.4	18.4-23.0	16.5-22.0
Lakha (2015)	**South African**	**4**	**15.0-20.0**	**12.0-18.0**	**15.0-20.0**	**12.0-18.0**
Memchoubi (2006)	Indian	4	-	16.0-18.0	-	16.0-18.0
Nemade *et al*. (2010)	Indian	2	16.0-21.0	15.0-20.0	17.0-20.0	16.0-20.0
Patel *et al.* (2011)	Indian	4	17.0-≥20	16.0-19.0	17.0-19.0	17.0-18.0
Paterson (1929)	British	2	≥21.0	≥19.0	≥21.0	≥19.0
Pryor (1923)	American	2	≥20.0	≥19.0	≥19.0	≥18.0
Sahni and Jit (1995)	Indian	5	-	≥16.0	-	≥16.0
Schmidt *et al.* (2008:a)	German	5	14.5-18.9	12.9-19.0	-	-
Schmidt *et al.* (2013)	German	4	15.2-26.9	15.0-26.0	-	-
Serin *et al.* (2016)	French	3	≥16.0	≥15.0	≥16.0	≥15.5
Sidhom and Derry (1931)	Egyptian	3	16.0-20.0	-	15.0-20.0	-
Stevenson (1924)	American	4	18.0-21.0	18.0-21.0	18.0-21.0	18.0-21.0

* Values in years

As demonstrated in Table 2.3, little variation exists between the epiphyseal plate fusion times of the distal radius and ulna of the same side. Stevenson (1924) stresses the close proximity of the timing of the epiphyseal plate fusion of the distal radius and ulna. Nonetheless, the current study will test for statistically significant differences in the fusion times of the distal radius and ulna on the same side.

According to Christensen and Crowder (2009), it is important to determine known or potential error rates through validation studies. In the field of forensic science, rigorous testing and validity of methods is of utmost importance when delivering expert testimony. Due to the variation of the human skeleton, it is important to evaluate and redefine standards using modern skeletal samples (Langley-Shirley and Jantz, 2010). The *Daubert* criteria ensure validation and reliability of a theory or method through the following (Fradella and O'Neill, 2004):

a) Objective testing of the theory or method.
b) Subjection to peer review and publication.
c) A known potential error rate.
d) The existence and maintenance of standards and controls.
e) General acceptance in the scientific world.

With regard to age estimation, forensic anthropologists are required to make both accurate and reliable age estimations. However, as age range estimations are narrowed to be more precise, it becomes increasingly more probable to eliminate true age and therefore, the presumptive identity of the given individual. Thus, forensic anthropologists make use of confidence intervals, allowing them to state that the true age of an individual is included in the age range estimate with a certain degree of confidence (typically 95%) (Dirkmaat and Cabo, 2012). Confidence intervals were not performed for any South African specific studies; therefore, this study provides a more robust statistical analysis for the estimation of age.

2.3.4 Sex differences

Sexual dimorphism refers to the morphological differences between the males and females of the same species (Christensen *et al.*, 2014). Females are documented to develop earlier than males; this difference tends to increase with age. Many authors have suggested a two year difference in the skeletal maturation of adolescents between sexes (Davies and Parsons, 1927; Spencer, 2002; Bokariya *et al.*, 2011). However, Pryor (1925) suggested a more marked difference of three to four years. Whereas Patel *et al.* (2011) reported a 12 month difference

in maturation. Difference in skeletal maturation is due to the earlier onset of puberty in females (8 to 13 years) compared to males (10 to 15 years) (Wells, 2007; Soliman *et al.*, 2014). The onset of puberty contributes approximately 15% of growth toward adult height, and also plays an important role in the fusion of epiphyseal plates (Cutler, 1997). Females undergo a rapid pubertal transformation (i.e. transformation takes place over a shorter period), as opposed to a longer growth period in males (Wells, 2007). Female skeletal growth peaks at around 11 to 13 years of age, while males only reach their skeletal growth peak between 13 and 15 years (Pryor, 1923; Hägg and Taranger, 1982; Dimeglio, 2001; Lewis and Flavel, 2006). Hägg and Taranger (1982) found that the rate of skeletal development in males surpasses the rate of development in females at the end of the pubertal growth spurt. Thus supporting the fact that skeletal growth in males take place over a longer period of time than in females, resulting in bigger and taller stature (Wells, 2007).

Levels of sexual dimorphism or sex differences within a population have been suggested to be related to environmental influences (Tanner, 1962). Many authors have reported female buffering which refers to the apparent higher level of sensitivity to environmental influences or stressors in males (Stinson, 1985). Researchers have credited this effect to the reproductive responsibility of females. Males have been found to be more susceptible to environmental changes with regard to growth and development (Stinson, 1985). In more adverse conditions, males display retarded growth; whereas the improvement of environmental conditions, lead to a more marked increase in skeletal maturation compared to their female counterparts. Studies have also suggested that developmental changes in response to environmental influences are more pronounced in males compared to females, as males undergo a longer period of growth, during which external factors may influence morphology (Hiernaux, 1968; Tobias, 1972; Stinson, 1985).

2.3.5 Population variation

Numerous studies have indicated variation in the timing of epiphyseal plate fusion among different population groups (Tables 2.3 and 2.4) (Mackay, 1952; Massé and Hunt, 1963; Marshall *et al.*, 1970; Brown and Grave, 1976; Roche *et al.*, 1978; Loder *et al.*, 1993; Matsuo, 1993; Ashizawa *et al.*, 1996; Ontell and Barlow, 1996; Jiménez-Castellanos *et al.*, 1996; Rikhasor *et al.*, 1999; Mora *et al.*, 2001; Schmidt *et al.*, 2008:a; Soegiharto *et al.*, 2008; Nemade *et al.*, 2010; Patel *et al.* , 2011; Dembetembe and Morris, 2012). Additionally, regional variations within the same population group have been described (Banerjee and Agarwal, 1998;

Nemade *et al.*, 2010). Some authors suggest that variation in ossification times are not resultant from population differences but rather the result of SES, such as the works of Abbie and Adey (1953) and Schmeling *et al.* (2000). Variation in skeletal maturation owing to population differences have been widely discussed and studies have been performed on multiple test populations.

Table 2.4: Comparative studies indicating differences in skeletal development among various population groups

Author	Population	Age group	Method	Result
Ashizawa *et al*. (1996)	Japanese	3-18	TW2	Japanese children reach skeletal maturity approximately one to two years earlier.
Brown and Grave (1976)	Australian Aborigines	5-20	GP and TW2	Developmental delay in males (up to 10 months) and females (up to 6 months).
Dembetembe and Morris (2012)	South African	13-22	GP	Black South African males displayed a two year delay in skeletal maturation.
Jiménez-Castellanos *et al*. (1996)	Spanish	0-14	GP	Males display a three-month delay, whereas female development correspond to the standards as published by Greulich and Pyle (1959).
Loder *et al.* (1993)	American	0-18	GP	black males and females are advanced compared to their chronological age, white males exhibited a developmental delay while the GP method is applicable to white females.
Mackay (1952)	East African	0-18	Ossification tables	East African Children presented with a one and a half to two year delay in skeletal development.
Marshall *et al.* (1970)	Jamaican	1-15	TW2	Jamaican children exhibit accelerated skeletal development up to the age of 13 years, beyond that Jamaican children were found to exhibit retarded skeletal growth.
Massé and Hunt (1963)	West African (Senegal)	0-15	GP	Developmental delay in both male and female Africans (delay increases with increasing age).
Matsuo (1993)	Japanese	1-19	GP	Japanese children displayed a one year advance in skeletal and sexual maturity.
Mora *et al.* (2001)	African-American and European-American	0-19	GP	European-American males displayed a developmental advance (three months) compared to African American males
Rikhasor *et al.* (1999)	Pakistani	1-18	GP	Despite developmental retardation during early childhood, skeletal maturity is reached earlier in Pakistani children.

* Values in years
** TW2 = Tanner-Whitehouse; GP = Greulich and Pyle

2.3.6 Socio-economic status

Socio-economic status refers to the social standing of an individual or group of individuals within a society. Socio-economic status can be measured as a combination of education, income, health and nutritional status (Cole, 2000; American Psychological Association, 2016).

Studies have reported that skeletal maturation is greatly influenced by the SES of a population (Cameron *et al.*, 1992; Schmeling *et al.*, 2000; Schmeling *et al.*, 2004:a; Schmeling *et al.*, 2004:b; Kellinghaus *et al.*, 2010). A delay in epiphyseal plate fusion has been observed in individuals from a lower SES. Additionally, growth rate is negatively influenced by poor living conditions, such as overcrowding (Cole, 2000). A study by Cameron *et al.* (1992), assessing the effect of SES on growth in black South African children, found that a lower SES, whether or not the individual subsided in a rural or urban setting, resulted in reduced growth, in both height and weight. Therefore, growth and development are not improved unless SES is improved. This is emphasised by the findings of Schmeling *et al.* (2006:b), where acceleration of skeletal maturation was observed in medically and economically developed regions and countries.

As indicators of low SES, health and nutrition play an important role in normal bone growth and development. Poor nutritional status negatively influences pubertal and skeletal maturation, thereby delaying growth (Nilsson *et al.*, 1994; Soliman *et al.* , 2014). Therefore, SES should be kept in mind as a confounding factor (Schmeling *et al.*, 2004:a; Kellinghaus *et al.*, 2010). Confounders are defined as factors that influence or distort the data and may lead to biased conclusions (Cronje *et al.*, 2015). The effects of SES, however, are beyond the scope of this study. Yet, SES was likely to be relatively equal for all individuals included in the current study as radiographic images were sourced from a private hospital. Therefore, differences observed are expected to be related to biological differences between population groups.

2.3.7 Secular trend

Secular trend refers to the continuous adaptation of humans over a long period of time, resulting in either positive or negative changes; therefore, secular trends are indicative of the ever-changing health and affluence of a population. The rate and direction of secular trends have been described as a reflection of the change in SES (Henneberg and Van den Berg, 1990). Positive trends result in the acceleration of a process, whereas a negative trend is indicative of

a decrease or deceleration of a developmental process. Neutral secular trends (weak or barely noticeable trends) have been suggested to occur due to the fact that the study population has either reached their phenotypic limit (for example maximum height limit as set by genetics) or no improvement in environmental and health conditions (Henneberg and Van den Berg, 1990). Secular trends are frequently the result of environmental influences and socio-economic factors and are therefore a reflection of the health and wealth of a population (Roche, 1979; Cole, 2000). Both menarche and growth in height and weight are greatly influenced by socio-economic factors (Cole, 2000).

Onset of menarche in females is a key indicator of maturity (Jones *et al.*, 2009). Studies on secular trends of menarcheal age provide essential information on the transitioning of a population, as earlier onset of menarche is indicative of a rise in SES; this statement is confirmed by the plateau in menarcheal age in developed countries (Roche, 1979; Jones *et al.*, 2009). A plateau in the age of menarcheal onset suggests a physiological lower limit for menarcheal onset, which may be delayed due to environmental factors (Cole, 2000). Age of menarcheal onset is strongly associated with the timing of puberty as well as body composition, where a higher body mass index (BMI) may result in the earlier onset of menarche (Rossouw *et al.*, 2012; Soliman *et al.* , 2014). The average age of menarcheal onset has declined in various population groups over the past few decades (Roche, 1979). Jones *et al*. (2009), found that the average age of menarcheal onset for a South African population has declined with 0.5 years per year for black South Africans, compared to 0.22 years per decade for white South Africans. Current age of menarcheal onset is averaged to be 12.4 and 12.5 years for blacks and whites, respectively (Jones *et al.*, 2009). This observation indicates accelerated maturation and thus earlier commencement of fusion of secondary ossification centres, as sexual and skeletal maturation are closely related (Roche, 1979; Onat and Ertem, 1974). During the onset of puberty, oestrogen plays an important role in the stimulation of GH and IGF-1, subsequently the growth spurt is initiated as discussed in section 2.3.1 (Shim, 2015). Hyperoestrogenemia (increased levels of oestrogen) has been associated with increased skeletal maturation (Satoh, 2015). Thus, age estimation standards for epiphyseal plate fusion will likely be affected.

Throughout history, growth in height and weight has shown both positive and negative trends, mainly due to environmental influences (Cole, 2000). In a South African study, black and coloured children were found to display a positive secular trend with regard to growth in height as well as weight from the 1960's to 2013. The causative factors behind this trend are

most likely linked to improvement in environmental conditions and SES at the end of Apartheid (Anholts, 2013).

However, an increase in weight is not always an indication of improved nutrition or SES. A recent study by Rossouw *et al.* (2012) stated that rural-to-urban transitioning may play a role in obesity, as there is an increased accessibility of energy-rich fast food and lower levels of physical activity. Obesity has been linked to advanced skeletal development in both males and females (Satoh, 2015). Several studies have raised concerns regarding the significant increase in obesity among South Africans over recent years (WHO, 2011; Armstrong, Lambert and Lambert, 2011).

2.4 SKELETAL MATURITY OF THE WRIST

Skeletal maturity is primarily assessed by the degree of fusion observed in secondary ossification centres; moreover, it is also the only maturity indicator present from birth to adulthood (Cox, 1997; Gilsanz and Ratib, 2005). Several maturity indicators exist, including general physical development (longitudinal growth and weight), secondary sexual traits and the appearance of secondary ossification centres in the hand and wrist. These indicators are often assessed in combination in order to determine whether an individual has reached age of majority and can therefore be held legally responsible (Uysal *et al.*, 2004; Bokariya *et al.*, 2011).

The hand and wrist are considered significant indicators of skeletal maturity as it commonly fuses between the ages of 14 and 20 years, which coincides with the age of majority and criminal capacity as stated by South African law (Schmeling *et al.*, 2005; Schmidt *et al.*, 2008; Baumann *et al.*, 2009). Generally, radiographic or X-ray images of the left hand and wrist are used to assess the distal epiphyseal plate of the radius and ulna in living individuals. Hand-wrist radiographs pose an advantage as the patient or individual is exposed to low-dose radiation (1-2 Milliradian) with minimal risk of contamination (Rikhasor *et al.*, 1999; Cameriere *et al.*, 2006; Introna and Campobasso, 2006; Schmeling *et al.*, 2008). Different combinations of hand-wrist skeletal indicators may be used for the assessment of an individuals' growth and development as such, various methods for skeletal age estimation of the hand and wrist exist. Specifically, the atlas method, bone-by-bone method, epiphyseal plate scoring and transition analysis.

a) **Atlas method**: The radiographic atlas method of Greulich and Pyle (1959) is the most commonly used method for skeletal age estimation. These standards were derived from a longitudinal study of white American children of high SES in the 1930's (Mora *et al.*, 2001; Suri *et al.*, 2013). The method involves comparison of the degree of epiphyseal plate fusion of all of the bones of the hand and wrist with the radiographic standards of different ages. The *Radiographic Atlas of Skeletal Development of the Hand and Wrist* by Greulich and Pyle (1959), illustrates standard hand-wrist radiographs from birth to maturity for both males (19 years) and females (18 years) whereby 'plate comparison' can be done. This method is quick and easy to apply and produces an actual age estimate (Baughan *et al.*, 1979; Dembetembe and Morris, 2012; Suri *et al.*, 2013). However, problems have been reported with regard to the over- and underestimation of age, as age predictions are presented as a point estimate rather than an age range (Schaefer *et al.*, 2018). The method has been criticised for its poor recognition of ancestral differences as well as existent variation; as the atlas depicts only the 'normal' standard (Lakha, 2015). Concerns with regard to the reproducibility of this method has also been raised as the large amount of elements may provide conflicting information, resulting in difficulty assigning one specific comparative plate (Bull *et al.*, 1999; Bunch *et al.*, 2017; Schaefer *et al.*, 2018). Finally, similarities may only produce a specific skeletal age and not truly chronological age, which proves difficult when this method is applied in order to estimate age in an individual whose age is unknown (Rylands-Monk, 2017).

b) **Bone-by-bone method**: The Tanner-Whitehouse (1975) method (TW2) was compiled from a longitudinal study of over 2000 British children. This method involves systematic radiographic assessment of all the bones of the hand and wrist, where each bone is assessed and scored individually, according to degree of maturity. A summation of the scores is calculated and an overall maturity score is obtained. These are then used to read skeletal age from the centile tables as published in *Assessment of Skeletal Maturity and Prediction of Adult Height* by Tanner *et al.* (1975). Although the TW2 method is more accurate and reliable, the GP method is still the preferred method due to the complexity and long examination times of the TW2 method (Tanner *et al.*, 1975; Rucci *et al.*, 1995; Bull *et al.*, 1999; Niemeijer *et al.*, 2003; Dembetembe, 2010). This method however, has been criticised for its 'rough' staging of the distal radius and ulna epiphyses (Rylands-Monk, 2017). 'Rough' staging refers to the lack of descriptive differentiation between the stages

of development for the distal radius and ulna, particularly concerning later stages of epiphyseal plate fusion (Schmidt *et al.*, 2008:b).

c) **Epiphyseal plate scoring:** An epiphyseal scoring method was first introduced by Moss and Noback (1958). Various authors have since utilised and modified this method, whereby the degree of epiphyseal plate fusion of a particular epiphysis is assessed. Generally, these scoring methods assess four to five different stages of fusion such as applied in the works of Schmeling *et al.*(2004:b), Schmidt *et al.* (2008:a) and Baumann *et al.*(2009). Some authors, however, only used a two-stage scoring system and scored the epiphyses as either 'fused' or 'unfused'(Pryor, 1923; Paterson, 1929; Flecker, 1932; Abbie and Adey, 1953; Banerjee and Agarwal, 1998; Nemade *et al.*, 2010). A two-stage scoring system is not advisable as the process of epiphyseal plate fusion occurs over an extensive period of time during which the epiphyseal surfaces undergo more complex morphological changes. Mostly, epiphyses are visualised through the use of X-rays, however, computed tomography (CT) (Kellinghaus *et al.*, 2010), sonography (Schmidt *et al.*, 2013) and magnetic resonance imaging (MRI) (Saint-Martin *et al.*, 2013; Serin *et al.*, 2016) techniques have been described. According to Schmidt *et al.* (2013), isolated consideration of the distal radius, compared to the atlas and bone-by-bone methods where the entire skeleton of the hand is assessed, is a more reliable technique as it reduces intra- and inter-observer variation. This method is sufficient for estimation of maturity and makes use of clearly defined radiomorphological characteristics in order to determine the stage of epiphyseal plate fusion. Furthermore, complexity involved in the identification of several landmarks is reduced (Soegiharto *et al.*, 2008; Schmidt *et al.*, 2013; Serin *et al.*, 2016).

d) **Transition analysis:** Transition analysis refers to the transition of a specific skeletal trait from one developmental stage to the next. Therefore, it aims to estimate the average age an individual transitions from one stage (*i*) to the next stage (*i* +1) (Boldsen *et al.*, 2002; Lottering *et al.*, 2015; Tangmose *et al.*, 2015). This method is similar to the above mentioned epiphyseal plate scoring method as it involves scoring the degree of maturity of the skeletal trait to determine age at transition. Boldsen *et al.* (2002), states that though individual variability of transition exists, the directionality remains fixed, due to the relatively unvarying manner of maturation and aging of skeletal elements. Transition analysis assumes that correlation between traits or indicators is only attributable to age (Tangmose *et al.*, 2015).

Population differences in the timing of epiphyseal plate fusion has been widely reported in literature, thereby indicating the need for population-specific standards. Related studies have been conducted on a South African population; however, the approach lacks the robust statistical component to make the method compliant with standards of best practice required of forensic methodology. Thus, the present study aimed at re-evaluating the age estimation standards for the epiphyseal plate fusion of the distal radius in ulna in a modern South African population.

Chapter 3 MATERIALS AND METHODS

3.1 ETHICAL CLEARANCE

Ethical clearance was obtained from the Faculty of Health Sciences Research Ethics Committee, University Pretoria (Ethics Reference No.:157/2017), as well as the Masters Committee of the Faculty of Health Sciences (Annexure A). Further permission was obtained for the use of hand-wrist radiographs from Director, Dr Johan Venter from Van Dyk and Partners Inc. at Mediclinic, Bloemfontein (Annexure B).

3.2 STUDY DESIGN

This study is a retrospective, cross-sectional study of hand-wrist radiographs. Cross-sectional studies are frequently used to determine prevalence of a given outcome in a population at a specific point in time (Mann, 2003). Due to the fact that cross-sectional studies provide more information on the variance of a population, these studies are more useful for age estimation than longitudinal studies (Ousley *et al.*, 2013; Stull *et al.*, 2014).

3.3 SOUTH AFRICAN POPULATION

The South African population contains cultural, linguistic and genetic diversity (Tishkoff and Williams, 2002); and consists of three major groups, namely South African black (80.7%), coloured (8.8%) and white (8.1%) individuals. The remaining 2.5% are comprised of Asian and Indian populations (Statistics South Africa, 2016). Each group constituting the South African population displays variation in origin and history (Liebenberg *et al.*, 2015). In addition, South Africa has undergone major socio-political changes over the last few decades. Apartheid was a policy which forced the segregation of individuals based on population group, favouring white individuals (Lakha, 2015). The enforcement of Apartheid resulted in lower SES, through limited access to resources of non-white individuals (Hawley *et al.*, 2009; Lakha, 2015). Furthermore, Apartheid influenced gene flow in modern South Africa, resulting in higher variability between some population groups (Stull *et al.*, 2014). Many skeletal differences have been noted between black and white South Africans (Stull *et al.*, 2014). Therefore, both black and white population groups were examined for potential differences in epiphyseal plate fusion times.

Mediclinic, Bloemfontein is a private hospital, and therefore patients admitted to this institution are likely to be of mid- to higher SES. As the inclusion of SES as a variable is

beyond the scope of the study, an attempt was not made to include individuals of lower SES as well. Thus, expected differences were based on population variation rather than SES.

3.3.1 Black South Africans

Black South Africans are mainly descendant from West and Central Africa (between Nigeria and Cameroon) (Huffman, 1982; Franklin *et al.*, 2007). Bantu-speaking groups are believed to have migrated towards sub-Saharan Africa during the first millennium A.D., ultimately settling in the eastern and southern parts of Africa (Huffman, 1982; Franklin *et al.*, 2007). 'Bantu' is a linguistic term referring to groups or tribes clustered by related languages. Due to different migratory paths, various sub-phylums have developed; black South Africans belong to the Niger-Congo linguistic group and further subdivisions, such as Natal Nguni, Cape Nguni and Sotho, have led to the existence of different ethnic groups and tribes among black South Africans, each with their own culture (Ribot, 2004; Stull *et al.*, 2014; Liebenberg *et al.*, 2015).

3.3.2 White South Africans

White South Africans are descendant of the 17th century European colonists who established the Cape colony along the trade route of the Dutch East India Company. The first settlements were established by Dutch and later French, British and German immigrants. However, due to Founder's effect, South African white individuals have been shown to differ morphologically from their parent populations (Steyn and İşcan, 1999; Greeff, 2007; Stull *et al.*, 2014; Liebenberg *et al.*, 2015). Founder's effect is the loss of genetic variation due to a small sample of genes being carried over to the new population (Understanding evolution, 2008).

3.4 STUDY SAMPLE

Hand-wrist radiographs were retrospectively obtained from Van Dyk and Partners Inc. at Mediclinic, Bloemfontein. The sample consisted of patients who had undergone hand-wrist radiographs at Mediclinic, Bloemfontein from January 2009 to August 2017. A total of 782 radiographic images of individuals between the age of eight and 30 years were obtained through convenience sampling, i.e. all radiographs that fit the selection criteria were used.

Chronological age for each individual was calculated from the date of birth to the date of X-ray (Cameriere *et al.*, 2012). Radiographs lacking a date of birth were excluded. Furthermore, radiographic images had to be clear and include both the distal radius and ulna. Radiographs must have been taken in an antero-posterior or postero-anterior view. Images were rejected in

the presence of severe trauma affecting the epiphyseal plates of the distal radius or ulna, any pathology, atypical skeletal growth, or the presence of foreign bodies in the field of view (Dembetembe and Morris, 2012; Lottering *et al.*, 2015; Davies *et al.*, 2016).

Patient name and surname were used to ascertain population group. According to the 2011 South African Census (Statistics South Africa, 2011), Sesotho is the most spoken home language (64.2%) in the Free State, followed by Afrikaans (12.7%). Free State population group distribution was found to comprise of 87.6% black and 8.7% white individuals (Statistics South Africa, 2011). Therefore, Afrikaans home language with a typically Afrikaans surname is suggestive of a white individual, while Sesotho home language with a typically Sesotho (or any other related South African language) surname will be more indicative of a black individual. Only black and white population groups were included to ensure adequate sample size. The application of surname and home language was used as hospitals do not record the ancestry of the patient. While it is acknowledged that many factors may influence the surname and language of an individual, a correlation exists with ancestry. However, the lack of confirmed records of ancestry is recognised as a limitation of the study.

Patient information was kept confidential by assigning a number to each radiograph. The first letter of each word in the name of the institution from which the radiographic images were received constituted the first alphanumerical letters of the image number (i.e. MB – Mediclinic, Bloemfontein), this was followed by numerical characters (starting with 001), as assigned by the researcher (Dembetembe, 2010). Patients' sex, date of birth and date of X-ray was recorded.

3.4.1 General distribution of the study sample

A total of 782 radiographs were assessed and scored. In all cases the radii could be scored; however, ten ulnae could not be scored due to lack of clarity of the skeletal element.

The study sample consisted of 203 black males (26.0%); 129 black females (16.5%); 233 white males (29.8%) and 217 white females (27.7%) between the ages of eight and 30 years (Figure 3.1; Annexure B, Table B1). There were notably fewer black female subjects available for use in this study. This might be attributable to a lower frequency of visits to the radiology department at Mediclinic, Bloemfontein; or a higher number of traumatic events encountered by the other groups compared to black females (Dembetembe, 2010).

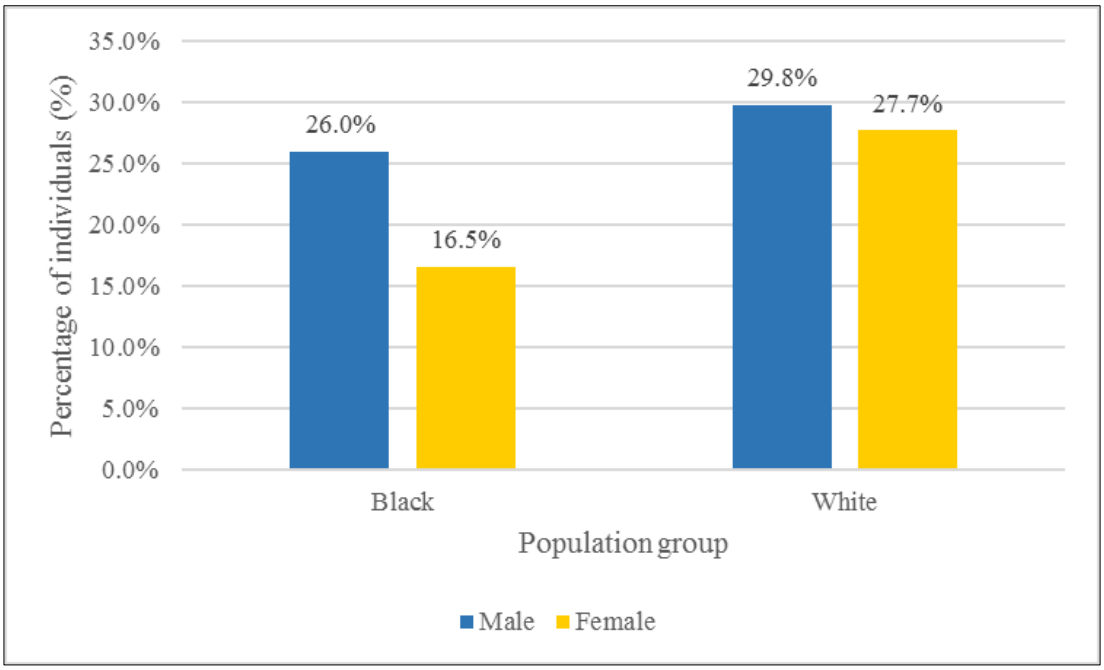

Figure 3.1:Composition of study sample by sex and population group

Figures 3.2 and 3.3 show the age distribution of the study sample by sex and population group. The younger and older age cohorts have smaller sample sizes compared to age cohorts in the middle of the age range. Within the male sample, the greatest number of individuals were found at 14, 15 and 22 years; whilst the greatest number of females were found at ages 13, 15 and 17 years.

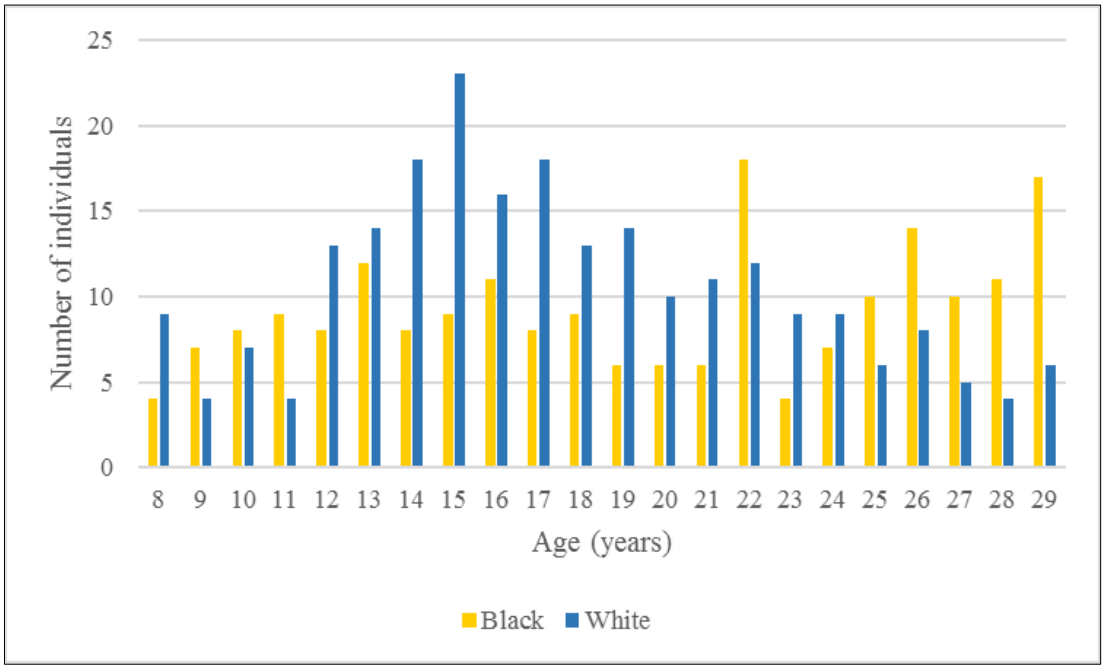

Figure 3.2: Age distribution of the study sample for males

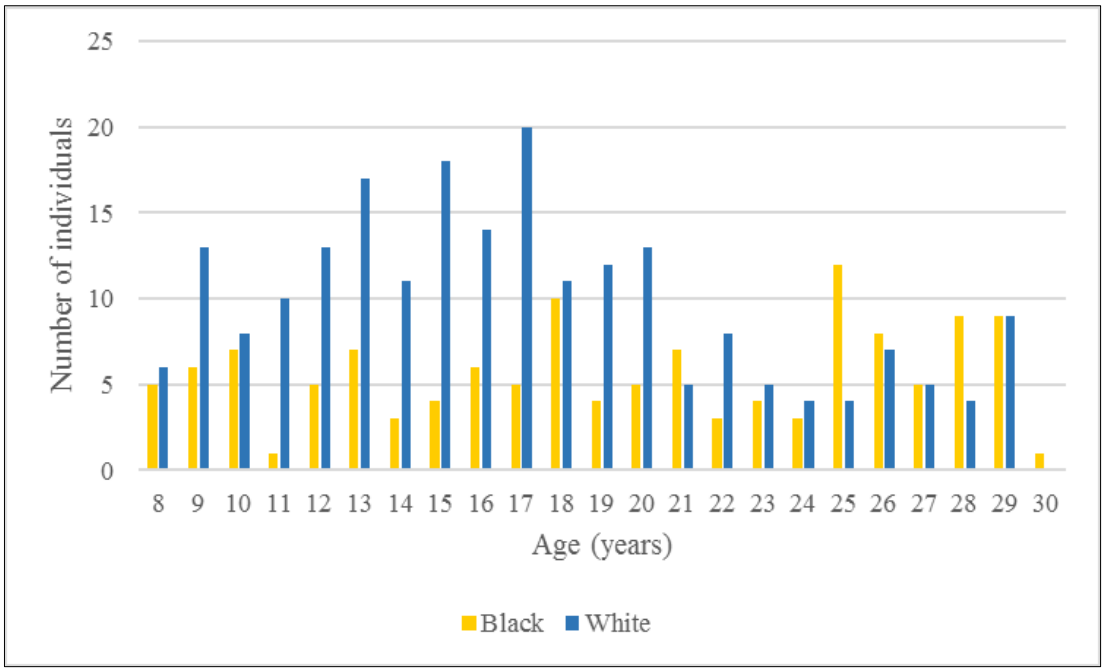

Figure 3.3: Age distribution of the study sample for females

3.5 METHOD

All hand-wrist radiographs were sourced from the *C-PACS* archive system and assessed and scored using *GE – Healthcare Centricity Universal Viewer* (version 6).

Each radiograph was analysed by the researcher to determine the stage of epiphyseal plate fusion of the distal radius and ulna. Because several years pass between the initiation of epiphyseal plate fusion and complete fusion of the epiphysis it is important to assess the various stages of epiphyseal plate fusion, rather than simply documenting whether an epiphysis is 'fused' or 'unfused'(Ubelaker, 1987). The degree of fusion was gauged using a four-stage scoring system, modified from multiple studies ranging from three to five stage scoring systems; where the following criteria determined the category of maturation allotted to each epiphysis (Schmeling *et al.*, 2004:a; Memchoubi, 2006; Nemade *et al.*, 2010; Saint-Martin *et al.*, 2013; Lottering *et al.*, 2015):

Stage I: Epiphyseal plate is classified as open; no sign of fusion is present (absence of bridging). A complete radiolucent line is visible throughout the length of the epiphyseal plate (Figure 3.4a).

Stage II: Fusion between the epiphysis and the metaphysis has commenced and partial union is visible (gap between contact surfaces is not continuous as bridging occurs).

Characterised by a radio-dense area in the middle or on either side of the epiphyseal and metaphyseal contact surfaces (less than 50%) (Figure 3.4b).

Stage III: Advanced fusion of the epiphyseal plate occurs. Characterised by the presence of a radio-dense area on more than 50% of the contact surfaces between the epiphysis and the metaphysis (Figure 3.4c).

Stage IV: Classified as the complete fusion of the epiphysis and metaphysis. The epiphyseal plate is characterised by the obliteration of the space between the epiphysis and metaphysis; thus, a radio-dense area is seen throughout the entire length of the epiphyseal plate. Additionally, no break in the continuity of the periosteum is visible and the epiphysis presents with the same density and architecture as the surrounding bone (Figure 3.4d).

Any epiphysis that could not be scored due to visibility was omitted from the sample. Additionally, comments were added with regard to the physical and morphological appearance of the epiphyses during various stages of fusion. Scores were entered into an *Excel* spreadsheet for further statistical analysis.

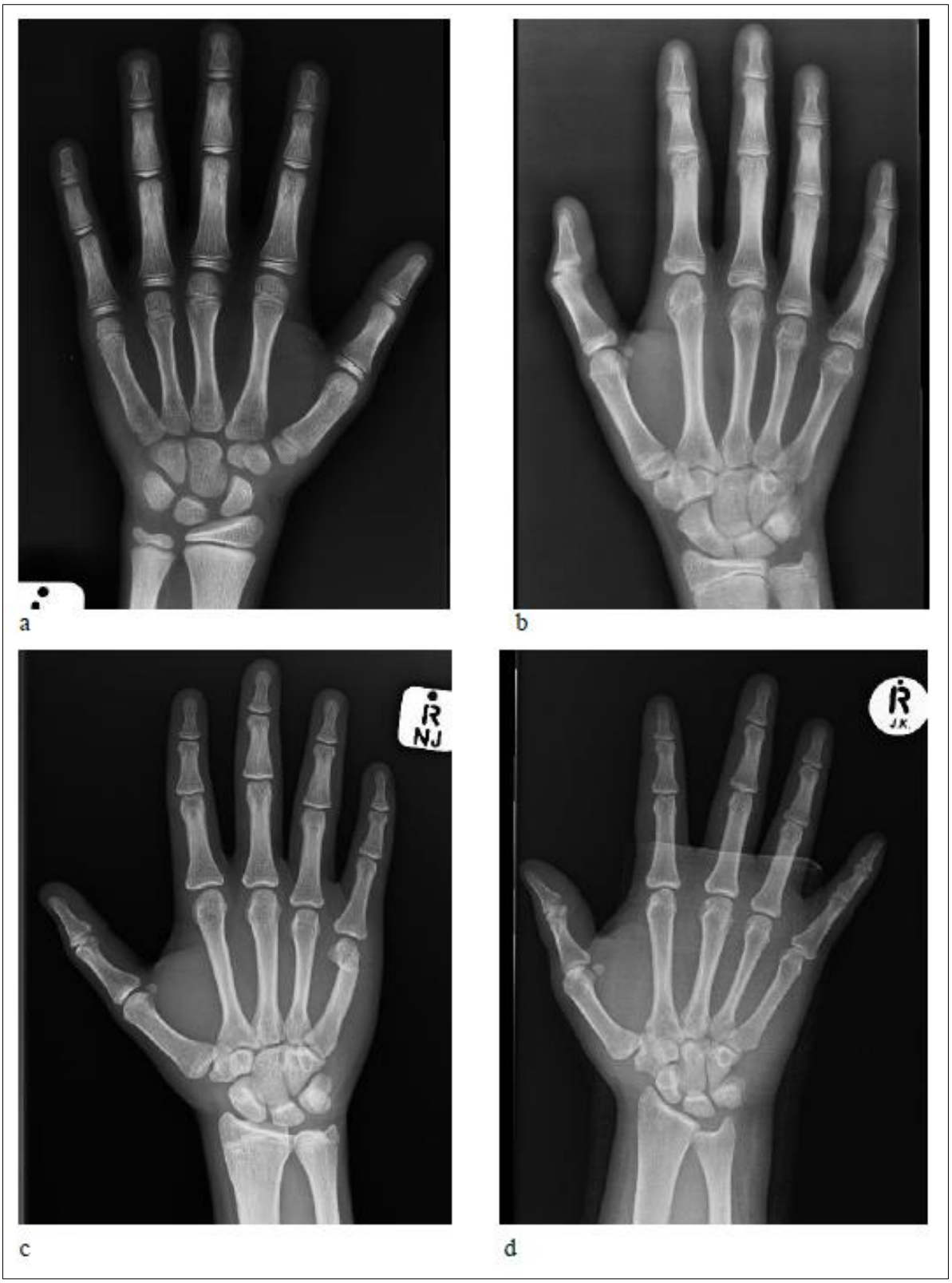

Figure 3.4: Four-stage scoring system of epiphyseal plate fusion

In addition to assessing the timing of epiphyseal plate fusion, the prevalence of persistent epiphyseal scars was evaluated. As mentioned previously, an epiphyseal scar is formed at the locus of fusion between the metaphysis and epiphysis. It is characterised by a radio-dense,

white, horizontal line when inspected on a radiograph. All radiographs of individuals older than the estimated age of complete fusion (as calculated with TA) were examined in order to determine whether an epiphyseal scar was still visible:

Absent (A): Epiphyseal scar absent.

Present (P): Epiphyseal scar visible.

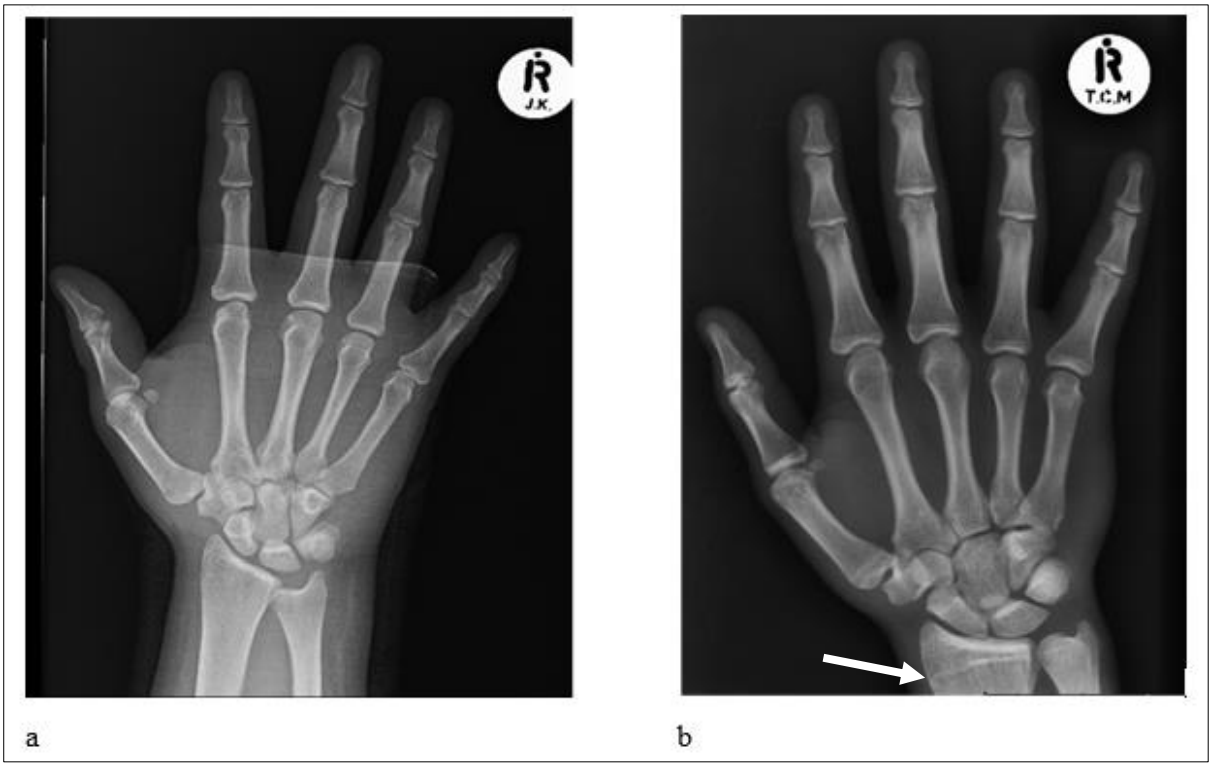

Figure 3.5: Evaluation of persistent epiphyseal scar: a) Absent b) Present

3.6 STATISTICAL ANALYSES

The scores of epiphyseal fusion allotted to each individual were used to calculate the frequency distribution of each stage for the radius and ulna, respectively. The frequency distributions were calculated separately for the sexes (males and females) and population groups (black and white South Africans) to assess the effect of sex and population group on fusion of the wrist.

Data were tested for normality using the Shapiro-Wilks test; as the data was found to be non-normal, non-parametric tests had to be used throughout. Possible mean age differences were tested with a Kruskal-Wallis test using fusion stage, population group and sex as independent variables, with tests for all two-way and the three-way interaction, using the 5% level of significance ($p < 0.05$). A Kruskal-Wallis test is a non-parametric test that does not

assume normality of the data (Laerd Statistics, 2017). Separate analyses were conducted for the radius and ulna. Additionally, a frequency distribution was calculated to assess the prevalence of a persistent epiphyseal scar. The prevalence of persistent epiphyseal scars between black and white males and females were calculated for individuals who had achieved stage IV fusion.

Transition analysis (TA) was conducted to obtain age ranges to model the South African population. Transition analysis models the passage, or transition, of an individual from one developmental stage to the next higher stage in an ordered sequence (Boldsen *et al.*, 2002). Essentially, TA provides a maximum likelihood estimate, which is the average age individual is most likely to transition from one phase to the next (Langley-Shirley and Jantz, 2010). Transition analysis has advantages over the more commonly employed percentile method, in that it is less sensitive to developmental outliers (e.g. very early fusion), sample size constraints and the effects of age mimicry in the sample (Shirley and Jantz, 2011). Using the cumulative logit function in *R*, the model employs logistic regression to fit the intercept and slope of the regression model to the data, which is then converted to the average age and standard deviation of the age an individual will move from one stage to the next (i.e. age of transition) (Konigsberg *et al.*, 2008). The TA was conducted using uniform priors; uniform priors reflect a more realistic approach as it makes no assumptions about the age distribution of the target population (Lottering *et al.*, 2015).

In addition to TA, Bayesian statistics were applied using the coefficient estimates calculated with TA to determine the posterior distribution of age across the population for each stage. Essentially, TA was used to estimate the average age individuals transition from one stage to the next, while the Bayesian statistics were used to calculate the likely age of an individual to whom a given phase is allotted (i.e. the Bayesian analysis will provide the average age associated with a stage and TA will provide the likely age an individual will enter or leave that stage). The combination of TA and Bayesian statistics was used to create the population-specific aging standards for application on black and white South African subadults.

In order to assess inter-observer variation, 20 randomly selected radiographs of individuals of variable age and population group were assessed and scored by an additional observer with anthropological experience (Observer 2). Intra-observer variation was assessed through a second evaluation of 20 randomly selected radiographs by the primary researcher (Observer 1). Selection of radiographs to be re-evaluated for inter- and intra-observer variation occurred

through computer-aided random selection using *Excel*. This process occurred two weeks after data collection had been completed.

Inter- and intra-observer variation were tested with Cohen's Kappa (κ) statistic. Kappa statistics generate a numerical value of how much agreement is present compared to how much agreement is expected to be present due to chance, and therefore provides information on the repeatability of the data or method (Sim and Wright, 2005; Zaiontz, 2016). The Kappa value is calculated as follows (Landis and Koch, 1977):

$$\kappa = \frac{observed\ agreement - chance\ agreement}{1 - chance\ agreement}$$

Kappa may take a negative value; a negative Kappa value is a rare occurrence and it implies that the agreement between the two observers is less than would be expected by chance (Simon, no date). However, we are only interested in the values that lie between zero and one (Zaiontz, 2016). Table 3.1 shows the scale demonstrating the level of agreement frequently used to interpret the calculated Kappa value, which ranges from no agreement to very good agreement (Landis and Koch, 1977).

Table 3.1: Scale for interpretation of κ value

	No agreement	**Poor**	**Fair**	**Moderate**	**Good**	**Very good**
κ	0	0.0–0.2	0.2–0.4	0.4–0.6	0.6–0.8	0.8–1.0

Chapter 4 RESULTS

4.1 OBSERVER VARIATION

Observer variation was tested with Cohen's Kappa statistic and single score interclass correlation coefficient (ICC). Table 4.1 indicates the quantification of inter- and intra-observer variation for both the radius and ulna. Intra-observer agreement was found to be almost perfect (following the Landis and Koch scale (1977)), with Kappa values calculated at 0.96 and 0.92 for the radius and ulna, respectively. Furthermore, ICC values greater than 0.9 were obtained for both the radius and ulna. Almost perfect agreement was also observed for the inter-observer variation of the radius ($\kappa = 0.80$ and ICC = 0.93); however, precision in the scoring of the ulna proved to be more difficult as only moderate agreement was achieved ($\kappa = 0.542$, ICC = 0.911). The results demonstrate satisfactory consistency and repeatability of the scoring method when applied to the radius.

Table 4.1: Quantification of observer variation with Cohen's Kappa statistic and interclass correlation coefficient for scores of the radius and ulna

	Skeletal element	**Kappa (κ)[a]**	**Single Score ICC[b]**	**95% CI**
Inter-observer variation	Radius	0.80	0.93	0.84-0.97
	Ulna	0.54	0.91	0.79-0.96
Intra-observer variation	Radius	0.96	0.98	0.96-0.99
	Ulna	0.92	0.97	0.93-0.99

[a] Kappa: 0.8-1.0 = almost perfect; 0.6-0.8 = substantial; 0.4-0.6 = moderate; 0.2-0.4 = fair; 0-0.2 = slight, <0.00 = poor (Landis and Koch, 1977).
[b] Interclass correlation coefficient, ICC: > 0.9 = excellent agreement (Koo and Li, 2016).

4.2 EXPLORATORY STATISTICS

4.2.1 Descriptive statistics

Descriptive statistics (means, standard deviations (SD) and age ranges) of each phase of epiphyseal plate fusion of the distal radius and ulna in a South African population are shown in this section.

4.2.1.1 Radius

The distribution of the sample per stage of epiphyseal plate fusion of the distal radius is depicted for the pooled sample (Table 4.2), separated by population group (Table 4.3), separated by sex (Table 4.4) and separated by population group and sex simultaneously (Table 4.5). The mean age per stage increases from stage I to IV (Tables 4.2 – 4.5), with the highest variability seen in stage IV throughout all groups, coinciding with a larger sample size. Furthermore, observed age ranges overlapped within each group, suggestive of individual variation in the rate of fusion (Figures 4.1 – 4.4).

Table 4.2: Mean age, SD and age range of the radius per phase of fusion for the pooled sample

Phase	n	Mean age	SD	Observed range
I	142	11.31	2.06	8-16
II	114	13.84	2.39	8-21
III	75	15.94	1.70	11-23
IV	451	22.82	4.27	12-30

*Values in years

Table 4.3: Mean age, SD and age range of the radius per phase of fusion separated by population group

Group	Phase	n	Mean age	SD	Observed range
Black	I	61	11.11	1.83	8-14
	II	36	13.71	2.07	8-17
	III	21	15.95	1.65	12-18
	IV	214	24.10	4.05	15-30
White	I	81	11.46	2.21	8-16
	II	78	13.90	2.53	8-21
	III	54	15.93	1.74	11-18
	IV	237	21.67	4.15	12-29

*Values in years

Table 4.4: Mean age, SD and age range of the radius per phase of fusion separated by sex

Group	Phase	n	Mean age	SD	Observed range
Female	I	50	10.56	1.78	8-15
	II	50	12.95	2.67	8-20
	III	42	15.36	1.93	12-18
	IV	204	22.35	4.53	12-30
Male	I	92	11.72	1.86	8-16
	II	64	14.53	1.89	9-17
	III	33	16.67	0.96	14-18
	IV	247	23.21	4.02	15-29

*Values in years

Table 4.5: Mean age, SD and age range of the radius per phase of fusion separated by population group and sex

Group	Phase	n	Mean age	SD	Observed range
BF	I	16	9.708	1.08	8-12
	II	13	12.55	1.91	8-16
	III	11	15.14	1.85	12-18
	IV	89	23.72	4.29	15-30
BM	I	45	11.61	1.79	8-14
	II	23	14.32	1.9	9-17
	III	10	16.84	0.76	15-18
	IV	124	24.38	3.88	15-29
WF	I	34	10.96	1.91	8-15
	II	37	13.09	2.9	8-20
	III	31	15.44	1.99	11-23
	IV	115	21.29	4.45	12-29
WM	I	47	11.82	2.35	8-16
	II	41	14.65	1.9	9-21
	III	23	16.60	1.04	14-18
	IV	123	22.03	3.82	13-29

* Values in years
** BF = black female; BM = black male; WF = white female; WM = white male

Figures 4.1 – 4.4 represent the progression of epiphyseal plate fusion from stage I (no fusion) to stage IV (complete fusion) for black females, black males, white females and white males, respectively. Figure 4.1 represents the progression of epiphyseal plate fusion of the distal radius in black females. Stage I ranged between the ages of eight to 12 years, overlapping with stage II, which extended to 16 years of age. Stage III began at 12 years and terminated at 18 years, where after 100% of black females had completed epiphyseal plate fusion. In black males (Figure 4.2), stage II only began at the age of nine years. Stage II overlapped with stage I up to the age of 14 years, where after stage I terminated. Stage III began at age 15 and terminated at age 18, followed by complete fusion of 100% of black males. Figure 4.3 shows that in white females, stage I ranged between the ages of eight and 15 years, stage II and III terminated at the age of 23 years. At the age of 24, 100% of white females had completed fusion of the distal radius. In white males (Figure 4.4), stage II only began at the age of nine years. Stage I and II terminated by the ages of 16 and 17 years, respectively. Complete fusion was displayed by 100% of white males over the age of 22 years.

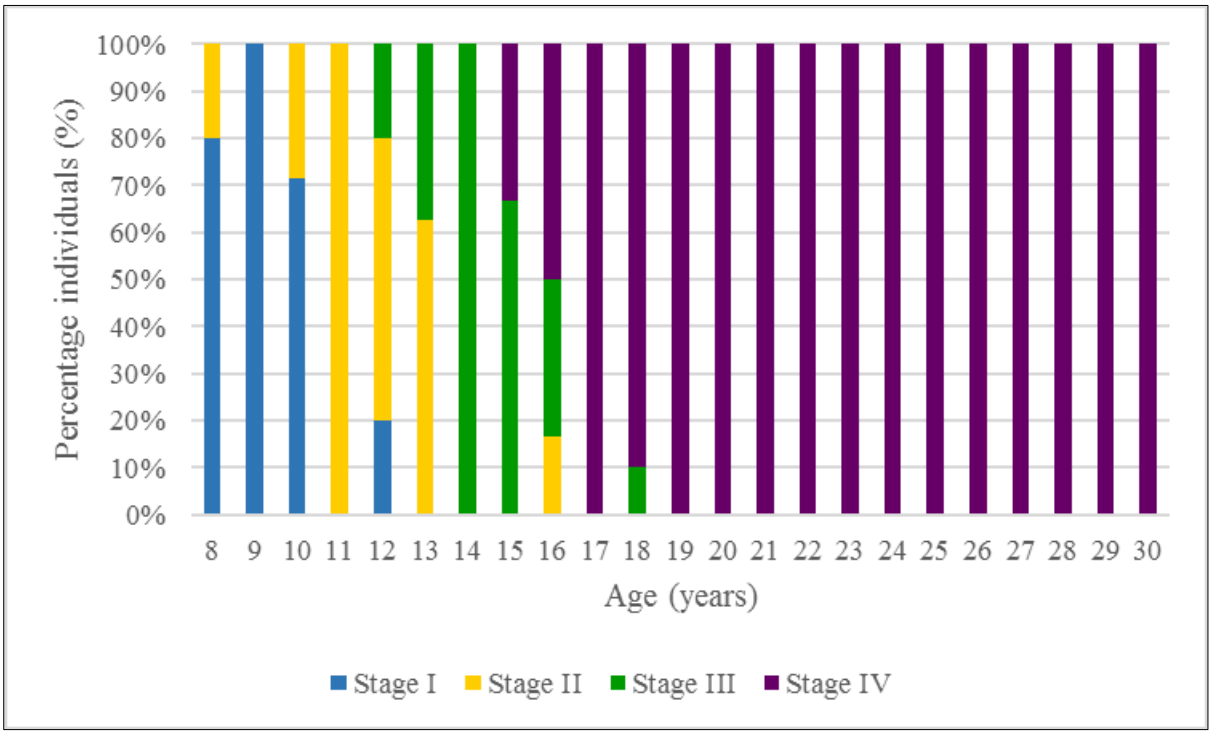

Figure 4.1: Progression of epiphyseal plate fusion of the distal radius in black females

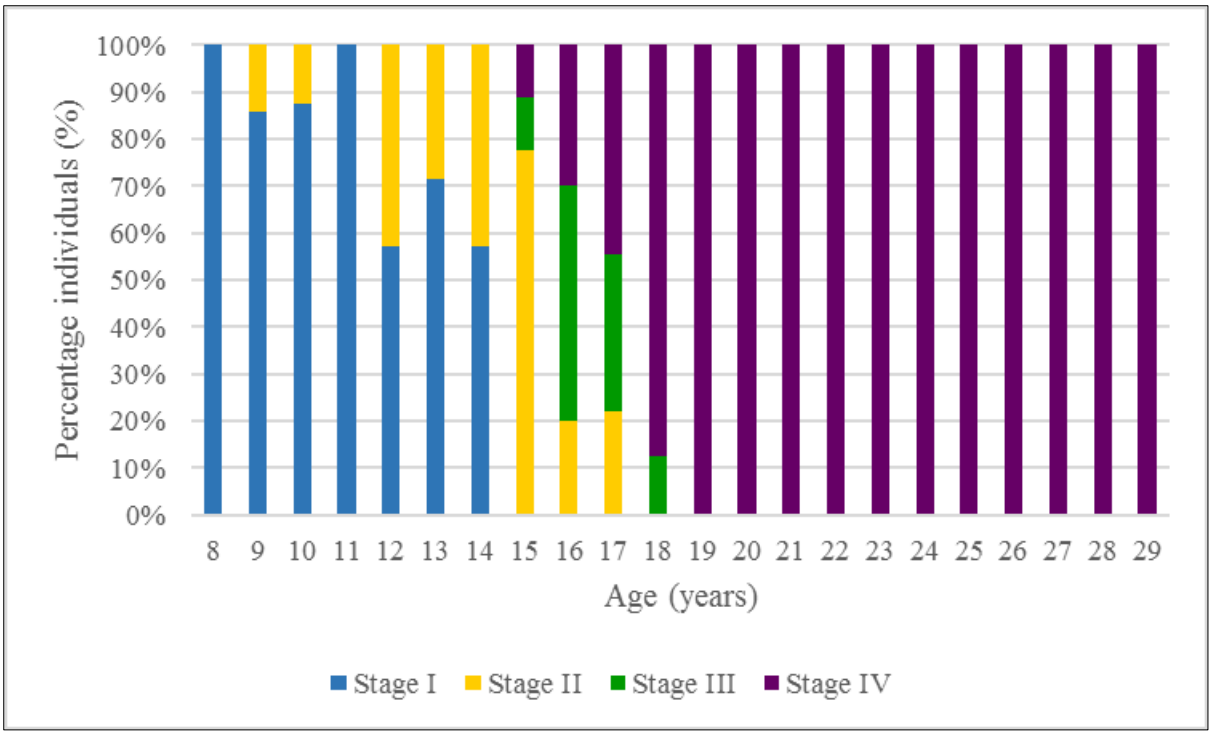

Figure 4.2: Progression of epiphyseal plate fusion of the distal radius in black males

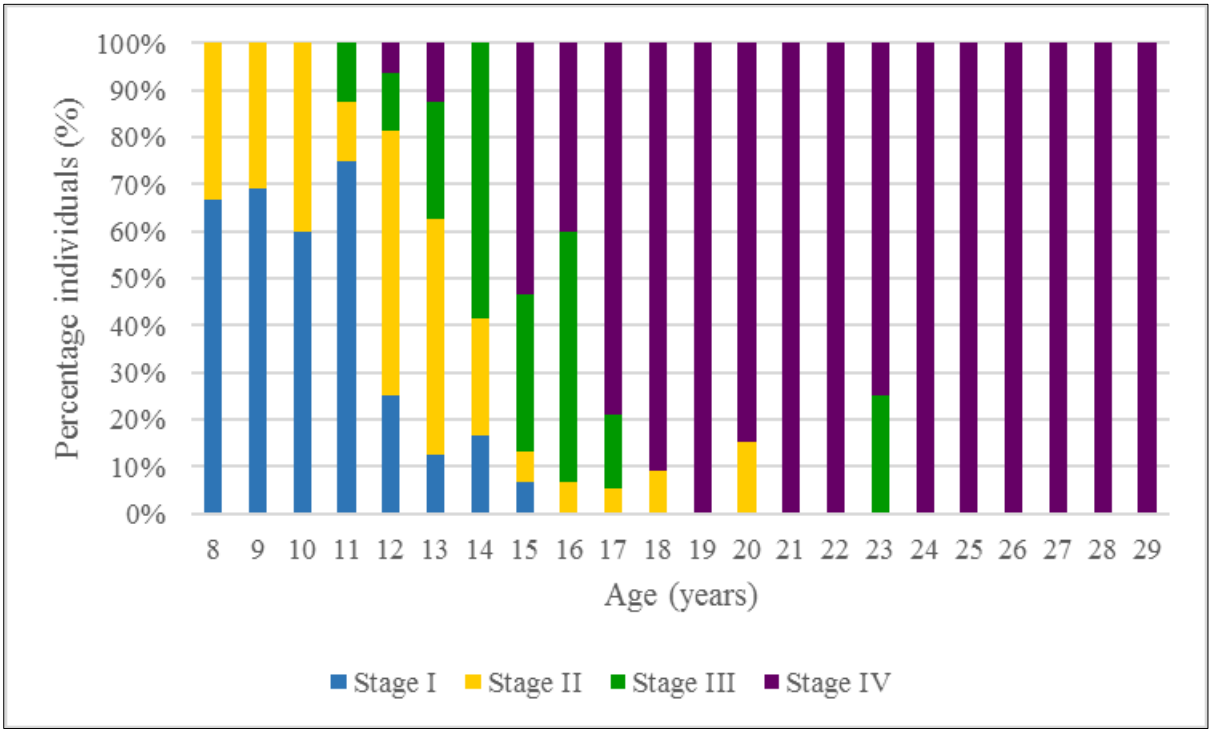

Figure 4.3: Progression of epiphyseal plate fusion of the distal radius in white females

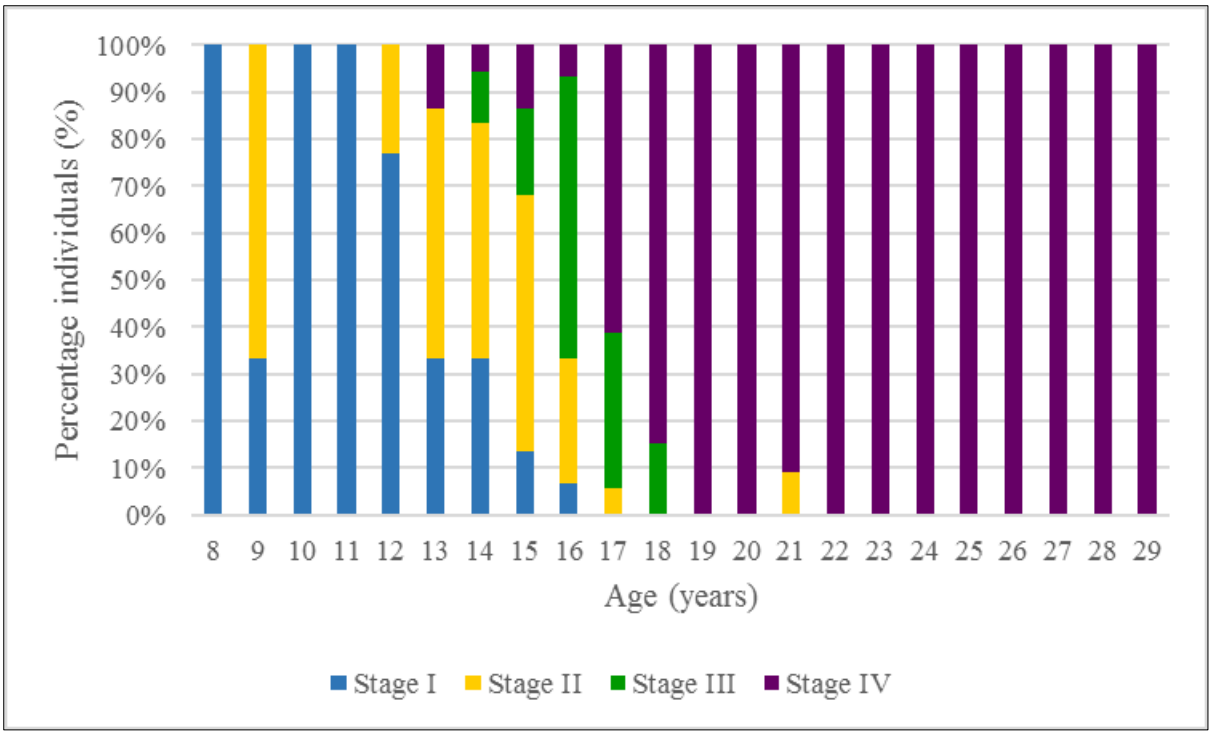

Figure 4.4: Progression of epiphyseal plate fusion of the distal radius in white males

4.2.1.2 Ulna

The distribution of the sample per stage of epiphyseal plate fusion of the distal ulna is depicted for the pooled sample (Table 4.6), separated by population group (Table 4.7), separated by sex (Table 4.8) and separated by population group and sex (Table 4.9). Similar to the radius, the mean age per stage increased from stage I to IV (Table 4.6 – 4.9), with highest variability again seen in stage IV throughout all groups. Furthermore, observed age ranges overlapped within each group, suggestive of individual variation in the rate of fusion (Figures 4.5 – 4.8).

Table 4.6: Mean age, SD and age range of the ulna per phase of fusion for the pooled sample

Phase	n	Mean age	SD	Observed range
I	162	11.30	2.01	8-16
II	69	14.76	2.24	9-21
III	76	15.47	1.85	11-23
IV	465	22.62	4.36	12-29

*Values in years

Table 4.7: Mean age, SD and age range of the ulna per phase of fusion separated by population group

Group	Phase	n	Mean age	SD	Observed range
Black	I	75	11.32	1.91	8-15
	II	22	14.97	1.69	12-17
	III	15	15.36	1.21	13-16
	IV	219	23.94	4.15	14-30
White	I	87	11.29	2.1	8-16
	II	47	14.66	2.47	9-21
	III	61	15.50	1.98	11-23
	IV	246	21.45	4.22	12-29

* Values in years

Table 4.8: Mean age SD and age range of the ulna per phase of fusion separated by sex

Group	Phase	n	Mean age	SD	Observed range
Female	I	66	10.70	1.72	8-14
	II	24	14.00	2.95	9-20
	III	38	14.71	2.00	11-23
	IV	216	22.01	4.63	12-30
Male	I	96	11.72	2.09	8-16
	II	45	15.16	1.65	12-21
	III	38	16.24	1.30	13-19
	IV	249	23.15	4.05	13-29

* Values in years

Table 4.9: Mean age, SD and age range of the ulna per phase of fusion separated by population group and sex

Group	Phase	n	Mean age	SD	Observed range
BF	I	23	10.24	1.50	8-13
	II	6	13.69	1.45	12-16
	III	7	14.36	1.02	13-15
	IV	93	23.42	4.44	14-29

BM	I	52	11.80	1.88	8-15
	II	16	15.38	1.61	12-17
	III	8	16.24	0.40	15-16
	IV	125	24.33	3.90	15-29
WF	I	43	10.94	1.79	8-14
	II	18	14.11	3.34	9-20
	III	31	14.78	2.17	11-23
	IV	123	20.95	4.50	12-29
WM	I	44	11.62	2.34	8-16
	II	29	15.03	1.69	12-21
	III	30	16.24	1.46	13-19
	IV	124	21.96	3.87	13-29

* Values in years
** BF = black female; BM = black male; WF = white female; WM = white male

Figures 4.5 – 4.8 represent the progression of epiphyseal plate fusion from stage I (no fusion) to stage IV (complete fusion) for black females, black males, white females and white males, respectively. Figure 4.5 represents the progression of ulnar fusion in black females. An open epiphyseal plate was present in all individuals up to the age of eleven years. Stage II ranged between 12 and 16 years. Stage III ranged between 13 and 15 years, complete fusion was first observed at age 14. After the age of 16, black females displayed complete fusion of the distal ulna. In black males (Figure 4.6), stage I continued up to the age of 15 years, overlapping with stage II from 12 to 15 years. Stage III ranged from 15 to 16 years of age. Complete fusion in black males was only observed at the age of 18 years. Figure 4.7 shows that initial fusion in white females occurred between nine and 16 years. Stage III overlapped with stage I, II and IV, ranging from 12 to 23 years of age. Complete fusion was first observed at the age of 12 years in two individuals; however, complete fusion of the entire white female sample was only observed at age 24. White males (Figure 4.8) entered stage II of fusion from the age of 12 years, overlapping with stage III from 13 to 17 years of age. Complete fusion (stage IV) of 100% of the white male sample was observed from age 23 onwards.

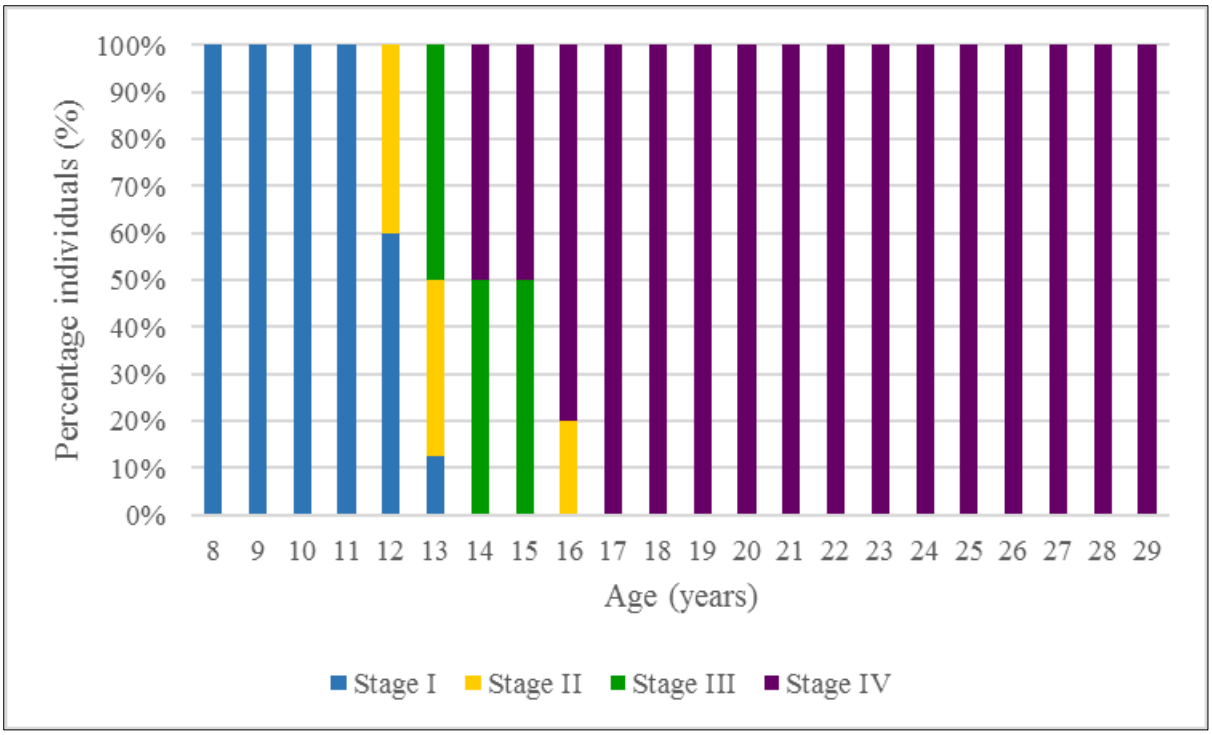

Figure 4.5: Progression of epiphyseal plate fusion of the distal ulna in black females

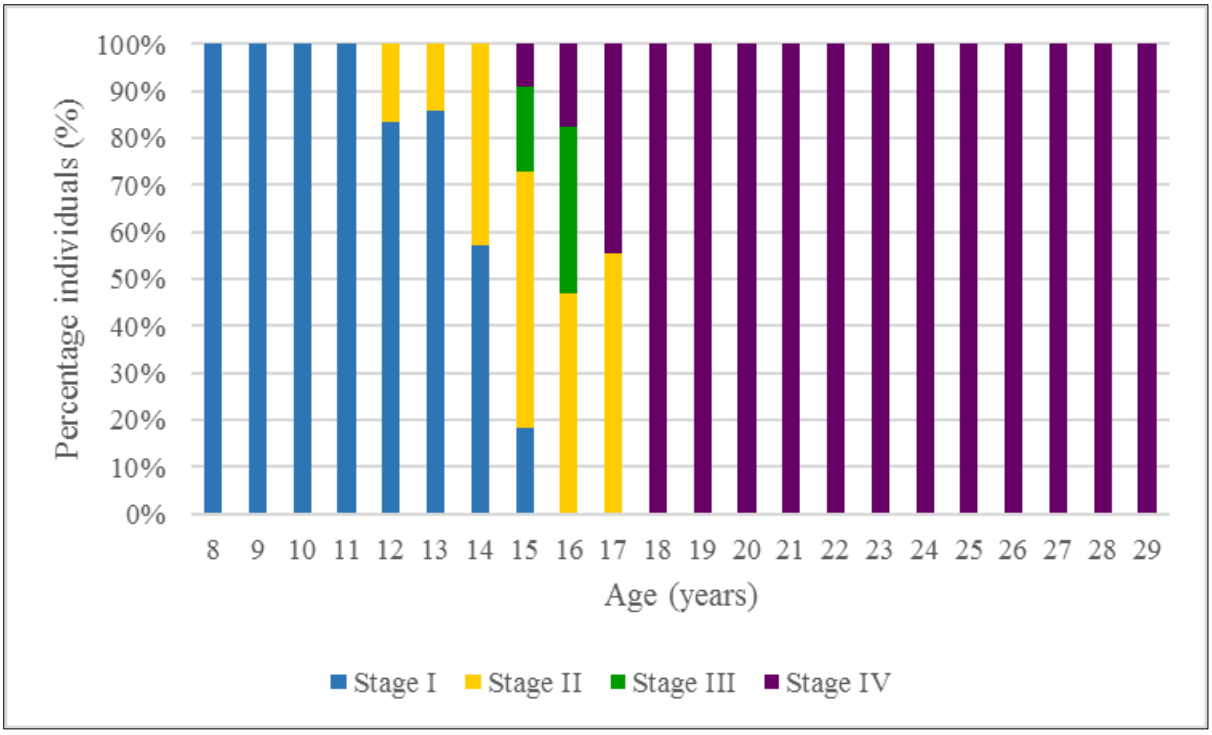

Figure 4.6: Progression of epiphyseal plate fusion of the distal ulna in black males

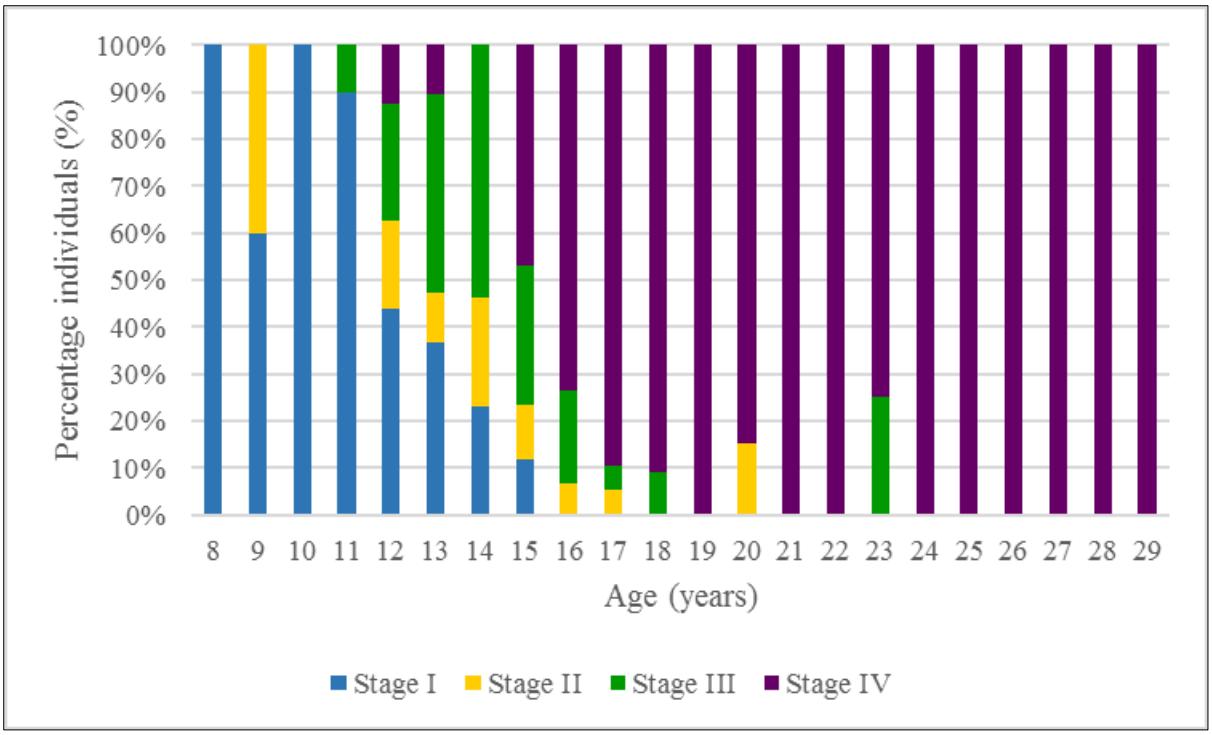

Figure 4.7: Progression of epiphyseal plate fusion of the distal ulna in white females

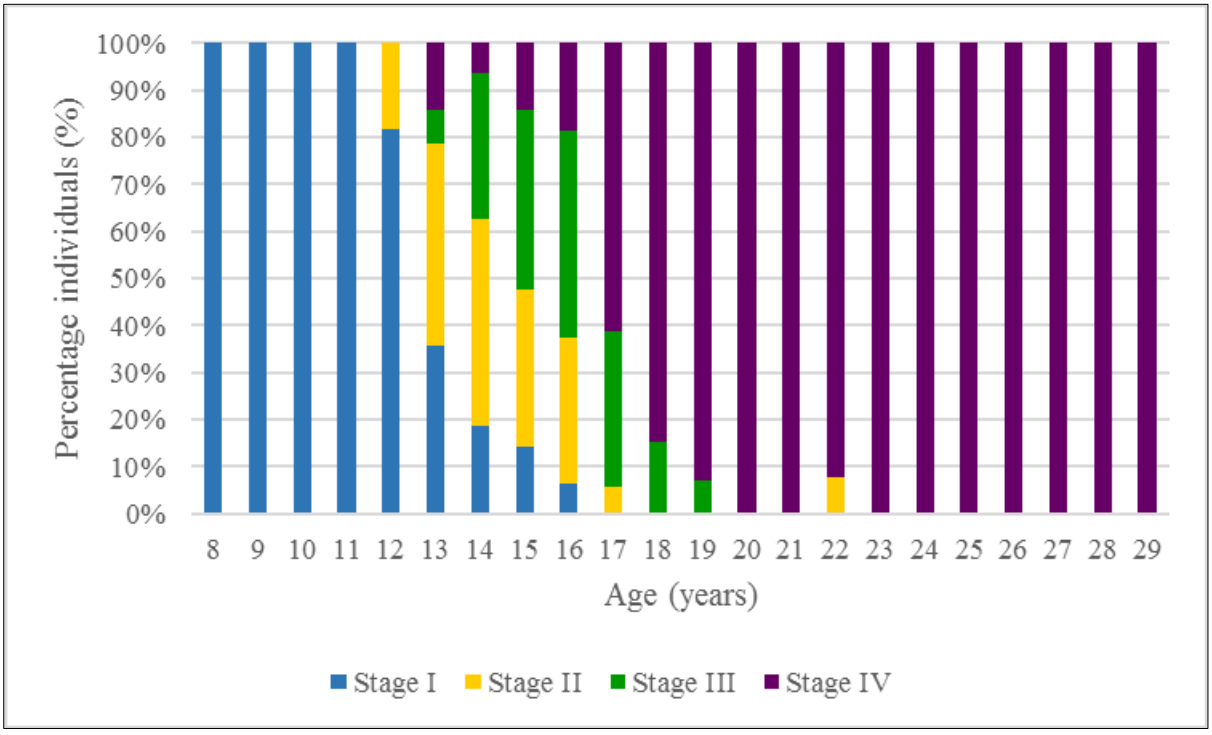

Figure 4.8: Progression of epiphyseal plate fusion of the distal ulna in white males

4.2.2 Exploratory analyses

A test for normal distribution of the data were performed using a Shapiro-Wilks test. Results indicated that the data distribution were non-normal (p-value < 2.2e-16); as such non-parametric statistical tests had to be employed. A Kruskal-Wallis test was performed to identify statistically significant differences between groups (Laerd Statistics, 2017), and showed statistically significant differences between the mean ages of distal epiphyseal plate fusion of the radius and ulna (Table 4.10).

Table 4.10: Results of the Kruskal-Wallis tests for evaluating statistically significant differences in epiphyseal fusion of the radius and ulna

	Comparison	**p-value**
Pooled sample	Radius – Ulna	**< 2.2e-16**
Radius	Black – White	**0.01288**
	Male – Female	0.14680
	Pooled	**0.01973**
Ulna	Black – White	0.07350
	Male – Female	0.13580
	Pooled	0.06394

*Statistically significant differences are highlighted in bold.

For the radius, statistically significant differences were detected between the fusion times of black and white South Africans as well as for the comparison within the pooled sample. However, no statistically significant differences were found between males and females for the chronological age of fusion (Table 4.10). For the ulna, no statistically significant differences were found between population groups or sexes (p>0.05) (Table 4.10).

A Kruskal-Wallis test is only indicative if whether or not a statistically significant difference is present; if more than two groups are compared, as is the case with the population-sex group of the radius, a post-hoc test is required to determine where the differences occur (*Dunn's test: Definition*, 2017). Thus, a *post-hoc* Dunn-test with a Benjamini-Hochberg adjustment was performed. Dunn's test revealed that statistically significant differences were present between black females and white females and black females and white males (Table 4.11).

Table 4.11: Results of the post-hoc Dunn-test evaluating statistically significant differences among the population-sex subgroups

Comparison	**p-value**
black female – black male	0.11493
black female – white female	**0.03804**
black male – white female	0.62315
black female – white male	**0.01408**
black male – white male	0.31993
white female – white male	0.54866

*Statistically significant differences are highlighted in bold.

4.3 TRANSITION ANALYSIS

Transition analysis was conducted to estimate the average age of transition from one stage of fusion to the next. Hereafter, Bayesian statistics were applied to estimate the average age at which an individual was found in a specific stage of epiphyseal plate fusion using different confidence intervals. Transition analysis was conducted for groups that displayed statistically significant differences ($p<0.05$). Therefore, TA models were run for the radius and ulna with all groups pooled, as well as for black and white individuals (with sexes pooled). No TA was run for the sexes as no statistically significant differences were observed.

4.3.1 Radius

Bayesian estimates for the radius indicating the mean age at transition between various stages of fusion are presented in Tables 4.12 and 4.13 for the pooled sample and the sample separated by population group. The age estimate increased as the individuals transitioned from one stage to the next in the ordered sequence. Overlap of the age ranges occurred between transition stage II-III and III-IV in the pooled sample (Table 4.12) as well as for black individuals (Table 4.13). White individuals transitioned from one stage to the next at a younger age compared to black individuals (Table 4.13). Population differences between transition stages are present for all stages of epiphyseal plate fusion. The peaks on the graphs for black individuals are higher and narrower, while the graphs for white individuals are flatter and wider (Figures 4.9 – 4.10). Thus, white individuals remain longer in one stage than black individuals.

Table 4.12: Bayesian estimates showing the average age-at-transition for the radius (pooled sample)

Transition stage	Estimate (±SEM)	SD
I-II	12.45 ± 0.66	
II-III	15.14 ± 0.76	1.18
III-IV	16.86 ± 0.81	

* Estimates in years

Table 4.13: Bayesian estimates showing the average age-at-transition for the radius per population group

Group	Transition stage	Estimate (±SEM)	SD
Black	I-II	12.81 ± 1.33	0.998
	II-III	15.35 ± 1.54	
	III-IV	16.95± 1.67	
White	I-II	12.19 ± 0.76	1.300
	II-III	15.04 ± 0.86	
	III-IV	16.85± 0.93	

* Estimates in years

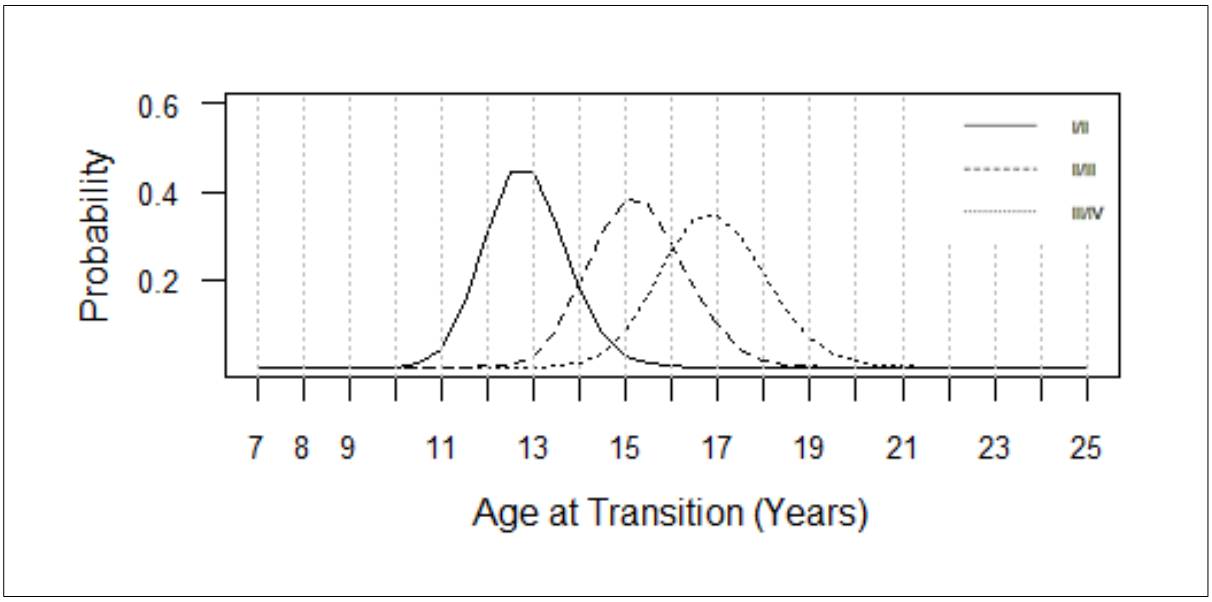

Figure 4.9: Probability density plot for age-at-transition distributions of the radius in black individuals

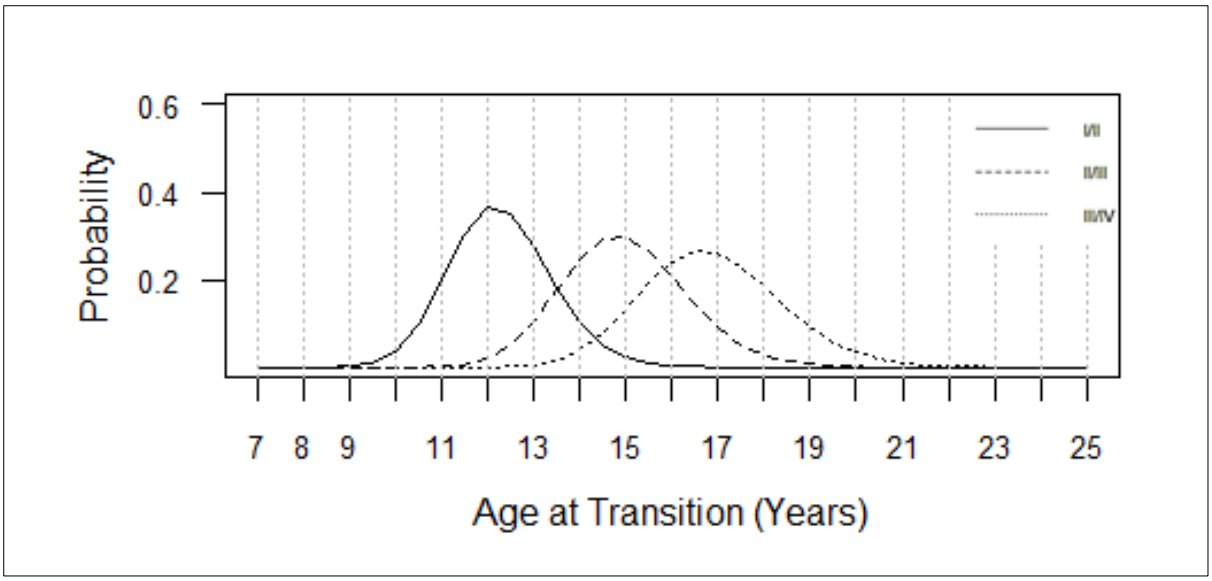

Figure 4.10: Probability density plot for age-at-transition distributions of the radius in white individuals

Tables 4.14 – 4.16 present posterior density estimates for the radius with confidence intervals demonstrating the mean age range of individuals within a specific stage of fusion. Thus, posterior mean values signify the estimated mean age associated with a given stage of development with a certain degree of confidence (95%, 90%, 75% and 68%, respectively). Confidence intervals represent the probability that an individual will fall within a particular age range if that individual displays morphological characteristics associated with a certain stage of epiphyseal plate fusion. Thus, CI's are reported alongside the posterior mean values in order to derive age estimates, with the 95% CI used most frequently (Lottering *et al.*, 2015).

Posterior distribution estimates regarding stage IV of epiphyseal plate fusion may provide misleading age estimates due to the fact that the upper age limit is designated by the upper age limit of the study sample (i.e. 30 years). Thus, using the 95% CI (Table 4.14), complete fusion of the distal radius, within the pooled sample, was achieved between the ages of 16.0 years and 18.6 years. Furthermore, an age estimate of ≤ 12.8 was afforded to an individual displaying stage I of epiphyseal plate fusion of the distal radius (95% CI).

Using the 95% CI, black individuals reached complete fusion of the distal radius earlier compared to white individuals. In contrast, stage I is associated with younger individuals in white South Africans than in black South Africans (Tables 4.15 – 4.16).

A wider age range was seen for stage I of fusion (Figure 4.11). Additionally, within stage IV of fusion, it is clear that white individuals display a wider age range at which fusion is completed (Figure 4.12).

Table 4.14: Posterior distribution estimates for the radius demonstrating the mean age per phase in the pooled sample

	Stage I	**Stage II**	**Stage III**	**Stage IV**
Mean CI	6.84	13.78	15.96	20.15
95% CI	≤ 12.81	11.02-16.53	13.49-18.55	≥15.97
90% CI	≤ 12.23	11.36-15.10	13.90-18.07	≥16.58
75% CI	≤ 11.09	12.15-15.43	14.53-17.45	≥17.353
68% CI	≤ 10.63	12.37-15.20	14.67-17.26	≥17.70

* Estimates in years

Table 4.15: Posterior distribution estimates for the radius demonstrating the mean age per phase in black South Africans

	Stage I	**Stage II**	**Stage III**	**Stage IV**
Mean CI	6.92	14.07	16.16	18.50
95% CI	≤ 13.21	11.66-16.48	13.97-18.28	≥ 15.91
90% CI	≤ 12.53	12.06-16.15	14.34-18.00	≥ 16.29
75% CI	≤ 11.42	12.66-15.51	14.83-17.43	≥ 16.85
68% CI	≤ 11.01	12.82-15.39	15.02-17.21	≥ 17.10

* Estimates in years

Table 4.16: Posterior distribution estimates for the radius demonstrating the mean age per phase in white South Africans

	Stage I	**Stage II**	**Stage III**	**Stage IV**
Mean CI	6.82	13.62	15.98	21.46
95% CI	≤ 12.84	10.59-16.64	13.22-18.67	≥ 16.16
90% CI	≤ 12.15	11.09-16.16	13.76-18.21	≥ 16.82
75% CI	≤ 10.88	11.86-15.48	14.37-17.55	≥ 17.89
68% CI	≤ 10.45	12.06-15.24	14.61-17.33	≥ 18.28

* Estimates in years

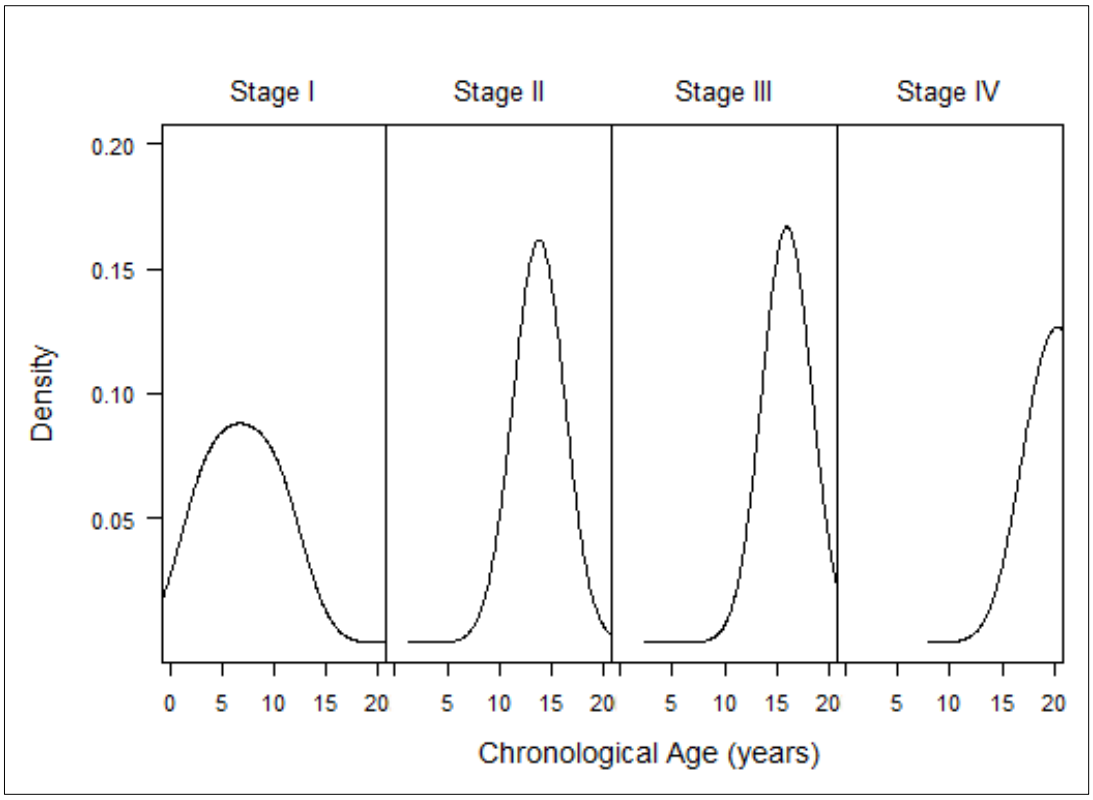

Figure 4.11: Probability distributions for the radius (pooled sample)

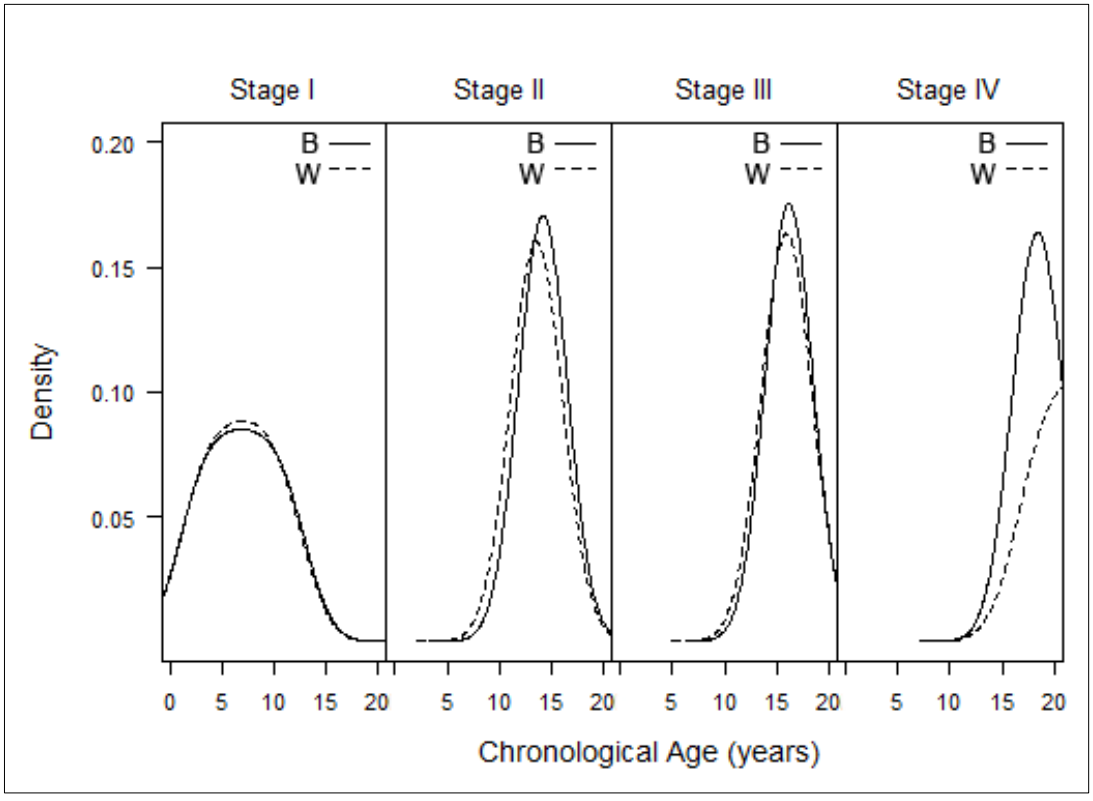

Figure 4.12: Probability distributions for the radius (per population group)

4.3.2 Ulna

Bayesian estimates for the ulna, indicating the mean age at transition between various stages of fusion, are presented in Table 4.17. The age estimate increased as the individuals transitioned from one stage to the next in the ordered sequence. No overlapping was observed between the transition stages. No statistically significant differences were observed for the ulna separated by population group (Table 4.10). However, for the sake of consistency, Bayesian estimates for the ulna separated by population group is shown in Annexure C (Table C1, Figures C5 – C6), as the information it provides proved to be of little use.

Table 4.17: Bayesian estimates showing the average age-at-transition for the ulna (pooled sample)

Transition stage	Estimate (±SEM)	SD
I-II	13.22 ± 0.78	
II-III	14.86 ± 0.84	1.11
III-IV	16.49 ± 0.90	

* Estimates in years

Table 4.18 presents posterior density estimates for the ulna with confidence intervals demonstrating the mean age range of individuals within a specific stage of fusion. Complete fusion (stage IV) of the ulna was attained between 15.6 and 18.2 years (95% CI). Furthermore, an age estimate of ≤ 13.5 years was afforded to an individual displaying stage I of epiphyseal plate fusion of the distal radius (95% CI).

Higher variability was found to be present for the first stage of epiphyseal plate fusion, due to the wider age range observed (Figure 4.13).

Table 4.18: Posterior distribution estimates for the ulna demonstrating the mean age per phase (pooled sample)

	Stage I	Stage II	Stage III	Stage IV
Mean CI	7.29	14.00	15.73	19.36
95% CI	≤13.50	11.69-16.21	13.34-18.21	≥ 15.59
90% CI	≤13.00	12.02-15.89	13.66-17.78	≥ 16.24
75% CI	≤11.77	12.54-15.38	14.36-17.20	≥ 17.09
68% CI	≤11.33	12.80-15.18	14.52-17.03	≥ 17.73

* Estimates in years

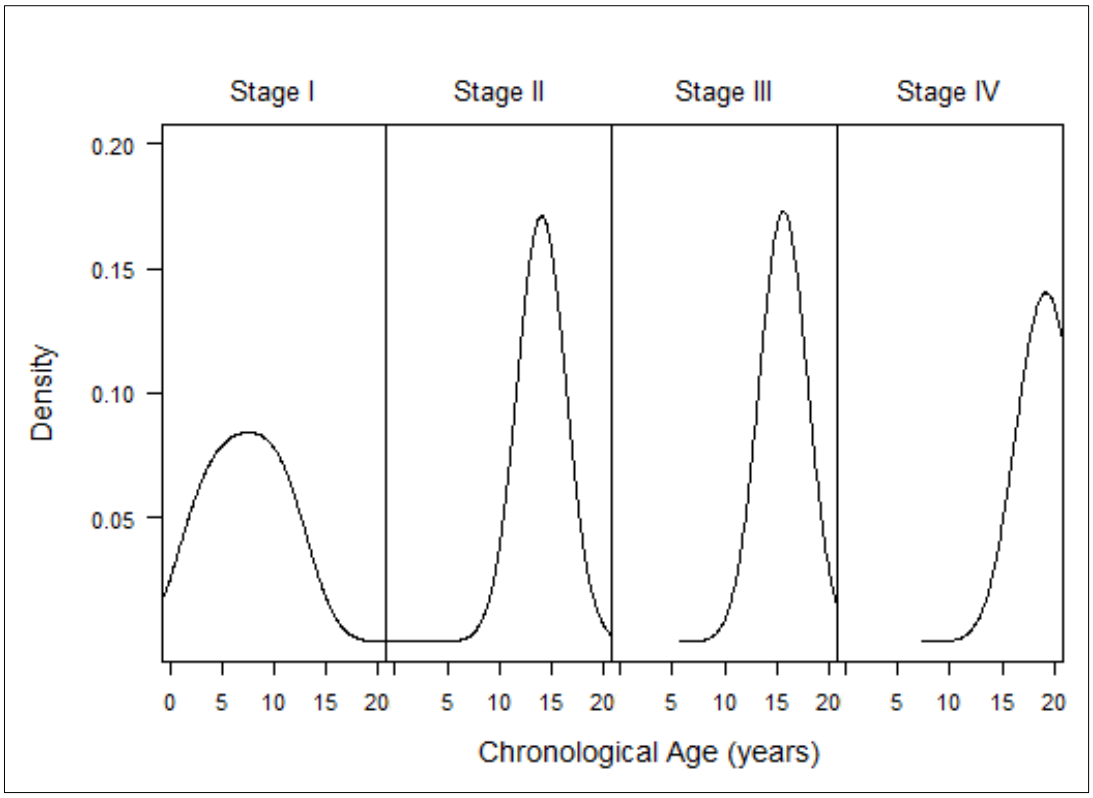

Figure 4.13: Probability distributions for the ulna (pooled sample)

4.4 MODEL ACCURACY

The accuracy of the four stage scoring system proved to be high when applying the 95% CI age estimates of the pooled sample to the current study sample. Model accuracy for the radius and ulna (all stages) was 83.9% and 85.1%, respectively. Accuracy per stage for each bone is reported in Table 4.19. Model accuracy was calculated by applying 95% CI posterior distribution estimates (pooled sample) for both the radius and ulna to each stage of epiphyseal plate fusion of the study sample. Therefore, accuracy represents the percentage (%) of individuals, displaying morphological characteristics of a specific stage, that fall within the predicted age range.

Table 4.19: Accuracy of the four stage scoring system using 95% CI

	Stage I	**Stage II**	**Stage III**	**Stage IV**
Radius	73.2%	77.2%	89.3%	95.8%
Ulna	84%	73.9%	85.5%.	96.8%

4.5 PERSISTENT EPIPHYSEAL SCAR

The presence of a persistent epiphyseal scar was evaluated using the estimated age of complete fusion of the pooled sample as calculated with TA. Using the 95% CI for the pooled sample (Tables 4.14 and 4.18), the radius and ulna were estimated to complete epiphyseal plate fusion by the age of 18.6 and 18.2 years, respectively. Persistent epiphyseal scars were found to be present in 89 individuals for the radius and eight individuals for the ulna (Table 4.19).

Table 4.20: Frequency of persistent epiphyseal scar

	n	**Epiphyseal scar present (n)**	**Epiphyseal scar absent (n)**	**Frequency (%)**
Radius	364	89	275	24.45
Ulna	377	8	369	2.12

Chapter 5 DISCUSSION

The current study is the first to report epiphyseal plate fusion standards of the distal radius and ulna in two South African populations with the application of TA and Bayesian statistics rather than merely employing mean data. The application of robust statistical analyses allows for the creation of more reliable age estimation standards for the distal radius and ulna that are specific to the South African population, and in so doing makes the method compliant with *Daubert* guidelines. This section will discuss the maturity of the wrist among other South African studies with respect to sample size differences, SES, as well as methodological differences. Furthermore, the practical application of the current method will be discussed, with emphasis on variation in statistical analyses and the advantages of TA.

5.1 MATURITY OF THE WRIST IN THE SOUTH AFRICAN POPULATION

With the implementation of TA and Bayesian analysis, the current investigation demonstrates that complete epiphyseal plate fusion of the distal radius occurs between ages of 15.9 and 18.3 years and 16.2 and 18.7 years in black and white South Africans, respectively (95% CI). Whereas, complete fusion of the ulna is attained from 15.6 to 18.2 years in the pooled sample. As the results did not identify major differences between the population groups, the use of the models created from the pooled datasets is recommended for practical application of the method.

Internationally recognised standards of Schaefer *et al.* (2009) and Scheuer and Black (2000), as well as numerous other studies (Davies and Parsons, 1927; Paterson, 1929; Uysal *et al.*, 2004; Baumann *et al.*, 2009) demonstrated a two-year delay in males for the onset of puberty, with resultant earlier epiphyseal plate fusion in females. Among South African groups, Lakha (2015) noted similar findings. Yet, the current study does not demonstrate distinct differences between the sexes (Figures 5.1 and 5.2). Females enter each stage of fusion slightly earlier compared to their male counterparts, which is consistent with variation observed by Patel *et al.* (2011), but the distinct two-year gap is not observed.

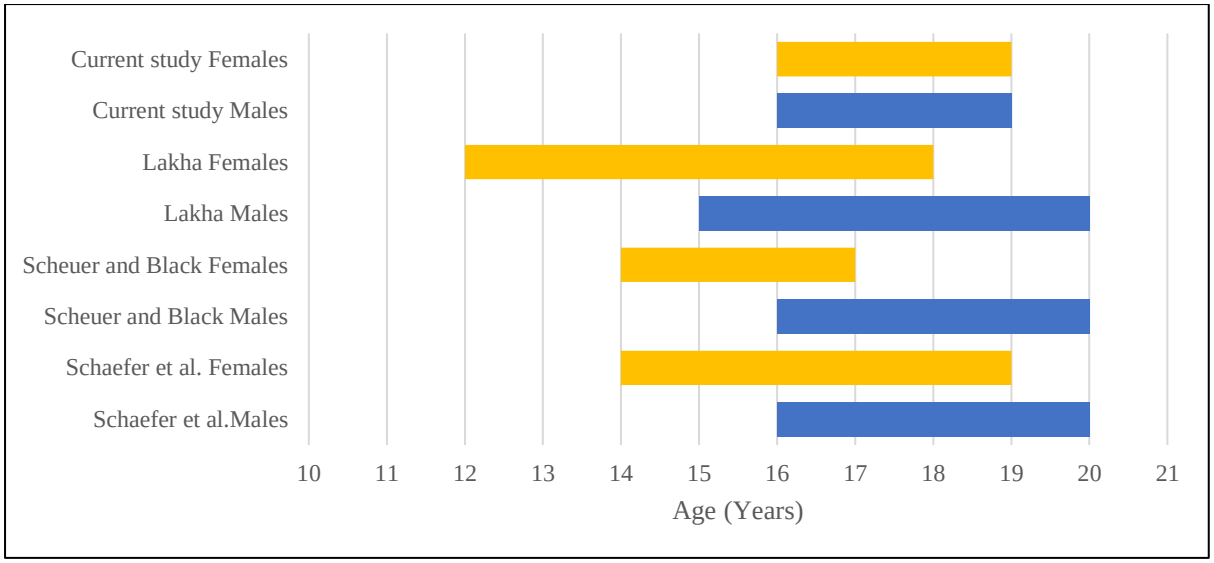

Figure 5.1: Comparative age estimation standards for the distal radius

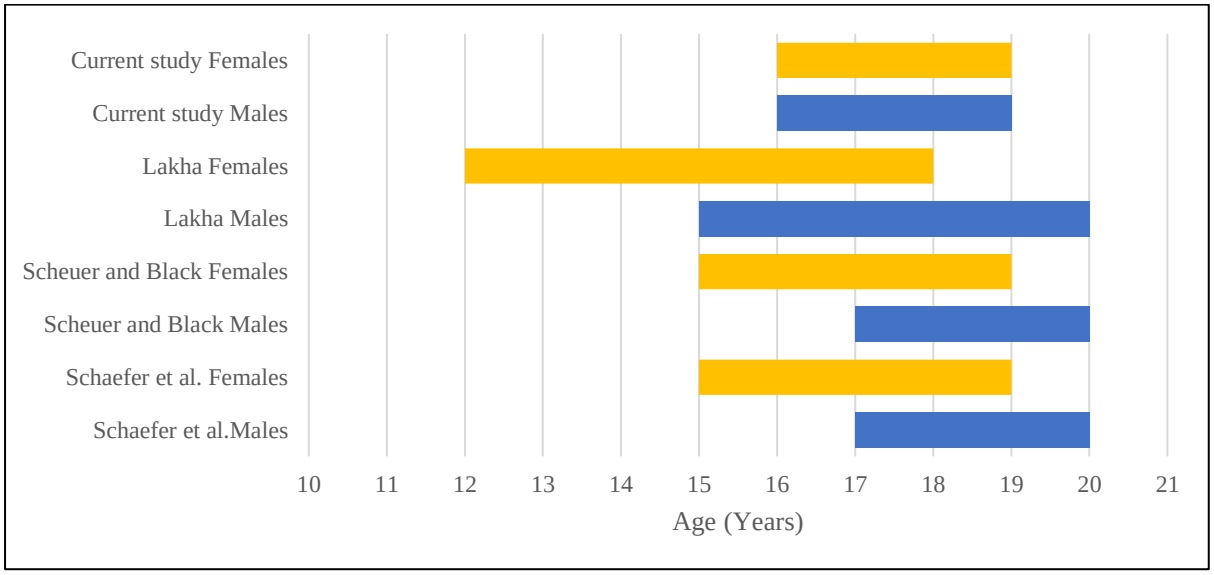

Figure 5.2: Comparative age estimation standards for the distal ulna

Population differences for epiphyseal plate fusion have been reported by various authors for the distal radius (Figure 5.3) and ulna (Figure 5.4) (Dvorak *et al.*, 2007; Lakha, 2015). Studies using four or five stage scoring systems for the degree of epiphyseal plate fusion of the distal radius and ulna were compared for different population groups, including a recent South African study (Stevenson, 1924; S. Schmidt *et al.*, 2008:a; Patel *et al.* , 2011; Lakha, 2015). In addition, differences within the same population group from different regions have been reported (Banerjee and Agarwal, 1998; Nemade *et al.*, 2010). Causative factors for the difference in timing of skeletal maturation among different population groups have been suggested to range from environmental factors such as SES, nutritional intake to biological

differences as well as individual variation within each population group (Christensen *et al.*, 2014; Lottering *et al.*, 2015).

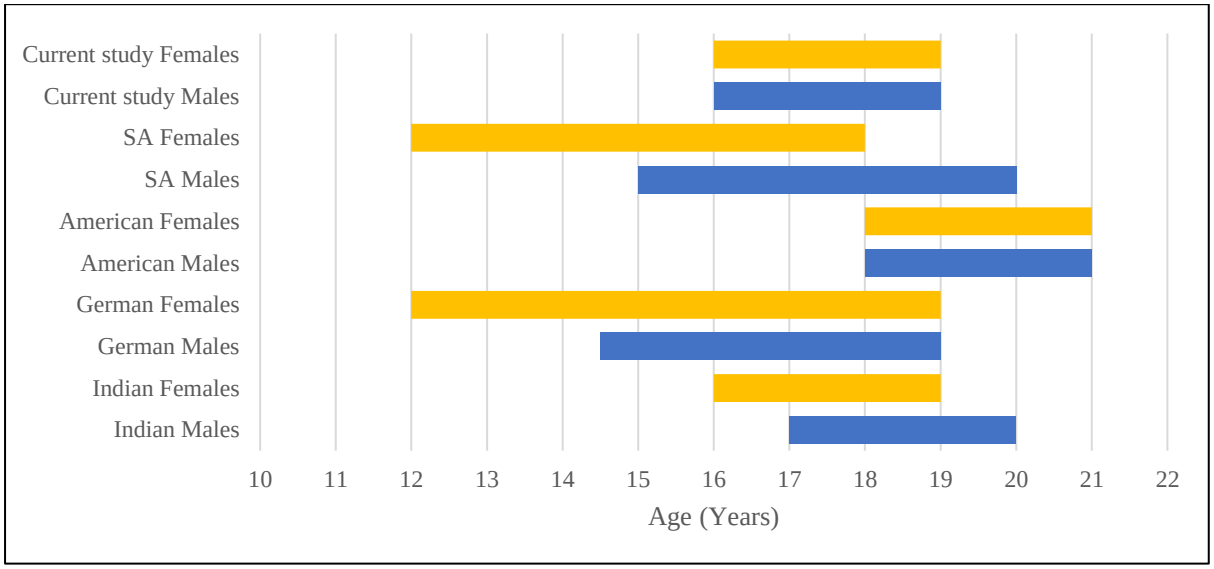

Figure 5.3: Comparative radiographic age ranges for the distal radius

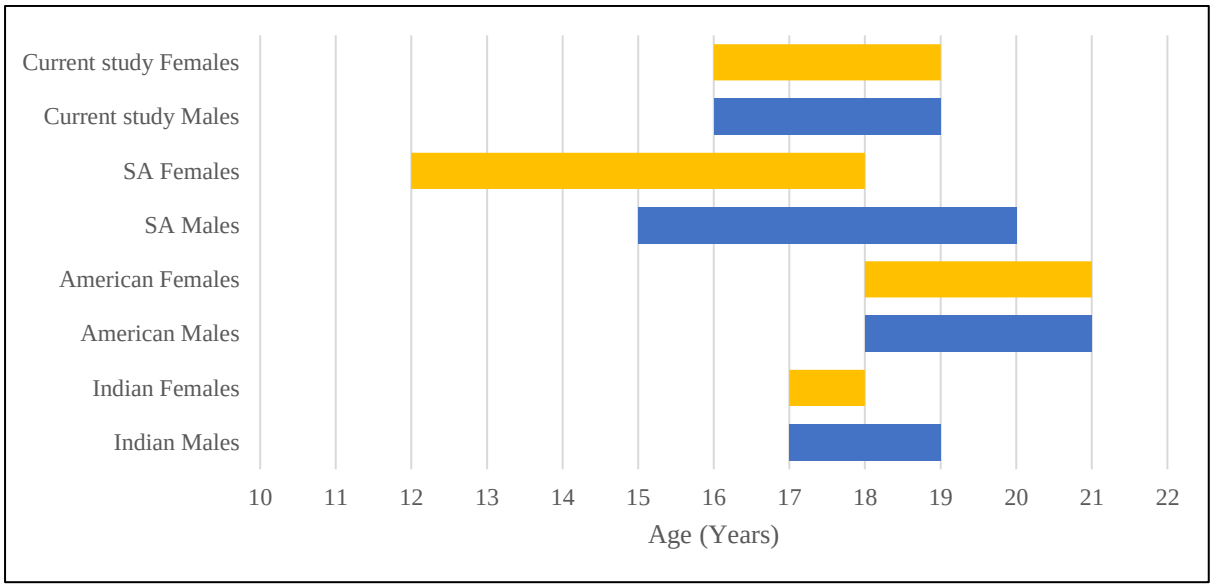

Figure 5.4: Comparative radiographic age ranges for the distal ulna

A narrower age range for epiphyseal plate fusion of the distal radius and ulna was noted in this sample when compared to similar South African studies. In other words, the process of fusion in the wrist (as observed with the current sample) appears to be fairly rapid. Lakha (2015) reported age ranges of 15 to 20 years and 12 to 18 years for the fusion of the distal radius and ulna for males and females, respectively. Whereas complete fusion of the wrist (inclusive of the distal radius and ulna) was attained at 21 years of age in a study by Dembetembe and Morris (2012). Thus, indicating earlier age of complete fusion for males.

Possible reasons for variation from the other studies include influences of SES, differences in sample size and composition as well as differences in scoring method and statistical analyses.

5.1.1 Socio-economic influences

Socio-economic status plays an important role in skeletal development. Higher SES of a sample may result in advanced skeletal development compared to samples, such as that of Lakha (2015), which consisted of individuals of low SES. The effect of SES may be observed in variation between sexes as well as among population groups.

Numerous studies have reported advanced environmental buffering in females, suggesting that males are more sensitive to environmental influences during growth and development. Advanced female buffering is thought to be due to the fact that females play a larger role in reproduction and should therefore be able to adapt more easily to changes in the environment (Stinson, 1985). Studies conducted to compare the effects of environmental stressors on males and females have found that males display retarded growth and skeletal maturation compared to females (Tanner, 1962). It has been suggested that the level of sexual dimorphism or sex differences within a population are related to environmental influences (Hiernaux, 1968). Therefore, in a low socio-economic environment, males display delayed growth and development when compared to their female counterparts; however, when socio-economic circumstances improve males display a more marked increase in growth and development (Tobias, 1972). Researchers have also suggested that males display more pronounced changes in response to environmental fluctuation than females. When considering the maturation of the distal radius and ulna in Lakha's (2015) study, individuals of low- and mid SES were included. As this sample was obtained from a private clinic, the individuals in the current study were of mid- to high SES. Lakha (2015) reported a delay in the timing of epiphyseal plate fusion in males compared to females, whereas no difference was observed for the current study. Thus, observed variation between the findings may be attributed to the differences in SES of the study samples. Lakha's (2015) sample consisted of individuals from lower SES and as males are more adversely affected by environmental influences, a delay in their development may have been observed. Whereas, the current study sample included individuals of a higher SES, therefore improved conditions potentially may have resulted in maturation rates comparable to females.

Significant population differences were observed for the epiphyseal plate fusion of the radius. Specifically, white South Africans reached each transitioning stage earlier compared

to black South Africans in the sample. Findings differ from a similar South African study where no population differences were noted for the maturation of various epiphyses, including the wrist, in black, coloured, Indian and white population groups (Lakha, 2015). Population differences observed in the current study may be attributed to the socio-political history of the South African population (Lakha, 2015). During the Apartheid regime, population groups were segregated in public, geographic locality and education (Anderson *et al.*, 2002). Each population group was assigned a certain set of rights and privileges (Anderson *et al.*, 2002). Further, each group had their separate educational and health care systems, with white individuals having access to superior institutions and resources (Lakha, 2015). Thus, directly affecting accessibility to resources such as health care and nutrition for non-white populations. With this regard, differences between black and white South Africans are influenced by two factors: (i) Forced segregation between population groups, resulting in decreased within-group variation, with between-group variation becoming more prominent; and (ii) Restricted accessibility to resources resulting in lower SES (Stull *et al.*, 2014; Lakha, 2015). As SES is determined by an array of factors such as education, income, health and nutritional status, it has been shown to influence skeletal maturity, either positively or negatively (Cole, 2000; American Psychological Association, 2016). Since the end of the Apartheid era in 1994, signs of increased SES have been observed (Hawley *et al.*, 2009). Factors include decrease in infant mortality rates, decrease in age of menarcheal onset as well as accelerated skeletal maturation (Hawley *et al.*, 2009). However, a decrease in age of menarcheal onset as a singular factor may not indicate SES. Hawley (2009) conducted a study to determine whether a secular trend is present for skeletal maturation in the South African population. Results found that both black and white South Africans displayed advanced skeletal maturity compared to results from a 1962 study, whilst black South Africans displayed a more marked advance in skeletal maturation than whites (Hawley *et al.*, 2009). Possibly indicating improved SES as a result of improved circumstances and access to resources post-apartheid (Hawley *et al.*, 2009). As no significant changes occurred in the SES of white individuals, no marked difference in skeletal maturation is expected.

The enforcement and subsequent cessation of Apartheid may have contributed to the difference in skeletal maturation between South African population groups. Yet, differences observed in the current study amount to only a few months. Therefore, the effects of Apartheid on population differences of skeletal maturation may have been reduced through improved nutrition, health care and social status (Hawley *et al.*, 2009). Indicating that our sample are of

higher SES, thereby reducing differences previously observed between segregated population groups. Observed differences are therefore attributed to biological differences between the population groups.

5.1.2 Sample size and composition

Sample size differences and composition may play a role in the observed variation among South African studies. Larger sample sizes, as utilised by Lakha (2015), result in greater variability sampled from the target population; possibly explaining the wider age range reported for her standards. A one to three-year delay is observed for the current sample (pooled sample) when compared to the proposed age ranges for males and females in the former study; i.e. commencement of epiphyseal plate fusion occurs later in the current sample. However, complete fusion in males is attained one year earlier in the current study. Additionally, the black females group for this study was substantially smaller compared to the other groups sampled. Lastly, lack of significant sex differences may be an expression of the lower variability sampled or the fact that the male sample was larger than the female sample (Cardoso *et al.*, 2014).

In contrast, Dembetembe and Morris (2012) employed a much smaller study sample. A smaller sample size may not be representative of the population under investigation, and may further be more severely influenced by outliers (Unite for sight, 2015). Moreover, Dembembe and Morris (2012) only assessed epiphyseal plate fusion among black South African males, thereby excluding females and other population groups.

5.1.3 Methodology and statistical analyses

Difference in scoring methods and statistical analyses may result in the variation expected in the timing of epiphyseal plate fusion within the same population. A greater variance for the epiphyseal plate fusion of the wrist is observed for males when compared to Dembetembe and Morris's (2012) results, reporting complete fusion of the wrist at the age of 21 years in black males. Differences may be attributed to the fact that Dembetembe and Morris (2012) utilised the GP standards compared to the four stage scoring system of the current study. The GP standards employ a 'plate comparison' method, whereby the hand and wrist are compared to a specific age standard. Multiple studies have reported problems associated with the application of GP standards to other population groups. Problems have been reported with regard to the over- and underestimation of age, poor recognition of ancestral differences as well as existent

variation; as the atlas depicts only the 'normal' standard (Lakha, 2015). Concerns with regard to the reproducibility of this method has also been raised as a great number of elements may provide conflicting information, resulting in difficulty assigning one specific comparative plate (Bull *et al.*, 1999; Bunch *et al.*, 2017; Schaefer *et al.*, 2018).

Regarding statistical analysis, the present study employs more robust statistical methods, thereby reducing the effects of outliers. The wider age range by Lakha (2015) as can be seen for the females, may be attributed to the fact that mean values were utilised to predict age ranges, a method that is prone to age mimicry of the reference sample. Mean values may be influenced by outliers and skewed data resulting in reliability and reproducibility issues. The mean values of the current study indicate earlier fusion in females by approximately 12 months; however, after the application of Bayesian statistics and TA, differences were reduced to non-significant. Therefore, even with the presence of high levels of variation within the South African population, the current method provides narrower and more precise age ranges for the epiphyseal plate fusion of the distal radius in a modern South African population, without compromising accuracy.

5.2 PRACTICAL APPLICATION

The standards proposed provide an accurate and reliable method for age estimation within a South African sample. The effects of outliers and age mimicry in the sample was reduced through the application of more robust statistical analysis (Langley-Shirley and Jantz, 2010; Lottering *et al.*, 2015). Furthermore, four different probabilities are provided (68%, 75%, 90% and 95%) for each stage, referred to as confidence regions rather than CI calculated around a mean value. The confidence regions hereby provide a probability that an individual will fall within a certain age range given that the distal radius or ulna display the radiomorphological characteristics associated with that given stage (Langley-Shirley and Jantz, 2010). The probability regions include conservative proportions of individual variability in the epiphyseal plate fusion times within the South African population, thereby contributing to more accurate estimates (Langley-Shirley and Jantz, 2010; Lottering *et al.*, 2015; Schaefer *et al.*, 2018). Anthropologists aim to provide age estimates which is both accurate and precise. However, individual variation as well as the effects of environmental influence on the skeleton makes this task difficult. Accuracy refers to a certain degree of confidence (for example 95%) that an individual will fall within the range produced. Confidence intervals (CI's) provide additional information on point estimates. By establishing CI's, mean and standard deviation of the study

sample are used to calculate minimum and maximum values. A 95% CI implies that the true mean of the sample is bound within the minimum and maximum value 95% of the time, i.e. an individual has a 95% chance of falling within the proposed range. Confidence intervals can subsequently be increased to increase accuracy; however, this would result in a broader range to accommodate a greater number of sample means. Conversely, CI's may be reduced (for example 68%), thereby increasing precision through a narrower range. Though, by employing a lower CI, anthropologists run the risk of misclassifying the age of an individual. By using the 95% CI with subsequent posterior distribution estimates, anthropologists are able to produce an accurate age range based on the degree of fusion in the distal radius and ulna.

Although statistically significant, the observed differences in the timing of epiphyseal plate fusion of the distal radius between black and white South Africans amount to only a few months. Thus, separate standards for black and white South Africans are not justified as the application thereof would negatively affect the practical application. Due to the small differences observed between the fusion times of black and white individuals, separate age ranges may result in confusion and difficulty in its application. When an age estimate is applied to a set of skeletal remains, the estimate would be rounded off to range, for example, between 16 and 19 years. As results indicate that age ranges overlap, identical age ranges would likely be reported for both population groups. Moreover; currently, no methods for estimating ancestral or population group in subadults exists; further supporting the application of standards for a pooled sample. Epiphyseal plate fusion in a pooled South African population was therefore found to occur between the ages of 16 and 19 years for the both the distal radius and ulna (95% CI). Using the 95% Bayesian probability distributions, it can be assumed South Africans displaying an open epiphyseal plate, characterised by a radiolucent line without bridging between the epiphyseal surfaces, are younger than 14 years. Individuals displaying less than 50% fusion between the epiphyseal surfaces, are suspected to be under the age of 17 years. Individuals that exhibit more than 50% radio-density within the epiphyseal plates, but not full fusion due a small radiolucent area within the epiphyseal plates, may be estimated to be younger than 19 years of age. The age limit of the final stage may be influenced by the upper age limit of the study sample. Thus, the estimate age range for complete fusion of the distal radius may be calculated using the upper limit of the preceding phase (stage III) and the lower limit of the final phase (stage IV). Therefore, it can be said that individuals displaying complete fusion of the distal radius or ulna have a 95% chance of being older than 16 years.

The four stage scoring system as used in the current study proved to be reliable and repeatable when applied to the distal radius. Issues with regard to inter-observer reliability were, however, encountered with the scoring of the distal ulna. This may be due to the anatomical position and movement associated with the distal radius and ulna. During pronation and supination of the hand, the distal portions of the radius and ulna rotates over one another. Therefore, slight movement or ill-positioning of the hand during an X-ray may compromise the view of either bone. Furthermore, the principal investigator was unable to score the ulna on multiple occasions. Therefore, it is recommended that when both bones are available for assessment, the radius be scored for a more reliable age estimate. However, during subadult forensic cases, the availability of skeletal elements may be scarce. In cases where only the ulna is available for assessment, the standards will be sufficient. Although differences in the fusion times of the distal radius and ulna were found to be significant in the current study, the differences amount to a few months. Stevenson (1924) emphasised the close proximity in time in which the distal radius and ulna attain complete fusion. Subsequently, many authors have proposed identical age ranges for the distal radius and ulna (Paterson, 1929; Abbie and Adey, 1953; Lakha, 2015). As with practicality issues regarding separate standards for black and white South Africans, separate standards for the use of forensic age estimation is not justified. Therefore, a standard ranging from 16 to 19 years for the epiphyseal plate fusion of the distal radius and ulna is proposed. When applying the 95% CI age ranges, as proposed by the current study, to both the radius and ulna; accuracy ranged from 73.2% to 96.8%.

The medico-legal application of age assessment is important when considering the age of the accused, especially in cases where the accused are under the age of 14 years or between the ages of 14 and 18 years. Penal laws differ with regard to accountability and sentencing of juvenile individuals (RSA, 1997, 2005; Cameriere *et al.*, 2012). By applying the proposed age estimation standards, individuals displaying non-fusion of either the distal radius or ulna, are estimated to be younger than 14 years of age and may not be arrested (RSA, 2008). The current study provides valid evidence that an individual displaying advanced to complete fusion of the distal radius and ulna, has reached a minimum age of 19 years, using the 95% CI. However, to establish age of majority, the proposed standards will not be sufficient when applied in isolation. A combination of age estimation methods, such as examination of dental status through X-ray of dentition and physical examination (body weight and height, constitutional type as well as examination for sexual maturity characteristics) be applied in order to establish whether or not age of majority has been reached (Schmeling *et al.*, 2008).

Based on the high levels of agreement and low error rates as found by Lakha (2015) for the application of standards for epiphyseal plate fusion derived from radiographs on dry skeletal remains in a South African population; proposed standards of the current study may be applied to dry skeletal remains for the purpose of age estimation (Schaefer *et al.*, 2018). However, findings should be interpreted with caution as concerns have been raised with standards derived from radiological methods being applied to dry bone (Ubelaker, 1987; Lakha, 2015). Thus, proposed standards should first be validated by applying the current method to a skeletal population of known age and population group. In addition, due to the fact that Lakha (2015) employed a low SES sample, the proposed method should be applied to both low and high SES samples to determine whether accurate age estimates may be achieved. Further, other age estimation techniques should be combined with the proposed method, such as dental analysis and the assessment of other available epiphyses. Epiphyses that have been reported to have high correlation with chronological age include the proximal humerus, medial epicondyle, femoral head, distal femur and iliac crest (Lakha, 2015).

The interchangeability of radiographic methods and other scanning modalities such as MRI and ultrasound have yielded controversial results (Schaefer *et al.*, 2018). Magnetic Resonance Imaging (MRI) provides a higher contrast and definition compared to radiographic images, resulting in differences in age estimations (Schaefer *et al.*, 2018). Whereas the use of ultrasound to assess the degree of epiphyseal plate fusion produces difficulties as the entire epiphyseal plate cannot be visualised or accessed (Schaefer *et al.*, 2018). Therefore, interchanging of the proposed standards with other scanning modalities without further research is not recommended as inaccurate age estimations may be obtained.

A persistent epiphyseal scar plays an important role in medico-legal settings as it indicates that epiphyseal plate fusion has completed, whether recently or not (Davies *et al.*, 2016). Thus, when applying the proposed standards, the presence of an epiphyseal scar indicates that an individual is older than 16 years of age. The highest age at which an epiphyseal scar was observed in the present study, was 29 years and 23 years for the radius and ulna, respectively. Using the 95% CI, complete fusion of both epiphyses occurs between the ages of 16 and 19 years. Indicating that an epiphyseal scar may be visible long after epiphyseal plate fusion has been completed. It is important to note that persistent epiphyseal scars may be present in much older individuals as maximum age of visible epiphyseal scars were limited by the age range as part of the inclusion/exclusion criteria of the study. Therefore, evaluating the presence or absence of an epiphyseal scar for age estimation based on the epiphyseal plate fusion of the

distal radius and ulna may result in an underestimation of a given individuals age. Similar observations have been reported in literature, for example Davies *et al.* (2016) reported no statistically significant differences between chronological age and the persistence of an epiphyseal scar in the radius and stated that a maximum age should not be applied to a persistent epiphyseal scar. Further, Stevenson (1924) cautioned against the use of an epiphyseal scar as a sign of recent fusion as it may result in overestimation of chronological age. Thereby concluding that a persistent epiphyseal scar should not be considered a criterion for skeletal maturation as correlation is insufficient to infer a relation.

Chapter 6 CONCLUSION

The estimation of subadult biological parameters for medico-legal purposes is an important and under developed field of research in South Africa. The current study aimed at producing more accurate and reliable radiographic age estimation standards for the epiphyseal plate fusion of the distal radius and ulna in a modern South African population. Related studies have been conducted on a South African population; however, posterior predictive statistics were applied in the present study, to provide more reliable age estimates with a known error rate, in order to comply with standards of best practice (Christensen and Crowder, 2009; Langley-Shirley and Jantz, 2010). Transition analysis and Bayesian statistics were applied to obtain the maximum likelihood age estimate and the average age of transition among the stages, respectively (Lottering *et al.*, 2015). This is in contrast to the use of mean values, which may yield a variety of difficulties, such as being influenced by outliers and resultantly being wrongfully interpreted. Data for the epiphyseal plate fusion show that complete fusion of the distal radius and ulna occur between the ages of 16 and 19 years in the pooled sample (95% CI). Further, results of this study show earlier age of complete fusion compared to previous South African studies, particularly for males.

The lack of sex differences may be due to a combination of improved SES of the current sample when compared to previous studies, as well as the fact that males tend to be more adversely affected by environmental influences. The present study likely included individuals from higher socio-economic circumstances, therefore males displayed an increase in skeletal maturation compared to results of a former South African study. Another factor to consider is that females are suggested to display a more marked buffering effect in response to environmental changes, thus the increase in SES may not have resulted in changes in female skeletal maturation. The results indicate that combined age estimation standards for the distal radius and ulna in both white and black males and females may be used.

Age estimation standards as proposed by the current study may be applied in clinical, academic and medico-legal purposes on both living individuals and skeletal remains. However, for application to forensic cases, the method must first be validated by applying the proposed standards to a skeletal sample. For medico-legal purposes, the use of the 95% CI is recommended as it demonstrates accurate and precise standards for age estimation in a South African population. Further, the current set of standards have been objectively tested and

reports a known potential error rate; thereby ensuring standards of best practise required for forensic application.

The next step for future research may be to gauge the effects of SES by comparing the results of the pooled sample to data obtained from multiple institutions with known ancestry records that showcase different levels of SES. Future research may comprise expansion of the current study by including multiple epiphyses in order to create a multifactorial model to increase accuracy of age estimation in subadults. Additionally, to assess the interchangeability of the current scoring system with other scanning modalities, such MRI and ultrasound. Finally, and most importantly, to assess the applicability of the current scoring system on dry skeletal remains in order to determine whether it may be applied to forensic cases where age is unknown.

REFERENCES

Abbie, A.A. and Adey, W.R. 1953. Ossification in a Central Australian tribe. *Human Biology*, *25*(4), pp.265-278.

American Psychological Association. 2016. *Education and Socioeconomic Status*. [online] Available at: http://www.apa.org/pi/ses/resources/publications/education.aspx [Accessed: 15 November 2016].

Anderson, B.A., Romani, J.H., Phillips, H.E. and Van Zyl, J.A. 2002. Environment, access to health care, and other factors affecting infant and child survival among the African and coloured populations of South Africa, 1989–94. *Population and Environment*, *23*(4), pp.349-364.

Anholts, A.C. 2012. *Secular trends in the height and weight of South African children aged 6 to 10 years*. PhD. University of Pretoria.

Armstrong, M.E., Lambert, M.I. and Lambert, E.V. 2011. Secular trends in the prevalence of stunting, overweight and obesity among South African children (1994–2004). *European Journal of Clinical Nutrition*, *65*(7), pp.835-840.

Ashizawa, K., Asami, T., Anzo, M., Matsuo, N., Matsuoka, H., Murata, M., Ohtsuki, F., Satoh, M., Tanaka, T., Tatara, H. and Tsukagoshi, K. 1996. Standard RUS skeletal maturation of Tokyo children. *Annals of Human Biology*, *23*(6), pp.457-469.

Banerjee, K.K. and Agarwal, B.B.L. 1998. Estimation of age from epiphyseal union at the wrist and ankle joints in the capital city of India. *Forensic Science International*, *98*(1-2), pp.31-39.

Baughan, B., Demirjian, A. and Levesque, G.Y. 1979. Skeletal maturity standards for French-Canadian children of school-age with a discussion of the reliability and validity of such measures. *Human Biology, 51*(3), pp.353-370.

Baumann, U., Schulz, R., Reisinger, W., Heinecke, A., Schmeling, A. and Schmidt, S. 2009. Reference study on the time frame for ossification of the distal radius and ulnar epiphyses on the hand radiograph. *Forensic Science International*, *191*(1-3), pp.15-18.

Bokariya, P., Chowdhary, D.S., Tirpude, B.H., Kothari, R., Waghmare, J.E. and Tarnekar, A. 2011. A review of the chronology of epiphyseal union in the bones at knee and ankle joint. *Journal of Indian Academy of Forensic Medicine*, *33*(3), pp.258-260.

Boldsen, J.L., Milner, G.R., Konigsberg, L.W. and Wood, J.W. 2002. Transition analysis: A new method for estimating age from skeletons. In: Hoppa, R.D. and Vaupel, J.W. (eds.). In: *Paleodemography: Age distributions from skeletal samples*, pp.73-106. Cambridge University Press.

Brown, T. and Grave, K.C. 1976. Skeletal maturation in Australian aborigines. *Journal of Paediatrics and Child Health*, *12*(1), pp.24-30.

Buikstra, J.E. and Rhine, S. 2010. *Age estimation of the human skeleton*. Charles C. Thomas Publisher.

Buikstra, J.E. and Ubelaker, D.H. 1994. Standards for data collection from human skeletal

remains. *Arkansas archaeological survey research series, 44*.

Bull, R.K., Edwards, P.D., Kemp, P.M., Fry, S. and Hughes, I.A. 1999. Bone age assessment: a large scale comparison of the Greulich and Pyle, and Tanner and Whitehouse (TW2) methods. *Archives of Disease in Childhood, 81*(2), pp.172-173.

Bunch, P.M., Altes, T.A., McIlhenny, J., Patrie, J. and Gaskin, C.M. 2017. Skeletal development of the hand and wrist: digital bone age companion—a suitable alternative to the Greulich and Pyle atlas for bone age assessment? *Skeletal Radiology, 46*(6), pp.785-793.

Burr, D.B. and Allen, M.R. 2013. Bone modelling and remodelling. In: Burr, D.B. and Allen, M.R. (eds.). *Basic and applied bone biology*, pp.75-93. Academic Press.

Cameriere, R., De Luca, S., Biagi, R., Cingolani, M., Farronato, G. and Ferrante, L. 2012. Accuracy of three age estimation methods in children by measurements of developing teeth and carpals and epiphyses of the ulna and radius. *Journal of Forensic Sciences, 57*(5), pp.1263-1270.

Cameriere, R., Ferrante, L., Mirtella, D. and Cingolani, M. 2006. Carpals and epiphyses of radius and ulna as age indicators. *International Journal of Legal Medicine, 120*(3), pp.143-146.

Cameron, N., Kgamphe, J.S., Leschner, K.F. and Farrant, P.J. 1992. Urban-rural differences in the growth of South African black children. *Annals of Human Biology, 19*(1), pp.23-33.

Cardoso, H.F., Pereira, V. and Rios, L. 2014. Chronology of fusion of the primary and secondary ossification centres in the human sacrum and age estimation in child and adolescent skeletons. *American Journal of Physical Anthropology, 153*(2), pp.214-225.

Cardoso, H.F., Vandergugten, J.M. and Humphrey, L.T. 2017. Age estimation of immature human skeletal remains from the metaphyseal and epiphyseal widths of the long bones in the post-natal period. *American Journal of Physical Anthropology*, *162*(1), pp.19-35.

Christensen, A.M. and Crowder, C.M. 2009. Evidentiary standards for forensic anthropology. *Journal of Forensic Sciences, 54*(6), pp.1211-1216.

Christensen, A.M., Passalacqua, N.V. and Bartelink, E.J. 2014. *Forensic Anthropology: Current methods and practice*. Elsevier.

Cole, T.J. 2000. Secular trends in growth. *Proceedings of the Nutrition Society, 59*(2), pp.317-324.

Cope, Z. 1920. Fusion-lines of bones. *Journal of Anatomy*, *55*(Pt 1), pp.36-37.

Cox, L.A. 1997. The biology of bone maturation and ageing. *Acta Paediatrica, 86*(S423), pp.107-108.

Cronje, H., Bam, R. and Joubert, G. 2015. *How to write a protocol*. Bloemfontein: University of the Free State.

Cutler Jr, G.B. 1997. The role of oestrogen in bone growth and maturation during childhood and adolescence. *The Journal of Steroid Biochemistry and Molecular Biology*, *61*(3-6), pp.141-144.

Davies, C., Hackman, L. and Black, S. 2014. The persistence of epiphyseal scars in the adult tibia. *International Journal of Legal Medicine*, *128*(2), pp.335-343.

Davies, C., Hackman, L. and Black, S. 2016. The persistence of epiphyseal scars in the distal radius in adult individuals. *International Journal of Legal Medicine*, *130*(1), pp.199-206.

Davies, D.A. and Parsons, F.G. 1927. The age order of the appearance and union of the normal epiphyses as seen by X-rays. *Journal of Anatomy*, *62*(Pt 1), pp.58-71.

Dembetembe, K. A. 2010. *Age estimation using epiphyseal closure at the wrist joint: An investigation of individuals of African origin, age 14 to 22 years*. PhD. University of Cape Town.

Dembetembe, K.A. and Morris, A.G. 2012. Is Greulich-Pyle age estimation applicable for determining maturation in male Africans? *South African Journal of Science*, *108*(9-10), pp.1-6.

Dimeglio, A. 2001. Growth in pediatric orthopaedics. *Journal of Pediatric Orthopaedics*, *21*(4), pp.549-555.

Dirkmaat, D.C. and Cabo, L.L. 2014. Anthropology: Embracing the New Paradigm. *A Companion to Forensic Anthropology*, pp.3-40.

Du Plessis, J. 2006. *Juvenile delinquency on the increase in SA, IOL*. [online] Available at: http://www.iol.co.za/news/south-africa/juvenile-delinquency-on-the-increase-in-sa-288359 [Accessed: 7 April 2016].

Dvorak, J., George, J., Junge, A. and Hodler, J. 2007. Age determination by magnetic resonance imaging of the wrist in adolescent male football players. *British Journal of Sports Medicine*, *41*(1), pp.45-52.

Emons, J., Chagin, A.S., Hultenby, K., Zhivotovsky, B., Wit, J.M., Karperien, M. and Sävendahl, L. 2009. Epiphyseal fusion in the human growth plate does not involve classical apoptosis. *Pediatric Research*, *66*(6), pp.654-659.

Emons, J., Chagin, A.S., Sävendahl, L., Karperien, M. and Wit, J.M. 2011. Mechanisms of growth plate maturation and epiphyseal fusion. *Hormone Research in Paediatrics*, *75*(6), pp.383-391.

Fischer, S. 2017. *Crimes committed by children have become far more violent, EWN*. [online]. Available at: https://www.google.co.za/amp/amp.ewn.co.za2017/09/23/cromes-committed-by-children-have-become-far-more-violent [Accessed: 23 February 2018].

Flecker, H. 1932. Roentgenographic observations of the times of appearance of epiphyses and their fusion with the diaphyses. *Journal of Anatomy*, *67*(Pt 1), pp.118-164.

Flecker, H. 1942. Time of appearance and fusion of ossification centres as observed by roentgenographic methods. *American Journal of Roentgenology*, *47*, pp.97-159.

Fradella, H.F., O'Neill, L. and Fogary, A., 2003. The impact of Daubert on forensic science. *Pepp. L. Rev.*, *31*, pp.323-362.

Franklin, D., Freedman, L., Milne, N. and Oxnard, C.E., 2007. Geometric morphometric study of population variation in indigenous southern African crania. *American Journal of*

Human Biology, *19*(1), pp.20-33.

Garvin, H.M., Nicholas, V., Passalacqua, N.M.U., Gipson, D.R. and Rebecca, S. 2012. Developments in Forensic Anthropology: Age-at-Death Estimation. In: Dirkmaat, D. (ED.). *A companion to forensic anthropology*, pp.202-223. Blackwell Publishing.

Gilbert, S.F. 2000. *Developmental Biology 6th Edition*. Sunderland (MA): Sinauer Associates.

Gilsanz, V. and Ratib, O. 2005. *Hand Bone Age: A digital atlas of skeletal maturity*. Springer Science & Business Media.

Greeff, J.M. 2007. Deconstructing Jaco: Genetic heritage of an Afrikaner. *Annals of Human Genetics*, *71*(5), pp.674-688.

Greulich, W.W., Pyle, S.I. and Todd, T.W. 1959. *Radiographic atlas of skeletal development of the hand and wrist*. Stanford: Stanford University Press.

Hägg, U. and Taranger, J. 1982. Maturation indicators and the pubertal growth spurt. *American Journal of Orthodontics*, *82*(4), pp.299-309.

Hansman, C.F. 1962. Appearance and fusion of ossification centres in the human skeleton. *American Journal of Roentgenology*, *88*, pp.476-482.

Hawley, N.L., Rousham, E.K., Norris, S.A., Pettifor, J.M. and Cameron, N. 2009. Secular trends in skeletal maturity in South Africa: 1962–2001. *Annals of Human Biology*, *36*(5), pp.584-594.

Henneberg, M. and Van den Berg, E.R. 1990. Test of socioeconomic causation of secular trend: stature changes among favored and oppressed South Africans are parallel. *American Journal of Physical Anthropology*, *83*(4), pp.459-465.

Hiernaux, J. 1968. Variabilite du dimorphisme sexuel de la stature en Afrique subsaharienne et en Europe. In: Fischer, S.G. (ed.). *Anthropologie and Humangenetik*, pp.42-50.

Huffman, T.N. 1982. Archaeology and ethnohistory of the African Iron Age. *Annual review of Anthropology*, *11*(1), pp.133-150.

Introna, F. and Campobasso, C. P. 2006. Biological vs legal age of living individuals. In: Schmitt, A., Cuncha, E., and Pinheiro, J. (eds.). *Forensic Anthropology and Medicine: Complementary Sciences From Recovery to Cause of Death*, pp.57-82. Humana Press.

Jiménez-Castellanos, J., Carmona, A., Catalina-Herrera, C.J. and Viñuales, M. 1996. Skeletal maturation of wrist and hand ossification centres in normal Spanish boys and girls: a study using the Greulich-Pyle method. *Acta Anatomica*, *155*(3), pp.206-211.

Johnston, C.A. 2008. Aging and sexing human remains from the Hopewell Site. In: *The Scioto Hopewell and Their Neighbors*, pp. 485-500. Springer.

Jones, L.L., Griffiths, P.L., Norris, S.A., Pettifor, J.M. and Cameron, N. 2009. Age at menarche and the evidence for a positive secular trend in urban South Africa. *American Journal of Human Biology*, *21*(1), pp.130-132.

Kellinghaus, M., Schulz, R., Vieth, V., Schmidt, S. and Schmeling, A. 2010. Forensic age estimation in living subjects based on the ossification status of the medial clavicular

epiphysis as revealed by thin-slice multidetector computed tomography. *International Journal of Legal Medicine*, *124*(2), pp.149-154.

Kemkes-Grottenthaler, A. 2002. Aging through the ages: Historical perspectives on age indicator methods. In: Hoppa, R.D. and Vaupel, J.W. (eds.). *Paleodemography: Age Distributions from Skeletal Samples*, pp.48-72. Cambridge University Press.

Konigsberg, L.W., Herrmann, N.P., Wescott, D.J. and Kimmerle, E.H. 2008. Estimation and evidence in forensic anthropology: Age-at-death. *Journal of Forensic Sciences*, *53*(3), pp.541-557.

Koo, T.K. and Li, M.Y. 2016. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *Journal of Chiropractic Medicine*, *15*(2), pp.155-163.

Laerd Statistics. 2017. *Kruskal-Wallis H test using SPSS statistics.*[online] Available at: https://statistics.laerd.com/spss-tutorials/kruskal-wallis-h-test-using-spss-statistics.php [Accessed: 9 October 2017].

Lakha, K.N. 2015. *Standards for epiphyseal union in South African children between the ages of 6 to 24 years using low dose X-ray (lodox).* PhD. University of Cape Town.

Lamprecht, M. 2015. *9 Crime facts you may have missed*, *News24*. [online] Available at: http://www.news24.com/SouthAfrica/News/9-crime-facts-you-may-have-missed-20151003 [Accessed: 7 April 2016].

Landis, J.R. and Koch, G.G. 1977. The measurement of observer agreement for categorical data. *Biometrics*, pp.159-174.

Langley-Shirley, N. and Jantz, R.L. 2010. A Bayesian approach to age estimation in modern Americans from the clavicle. *Journal of Forensic Sciences*, *55*(3), pp.571-583.

Lewis, M.E. and Flavel, A. 2006. Age assessment of child skeletal remains in forensic contexts. In: Schmitt, Cuncha, E. and Pinheiro, J. (eds.). *Forensic Anthropology and Medicine: Complementary Sciences From Recovery to Cause of Death*, pp.243-257. Humana Press.

Liebenberg, L., L'Abbé, E.N. and Stull, K.E. 2015. Population differences in the postcrania of modern South Africans and the implications for ancestry estimation. *Forensic Science International*, *257*, pp.522-529.

Loder, R.T., Estle, D.T., Morrison, K., Eggleston, D., Fish, D.N., Greenfield, M.L. and Guire, K.E. 1993. Applicability of the Greulich and Pyle skeletal age standards to black and white children of today. *American Journal of Diseases of Children*, *147*(12), pp.1329-1333.

Lottering, N., MacGregor, D.M., Alston, C.L. and Gregory, L.S. 2015. Ontogeny of the spheno-occipital synchondrosis in a modern Q ueensland, A ustralian population using computed tomography. *American Journal of Physical Anthropology*, *157*(1), pp.42-57.

MacKay, D.H. 1952. Skeletal maturation in the hand: A study of development in East African children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *46*(2), pp.135-150.

Magqibelo, L., Londt, M., September, S. and Roman, N. 2016. Challenges faced by unaccompanied minor-refugees in South Africa. *Social Work*, *52*(1), pp.73-89.

Mann, C.J. 2003. Observational research methods. Research design II: Cohort, cross sectional, and case-control studies. *Emergency Medicine Journal*, *20*(1), pp.54-60.

Maromo, J. 2015. *African nations to blame for influx: Zuma*, *IOL*. [online] Available at: http://www.iol.co.za/news/politics/african-nations-to-blame-for-influx-zuma-1850812 [Accessed: 24 August 2016].

Marshall, W.A., Ashcroft, M.T. and Bryan, G. 1970. Skeletal maturation of the hand and wrist in Jamaican children. *Human Biology*, pp.419-435.

Massé, G. and Hunt, E.E. 1963. Skeletal maturation of the hand and wrist in West African children. *Human Biology*, *35*(1), pp.3-25.

Matsuo, N. 1993. Skeletal and sexual maturation in Japanese children. *Clinical Pediatric Endocrinology*, *2*(Supple1), pp.1-4.

Memchoubi, P. 2006. Age determination of Manipuri girls from the radiological study of epiphyseal union around the elbow, knee, wrist joints and pelvis. *Journal of Indian Academy of Forensic Medicine*, *28*(2), pp.55-59.

Moore, K.L., Dalley, A.F. and Agur, A.M. 2014. *Clinically Oriented Anatomy 7th Edition*. Lippincott Williams & Wilkins.

Mora, S., Boechat, M.I., Pietka, E., Huang, H.K. and Gilsanz, V. 2001. Skeletal age determinations in children of European and African descent: Applicability of the Greulich and Pyle standards. *Pediatric Research*, *50*(5), pp.624-628.

Moss, M.L. and Noback, C.R. 1958. A longitudinal study of digital epiphyseal fusion in adolescence. *The Anatomical Record*, *131*(1), pp.19-32.

Nemade, K.S., Kamdi, N.Y. and Parchand, M.P. 2010. Ages of epiphyseal union around wrist joint-A radiological study. *Journal of the Anatomical Society of India*, *59*(2), pp.205-210.

Niemeijer, M., van Ginneken, B., Maas, C.A., Beek, F.J. and Viergever, M.A. 2003. Assessing the skeletal age from a hand radiograph: Automating the Tanner-Whitehouse method. *International Society for Optical Engineering*, *5032*, pp.1197-1206.

Nilsson, A., Ohlsson, C., Isaksson, O.G., Lindahl, A. and Isgaard, J. 1994. Hormonal regulation of longitudinal bone growth. *European Journal of Clinical Nutrition*, *48*(1), pp.150-158.

Noback, C.R. 1954. The appearance of ossification centres and the fusion of bones. *American Journal of Physical Anthropology*, *12*(1), pp.63-70.

Onat, T. and Ertem, B. 1974. Adolescent female height velocity: Relationships to body measurements, sexual and skeletal maturity. *Human Biology*, *46*(2), pp.199-217.

Ontell, F.K., Ivanovic, M., Ablin, D.S. and Barlow, T.W. 1996. Bone age in children of diverse ethnicity. *American Journal of Roentgenology*, *167*(6), pp.1395-1398.

Ousley, S.D., Daly, S., Frazee, K. and Stull, K. 2013. *A Radiographic database for estimating biological parameters in modern subadults*. Mercyhurst University.

Patel, D.S., Agarwal, H. and Shah, J.V. 2011. Epiphyseal fusion at lower end of radius and

ulna valuable tool for age determination. *Journal of Indian Academy Forensic Medicine, 33*(2), pp.125-129.

Paterson, R.S. 1929. A radiological investigation of the epiphyses of the long bones. *Journal of Anatomy, 64*(Pt 1), pp.28-46.

Pryor, J.W. 1923. Differences in the time of development of centres of ossification in the male and female skeleton. *The Anatomical Record*, *25*(5), pp.257-273.

Pryor, J.W. 1925. Time of ossification of the bones of the hand of the male and female and union of epiphyses with the diaphyses. *American Journal of Physical Anthropology, 8*(4), pp.401-410.

Ribot, I. 2004. Differentiation of modern sub-Saharan African populations: craniometric interpretations in relation to geography and history. *Bulletins et Mémoires de la Société d'Anthropologie de Paris*, (16 (3-4)), pp.143-165.

Rikhasor, R.M., Qureshi, A.M., Rathi, S.L. and Channa, N.A. 1999. Skeletal maturity in Pakistani children. *The Journal of Anatomy, 195*(2), pp.305-308.

Rissech, C., Wilson, J., Winburn, A.P., Turbón, D. and Steadman, D. 2012. A comparison of three established age estimation methods on an adult Spanish sample. *International Journal of Legal Medicine, 126*(1), pp.145-155.

Roche, A.F. 1979. Secular trends in stature, weight, and maturation. *Monographs of the Society for Research in Child Development 44*(3/4), pp.3-27.

Roche, A.F., Roberts, J. and Hamill, P.V. 1978. Skeletal maturity of youths 12-17 years; racial, geographic area, and socioeconomic differentials, *United States, 1966-1970. Vital and Health Statistics series 11*, 167, pp.1-98.

Rossouw, H.A., Grant, C.C. and Viljoen, M. 2012. Overweight and obesity in children and adolescents: The South African problem. *South African Journal of Science*, *108*(5-6), pp.31-37.

RSA (Republic of South Africa). 1997. Criminal Law Amendment Act 105 of 1997. Cape Town: Government Printer.

RSA (Republic of South Africa). 2005. Children's Act 38 of 2005. Cape Town: Government Printer.

RSA (Republic of South Africa). 2008. The Child Justice Act 75 of 2008. Cape Town: Government Printer.

Rucci, M., Coppini, G., Nicoletti, I., Cheli, D. and Valli, G. 1995. Automatic analysis of hand radiographs for the assessment of skeletal age: a subsymbolic approach. *Computers and Biomedical Research, 28*(3), pp.239-256.

Rylands-Monk, F. 2017. Bone age evaluations raise complex issues. *European Society of Radiology*, pp.1-4.

Sahni, D. and Jit, I. 1995. Time of fusion of epiphyses at the elbow and wrist joints in girls of Northwest India. *Forensic Science International*, *74*(1-2), pp.47-55.

Saint-Martin, P., Rérolle, C., Dedouit, F., Bouilleau, L., Rousseau, H., Rougé, D. and Telmon, N. 2013. Age estimation by magnetic resonance imaging of the distal tibial epiphysis and the calcaneum. *International Journal of Legal Medicine*, *127*(5), pp.1023-1030.

Satoh, M. 2015. Bone age: assessment methods and clinical applications. *Clinical Pediatric Endocrinology*, *24*(4), pp.143-152.

Schaefer, M., Black, S.M. and Scheuer, L. 2009. *Juvenile Osteology*. Academic Press.

Schaefer, M., Geske, N. and Cunningham, C. 2018. A decade of development in juvenile ageing. In: Latham, K.E., Bartelink, E.J., and Finnegan, M. (eds.). *New Perspectives in Forensic Human Skeletal Identification*, pp.45-60. Academic Press.

Scheuer, L. 2002. Application of osteology to forensic medicine. *Clinical Anatomy*, *15*(4), pp.297-312.

Scheuer, L. and Black, S. 2000. *Developmental Juvenile Osteology*. Academic Press.

Scheuer, L. and Black, S. 2004. *The Juvenile Skeleton*. Academic Press.

Schmeling, A., Baumann, U., Schmidt, S., Wernecke, K.D. and Reisinger, W. 2006. Reference data for the Thiemann–Nitz method of assessing skeletal age for the purpose of forensic age estimation. *International Journal of Legal Medicine*, *120*(1), pp.1-4.

Schmeling, A., Grundmann, C., Fuhrmann, A., Kaatsch, H.J., Knell, B., Ramsthaler, F., Reisinger, W., Riepert, T., Ritz-Timme, S., Rösing, F.W. and Rötzscher, K. 2008. Criteria for age estimation in living individuals. *International Journal of Legal Medicine*, *122*(6), pp.457-460.

Schmeling, A., Olze, A., Reisinger, W. and Geserick, G. 2004. Forensic age diagnostics of living people undergoing criminal proceedings. *Forensic Science International*, *144*(2-3), pp.243-245.

Schmeling, A., Reisinger, W., Geserick, G. and Olze, A. 2005. The current state of forensic age estimation of live subjects for the purpose of criminal prosecution. *Forensic Science, Medicine, and Pathology*, *1*(4), pp.239-246.

Schmeling, A., Reisinger, W., Loreck, D., Vendura, K., Markus, W. and Geserick, G. 2000. Effects of ethnicity on skeletal maturation: consequences for forensic age estimations. *International Journal of Legal Medicine*, *113*(5), pp.253-258.

Schmeling, A., Schulz, R., Danner, B. and Rösing, F.W. 2006. The impact of economic progress and modernization in medicine on the ossification of hand and wrist. *International Journal of Legal Medicine*, *120*(2), pp.121-126.

Schmeling, A., Schulz, R., Reisinger, W., Mühler, M., Wernecke, K.D. and Geserick, G. 2004. Studies on the time frame for ossification of the medial clavicular epiphyseal cartilage in conventional radiography. *International Journal of Legal Medicine*, *118*(1), pp.5-8.

Schmidt, S., Baumann, U., Schulz, R., Reisinger, W. and Schmeling, A. 2008. Study of age dependence of epiphyseal ossification of the hand skeleton. *International Journal of Legal Medicine*, *122*(1), pp.51-54.

Schmidt, S., Nitz, I., Schulz, R. and Schmeling, A. 2008. Applicability of the skeletal age determination method of Tanner and Whitehouse for forensic age diagnostics. *International Journal of Legal Medicine*, *122*(4), pp.309-314.

Schmidt, S., Schiborr, M., Pfeiffer, H., Schmeling, A. and Schulz, R. 2013. Age dependence of epiphyseal ossification of the distal radius in ultrasound diagnostics. *International Journal of Legal Medicine*, *127*(4), pp.831-838.

Serin, J., Rérolle, C., Pucheux, J., Dedouit, F., Telmon, N., Savall, F. and Saint-Martin, P. 2016. Contribution of magnetic resonance imaging of the wrist and hand to forensic age assessment. *International Journal of Legal Medicine*, *130*(4), pp.1121-1128.

Serrat, M.A., Williams, R.M. and Farnum, C.E. 2009. Temperature alters solute transport in growth plate cartilage measured by in vivo multiphoton microscopy. *Journal of Applied Physiology*, *106*(6), pp.2016-2025.

Shim, K.S. 2015. Pubertal growth and epiphyseal fusion. *Annals of Pediatric Endocrinology & Metabolism*, *20*(1), pp.8-12.

Shirley, N.R. and Jantz, R.L. 2011. Spheno-occipital synchondrosis fusion in modern Americans. *Journal of Forensic Sciences*, *56*(3), pp.580-585.

Sidhom, G. and Derry, D.E. 1931. The dates of union of some epiphyses in Egyptians from X-ray photographs. *Journal of Anatomy*, *65*(Pt 2), pp.196-211.

Sim, J. and Wright, C.C. 2005. The kappa statistic in reliability studies: Use, interpretation, and sample size requirements. *Physical Therapy*, *85*(3), pp.257-268.

Simon, S. 2016. *What is a Kappa coefficient? (Cohen's Kappa)*, *STATS*. [online] Available at: http://www.pmean.com/definitions/kappa.htm [Accessed: 11 July 2016].

Soegiharto, B.M., Cunningham, S.J. and Moles, D.R. 2008. Skeletal maturation in Indonesian and white children assessed with hand-wrist and cervical vertebrae methods. *American Journal of Orthodontics and Dentofacial Orthopedics*, *134*(2), pp.217-226.

Soliman, A., De Sanctis, V. and Elalaily, R. 2014. Nutrition and pubertal development. *Indian Journal of Endocrinology and Metabolism*, *18*(Suppl 1), pp.39-47.

Spencer, R.P. 2002. Pubertal height gain: male–female and interpopulation comparisons. *Medical Hypotheses*, *59*(6), pp.759-761.

Statistics how to. 2017. *Dunn's test: Definition*. [online] Available at: http://www.statisticshowto.com/dunns-test/ [Accessed: 17 April 2017].

Statistics South Africa. 2011. *Census 2011 - Provincial profile: Free State*. [online] Available at: http://www.statssa.gov.za/publications/Report-03-01-73/Report-03-01-732011.pdf [Accessed: 9 March 2017].

Statistics South Africa. 2016. *Mid-year population estimates*. [online] Available at: https://www.statssa.gov.za/publications/P0302/P03022016.pdf [Accessed: 9 March 2017].

Stevenson, P.H. 1924. Age order of epiphyseal union in man. *American Journal of Physical Anthropology*, *7*(1), pp.53-93.

Steyn, M. and İşcan, M.Y. 1999. Osteometric variation in the humerus: sexual dimorphism in South Africans. *Forensic Science International*, *106*(2), pp.77-85.

Stinson, S. 1985. Sex differences in environmental sensitivity during growth and development. *American Journal of Physical Anthropology*, *28*(S6), pp.123-147.

Stull, K.E., Kenyhercz, M.W. and L'Abbé, E.N. 2014. Ancestry estimation in South Africa using craniometrics and geometric morphometrics. *Forensic Science International*, *245*, pp.206.e1-206.e7.

Stull, K.E., L'Abbé, E.N. and Ousley, S.D. 2014. Using multivariate adaptive regression splines to estimate subadult age from diaphyseal dimensions. *American Journal of Physical Anthropology*, *154*(3), pp.376-386.

Suri, S., Prasad, C., Tompson, B. and Lou, W. 2013. Longitudinal comparison of skeletal age determined by the Greulich and Pyle method and chronologic age in normally growing children, and clinical interpretations for orthodontics. *American Journal of Orthodontics and Dentofacial Orthopedics*, *143*(1), pp.50-60.

Tangmose, S., Thevissen, P., Lynnerup, N., Willems, G. and Boldsen, J. 2015. Age estimation in the living: Transition analysis on developing third molars. *Forensic Science International*, *257*, pp.512.e1-512.e7.

Tanner, J.M. 1962. *Growth at Adolescence 2nd Edition*. Oxford: Blackwell Publishing.

Tanner, J.M., Whitehouse, R.H., Marshall, W.A. and Carter, B.S. 1975. *Assessment of skeletal maturity and prediction of adult height (TW2 method)*. Academic Press.

Tishkoff, S.A. and Williams, S.M. 2002. Genetic analysis of African populations: Human evolution and complex disease. *Nature Reviews Genetics*, *3*(8), p.611-621.

Tobias, P. 1972. Growth and stature in South African populations. In: Vorster, D. (ed.). *Human Biology of Environmental Change*, pp.96-104. London: International Biological Program.

Ubelaker, D.H. 1987. Estimating age at death from immature human skeletons: An overview. *Journal of Forensic Science*, *32*(5), pp.1254-1263.

Ubelaker, D.H. 2005. Estimating age at death. In: Rich, J., Dean, D. and Powers, R. *Forensic Medicine of the Lower Extremity*, pp.99-112. Humana Press.

Ubelaker, D.H. and De La Paz, J.S. 2012. Skeletal indicators of pregnancy and parturition: A historical review. *Journal of Forensic Sciences*, *57*(4), pp.866-872.

Understanding evolution. 2008. *Bottlenecks and founder effects*. [online] Available at: https://evolution.berkeley.edu/evolibrary/article/bottlenecks_01 [Accessed 17 Nov. 2017].

Unite for sight. 2015. *The importance of quality sample size*. [online] Available at: http://www.uniteforsight.org/global-health-university/importance-of-quality-sample-size [Accessed: 10 July 2017].

United Nations High Commissioner for Refugees (UNCHR). 2015. *Global Appeal Report: South Africa*. [online] Available at: http://www.unhcr.org/afr/publications/fundraising/5a0c05027/unhcr-global-appeal-2018-

2019-full-report.html?query=global%20appeal%20report [Accessed: 10 July 2018].

Uysal, T., Sari, Z., Ramoglu, S.I. and Basciftci, F.A. 2004. Relationships between dental and skeletal maturity in Turkish subjects. *The Angle Orthodontist*, *74*(5), pp.657-664.

Weise, M., De-Levi, S., Barnes, K.M., Gafni, R.I., Abad, V. and Baron, J. 2001. Effects of oestrogen on growth plate senescence and epiphyseal fusion. *Proceedings of the National Academy of Sciences*, *98*(12), pp.6871-6876.

Wells, J.C. 2007. Sexual dimorphism of body composition. *Best Practice & Research: Clinical Endocrinology & Metabolism*, *21*(3), pp.415-430.

White, T.D. and Folkens, P.A. 2005. *The Human Bone Manual*. Academic Press.

World Health Organisation. 2011. *Global strategy on diet, physical activity and health. What is overweight and obesity?* [online] Available at: http://www.who.int/dietphysicalactivity/en/ [Accessed: 22 March 2018].

Zaiontz, C. 2016. *Real statistics using Excel*. [online] Available at: http://www.real-statistics.com/reliability/cohens-kappa/ [Accessed: 11 July 2016].

ANNEXURES

ANNEXURE A: ETHICAL APPROVAL

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

6/07/2017

Approval Certificate
New Application

Ethics Reference No.: 157/2017

Title: Transition analysis of age-related changes to the distal radius and ulna in a modern South African population

Dear Nastasha Coetzee

The **New Application** as supported by documents specified in your cover letter dated 30/03/2017 for your research received on the 30/03/2017, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 26/04/2017.

Please note the following about your ethics approval:
- Ethics Approval is valid for 2 years
- Please remember to use your protocol number (**157/2017**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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ANNEXURE B: PERMISSION LETTER

LETTER OF PERMISSION TO CONDUCT RESEARCH

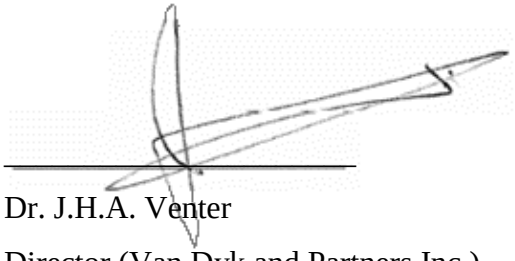
To whom it may concern.

A request was received from **Nastasha Coetzee** to conduct the following research study:

TITLE	Transitional analysis of age-related changes to the distal radius and ulna in a modern South African population.
RESEARCHER	Nastasha Coetzee
PROMOTORS	Ms Leandi Liebenberg & Prof Ericka N. L'Abbé
INSTITUTIONS INVOLVED	University of Pretoria (Department of Anatomy) & Mediclinic Bloemfontein (Van Dyk and Partners Inc.)
DESCRIPTION OF STUDY	This study will be a cross sectional, descriptive study of retrospective hand-wrist radiographs of black and white males and females between the ages of 8 and 30 years. Stage of epiphyseal plate fusion will be determined for both radius and ulna in order to evaluate and possibly redefine age estimation standards within a South African population.
DURATION	Two years
PUBLICATION OF RESULTS	The outcomes of the study will be published in a reputable and accredited journal.
CONTACT DETAILS	(051) 401 7281 ncoetzee123@gmail.com

Hereby, I grant permission to **Nastasha Coetzee** to conduct the abovementioned research study within the Department of Radiology, Mediclinic Bloemfontein (Van Dyk and Partners Inc.).

Yours Faithfully,



12 January 2017

Dr. J.H.A. Venter — Date

Director (Van Dyk and Partners Inc.)

ANNEXURE C: DEMOGRAPHC INFORMATION

Table C.1: Age, sex and population distribution of study sample

Age range (years)	Black		White		Total
	Male	Female	Male	Female	
8	4	5	9	6	24
9	7	6	4	13	30
10	8	7	7	8	30
11	9	1	4	10	24
12	8	5	13	13	39
13	12	7	14	17	50
14	8	3	18	11	40
15	9	4	23	18	54
16	11	6	16	14	47
17	8	5	18	20	51
18	9	10	13	11	43
19	6	4	14	12	36
20	6	5	10	13	34
21	6	7	11	5	29
22	18	3	12	8	41
23	4	4	9	5	22
24	7	3	9	4	23
25	10	12	6	4	32
26	14	8	8	7	37
27	10	5	5	5	25
28	11	9	4	4	28
29	18	9	6	9	42
30	0	1	0	0	1

ANNEXURE D: ADDITIONAL RESULTS

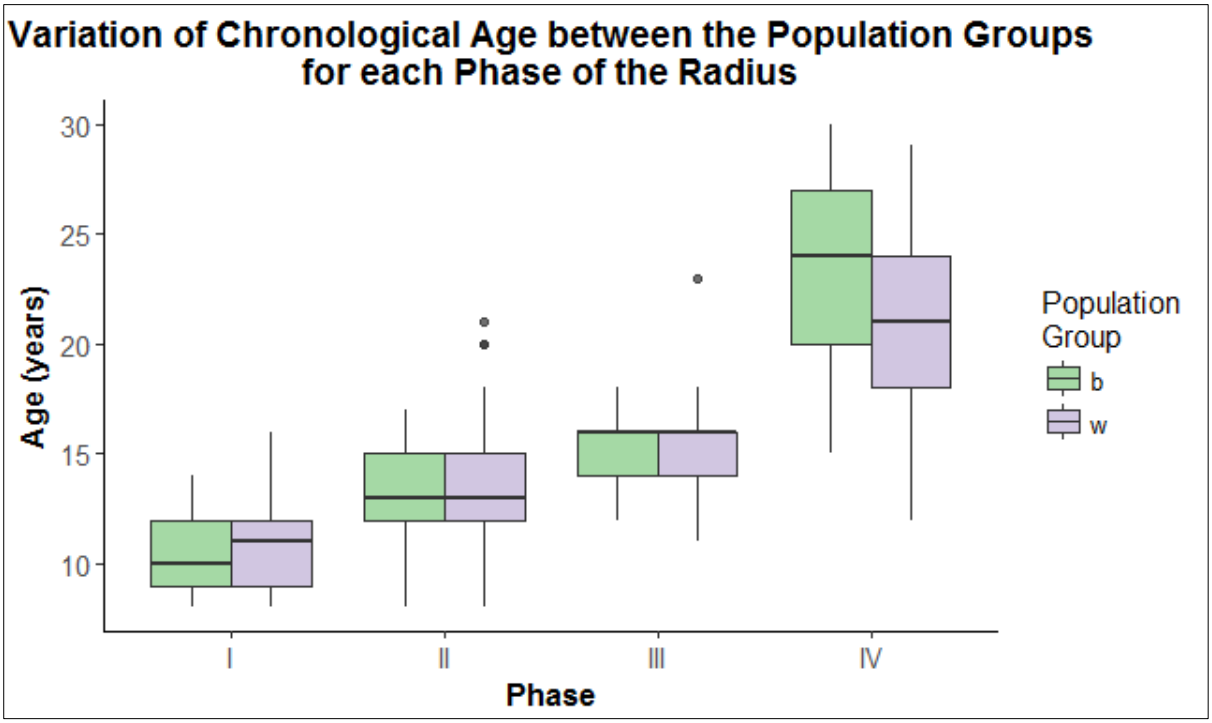


Figure D.1: Boxplot for the variation of chronological age between population groups for each stage of fusion for the radius

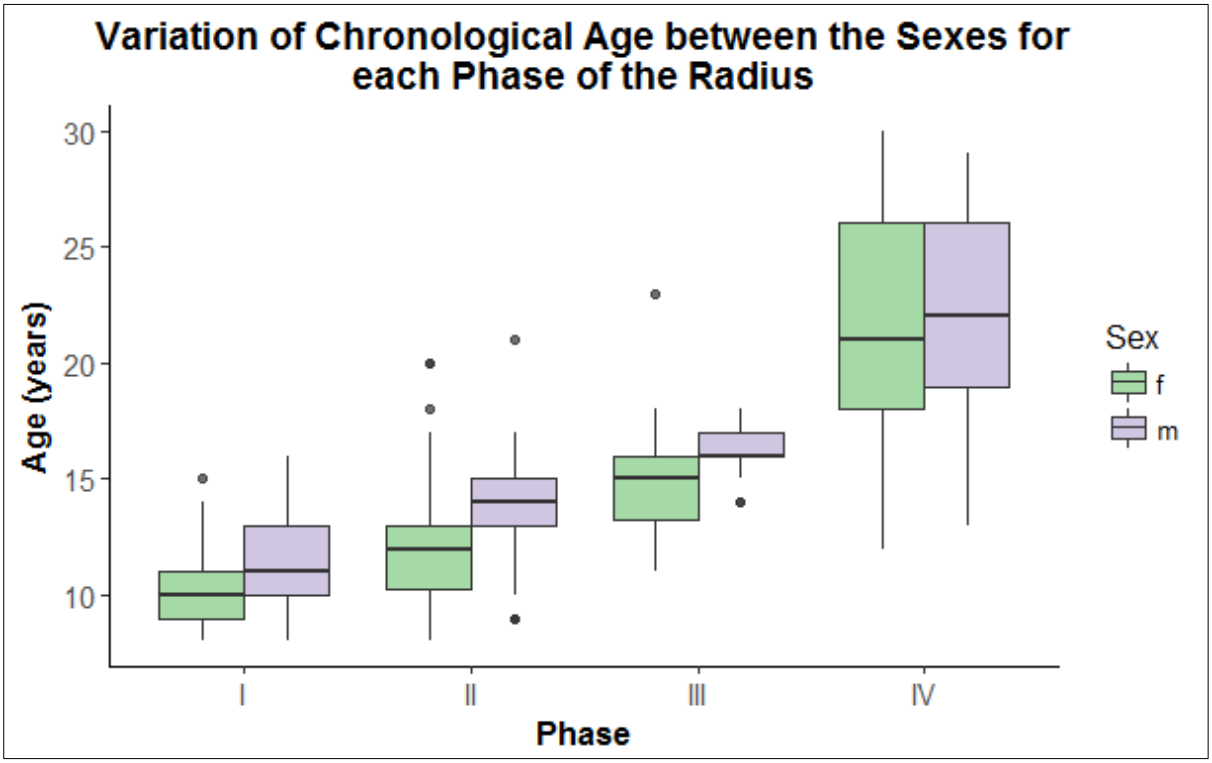


Figure D.2: Boxplot for the variation of chronological age between sexes for each stage of fusion for the radius

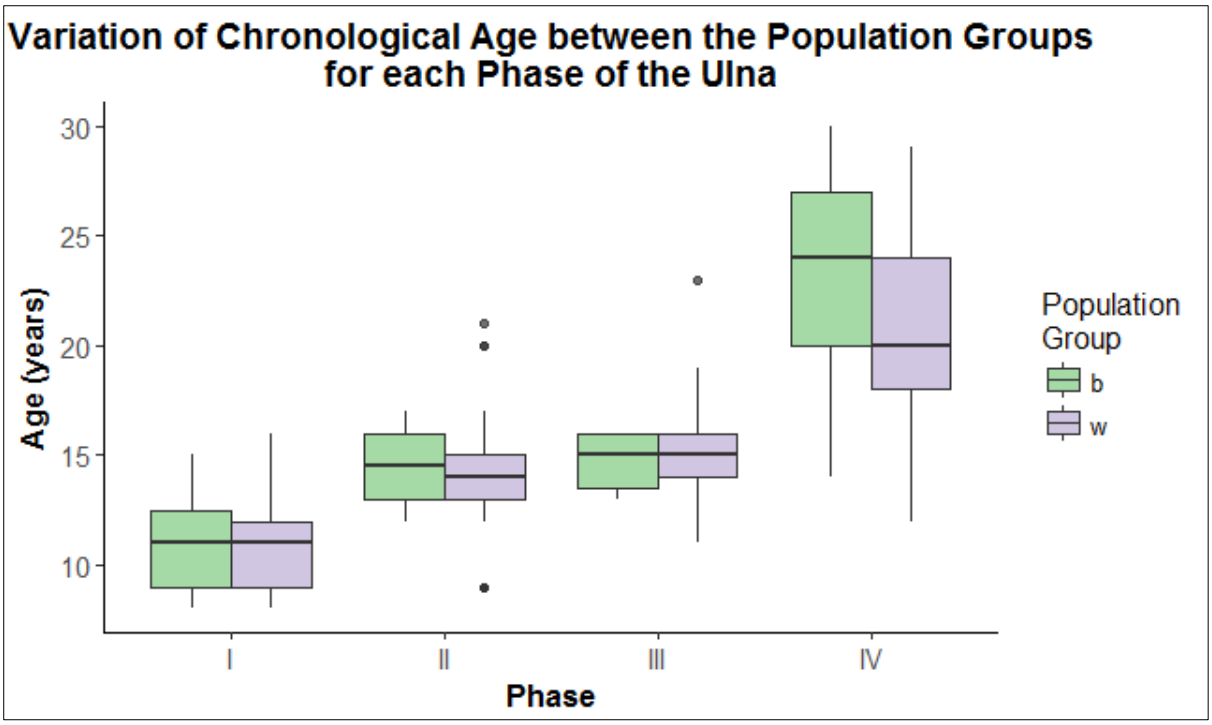


Figure D.3: Boxplot for the variation of chronological age between population groups for each stage of fusion for the ulna

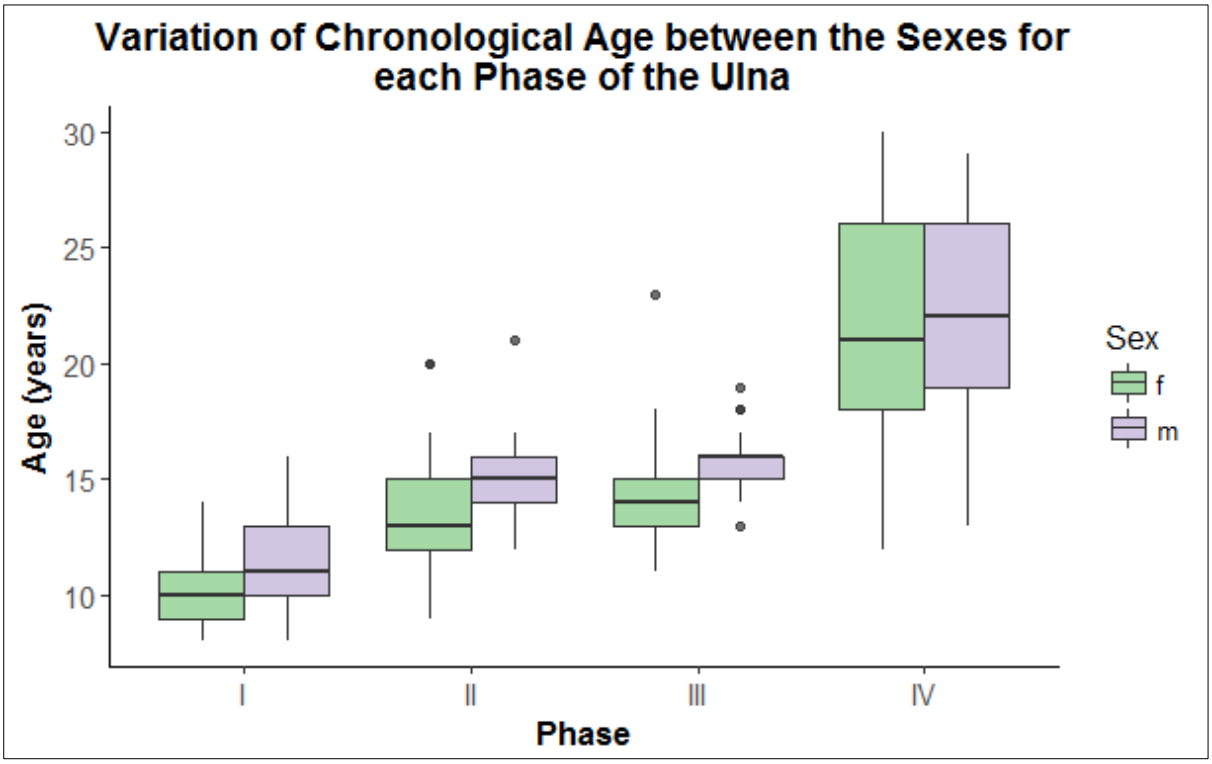


Figure D.4: Boxplot for the variation of chronological age between sexes for each stage of fusion for the ulna

Table D.1: Bayesian estimates showing the average age-at-transition for the ulna (pooled sample)

Group	Transition stage	Estimate (±SEM)	SD
Black	I-II	13.93 ± 1.94	0.83
	II-III	15.46 ± 2.10	
	III-IV	16.50 ± 2.20	
White	I-II	12.75 ± 0.85	1.25
	II-III	14.54± 0.91	
	III-IV	16.51± 0.98	

* Estimates in years

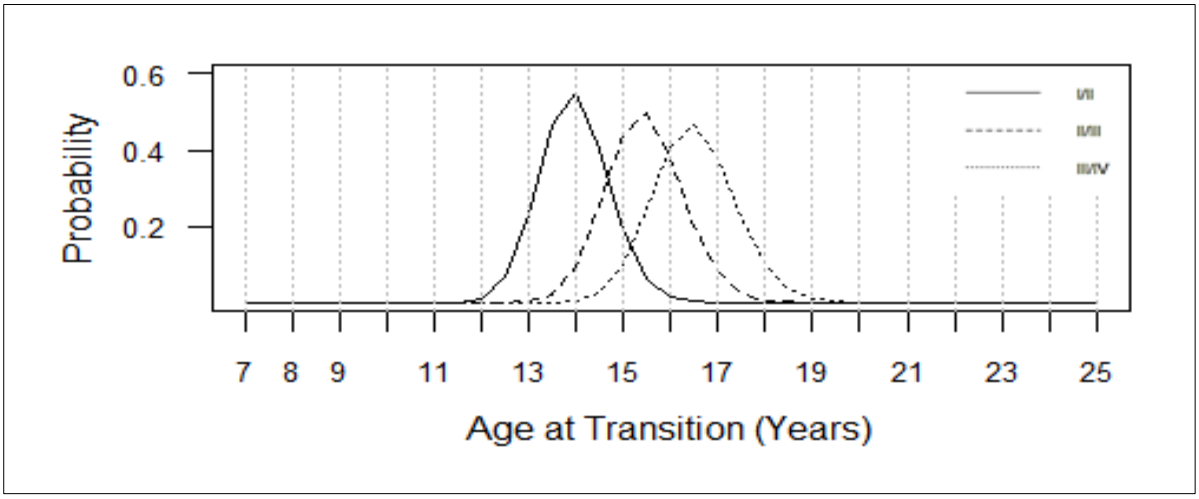


Figure D.5: Probability density plot for age-at-transition distributions of the ulna in black individuals

Figure D.6: Probability density plot for age-at-transition distributions of the ulna in white individuals

Table D.2: Posterior distribution estimates for the ulna demonstrating the mean age per phase in black South Africans

	Stage I	**Stage II**	**Stage III**	**Stage IV**
Mean CI	7.42	14.70	15.98	16.56
95% CI	≤13.92	12.77-16.47	14.26-17.63	≥ 14.85
90% CI	≤13.44	13.15-16.19	14.56-17.35	≥ 15.17
75% CI	≤12.37	13.60-15.80	14.96-16.95	≥ 15.62
68% CI	≤11.82	13.76-15.66	15.10-16.79	≥ 15.74

* Estimates in years

Table D.3: Posterior distribution estimates for the ulna demonstrating the mean age per phase in white South Africans

	Stage I	**Stage II**	**Stage III**	**Stage IV**
Mean CI	7.01	13.66	15.50	20.78
95% CI	≤ 13.08	10.79-16.35	12.81-18.22	≥ 15.82
90% CI	≤ 12.50	11.30-15.89	13.32-17.71	≥ 16.41
75% CI	≤ 11.34	12.13-15.28	13.95-17.03	≥ 17.40
68% CI	≤ 10.91	12.34-15.04	14.16-16.83	≥ 17.74

* Estimates in years

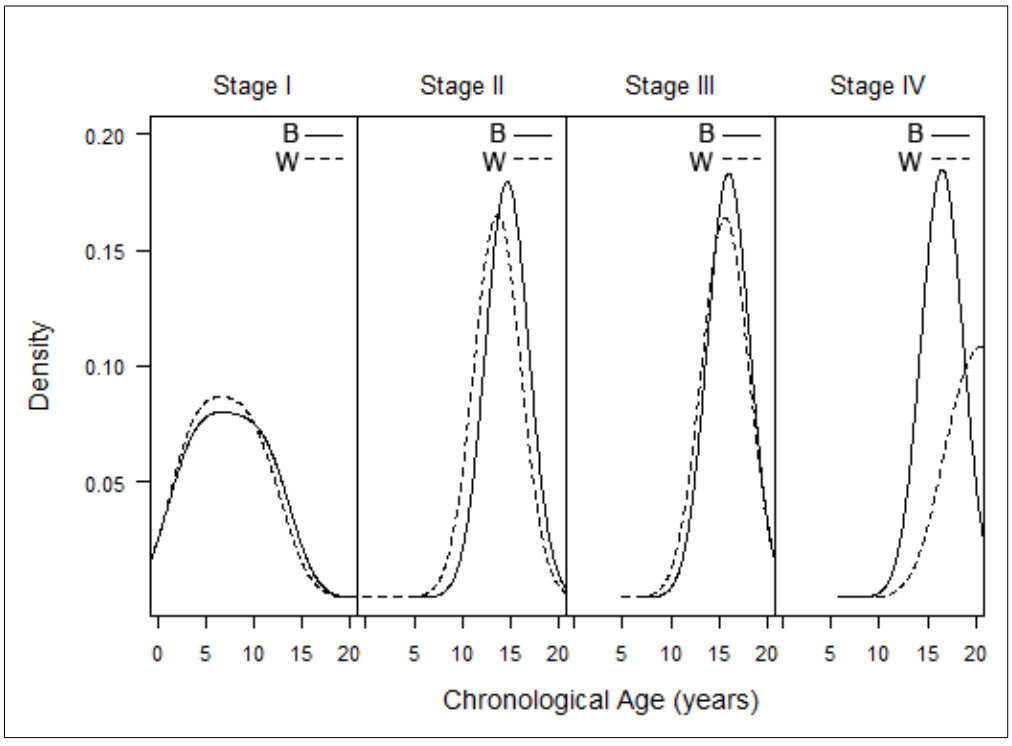


Figure D.7: Probability distributions for the ulna (per population group)