Nasal Aperture Shape and its Application for Estimating Ancestry in Modern South Africans

By

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DECLARATION

I, Jennifer McDowell, declare that this dissertation is my own work. It is being submitted for the degree of Masters of Science in Anatomy at the University of Pretoria. It has not been submitted before for any other degree or examination at this or any other University.

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ABSTRACT

With both a heterogeneous population and a large number of unidentified persons in South Africa, an accurate method to estimate ancestry is needed. The purpose of this study was to evaluate variation in nasal aperture shape in black, white and coloured South Africans, using linear measures and geometric morphometrics (GM), the latter which includes both procrustes analysis (GPA) and elliptical fourier analysis (EFA). To test statistical significance among groups, discriminant function analysis (DFA) and principal component analysis (PCA) was used.

A total of 310 (164 male, 145 female) crania of black, white and coloured South Africans were used. Thirteen standard landmarks, namely, glabella, nasion, nasale superior, dacryon, nasale inferius, alare, most inferior nasal border and subspinale, were digitised with a MicroScribe G2™ (Immersion: San Jose, CA). Five linear measures, nasion-dacryon angle (NDA), nasal breadth (NLB), nasal height (NLH), inter-orbital breadth (DKB) and nasion-dacryon subtense (NDS), were calculated. For EFA, photographs were taken in a frontal plane of skulls that had been positioned in the Frankfort horizontal plane on a craniophore.

All classification accuracies for all groups were better than chance. Using linear measures and GPA, black South Africans classified 55-71% correctly, coloured classified 53-61% correctly and whites classified 85-95% correctly. Black and coloured South Africans demonstrated bell-shaped nasal apertures with nasal spines superior to the inferior nasal border. White South Africans had pear-shaped nasal apertures with a nasal spine inferior of the inferior nasal border. Using EFA black South Africans classified 62% correctly. While coloured South Africans only classified 39% correctly, which demonstrates high within group variability. Due to their unique historical development, large variation (heterogeneity) within the coloured group was expected. White South Africans had the highest correct classification accuracy of 85%.

For all methods, misclassification rarely occurred between white and non-white (black and coloured) groups and most difficulties arose in distinguishing non-white groups from each other. High rates of misclassification was also noted between sex designations within a group, which suggests less or an absence of sexual dimorphism for these variables The distinct separation of white South Africans may reflect the mid-to late 20th century political and social separation of white and non-white groups in South Africa.

Nasal aperture shape, alone, is less useful for separating groups such that all groups have relatively intermediate nasal aperture shapes; however the pinched nasal bone structure of white South Africans clearly separates them from the other groups. When using nasal bone and aperture landmarks, linear measures are as accurate as the modern geometric techniques in distinguishing groups.

All methods are feasible to use in the estimation of ancestry on modern South Africans, with craniometry a sensible solution as the data can be rapidly collected, accurately analysed and compared to current reference samples.

Keywords: Geometric Morphometrics, elliptical Fourier Analysis, Craniometrics, Black South African, White South African, Coloured South African, Procrustes Analysis, Canonical Variate Analysis, Physical Anthropology, Mid-face.

ABSTRAK

Suid-Afrika, met ‘n heterogene bevolking en hoë aantal ongeïdentifiseerde liggame, benodig ‘n akkurate metode om afkoms te bepaal. Die doel van hierdie studie was om die variasie van die nasale opening in kleurling, swart en wit Suid-Afrikaners te evalueer deur gebruik te maak van liniêre afmetings en geometriese morfometrie (GM). Geometriese morfometrie sluit in beide prokrustes ontledings (GPA) en elliptiese fourier ontleding (EFA). Om statisties betekenisvolheid tussen groepe te toets, is diskriminante funksie ontleding (DFA) en hoof component ontleding (PCA) gebruik.

‘n Totaal van 310 skedels (164 manlik en 145 vroulik) van swart, wit en kleurling Suid-Afrikaners is gebruik. Dertien standaard landmerke, naamlik glabella, nasion, nasale superior, dakrion, nasale inferius, alare, mees inferior nasale grens en subspinale is gedigitaliseer met ‘n MicroScribe G2™ (Immersion: San Jose, CA), en vyf liniêre afmetings, nasion-dakrion hoek (NDA), nasale breedte (NLB), nasale hoogte (NLH), inter-orbitale breedte (DKB) en nasion-dakrion koord (NDS), is bereken.

Klassifikasie akkuraatheid vir al drie groepe was beter as ewekansig. Met liniêre afmetings en GPA, het swart Suid-Afrikaners 55-71%, kleurlinge 53-61% en wit Suid-Afrikaners 85-95% akkuraat geklassifiseer. Swart en kleurling Suid-Afrikaners vertoon klokvormige nasale openinge met nasale spinas superior tot die inferior nasale grens. Wit Suid-Afrikaners het peervormige nasale openinge met ‘n nasale spina inferior tot die inferior nasale grens. Met EFA klassifiseer swart Suid-Afrikaners 62% korrek, terwyl kleurling Suid-Afrikaners slegs 39% akkuraat klassifiseer, wat op hoë variëring binne die groep dui. Weens hul unieke historiese ontwikkeling, word hoë variasie (heterogeniteit) binne die kleurling groep verwag. Wit Suid-Afrikaners het die hoogste korrekte klassifikasie akkuraatheid met 85%.

Met al die metodes kom misklassifikasie tussen wit en nie-wit (swart en kleurling) groepe min voor. Die grootste probleme kom voor met die onderskeid tussen swart en kleurling. Misklassifikasie was ook hoog tussen geslagte binne ‘n groep, wat dui op afwesige of lae seksuele dimorfisme vir hierdie veranderlikes. Die duidelike afsondering van wit Suid-Afrikaners weerspieël moontlik die mid tot laat 20ste eeuse polities en sosiale afsondering van die drie groepe in Suid-Afrika.

Op sy eie is die vorm van die nasale opening minder bruikbaar omdat al drie groepe relatief intermediêre nasale opening vorms het; maar die geknypte nasale been struktuur van wit

Suid-Afrikaners onderskei duidelik van die ander groepe. Met die gebruik van landmerke van die nasale been en opening, is liniêre afmetings so akkuraat soos moderne geometriese tegnieke om groepe te onderskei.

Alle metodes is uitvoerbaar om te gebruik in die beraming van afkoms van moderne Suid-Afrikaners, met kraniometrie as ‘n praktiese oplossing omdat die data vinnig gekry word, akkuraat ontleed word en vergelyk word met huidige verwysingsdata.

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CHAPTER ONE: INTRODUCTION

Forensic anthropologists are often employed to construct a biological profile from unknown skeletal remains. A part of this profile is the estimation of peer-perceived social identification (Hefner, 2009). Traditionally, anthropologists often referred to social identification as race, which was associated with the idea of biologically distinct groups within the human species (Wheat 2009). However, recent genetic and geographical research into human variation has shown that the idea of distinct biological races does not exist (c.f. Lewontin, 1972; Long et al., 2009).

Sauer (1992) posed the question "If race does not exist, why are forensic anthropologists so good at identifying them?" The simple answer is that physical anthropologists are good at translating morphological traits into a culturally constructed labelling system (Sauer, 1992). Irrelevant of biology, clinal morphological variation has been shown to exist across geographical distances and this clinal variation shows "patterned variation when [humans are] classified as groups" (Ousley et al., 2009:73). Despite overlap and significant within group variation, Ousley et al. (2009) showed that with the use of morphological variation based on geographical patterning individuals and groups could be classified into defined population groups at a much better rate than chance.

However, geographic variation does not support the theory of distinct biological races. If this were the case, a large number of races would be classified using craniometrics, alone, and that number would multiply if variation beyond craniometrics was introduced into the criteria (Ousley et al., 2009).

To eliminate confusion surrounding the definition of race and to remove the idea of distinct biological differences, Sauer (1992) suggested that the term ancestry be used for associating morphological variation to population groups. Ancestry is concentrated on the principles of geographical origin. On a worldwide scale, humans exhibit geographic clinal patterning in morphology when classified as groups (Ousley et al., 2009).

Cartmill (1998) argued that population specific studies often collected their data from "so-called black, white and Asian individuals who had been born in the same geographical area" (Cartmill, 1998:652). This, according to Cartmill, suggests that ancestry was not based on geography and that the whole concept was a fallacy (Cartmill, 1998). What Cartmill (1998)

failed to acknowledge was that the estimation of ancestry was not based on the geographical origin of the individual being analysed but on the geographical origin of that unknown person's ancestors. A few hundred years of migration, particularly in societies which historically restricted interracial mating, is not enough time to eliminate the geographic variation caused by thousands of years of evolutionary influence (Ousley et al., 2009).

Dr. Hooten and his postgraduate students, particularly Stanley Rhine, were staunch advocates for morphological variation among race groups (c.f. Hooten, 1947; Rhine, 1990). Their work strongly focused on categorising distinct morphological traits between population groups and is the main influence for the use of discrete traits in analyses of unknown remains. Non-metric analysis is based on categorising a series of graded discrete traits, such as presence, absence, shape and level of protrusion, to classify an unknown cranium into a broad racial category (Hefner, 2009). However, the validity and reliability of these traits have only been recently tested.

With regard to validity, studies have shown that the distribution of discrete traits, as claimed by Rhine (1990), varies considerably within and between population groups. While a statistically significant relationship was noted between ancestry and several of these skeletal traits, Hefner (2009) showed that only 17-51% of people in a defined population have all the skeletal traits previously associated to their broad population group. Therefore, the distribution of these traits is far more variable than suggested in previous studies. These findings do not invalidate non-metric analysis but demonstrate that the technique, in the present form, is unreliable. Non-metric analysis needs to follow other morphological research and studies and data needs to be collected from many population groups. By improving the reference samples and studying clinal variation, a better understanding of patterned variation and its relationship to ancestry can be established and a more accurate non-metric methodology can be formulated.

In addition to inaccurate trait distribution, the reliability of non-metric analysis is plagued with observer bias such that it has often been referred to as more of an art than a science (Hefner, 2009). Standardised guidelines, illustrations, frequency scales and distributions for non-American samples have both improved the method and demonstrated its limitations (c.f. Hefner, 2009; L'Abbé et al., 2011). Hefner (2009) found that previous definitions and line drawings of described traits did not encompass the range of variability present in modern populations. Hefner (2009) addressed the issue of standardising features and applying robust

statistical tests to these traits. With an improved testing strategy, Hefner (2009) used mid-facial morphological variation to estimate ancestry, as it has found to be a good discriminator for estimating ancestry (c.f. Hooten, 1947; İşcan & Steyn, 1999; Rhine, 1990). Hefner (2009) was able to classify an individual to an ancestral group with 84-93% classification accuracy, depending on the number of variables.

However, L'Abbé et al (2011) used these new standardised traits on a South African sample and found the distribution of traits to vary from North American groups. Results from L'Abbé et al. (2011) and Hefner (2009) highlight the importance of population specific research and sampling of discrete morphological traits, similar to what has been done with craniometric analysis (c.f. Howells, 1973; İşcan & Steyn, 1999; Ousley et al., 2009).

Craniometric analysis, also known as osteometrics, is an alternative method, based on measuring continuous traits from the skeleton. The measurements and landmarks used in traditional metric analysis come from the standards created at the Frankfurter Verstandigung in 1882. Osteometrics are considered more reliable because the method does not rely on observer interpretation, uses standardised measures, and has known standard error estimates (Wheat, 2009).

An additional benefit to craniometrics is the ability to use multivariate statistics to analyse variation, such as discriminant function analysis (DFA). Giles & Elliot (1962) were the first to use DFA to analyse differences in cranial morphology. This and subsequent studies concentrated on American samples and populations. DFA has since become a standard tool for analysing variation significance in morphological studies. W.W. Howells was the first to incorporate multivariate analysis with craniometric analysis on a worldwide sample and collected data from 28 population groups. Howells' goal was to analyse shape variation among different population groups as a means to find an objective method for comparing morphological variation (Howells, 1973). While Howells' data set has become a key referencing sample for many anthropological studies there are many modern populations not represented or represented adequately, such as South Africans.

In Howells' data set, only Zulu and Bushman are represented, which is not an adequate representation of modern South African groups. South Africa has a population of more than 49 million people of various ancestral and social identities. With such diversity, South Africa is an ideal country to evaluate human skeletal variation among ancestral groups. Despite the emergence of democracy in 1994, past segregation, laws and social behaviour have affected

the social interactions and gene flow of South Africans. South Africa also has a large number of unidentified persons. Gauteng mortuaries alone report more than 1000 bodies left unidentified in 2009 (SAPA, 2010), which demonstrates a need for accurate, reliable and efficient methods for personal identification from skeletal remains.

Previous research on ancestry in South Africa has shown that South Africans are unique. İşcan & Steyn (1999) found that South African black and white crania varied from American equivalents enough to make current American osteometric standards inadequate for use on South African populations. L'Abbé et al. (2011) found statistically significant relationships between discrete variable distribution and ancestry, but these differences, using current scoring methods, could not be accurately used for differentiating among South African groups.

In response to those findings this study uses a combination of craniometric and shape analysis with geometric morphometric (GM) techniques in an attempt to quantify and accurately differentiate what was visually observed with non-metric analysis. This study is designed to combine multivariate geometric analysis and skeletal morphology to assess variation in mid-facial size and shape among black, white and coloured South African groups. Using multivariate morphometric analysis allows for a more comprehensive and quantifiable evaluation of shape than observer analysis alone (Brues, 1977; Ousley et al., 2009).

While there may be statistically significant variations between the three population groups studied, this variation needs to be addressed within real world applicability. Recent research on South African groups has highlighted the importance of ancestry on other biological estimations. Patriquin et al. (2003) found that, in a South African population, an individual's ancestry affects the accuracy of sex estimates. Patriquin et al. (2003) showed that South African groups are morphologically different from their North American counterparts and advocated for population specific standards. Their research also highlighted that a person's ancestry may dictate which method for sex estimation would provide the highest accuracy. Patriquin et al. (2003) found that the sciatic notch had little sexual dimorphism among white South Africans and therefore cannot be accurately used to estimate sex. However the sciatic notch has shown to be an accurate sex estimator for black South African populations with 91% of black males exhibiting a typically male narrow sciatic notch. Therefore, ancestry can potentially affect the accuracy of other indicators of the biological profile such as sex, stature and age at death.

The purpose of this study is to quantify size and shape variation in nasal bone and nasal aperture morphology among black, white and coloured South Africans using craniometrics (linear measures) and GM, including elliptical fourier analysis (EFA) and general procrustes analysis (GPA). DFA and principal components analysis (PCA) is used to further describe variation and to predict classification accuracy among the three social groups. Comparing the linear measures, which is a more widely used method in forensic contexts, to the GM techniques allows a direct comparison of the accuracy and validity of using craniometrics in estimating ancestry.

CHAPTER TWO: LITERATURE REVIEW

2.1 Defining a species

Humans are a single species (*Homo sapiens sapiens*). What is not common knowledge is the endless debate over the scientific meaning of this statement (c.f. Coppinger & Schneider, 1995; Darwin, 1871/1981; Hooten, 1947; Pritchard et al., 1999). While historical views of speciation may appear peripheral to a discussion of physical anthropology and ancestry, it forms the foundation from which scientists began to categorise humans into biological groups.

2.1.1 Single versus multiple species theories

In the 18th century, scientists suggested that humans were a single species comprised of different race[1] groups, which were defined by visual characteristics such as skin colour, hair colour and behaviour patterns (Wheat, 2009). By the 19th century, civil war, immigration and colonization had shifted the general opinion into one that considered humans to be separate species (Wheat, 2009).

The most important contributor to the separate, or multiple, species theory was Samuel Morton (1799-1851). In 1839, Morton suggested that each human race had an independent origin; by 1851, he had openly stated that different population groups were formulated from separate species (Michael, 1988). Morton cited cranial capacity as the best discriminator for determining a species and differences among species (Gould, 1978; Michael, 1988), but he used skin colour, hair type, overall skull shape and face size to categorise individuals into populations during life (Wheat, 2009). Gould claimed Morton regarded cranial capacity as an "index of overall intelligence" and used this to defend the racist tenet that the Caucasian species was superior to all other groups (Gould, 1978:503). Aside from his theory of speciation, Morton has often been referred to as the father of scientific racism, as much of his work was used to validate slavery during the American Civil War (1861-1865) (Dobzhansky, 1973).

In contrast, Charles Darwin (1809-1882) advocated for the single origins of humans. In his publication, *The Descent of Man*, Darwin stated that it was pointless to debate human speciation until an agreed upon definition of a species could be formulated (Darwin,

[1] During this period the term *race* implied different subspecies

1871/1981:228). At the time, there was great disparity amongst researchers as to the number of species and races[2] within the human population (Dobzhansky, 1973). Two, five, twenty-two, and up to sixty-three population divisions existed in scientific literature of the time (Dobzhansky, 1973).

Darwin (1871/1981) argued for a common ancestor, from whom modern humans evolved through mutation and natural selection (Darwin, 1871/1981:386). He claimed that biological similarities between human race groups were too great for a multiple species origin to be plausible. According to Darwin, the lack of continuity among researchers as to the number of species demonstrated that human variation was clinally distributed. Darwin stated that it was not possible to identify any distinctive characteristics between or within human groups that warranted species differentiation (Darwin, 1871/1981:226). However Darwin (1871/1981) did believe that humans could be categorised into race groups based on these clinal variations.

While Darwin did find success and notoriety with his work, he was continually criticised because of his extreme and naturalist views. Now he is considered to be among one of the most illustrious names in the world (Wenley, 1909). He has "revolutionized our understanding of life [and] the relationship of humanity to all creatures in the world" (25 greatest science books of all time, 2006).

2.1.2 Morphology versus genetics

Morton and Darwin, despite their difference of opinion, highlight a seemingly obvious but important question: what defines a species and, in turn, within species variation?

Morton's definition of a species challenged and contradicted the widely accepted theory of inter-species infertility (Michael, 1988). The "official"[3] definition of the term species is a group of organisms having many characteristics in common and/or the members of which can breed to produce fertile offspring. Commonly this definition is known as the biological species concept; animals may look the same but if they do not interbreed then they are separate species. Incidentally, animals may look completely different but also be of the same species. They look different because of either environmental differences or because they

[2] From this point forward 'race', when not specifically referred to as biological or social, is being used as a vague term associated to human population groups for the purpose of explaining the history of the concept.

[3] The word "official" must be read with caution as the definition of a species changes depending on the discipline involved. The definition of species used in this text comes from, Collins (2003), Oxford (2010) and The American Heritage Science (2005) dictionaries.

fulfill different roles within the group (Evolution 101, 2006). This definition is not used within all scientific disciplines as it does not address the issue of asexuality or account for genetic variation (Evolution 101, 2006). There are many other species definitions that have been proposed over the years, such as, recognition species concept, phonetic species concept and phylogenetic species concept, to name a few (Evolution 101, 2006). An in depth discussion and comparison of these theories is beyond the scope of this research.

Lewontin (1972) studied genetic variation among humans and found significant overlap in all populations, with the highest level of variation noted between individuals. Variation among other species has also brought out interesting points regarding morphological similarities and genetic relationships. For example, Avise et al. (1990) found that the majority of mallard ducks have mitochondrial DNA (mtDNA) that is more closely related to black ducks than their own species. Likewise, Coppinger & Schneider (1995) noted greater mtDNA differences between dog breeds, such as, Doberman-Pinscher and Poodle, than between dogs and wolves. In fact, less mtDNA differences were observed between dog, wolf and coyote species than between various human population groups (Coppinger & Schneider, 1995). Also, despite obvious morphological differences among Lycaeides butterflies, neither mtDNA nor allozyme alleles can distinguish between the different species (Nice & Shapiro, 1999). Similar results were noted among Redpoll finches which have distinct phenotypes but undifferentiated DNA (Seutin et al., 1995). The reasons for this, seemingly paradoxical, genetic variation is relatively straightforward; natural selection does not affect the entire genome (Frost, 2008).

Only a small fraction of the genome is changed when a population differentiates in response to natural selection (Frost 2008). Most genes are little more than junk DNA, their presence or absence does little for the survival of the species. Therefore, they are not selected for or against (Frost, 2008). Also, many genes code for traits that are equally useful in a wide range of environments, so many species will have the same coding. This pattern is most common in species which have only recently differentiated (Frost, 2008). Eventually as a species differentiates, the genome steadily becomes variable but this requires reproductive isolation and time (Frost, 2008). The effect of natural selection on the genome can also explain how a single species can have high genetic variation, but little morphological differences. Again, this is because natural selection acts on such a small portion of the genome, that as a whole, the genome may appear vastly different without causing morphological or physiological

differences in the animals (Frost, 2008). This vast variation may be explained by a species' common ancestor (Pritchard et al., 1999).

The general view of human evolution is that we evolved from a common ancestor shared with the great apes. However, there has been more than one common ancestor between then and the appearance of modern humans. Our most recent common ancestors (approximately 120 000 years ago) are of interest because they may provide information about the size and structure of ancestral populations, specifically our gene pool (Pritchard et al., 1999). Couple this information with demographic expansion and it may provide answers to the similarities and differences within the modern human genome (Pritchard et al., 1999).

In summary, the word "species" has an arbitrary definition that is dependent on the discipline using it. In Western culture, the meaning has been shaped by the concept of human evolution such that a species is defined by both a historical and morphological relationship rather than its mere genetic constitutes (Frost, 2008). If we were to change this view, for example, the wolf would become a species of dog and the chimpanzee would become a hominin (Frost, 2008). This same justification can be used when discussing the concept of race, as the definition has also changed through time and among different groups, both in the scientific and social communities.

2.2 The concept of race

Race is a highly ambiguous and emotionally charged term and, in terms of human history, is a relatively new concept (Brace, 1995). In Europe, race, and subsequently racism, appeared in the 15th century with the discovery of the New World and as a result of the growing political need to categorise and govern people on various continents (Brace, 1995). In present-day countries, such as South Africa, race has become an established and documented political identity and, in turn, has become a living construct (Brace, 1995; Menand, 2001; Sauer, 1992).

In terms of defining human populations, race has been debated for nearly 200 years but without a legal or consistent definition (Ousley et al., 2009). Socially and politically, race is a term associated to racist tenets and segregation (Brace, 1995). Scientifically, the term is used to refer to biologically distinct groups. In other contexts, race has been used to link different human populations based on similarities in morphological variation; to describe

human subspecies, clinal variation and distinct population groups; and to associate people of a similar skin colour, facial morphology, accent, behaviour and religion (Hooten, 1947; Wheat, 2009). Based on the context in which the term race has been used, it leaves one to question: what is race and does it actually exist?

2.2.1 Pre-twentieth century

In the 18th century, humans were classified as a single species but were subdivided into distinct biological race groups. According to Wheat (2009), Linnaeus (1759) used skin colour, soft tissue morphology and behaviour to divide humans into four races, which were referred to as subspecies and included *Homo sapiens europeaus*, *H. sapiens americanus*, *H. sapiens asiaticus* and *H. sapiens africanus*. Blumenbach (1775), a contemporary of Linnaeus, took a more environmental approach to categorising humans. According to Wheat (2009), Blumenbach (1775) originally believed that all humans had an equal potential to achieve superiority, but climate, nutrition and mode of life over many generations had caused morphological variation that had led to different race groups. Blumenbach claimed the existence of five races which included Caucasoid, Asian, African, Aboriginal American and Malay (Wheat, 2009).

While Darwin (1871/1981) supported the idea of a single species, he suggested that biology created different race groups. Regarding race, he made two important conclusions about human groups. First, Darwin argued that phenotypic characteristics, such as skin colour, were superficial and were not useful in distinguishing one race from another (Darwin, 1871/1981:214-253). Dobzhansky (1973) later substantiated this point with genetic research in which skin colour was shown to not be directly linked to any other racial traits. Genes that control for skin pigmentation are independent of those which influence physical appearance, body proportion and skull shape (Dobzhansky, 1973). Second, Darwin suggested that race groups graduated into each other and thus should be viewed as clines and not discrete biological clusters (Darwin, 1871/1981). In *The Descent of Man*, Darwin (1871/1981) did not provide a set of criteria to define a race but he did group people based on their country of origin, physical and cultural similarities. At the time, the majority of scientists viewed Darwin's race criteria as too extreme to be accepted, but now the concept of grouping people based on geographic origin or ancestry is at the forefront of population studies within physical anthropology (c.f. Brace, 1995; Ousley et al., 2009).

2.2.2 The twentieth century

2.2.2.1 Disproving the existence of biological race

The definition of biology pre-20th century is vague and inclusive of many types of variation (c.f. Darwin, 1871/1981). Since genetics or hereditary factors were not understood; ideas on biological variation were commonly lumped into a heading under natural selection. In many cases, physical and behavioural differences were also considered as biological features (Dobzhansky, 1973; Wheat, 2009). During the early 20th century, race was determined based on the observable characteristics of human morphology (Hooten, 1947). With advancements in genetic research in the middle of the 20th century, biology came to be referred to as genetics (c.f. Hooten, 1947). Anthropologists at the time began to realise that for physical observations of race to be "valid and meaningful, [they] must be brought in line with the discovery of modern genetics" (Hooten, 1947:441). Race needed to be shown to be biological and to be observable.

E.A. Hooten, an early 20th century Harvard anthropologist, postulated that pure races, in a biological sense, did not exist due to continual inter-breeding among humans. However, Hooten argued that consistent and directional inter-breeding produced its own biologically and morphologically distinct race groups (Hooten, 1947). Hooten based the division of human population on differential geographic origins and genetic isolation and created three primary races, namely: Caucasoid, Negroid and Mongoloid [sic] (Hooten, 1947). In order to explain possible admixture among primary race groups, Hooten (1947) sub-divided the primary races into, sub, secondary and composite races. These sub-groups had distinct territorial distribution within the area of a primary race or some varied physical features as a result of inter-breeding with other groups, for example, Ainu, Keltic, Indonesian, Australian, Bushman-Hottentot [sic] (Hooten, 1947).

In his 1947 revised edition of *Up from the Ape,* Hooten attempted to explain how genetics and environmental factors influence physical characteristics.

> "The essential characters of any animal, morphological or physiological, may safely be assumed to be due to hereditary factors...the range of [morphological] variation is limited by the nature of the genes that are peculiar to the human species…the ultimate differences in phenotypes are, to some extent, genetically determined but subject to modification by the impact of environment upon the individual…it seems improbable

> that any hereditary character is completely emancipated from environmental interference" (Hooten, 1947:443-44)

Hooten viewed environmental influence as a biological variable. Hooten claimed it both feasible and appropriate to categorise humans into races using morphological features because the expression of these features are genetic (Hooten, 1947). Hooten explained population variation by claiming environmental influences contributed to *within* group variation (Hooten, 1947:444). Hooten created a list of morphological characteristics that could be used to distinguish between different race groups; and, over time, these traits became known as the "Harvard list" (Wheat, 2009). Modern physical anthropologists still use and study the "Harvard list" in various modified forms (c.f. Hefner, 2009; Rhine, 1990; Wheat, 2009).

In 1972, Lewontin analysed blood groups and protein alleles as a means to define the biological legitimacy of human races. He found no evidence to support the concept of biological race. In fact, Lewontin found that 85% of the genetic variation was observed between individuals and not between population groups (Lewontin, 1972). Between racial groups, only 6.3% of the total variation was observed (Lewontin, 1972). Lewontin stated that these findings give a reason to encourage banning the use of the word 'race' in relationship to humans" (Lewontin, 1972).

As a result of genetic research, in 1996, the American Association of Physical Anthropology (AAPA) released a statement on biological aspects of race, stating:

- All humans living today are a single species evolved from a common ancestor.
- The geographic pattern of genetic variation presents no major discontinuity.
- Pure races, in terms of genetically homogenous population groups, do not exist today, nor is there any evidence they existed in the past.

The findings from more recent genetic studies are similar to those of Lewontin (1972) and support the findings of the AAPA (1996). Rosenberg et al. (2002) found only 4.3% genetic variation between population groups, while Excoffier & Hamilton (2003) found 9.2% diversity between populations. A study by Serre & Pääbo (2004) and Long et al. (2009) concluded that genetic variation, on a global scale, is continuous and there are no discrete patterns which would give evidence for separate racial groups.

Research has found that genetic discontinuities occur on a more local scale, often in relation to allele frequencies relating to disease susceptibility (Brace, 1995; Serre & Pääbo, 2004). The most commonly discussed is the frequency of haemoglobin S in high risk areas for malaria. While these types of discontinuities occur, they are not viewed as racial differences since this type of variation is based on history and culture (Brace, 1995; Serre & Pääbo, 2004). Malaria pays no attention to race or regional boundaries (Brace, 1995). If a race group moved from a high malaria area the frequency of haemoglobin S would subsequently decrease in that group, indicating an environmental adaptation for survival and not a biological trait (Brace, 1995).

2.2.2.2 Morphological human variation

Despite biological (or genetic) race being shown to be invalid, many physical anthropologists suggest that geographic, morphological and social race exists among groups.

In the AAPA (1996) statement, the authors acknowledged that morphological differences did exist between population groups living in different geographical areas. This concept comes from observations that individuals in varying geographical locations vary in their frequency distributions for certain physical traits (Edgar & Hunley, 2009). The variable distribution and expression of a trait in modern populations are largely based on the observations of Hooten (1947) and Rhine (1990). Rhine (1990) did not argue for distinct biological races, but he did believe that racial categorisation was valid.

Rhine (1990) classified humans into the same three primary groups as Hooten (1947): Caucasoid, Negroid and Mongoloid. Also, Rhine's (1990) criteria were adapted from Hooten's "Harvard list". The aim of Rhine's study was to assess which morphological traits were 'expected' in each of the three defined groups (Rhine 1990). However, the study design and skeletal sample used make Rhine's (1990) conclusions suspect, at best. He used a small sample of 87 crania, with only 7 Negroid skulls; 2 of which were casts, and 12 Mongoloid skulls; of these only 3 were from a modern human sample (Rhine, 1990). Rhine states that the 45 non-metrics traits selected for categorising race groups are those "identified as being useful", but he does not explain the extent of their usefulness, their relationship with other traits or provide statistical data to support his theory (Rhine, 1990). Rhine (1990) offered no definition of the traits examined; only typological skull illustrations exist for each of the three defined groups.

Giles & Elliot (1962) were the first to use classification statistics, DFA[4], to analyse differences in cranial features and linear measures. It wasn't until 1984 that follow-up studies were conducted by Gill (citied in Krogman & İşcan, 1986), then Gill et al. (1988) and Gill & Gilbert (1990). Like Rhine (1990), these studies were concentrated on American samples and populations. Giles & Elliot (1962) analysed eight linear cranial measures from white Americans, Native Americans and black Americans. They found that the mid-facial region, which includes the nasal region and facial height, were the best to discriminate these groups (Giles & Elliot, 1962). Gill et al. (1988) added an Inuit sample and observed that there was a high accuracy in differentiating between white and non-white groups but a low accuracy when trying to differentiate non-white groups. Gill & Gilbert (1990) analysed three linear measures from the mid-facial region of black, white and Native American samples, to re-evaluate the results of Giles & Elliot (1962). All these studies concluded that DFA could be used to accurately distinguish between the different population groups with up to 90% accuracy, with the best accuracy between white and non-white populations (Gill & Gilbert, 1990; Krogman & İşcan, 1986).

Since Giles and Elliot (1962), many studies, including this one, have re-evaluated the validity of osteometric methodology in differentiating between population groups using multivariate analysis.

2.2.3 Current anthropological views on race

Biological race and the concept of race in humans are considered fallacies among geneticists and anthropologists. While most physical anthropologists do not deny the existence of systematic morphological variation among populations (Edgar & Hunley, 2009; Sauer, 1992), many refuse to associate this variation to the concept of race (Goodman, 1997; Smay & Armelagos, 2000). They suggest that continuing to use the term race is "lending credence" to the fallacy of public opinion that it is related to our genetics (Smay & Armelagos, 2000:23). Part of the problem is that the term 'race' has strong political and historic ties. Race has been used as a basis for segregation, institutional racism, unequal justice and genocide (Brace 1995; Ousley et al., 2009). For this reason, even when the term is used by well-meaning groups; such as physical anthropologists, it acts to legitimise and perpetuate scientific racism (Goodman, 1997; Smay & Armelagos, 2000).

[4] A full description of DFA is available in chapter two, page 26

Some anthropologists recognise that race, no matter its scientific invalidity, is a social construct (Sauer, 1992). Even Hooten recognised that biological affinity comes second to any cultural or social identification in terms of practical relevance (Hooten, 1947). For many people, membership into a race group forms a significant part of their self-image and social identity (Cartmill, 1998). In countries where racial identification is a mandatory requirement, the concept goes beyond social recognition to become a legal identity. The issue that arises is: if physical anthropologists conform to the political requirement of categorisation and assign race estimation to an unidentified person, does this validate the idea that different discrete human groups exists?

Sauer (1992) posed the question "If race does not exist, why are forensic anthropologists so good at identifying them?" This question sparked numerous debates amongst social and physical anthropologists (c.f. Brace, 1995; Goodman, 1997; Konigsberg et al., 2009; Ousley et al., 2009). The simple answer is that physical anthropologists are good at identifying race because they translate morphological traits into a culturally constructed labelling system (Sauer 1992). Races are ideas which "are provisionally useful for naming groups of interacting individuals" (Menand, 2001:123). Physical variation does not validate biological race, because in any given population at any given time physical characters are altered by the environment (Dobzhansky, 1973). Therefore, skeletal analysis does not provide a direct assessment of skin colour or culture, but it does allow an accurate estimation of geographical origin (Brace, 1995). Forensic anthropologists use the term race as a means to differentiate among common social labels (Sauer, 1992). The term is arbitrarily given for the sake of convenience to a group of individuals resembling each other, either physically, culturally or religiously (Menand, 2001). Essentially, it does not differ from the word 'variety' (Menand, 2001).

2.2.4 Replacing the concept of race with ancestry

Despite his firm belief in biological races, Hooten (1947) made an excellent point surrounding the issue of defining race and race groups. The word 'race' has been commonly used in a variety of social contexts and has lost any valid meaning (Hooten, 1947). For example, the word race has at different times been used to refer to "white", "Jewish", "Latin" and "Irish" peoples. This implies that race can refer to skin pigmentation, religious affiliation, linguistics or geographic origin, showing that the term is totally arbitrary (Hooten, 1947). With this type of ambiguity any research on race loses its value and credibility as it is overshadowed by the debate over the meaning of the word itself. Brace (1995) describes race

as an ever evolving term broadly linked to human variation, but because of its historical connections to segregation the term has become too divisive to be of any scientific value.

Irrelevant of biology, social race exists and a demand exists for physical anthropologists to provide this information to the remains of an unknown individual (Sauer, 1992). Clinal morphological variation has been shown to exist across population groups (Ousley et al., 2009), and is often due to cultural, geographic and linguistic barriers (Brace, 1995; Sauer, 1992). A concordance between social race and cranial morphology exists that can be used to accurately distinguish between different population groups (Giles & Elliot, 1962; Ousley et al., 2009). The remaining question is how to recognise and use this variation without associating it to the concept of race? Sauer (1992) suggested the term race be replaced with ancestry.

Ancestry is concentrated on the principles of geographical origin and is based on the idea that morphological traits may be translated into a culturally constructed labelling system (Sauer, 1992). Ancestry focuses on the idea that morphological variation is a cline and not bound to discrete boundaries (Ousley et al., 2009). Brace describes ancestry as an indicator of "regional kinship… on an expanded scale" (Brace, 1995:174). On a worldwide scale, humans exhibit geographic patterning in morphology when classified as groups (Ousley et al., 2009). The likely reason for morphological differences is because black and white population groups originated from different continents (Ousley et al., 2009). Europeans and Africans had been separated for thousands of years and experienced different evolutionary influences prior to migrating together (Ousley et al., 2009).

In addition to different geographic origins, social identity also limits the extent of gene flow among groups (Ousley et al., 2009). Social identity is the product of its bearers; it is cultural in nature, in that, it is learnt and moulded through experience and interaction (Adhikari, 2005). By its very nature, social grouping adds dimensions and boundaries between populations that in reality do not exist (Brace, 1995). Due to cultural and language barriers as well as institutional racism, political segregation and popular opinion, social race has greatly influenced mating habits among modern humans (Ousley et al., 2009).

Ancestry is a prediction of geographic origin that is based upon skeletal morphology and is translated into the likely social label that would have been assigned to an individual when alive (Sauer, 1992). To be of value, any label given to an unidentified individual by a forensic anthropologist must reflect the terminology used by the society which they interact

with (Sauer, 1992). Often the labels used and researched by forensic anthropologists are limited to those which appear on a missing person's report, which the government usually controls with recognition of certain populations within its borders (Sauer, 1992). Ancestry is not concerned with genetic variability, as any morphological variable used to distinguish between populations is arbitrary (Ousley et al., 2009). What is important is how that variable is related to the different origins, histories and environments of the population's concerned (Ousley et al., 2009).

Smay & Armelagos (2000) suggested that making assumptions on an individual's ancestry was the same as assuming a person's race and, therefore, validated the existence of geographical races. Sauer (1992:107) states that using the term ancestry is not a "vindication of the race concept"; it is actually translating information about physical traits to a cultural labelling system.

Cartmill (1998) claimed that the assumption of geographical origin had little to do with the use of the terms race or ancestry. He suggested that population specific studies often collected their data from "so-called black, white and Asian individuals born in the same geographical region" (Cartmill, 1998:652). If these groups are different, then he suggested that race and ancestry were not based on geography and the whole concept of either race or ancestry was a fallacy (Cartmill, 1998). What Cartmill (1998) failed to acknowledge was that ancestry estimation was not based on the geographical origin of the individual being analysed but on the geographical origin of that unknown person's ancestors. A few hundred years of migration, particularly in societies which historically restricted interracial mating, is not enough time to eliminate the geographic variation caused by thousands of years of evolutionary influence (Ousley et al., 2009).

2.3 Current methodology for assessing ancestry

2.3.1 Morphoscopic evaluation

This method, also known as non-metric, is based on evaluating discrete skeletal traits into various grades such as presence, absence, degree of presence, or shape. The traditional approach of this method relies heavily on observer experience, and has produced a method that is as much an art as it is a science (Rhine, 1990). As a consequence, the technique is often subjected to high inter-observer variation due to differing opinion on the definition and

expression of the trait (Wheat, 2009). Although this method is subjective, it remains important as forensic anthropologists often receive fragmented remains for which osteometric analysis may not be possible (Hefner, 2009). However, there are significant problems regarding the reliability and validity of morphoscopic analysis.

Poor reliability of the method is based on the ambiguity of trait definitions and terminology, such as 'moderate' or 'rounded' which have never been adequately defined (Hefner, 2009). With his Harvard List, Hooten developed these traits in the early twentieth century. While he did not publish this list, it survived through his pupils (Hefner, 2009). In 1990, Rhine published a list of "expected" traits which were derived from Hooten's work. As mentioned previously, this study was plagued with small sample sizes and poor statistical analyses. Despite these facts, Rhine's 1990 publication has been used worldwide for estimating ancestry, or race, of an unknown person (Wheat, 2009). Hefner (2009) re-examined many of the traits defined by Rhine (1990) and has provided definitions and line drawings for each trait to try and improve testing consistency and accuracy.

Scientific validity of non-metric traits has been more difficult to assess. Research has focused on improving the validity of non-metric analysis by developing standardised guidelines, frequency illustrations and scales, and frequency distributions for non-American samples (c.f. Hefner, 2009; L'Abbé et al., 2011).

Hefner (2009) tested mid-facial morphological variation and was able to classify an individual to an ancestral group with 84-93% classification accuracy, which was dependant on the number of traits used. Hefner (2009) found that previous definitions and line drawings did not encompass the range of variability present in modern populations. The purpose of his paper was to address the standardisation issues of Hooten's 1926 research, as the research had not been statistically analysed before the methods became commonly used amongst anthropologists.

Hefner (2009) used a sample of 747 skeletons which he divided into four groups based on geographic origin (African, Asian, European, and Native American). He found statistically significant differences among groups for 10 of the 11 traits tested, but also found the range of variation within groups far exceeded previous assumptions.

Similarly, L'Abbé et al. (2011), following the principles of Hefner (2009), examined the accuracy and usefulness of non-metric traits among black, white and coloured South Africans.

Using 13 discrete traits, L'Abbé et al. (2011) found the relationship between mid-facial morphology and ancestry to be statistically significant but highly variable. This study noted that discrete traits were difficult to repeat and were also affected by sex and age at death (L'Abbé et al., 2011). Unlike Hefner (2009), L'Abbé et al. (2011) concluded that mid-facial morphological analysis was not an accurate tool for differentiating among South African groups unless other factors and analysis were available.

2.3.2 Osteometric evaluation

The metric method of evaluating ancestry is based on measuring continuous traits from the skeleton. When referring to cranial traits it is referred to as craniometry. The measurements and landmarks used in traditional metric analysis come from the standards created at the Frankfurter Verstandigung in 1882. While some researchers advocate for non-metric analysis others suggest that craniometry is the more accurate method, as it is more reliable (Ousley et al., 2009). Osteometrics are considered more reliable because it limits bias, uses standardised measures and has known standard error estimates (Wheat, 2009).

The classic approach is to measure distances between specific landmarks using tools such as sliding or spreading callipers. Recent advancements in craniometric analysis include computer software, digitisers (such as a MicroScribe™ G2) and three dimensional scanners. These new tools are used to digitally record landmarks on the crania that can then be used to analyse two dimensional linear measurements or three dimensional structures (Slice, 2007). The benefit of traditional metric analysis to traditional non-metric analysis is that multivariate analysis is objective, precise and statistically quantifiable (Ousley et al., 2009).

W.W. Howells (1973) was the first to incorporate multivariate analysis with craniometric analysis on a worldwide modern sample. Howells' goal was to analyse shape variation among different population groups to find an objective method comparing, taxonomically, different population groups (Howells, 1973). Before the 1970's there was no method that utilised craniometric analysis, which could consistently be used with confidence, to compare shape differences, or to rate such differences in terms of variation among modern populations (Howells, 1973). Howells (1973) used 1927 individual crania from five major geographic regions: America, Europe, Africa, Asia and the Pacific. He further divided these groups into 17 population groups and used 70 variables to analyse cranial shape among them (see Howells (1973) for full details). With the use of DFA, Howells (1973) found population

specific cranial variation and was able to correctly classify an unknown individual with 92-93% accuracy.

2.3.3 FORDISC 3.0

FORDISC 3.0 (FD3) is an interactive computer programme designed to classify ancestry, sex and stature of an unknown adult from skeletal remains based on the reference samples in its database (Freid et al. 2005). FD3 combines a modern data set with osteometric measures and the power of multivariate discriminant analysis to assign group membership (Ousley & Jantz, 1998). Currently 11 population samples from the Forensic Data Bank and 28 population samples from W.W. Howells (1989) worldwide data base are represented in FD3 (Freid et al., 2005).

FD3 uses multivariate DFA to categorise an unknown skull. DFA is "a family of statistical procedures for the optimal separation of groups and classification of unknowns using measurements" (Jantz & Ousley, 2005). It is important to note that with DFA the data supplied is interpreted in terms of its similarities to the variables available for comparison, which means that a data set is never 'rejected' from belonging (Giles & Elliot, 1962). Thus, if data from a black South African were added to the DFA used by Giles & Elliot (1962) it would be forced to be either American black or white because these are the only variables available to that DFA for comparison.

DFA should be initially run using all possible groups that an unknown individual may classify into, then the most dissimilar groups are removed and the test run again (Jantz & Ousley, 2005). A DFA that uses up to five groups will be more accurate than using many groups; however the analysis must first be done using many groups to establish the best candidate groups (Jantz & Ousley, 2005). The researcher guides the analysis in FD3 as they choose the comparative reference groups (Freid et al., 2005); for this reason, issues of validity centre on the correct use of the programme. One of the advantages of FD3 is that it has reference samples from populations that may be unfamiliar to many forensic anthropologists and therefore it eliminates the effect that observer expertise may have on the analysis (Ousley & Jantz, 1998). FD3 also offers a standardised protocol for data collection which means that the data can be more widely shared, enhancing comparability (Ousley & Jantz, 1998).

However problems arise when an unknown person is not represented within the database of the 28 groups (Ousley et al., 2009). Many researchers have argued this point and attempted

to show FD3 to be an invalid tool (c.f. Belcher et al., 2002; Campbell & Armelagos, 2007; Williams et al., 2005). Some physical anthropologists argue FD3 is an invalid tool because ancestry is not biological and therefore cannot be estimated from skeletal remains (Smay & Armelagos, 2000). But human variation is authentic and can be quantified (Ousley et al., 2009). Not only this but a social demand exists for skeletal remains to be identified and an individual's ancestry forms a major part of their social identification (Menand, 2001).

2.4 South Africa's populations

South Africa is a poly-linguistic society (10 official indigenous languages and English) with over 49 million people (Census, 2010). According to statistics South Africa's mid-2010 estimates, coloured South Africans comprise 8.8% (4.4 million) of the population, white South Africans 9.2% (4.6 million), black South Africans are 79.4% (39.7 million), respectively, and other groups make up 2.6% (1.2 million) of the population (Census, 2010).

Despite the emergence of democracy in 1994, past segregation, laws (Group Areas Act, 1950) and social behaviour have affected the actions and ideals of South Africans. Segregation covered all aspect of a person's life and resulted in race groups being forced into designated areas where people could live, work and attend school (Jacobson et al., 2004). Inter-racial marriage was illegal and remained so until 1990. In 2004, a cultural survey found that 75% of whites, 35% of coloured and 27% of black South Africans would not wish their children to marry someone of a different race (Washington Post, 2004). While these ideas are socially entrenched, they are to affect gene flow and behaviour within the country. Thus social and language barriers coupled with political segregation inhibited any significant gene flow, meaning that distinct morphological variation still exists among the different modern population groups of South Africa.

2.4.1 White South Africans

White South Africans are widely distributed across South Africa. They are primarily descendants of Dutch, German, French and British settlers who came to the country from the 17th century onwards (Steyn et al., 2004). Other immigrants from Europe include Greek, Portuguese and Hungarians (Census, 2001). Due to founder's effect and likely admixture, South African whites are osteologically distinct from their European counterparts (Steyn et al., 2004).

2.4.2 Coloured South Africans

In South Africa, "coloured" has a unique meaning that is distinct from its use in other countries. The term refers to a "phenotypically varied social group of highly diverse social and geographical origin" (Adhikari, 2005:1). Coloured people are largely descendants of slaves brought to the country from other African countries, Indonesia, India and Malaysia (Adhikari, 2005; Carstens, 1996). They are also descendants of indigenous Khoi and San, Asian and European people who assimilated into the late nineteenth century Cape colonial society (Adhikari, 2005). The South African coloured population has three broad groupings namely, Cape coloureds, the Griquas and the Cape Malays (Census, 2001). The term Cape coloured is specifically associated with their origin, which is around the city of Cape Town (Carstens, 1996). Approximately 85% of coloured people live in the South-west part of the country, of which two thirds live in the Western Cape, and over 40% of those living in the Cape Town area (Adhikari, 2005; Census, 2010). The term "coloured" remains a difficult group to describe. Anthropologically it is often used to mean a person of "mixed" ancestral origin but socially it has a more specific history and meaning (Adhikari, 2005).

2.4.3 Black South Africans

Among the black South African population, there are many groups, each with their own language, culture and previously designated homeland. In South Africa, the Nguni and Sotho-Tswana, Tsonga, and Venda form the black, or African, population of South Africa (Pauw, 2002). These groups are classified as 'Bantu-speaking' because their languages have linguistic similarities compared to other African languages (Pauw, 2002). Together, they form the densest distribution of people in South Africa (Census, 2001).

Due to historical events and migrations various subdivisions were created within the broad Nguni and the Sotho-Tswana groups. The Nguni include the Zulu from Natal (South Africa); Xhosa from the Eastern Cape (South Africa); the Swazi from Swaziland; and the Ndebele from Gauteng (South Africa) (Census, 2001; Nguni, 2000). Similarly, Sotho-Tswana groups are also found in South Africa as well as Lesotho and Botswana. While their language is similar to the Nguni, they are separated via customs and social organisation (Van Schalkwyk, 2002). Sotho-Tswana include the Southern and Northern Sotho and the Western Tswana people (Census, 2001). The Northern Sotho incorporates the Setswana and Sepedi people and the Southern Sotho incorporates the Sesotho speaking people (Van Schalkwyk, 2002). According to the 2001 South African census, there are 3.5 million Setswana, 3.8 million

Sepedi and 3.2 million Sesotho speaking people in South Africa, which makes Sotho-Tswana the second largest language group in the country.

The Tsonga include the Shangaan, Thonga, Tonga and several smaller groups (Brynes, 1996). The name Tsonga comes from the Mozambican word "ronga' meaning from the east (Boonzaaier, 2002). Together they number approximately 1.9 million people in South Africa (Census, 2001). A Tsonga homeland, Gazankulu, was established by the South African government during the 1960s and was granted self-governing status in 1973 (Brynes, 1996). At the end of Apartheid in 1994, Gazankulu was made part of the modern day Limpopo and Mpumalanga provinces (Boonzaaier, 2002).

With a different linguistic origin, the Venda are considered the outliers of South African populations. They primarily occupy the area in and around the Soutpansberg Mountains in the north eastern region of South Africa (Hanisch, 2002). According to the 2001 South African census, approximately 1 million people identified themselves as Venda, the majority of which live in the Northern part of the country (Hanisch, 2002).

2.5 Recent ancestry research in South Africa

L'Abbé et al. (2011) examined the accuracy and usefulness of non-metric traits for estimating ancestry from the mid-facial region in South African populations. Using 13 discrete traits, and a black, white and coloured South African sample, they found that the relationship between mid-facial morphology and ancestry to be statistically significant, but highly variable. L'Abbé et al. (2011) noted that some discrete traits were difficult to repeat and were also affected by sex and age at death. Mean nasal form was found less variable than previously stated by Rhine (1990), with the majority of individuals having "intermediate" nasal forms. L'Abbé et al. (2011) concluded that non-metric traits from the mid-facial region could not be used, in their present format, to accurately differentiate between South African groups.

The observation of sexual dimorphism in the mid-facial region by L'Abbé et al. (2011) supports earlier studies by de Villiers (1968) who found sexual dimorphism in their morphological evaluation of South African population groups. L'Abbé et al. (2011) also observed that black South African males had a greater tendency for "intermediate" or "wide" nasal aperture width and inter-orbital breadth than black South African females. This finding

contradicts other mid-facial studies by Hefner (2009) and Wheat (2009), who found no sexual dimorphism in the mid-facial shape of American samples. However it is noted that males had consistently larger nasal apertures and these sexual differences are caused largely by size rather than shape.

The research by Oettlé & Steyn (2000) and Patriquin et al. (2005) did not focus on ancestry but they noted that the results of their study differed based on ancestry of the sample. For example, Oettlé & Steyn (2000) found that the current sternal rib end aging method under-aged young black South African males and over-aged older (+30 years) black males. The opposite result was noted among black South African females, where irregular bony outgrowths, which are indicative of much older individuals (age 56+ years), was noted in persons less than 30 years of age (Oettlé & Steyn, 2000).

Patriquin et al. (2005) found that the sciatic notch has little sexual dimorphism among white South Africans and therefore cannot be accurately used in sex estimation. However the sciatic notch has shown to be an accurate sex estimator for black South African populations, with 91% of black males exhibiting a typically male narrow sciatic notch versus only 33% of white males.

From these studies it is clear that standards based on black and white American samples are not suitable for interpreting variation in other geographical groups. Using size free geometric analysis will allow a more accurate evaluation of sexual dimorphism than the "eye balling" approach. Further research into ancestry using this type of analysis is important, as the level of accuracy in sexing and aging a South African population is dependent on ancestry.

2.6 Statistical techniques utilised in this study

2.6.1 Geometric Morphometrics

Morphometrics examine the central tendencies of shape[5] and shape variation (Slice, 2007). Traditional morphometrics examined shape using distance ratios, angles and indices. Because early morphometric methods were based on limited linear measures, they often failed to capture the full spatial relationship between samples (Slice, 2007). GM was developed in the 1980's with the invention of coordinate based methodology and Corti

[5] Shape is defined as being the geometric properties of an object irrelevant to position, orientation and size (Slice, 2007).

introduced the method to physical anthropology in 1993 (Slice, 2007). The name originates from the fact that this method makes use of landmark coordinates to form the shape of an object or region and as such it preserves the geometry of the landmarks, thereby retaining all the spatial information throughout the analysis (Slice, 2007). This permits "the exploration and visualisation of large high-dimensional data sets along with exact [multivariate] statistical tests" (Mitteroecker & Gunz, 2009:236).

GM is a statistical analysis that scales data to form a geometric sample mean based on Kendall's definition of shape space[6] (Mitteroecker & Gunz, 2009). From the geometric mean, procrustes coordinates may be derived to show how each specimen deviates from this mean.

2.6.1.1 Generalised Procrustes analysis

Procrustes methods are a widely used configuration for morphometric analysis. This form of analysis is named after the Greek giant Procrustes who would stretch or shrink his victims to fit his bed (Slice et al., 2009). Mitteroecker & Gunz (2009:238) define this methodology as "the translation of the landmark configuration of all objects so they share the same centroid". The landmark configurations are centred and scaled to have the same centroid size and then rotated around the mean centroid to where the sum distance is minimal as depicted in Figure 2.1. It is the deformation of each specimen from this mean shape which is used to analyse shape variations (Mitteroecker & Gunz, 2009). In 1962 Hurley and Cattell, first used Procrustes with the idea of superimposition (Slice et al., 2009). However, according to Slice et al. (2009), Cole (1996) argues that Boas in 1905 proposed the fundamental idea of using a method of least difference.

[6] The space induced by a set of shape coordinates. For greater description see Slice et al. (2009).

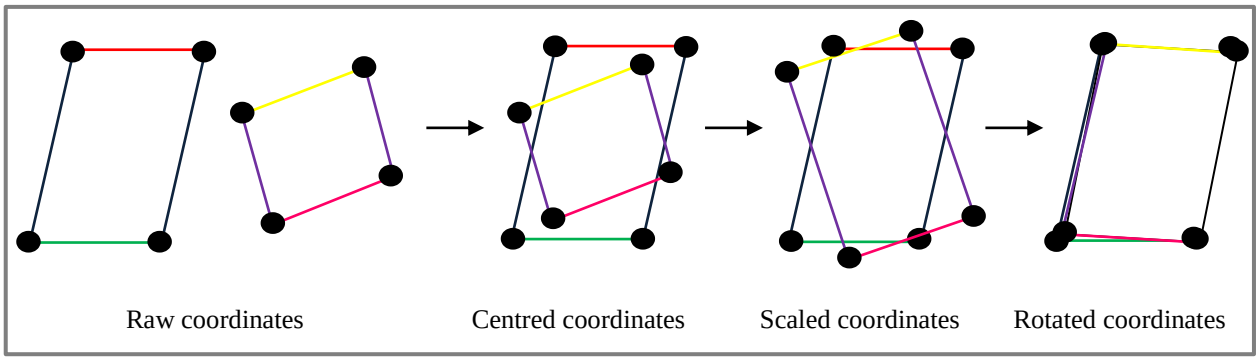

Figure 2.1. *The three steps of using procrustes superimposition to scale samples for shape analysis. First the landmark configurations are centred to the mean centroid, then scaled to give each configuration the same centroid size and rotated to the least distance between all coordinates. Taken and modified from Mitteroecker & Gunz (2009:239).*

2.6.1.2 Elliptical Fourier analysis

EFA is another morphometric approach to shape comparison (Mitteroecker & Gunz, 2009). The method defines a shape's outline through a series of best-fit ellipses, known as harmonics. These outlines are based on photographs, which are coordinate free. The computer programs, in this case Shape 1.3 (Iwata & Ukai, 2002), used to analyse the outlines generate the unique coordinates. The first harmonic accounts for the majority of the object's shape but may not adequately define it. Including additional harmonics will, in turn, better define the outline shape.

Michael Kenyhercz[7] explains EFA by comparing the shapes of a peanut and a rugby ball. You can place an ellipse (also known as a harmonic) on the outline of the ball that will account for most of its shape. That same ellipse will account for most of the outline shape of the peanut, but peanut outlines are different from a rugby ball. A peanut looks more like an infinity sign, so by adding two more ellipses this shape can be more accounted for. The more ellipses added, the better account created for any shape. Through these harmonics, a mean form is calculated and from this mean form an output is generated that will visually show the variation in shape from the mean.

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2.6.2 Linear measure

'Linear measure' is a traditional osteometric technique that uses linear measurements to calculate size, indices and/or angles of an object or region. Often the term linear measure, osteometrics and craniometrics are interchanged and used to describe the same form of analysis. Unlike GM, linear measures do not eliminate size from the analysis. Linear measurements may be taken using traditional tools such as sliding and spreading callipers or more advanced tools such as a digitiser.

2.6.3 Discriminant Function analysis

DFA is a statistical analysis which allows investigators to assess the osteometric similarities within population groups. By doing so, an unknown individual can be classified based on their similarities to the variables available for comparison. Therefore, a data set is never 'rejected' from belonging (Giles & Elliot, 1962). In other words, if you have the remains of a Native American but you have only black and white samples in your database the DFA will predict that individual to be black or white, as Native American is not an option. This is why DFA must be used wisely, to ensure that an appropriate number of populations as well as appropriate population samples are used during any analysis. The possibility of false or forced identification decreases with an increase in the number of variables available.

2.6.4 Principal Components analysis

PCA is another associated statistical procedure to morphometric analysis. PCA is a dimension reducing or data compression technique; often used in morphometrics because the original data set can be large and complex (Slice, 2007). PCA identifies patterns in these data sets in such a way as to highlight any co-variation among shapes (Smith, 2002). PCA can simplify complex data by identifying orthogonal [8] linear combinations of the original variables that most effectively account for sample variability (Slice, 2007).

The first principal component (PC) accounts for as much of the variability as possible and each succeeding PC accounts for as much of the remaining variation as possible until the pooled PC account for 100% of the variation (Smith, 2002). This combines correlated variables to reduce data noise and results in non-correlated factors which represent the most dynamic variations among the sample group (Shlens, 2003).

[8] Mutually independent, non-redundant, non-overlapping or irrelevant.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Materials

A total of 310 crania of self-identified black, white and coloured South Africans were used (Table 3.1). The crania were collected from the Pretoria Bone Collection (University of Pretoria), the Raymond A. Dart Collection (University of the Witwatersrand) and the Kirsten Collection (Stellenbosch University). All these collections are in South Africa and consist of known individuals whose biological information, such as sex and ancestry, is recorded from the individual's identification documents.

Table 3.1. Demographics and sample size (n) of South African crania used for study*.

	Sex		
Population group	*Male*	*Female*	*Total*
White	42	42	84
Coloured	23	13	36
Black	99	91	190
Total	164	145	310

Exclusion criteria included:

- Persons younger than 18 years of age; this was to eliminate the potential effect that growth and development have on the size and shape of the mid-face.
- Macroscopic pathology, ante- and post-mortem fractures, as this does not represent normal variation within each of the sample groups.
- Extensive ante-mortem tooth loss (less than 6 teeth remaining), as tooth loss can cause changes to facial shape resulting from alveolar resorption (Reichs et al., 2011).

3.1.1 Research collections

3.1.1.1 Pretoria Bone Collection

The Pretoria Bone collection (PBC) is housed in the Department of Anatomy at the University of Pretoria in Pretoria, South Africa. The acquisition of skeletal material began in 1942 with the opening of the Department of Anatomy and the Medical School at the University (L'Abbé et al., 2005). In 1987, a research collection was started and is now a

* A full list of the sample group can be found in appendix A

well-documented research resource. All the skeletons are of known age, sex and self-assigned population group (L'Abbé et al., 2005). The PBC continues to grow and provides a good representative of modern South African groups (L'Abbé et al., 2005).

As of 2012, the PBC contains 1135 complete crania, 816 postcrania, and 399 incomplete remains and continues to grow at a rate of 40 to 50 skeletons per year (L'Abbé & Steyn, 2012). Approximately 67% of the collection is aged between 20 and 70 years (L'Abbé et al., 2005). The mean age for white South Africans is 68 years and 53 years for black South Africans (L'Abbé et al., 2005). The crania sample is mainly comprised of males (78%) with less than a quarter females (21%). The majority (70%) are black South Africans, followed with 27% white South Africans and an unknown self-identification at 3% (L'Abbé & Steyn, 2012).

The skeletal material comes from donors and unclaimed, but not unknown, bodies from local hospitals in the Gauteng Province. While the collection includes numerous black South African ethnic groups, such as Zulu, Xhosa, Pedi, and Sotho, many skeletons were simply assigned a label of "black" (Patriquin et al., 2003).

3.1.1.2 Raymond A. Dart collection

The Raymond A. Dart Collection (RDC) is housed in the School of Anatomical Sciences at the University of the Witwatersrand, Johannesburg, South Africa. When Raymond A. Dart became head of the Anatomy department in the 1920's, he initiated the collection (Dayal et al., 2009). Now, it is one of the largest documented cadaver-based samples in the world. In the 1960's, many of the skeletons were accidentally commingled due to a flood in the basement of the Department where the remains were housed (Dayal et al., 2009). The skeletons were washed out of their boxes, and since they were not labelled, it was difficult to determine which bones belonged to which person. For this reason, many of the skeletons were later de-accessioned in the 1990's (Dayal et al., 2009).

In 2009, the collection consisted of 2605 skeletons with approximately 57% of the individuals between the ages of 20 and 70 years. The RDC consists mostly of white, black and Indian South Africans. Those in the collection labelled coloured were people who identified as having "mixed" ancestry, and were not necessarily true coloured South Africans[9]; for this reason they were not used in this study. The collection is made up of

[9] Refer to Chapter Two; page 21 for a full definition of the coloured South African population.

bequeathed and unclaimed bodies mainly from the Gauteng province and are of known age at death, sex and ancestry (Dayal et al., 2009).

3.1.1.3 Kirsten collection

The Kirsten collection is housed in the Department of Anatomy at Stellenbosch University. The skeletons in the collection consist of cadaver material and donated bodies. While the sample adequately represents populations of the South-western Cape, little history is available on the collection. However, all specimens are of known age, sex, ancestry and cause of death. All crania of self-identified coloured South Africans were collected from the Kirsten collection.

3.2 Methodology

Thirteen landmarks of the mid-facial region were digitised, using a MicroScribe™ G2. In this study mid-facial refers to the skeletal region that consists of the nasal bones and nasal aperture. Using a digitiser to collect data eliminates many of the errors associated with traditional craniometrics (Ousley & McKeown, 2001). For example, using a single instrument decreases the possibility of incorrect recordings. When using multiple instruments, the possibility of taking an incorrect reading is increased through observer error or from one or more of the instruments not being correctly calibrated. With digitising landmarks are only recorded once, eliminating coordinate variability with frequently used landmarks (Ousley & McKeown, 2001). The computer records and rounds each measurement, keeping the recording method consistent between data collectors (Ousley & McKeown, 2001). In providing detailed definitions of each landmark, inconsistencies in locating landmarks have been minimised.

The digitised landmarks are illustrated in Figure 3.1, with specific definitions of each landmark in Table 3.2. The landmarks include glabella [1], nasion [2], nasale superior left and right [3,13], dacryon left and right [4,12], nasale inferius left and right [5,11], alare left and right [6,10], most inferior nasal border left and right [7,9] and subspinale [8]. The computer programme 3Skull (Ousley 2004) was used to collect the raw coordinate data for GM, as well as to calculate standard craniometric measures for analysis of the linear measures from the coordinate data collected. Namely:

- Nasion-dacryon angle (NDA); measure of angle between the dacryon and nasion.

- Nasion-dacryon subtense (NDS); measure of the subtense between dacryon and nasion.
- Nasal breadth (NLB); distance between left and right alare.
- Nasal height (NLH); distance from nasion to lower inferior border of the nasal aperture.
- Inter-orbital breadth (DKB); distance between the left and right dacryon.

Photographs were taken of each skull in the frontal plane using a craniophore. The midpoint of the camera was focused on the nasion of each cranium. The same camera was used to take each photograph. Each photograph was taken at a distance of 46 cm from the tripod shaft to the back legs of the craniophore. The outlines of the nasal apertures were traced on each photograph and used in EFA.

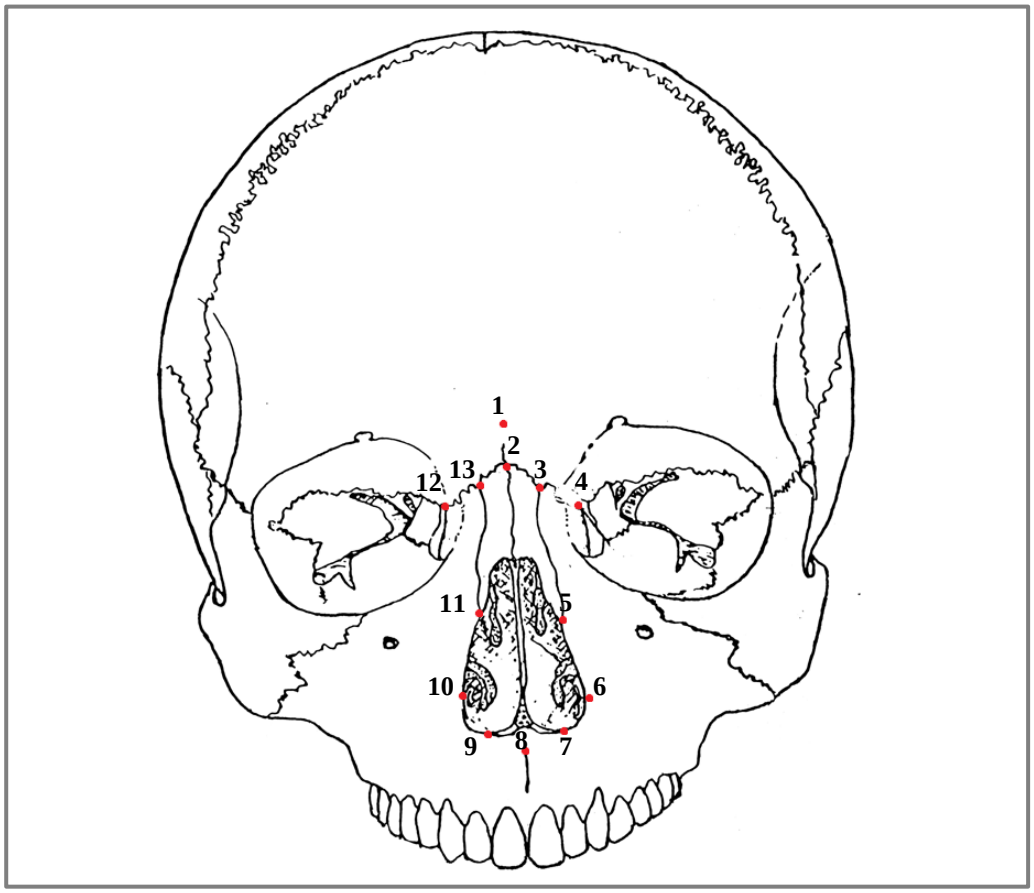

Figure 3.1. *Landmarks digitised with MicroScribe™ G2. Taken and modified from Ousley & McKeown (2001).*

Table 3.2. Definition of landmarks digitised from mid-facial region depicted in Fig. 3.1.

Assigned #	Landmark	Definition
1	Glabella	*Most forward projecting point of frontal bone in the midline of the supraorbital ridges
2	Nasion	*Midline junction of the nasal and frontal sutures
3	Nasale superius L	*Point at junction of left nasomaxilla and frontal sutures
4	Dacryon L	*Point where lacrimal, frontal and maxillary bones meet on medial wall of left orbit
5	Nasale inferius L	*Most inferior point on left nasomaxilla suture
6	Alare L	*Most lateral point on left side of nasal aperture
7	Most inferior nasal boarder L	*Most inferior point on left border of nasal aperture
8	Subspinale	*Most posterior midline point on premaxilla between the anterior nasal spine and prosthion
9	Most inferior nasal boarder R	*Most inferior point on right border of nasal aperture
10	Alare R	*Most lateral point on right side of nasal aperture
11	Nasale inferius R	*Most inferior point on right nasomaxilla suture
12	Dacryon R	*Point where lacrimal, frontal and maxillary bones meet on medial wall of right orbit
13	Nasale superius R	*Point at junction of right nasomaxilla and frontal sutures

3.2.1 Statistical methods

For GPA, the raw 3D coordinate data was prepared for use in MorphoJ (Klingenberg, 2011). MorphoJ uses Procrustes Fit to scale each specimen to the sample's geometric mean through scaling the landmark's raw coordinates and rotating to minimise the squared distances between landmarks. Each landmark is given Procrustes coordinates based on the mean for that landmark's coordinates from the entire sample. The deviation of each landmark from that landmarks mean was quantified. The differences in Procrustes Coordinates were also analysed through PCA. DFA was used to examine inter- and intra-group differences and as a means of group classification.

The photographs were used to draw outlines of each specimen's nasal aperture for EFA. The outlines generated were imported into the program Shape 1.3 (Iwata & Ukai, 2002). The number of harmonics necessary to define a shape adequately varies and depends on the complexity of the shape. Shape 1.3 requires that each outline is black on a white background or vice versa (Iwata & Ukai, 2002). Each specimen's outline was entered into the program and aligned on its long axis in anatomical position. Each outline was scaled to match the size of the first harmonic applied to the first specimen in the dataset, and removed gross size. A mean shape was generated from the entire sample, independent of biological parameters, and ten effective[10] PC of shape were derived to quantify the deviation from this mean shape. The PC scores were then linked to the related specimen number and demographic information.

Linear measure analysis was used to evaluate the form of the nasal aperture. The raw coordinate data was formatted to create craniometric measures. The five linear measurements possible from the 13 digitised landmarks; NDA, NDS, NLB, NLH, DKB, were calculated and then the best discriminators were selected using FD3 (Jantz & Ousley, 2005). For all methods, the data was formatted for use in a FD3 custom South African database. FD3 was used to calculate DFA and the Mahalanobis' Generalized Distances (D^2) for each specimen, as well as to examine the mean D^2 for each population group and its significance. Lastly FD3 was used to perform canonical variate analysis (CVA) and to visualise group differences in the EFA and linear analysis.

DFA was used in each method in either a three or six way analysis to classify all individuals into a reference group. In each method, the variables selected for DFA were forward

[10] Meaning that each principal component contributed Eigen values of greater than 1/n.

stepwise selected. DFA validity was judged by its classification accuracy. All results were cross-validated using leave-one-out-cross-validation (LOOCV) (Jantz & Ousley, 2005).

In LOOCV, upward bias towards greater classification accuracy is avoided as the individual being evaluated is removed from the reference group and tested against all other members. The parameters are recalculated and the individual being evaluated is then classified into one of the reference groups. The individual is then re-added to the reference sample, the next individual removed and so on until all individuals have been classified (Jantz & Ousley, 2005).

Inter- and intra-observer error rates were calculated from the re-digitising of 40 crania by the researcher and another anthropologist, from the Department of Anatomy, at the University of Pretoria.

3.3 Ethical approval

Ethical approval (S111/2011) was obtained on 24 June 2011 from the Faculty of Health Sciences Student Research Ethics Committee at the University of Pretoria.

A letter of permission to use each of the collections, stated above was obtained.

All data collecting procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

CHAPTER FOUR: RESULTS

Three main multivariate methods, linear measures, GPA and EFA, were used to examine the morphological variation in nasal aperture shape in three major South African populations. Up to 332 digitised crania of known individuals, from white, black and coloured South African populations, were analysed. This chapter reports the results from each methodology in detail, examining and comparing shape and/or size variation.

4.1 Linear analysis

4.1.1 Standard linear measures analysis

The 13 digitised landmarks (Figure 3.1) were used to create five linear measurements: NDA, NDS, NLB, NLH, and DKB.

4.1.1.1 Ancestry analysis

A three-way DFA of ancestry, using the five above mentioned measures, resulted in a total correct classification of 66%. Coloured South Africans were classified 54% correctly, black South Africans 57% and white South Africans 93% correctly (Table 4.1). When comparing D^2 groups, the distance for whites was statistically significant from blacks and coloureds ($p<0.001$) (Table 4.2). However, blacks' and coloureds' distances were not statistically significant from each other ($p=0.076$). Misclassification was observed most frequently between black and coloured South Africans. Both groups rarely misclassified as white. White South Africans only misclassified into coloured or black 6 out of 83 times (Table 4.1).

Table 4.1. Three-way ancestry DFA results using five linear measures: DKB, NDA, NDS, NLB and NLH.

From group	Total (n)	Into Group			% correct
		Black	**Coloured**	**White**	
Black	188	107	78	3	56.9
Coloured	37	13	20	4	54.1
White	83	2	4	77	92.8

Total Correct: 204 out of 308 (66.2%) Cross-validated

Table 4.2. Mahalanobis D^2 distance and statistical significance from a three-way ancestry DFA of five linear variables: DKB, NDA, NDS, NLB and NLH.

Group	**Black**	**Coloured**	**White**
Black	---	0.33	9.95*
Coloured	0.33	---	8.38*
White	9.95*	8.38*	---

*p<0.001

When represented as a scatter plot (Figure 4.1) the following variation can be visualised:

- greater variation is noted between white and non-white groups;
- less variation is noted between black and coloured South Africans; and
- as a group, coloured South Africans (illustrated with the blue dots) were more variable than either blacks or whites.

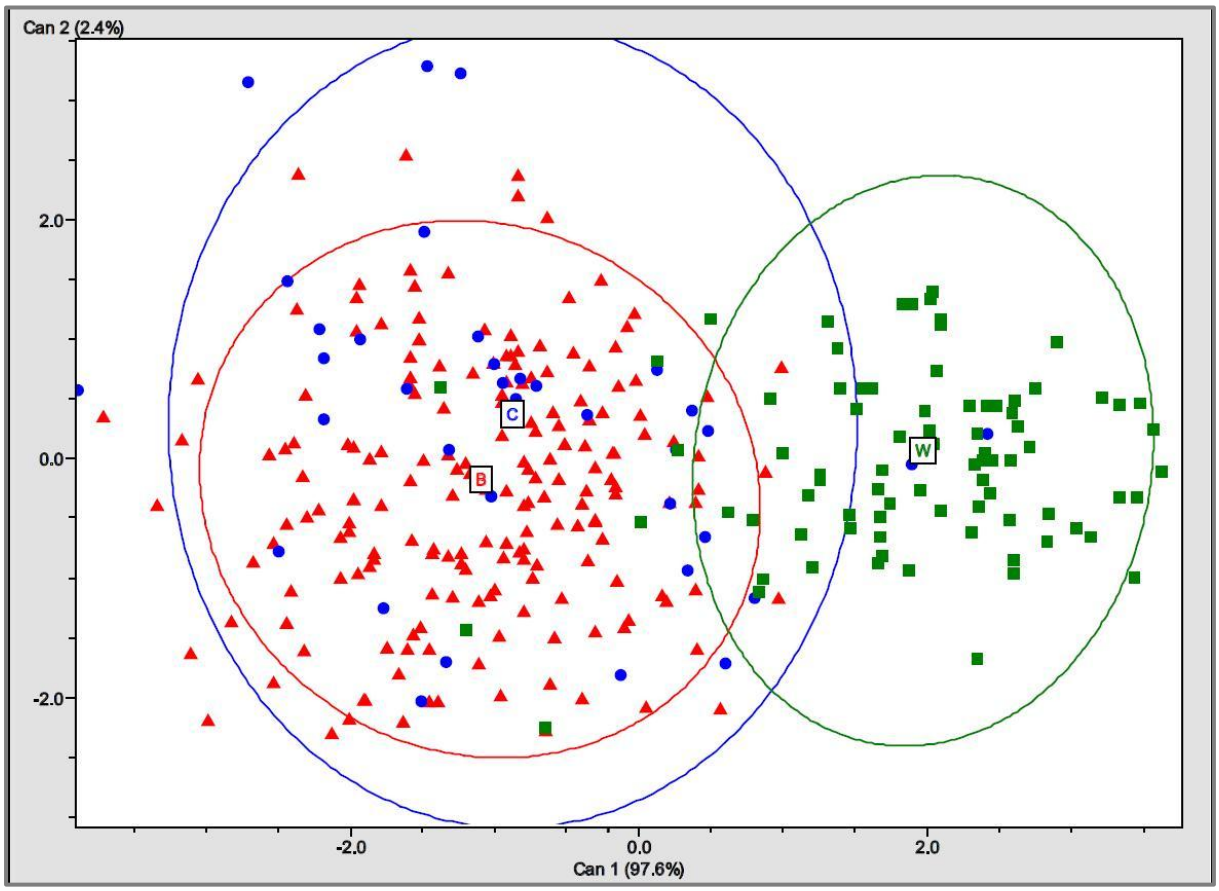

Figure 4.1. *A canonical variate scatter plot using five linear variables: DKB, NDA, NLH, NLB, NDS, accounting for 100% of the variation, shows results of a three-way ancestry DFA.*

In Table 4.3, group means for the selected linear variables demonstrate general size differences. On average, white South Africans have a greater NLH and NDS dimensions than black and coloured groups. White South Africans also have smaller NLB and DKB and a sharper NDA than the other two groups. The largest variation within and between the groups was in NDA with the mean for whites being 20 mm less than blacks or coloureds.

Table 4.3. Group means and standard deviations (mm) for five linear measures DKB, NDA, NLH, NLB and NDS used in three-way ancestry DFA.

	DKB	**NDA**	**NLH**	**NLB**	**NDS**
Black (n=188)	23.24(2.44)	103.20(9.29)	47.40(2.82)	27.77(2.31)	9.28(1.64)
Coloured (n=38)	22.27(2.21)	100.86(10.95)	46.43(3.93)	26.68(3.31)	9.30(1.84)
White (n=84)	19.48(2.19)	80.13(10.23)	50.84(2.86)	23.77(2.10)	11.65(1.54)

Total n=310, degrees of freedom = 3

4.1.1.2 Sex and ancestry analysis

A six-way DFA of sex and ancestry, using NDA, NLH, DKB, NDS and NLB, resulted in a cross-validated classification of 52%. Black females classified 38% correctly with the most frequent misclassifications being black male, coloured female and coloured male. Black males classified 55% correctly with the most frequent misclassification being coloured male, coloured female and black female (Table 4.4). Differences between black males and females were statistically significant at $p<0.001$ ($D^2 =1.51$).

Coloured females classified 46% correctly and only misclassified as black females. Coloured males classified 42% correctly and misclassified most frequently as either black females or coloured females (Table 4.4). Differences between coloured males and females was statistically significant at $p<0.05$ ($D^2 =1.81$).

White females classified 71% correctly and only misclassified as white males. White males classified 66% correctly and only misclassified as white females (Table 4.4). Differences between white males and females were statistically significant at $p<0.001$ ($D^2 =3.84$).

Table 4.4. Six-way sex and ancestry DFA results using five linear measures: DKB, NDA, NDS, NLB and NLH.

From group	Total (n)	Into Group						% correct
		BF	**BM**	**CF**	**CM**	**WF**	**WM**	
BF	89	34	20	17	15	2	1	38.2
BM	99	9	54	17	17	0	2	54.5
CF	13	4	3	6	0	0	0	46.2
CM	24	4	3	4	10	1	2	41.7
WF	42	0	0	1	4	30	7	71.4
WM	41	0	1	0	2	11	27	65.9

Total Correct: 161 out of 308 (52.3%) Cross-validated

D^2 distances were statistically significant at $p<0.05$ or greater (Table 4.5) for the majority of groups, except coloured males and black females ($p=0.201$) and coloured females and black females ($p=0.052$).

Table 4.5 Mahalanobis D^2 distance and statistical significance from a six-way sex and ancestry DFA of five linear variables: DKB, NDA, NDS, NLB and NLH.

Group	**BF**	**BM**	**CF**	**CM**	**WF**	**WM**
BF	---	1.51**	0.99	0.39	9.36**	11.89**
BM	1.51**	---	2.06**	1.47**	12.18**	12.99**
CF	0.99	2.06**	---	1.81*	12.71**	16.94**
CM	0.39	1.47**	1.81*	---	6.66**	8.41**
WF	9.36**	12.18**	12.71**	6.66**	---	3.84**
WM	11.89**	12.99**	16.94**	8.41**	3.84**	---

* $p<0.05$ **$p<0.001$

When examining the scatter plot in Figure 4.2 the following can be visualised:

- with a clear separation between white and non-white South Africans;
- significant overlap between group sexes, despite the statistical significance found; and
- less variation can be noted between group sexes than ancestry.

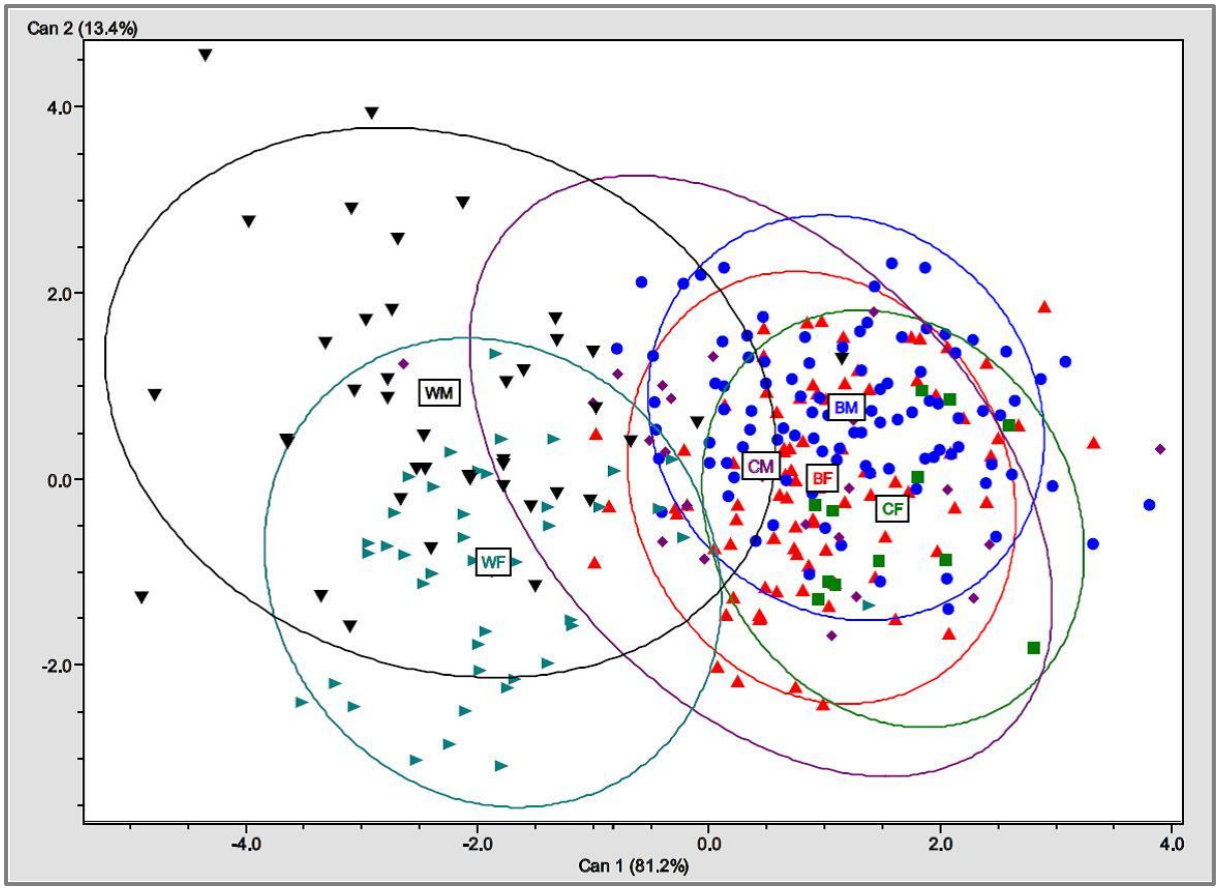

Figure 4.2. *A canonical variate scatter plot accounting for 94.6% of the variation showing the results of a six-way sex and ancestry DFA using five linear variables: DKB, NDA, NLH, NLB and NDS.*

When comparing group means for the selected linear variables (Table 4.6), whites (male and female) were less variable in size than blacks or coloureds (male and female). Black males had the widest DKB, which when combined with a wide NDA, formed flat nasal bones. In contrast, white females had the narrowest DKB, which when combined with a narrow or steep NDA, formed high and tightly pinched nasal bones.

As with the above-mentioned three-way analysis (Table 4.3), NDA had the greatest within and between group variations; white females had the smallest standard deviations in NDA whereas black and coloured females had the largest standard deviations (Table 4.6). Therefore, black and coloured females had larger differences in mean values and hence greater variation in NDA than white females.

NLB was similar in size between black and coloured male and female South African groups but it was much narrower in white males and females (Table 4.6). White males and females also displayed a higher NLH. The combination of a high NLH and a narrow NLB created a long and narrow nasal aperture. In contrast, non-white groups coloured and black females were wider for NLB and shorter in NLH (Table 4.6). This combination creates a circular, nasal aperture.

Table 4.6. Group means and standard deviations (mm) for five linear measures: DKB, NDA, NDS, NLB and NLH used in a six-way sex and ancestry DFA.

	n	DKB	NDA	NDS	NLB	NLH
BF	89	22.03(2.43)	103.46(10.19)	8.76(1.64)	27.07(2.28)	46.73(2.64)
BM	99	24.33(1.88)	102.96(8.45)	9.75(1.51)	28.39(2.17)	48.00(2.85)
CF	13	22.62(2.18)	103.38(8.21)	9.00(1.47)	27.15(1.72)	44.38(2.72)
CM	24	22.08(2.24)	99.50(12.13)	9.46(2.02)	26.42(2.57)	47.54(4.09)
WF	42	18.74(2.02)	80.62(10.15)	11.10(1.27)	23.12(1.94)	49.36(2.64)
WM	41	20.24(2.12)	79.63(10.43)	12.22(1.60)	24.44(2.07)	52.37(2.22)

4.1.2 Principal component analysis of linear measures

In Table 4.7, PCA for the linear measures is presented. The most heavily loaded variables are highlighted in bold and include NDA, NLH and NLB. Principal component (PC)1 accounts for 54% of the variation, PC2 26%; PC3 12%; PC4 7% and PC5 0.01%.

Table 4.7. Principal component loadings from linear measures.

	PC1	PC2	PC3	PC4	PC5
DKB	-0.418	-0.521	-0.299	0.574	0.366
NDA	**-0.585**	0.099	0.241	0.252	**-0.725**
NDS	0.466	-0.445	-0.493	0.050	-0.583
NLB	-0.429	-0.472	-0.055	**-0.768**	-0.003
NLH	0.285	**-0.545**	**0.779**	0.121	-0.004

4.1.2.1 Principal component analysis for ancestry

In a three-way DFA (sexes pooled) PC1, PC2 and PC3 were selected as the best discriminators among the groups and resulted in a cross-validated classification of 66% (Table 4.8). All groups means for D^2 were statistically significant at $p<0.05$ (Table 4.9).

Black South Africans classified 55% correctly and coloured South Africans classified 57% correctly. Both groups most frequently misclassified into each other. Whites South Africans classified 93% correctly and only misclassified 6 out of 83 times as either black or coloured.

Table 4.8. Results from a three-way ancestry DFA using three PC variables: PC1, PC2 and PC3, for linear measures.

From Group	Total Number	Into Group			% Correct
		B	**C**	**W**	
B	188	104	79	4	55.3
C	37	11	21	5	56.8
W	83	2	4	77	92.8

Total Correct: 202 out of 308 (65.6%) Cross-validated

Table 4.9. Mahalanobis D^2 distance and statistical significance from a three-way ancestry DFA using three PC variables: PC 1, PC 2 and PC3.

Group	**Black**	**Coloured**	**White**
Black	---	0.32*	9.75**
Coloured	0.32*	---	8.23**
White	9.75**	8.23**	---

*p<0.05 ** p<0.001

In a scatter plot (Figure 4.3) the following can be observed:

- greater variation in nasal dimensions in coloured and black groups when compared to whites;
- the largest between group differences are with black and white South Africans;
- whereas coloured South Africans demonstrated the greatest variability.

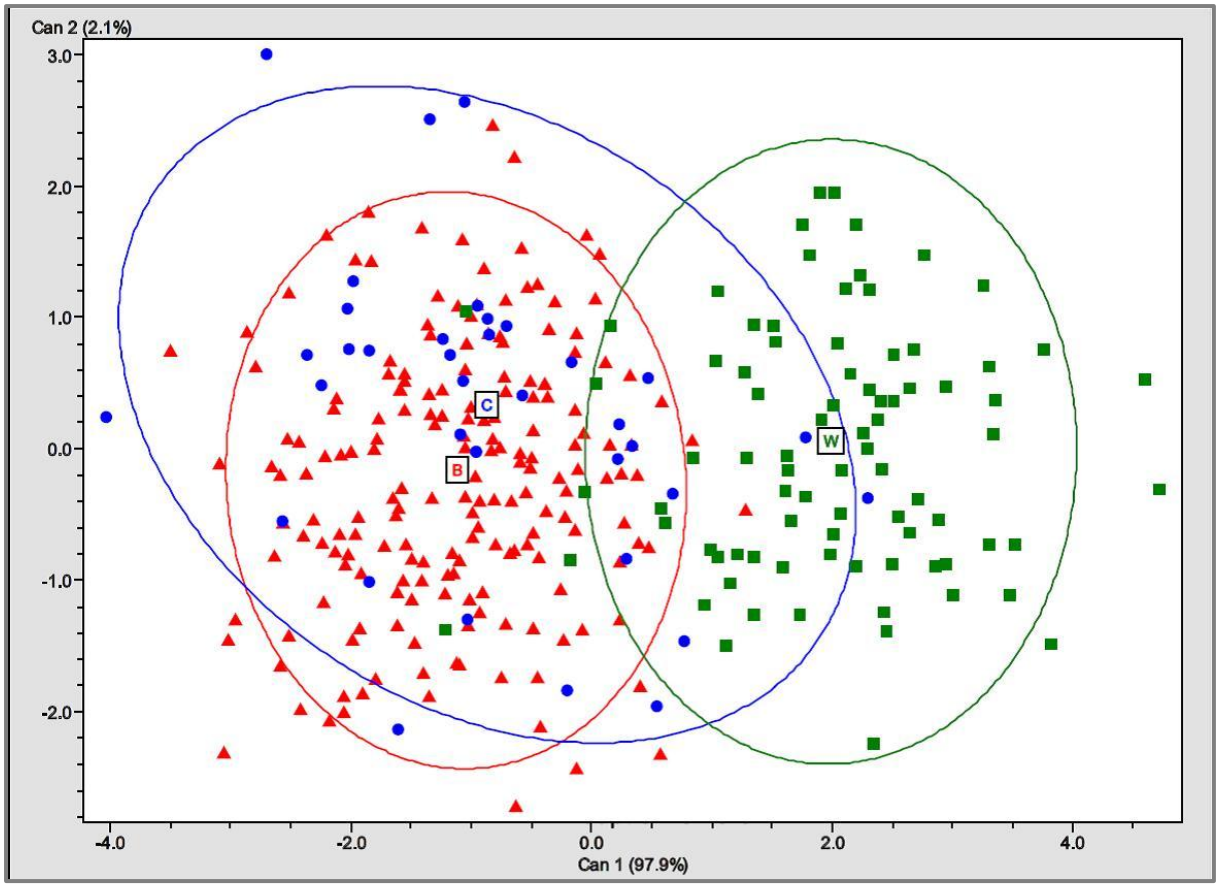

Figure 4.3. *A canonical variate scatter plot for PCA, accounting for 100% of the variation from a three-way ancestry DFA using three variables: PC1, PC2 and PC3.*

4.1.2.2 Principal component analysis for sex and ancestry

In a six-way DFA of sex and ancestry five PCs, PC1, PC2, PC3, PC4 & PC5, were selected and resulted in an overall correct classification of 53% (Table 4.10). All groups' D^2 were statistically significant at $p<0.05$, except for black females and coloured females ($p=0.052$) and black females and coloured males ($p=0.201$), (Table 4.11). As in the above linear analysis, the sexes within each group were found to be statistically different ($p<0.05$), with the greatest variation found among the white South African group (Table 4.11).

Black females classified 38% correctly and frequently misclassified as black males. Black males classified 55% correctly and often misclassified as either coloured males or females. Black males never misclassified as white females (Table 4.10).

Coloured females classified 46% correctly and misclassified frequently as black females. They did not misclassify as coloured males, white females or white males. Coloured males

classified 43% correctly and often misclassified as either black females or coloured females (Table 4.10).

White females classified 71% correctly and misclassified as white males and coloured males. They did not misclassify as either black males or females. White males classified 66% correctly and often misclassified as white females; they never misclassified as black females or coloured females (Table 4.10).

Table 4.10. Six-way sex and ancestry DFA results from PCA of linear measures, using five variables: PC1, PC2, PC3, PC4 and PC5.

From Group	Total Number	Into Group						% correct
		BF	**BM**	**CF**	**CM**	**WF**	**WM**	
BF	89	34	20	17	15	2	1	38.2
BM	99	9	54	17	17	0	2	54.5
CF	13	4	3	6	0	0	0	46.2
CM	24	4	3	4	10	1	2	41.7
WF	42	0	0	1	4	30	7	71.4
WM	41	0	1	0	2	11	27	65.9

Total Correct: 161 out of 308 (52.3%) Cross-validated

Table 4.11. Mahalanobis D^2 distance and statistical significance from a six-way sex and ancestry DFA of five PC variables: PC1, PC2, PC3, PC4 and PC5.

Group	**BF**	**BM**	**CF**	**CM**	**WF**	**WM**
BF	---	1.51**	0.99	0.39	9.36**	11.89**
BM	1.51**	---	2.06**	1.47**	12.18**	12.99**
CF	0.99	2.06**	---	1.81*	12.71**	16.94**
CM	0.39	1.47**	1.81*	---	6.66**	8.41**
WF	9.36**	12.18**	12.71**	6.66**	---	3.84**
WM	11.89**	12.99**	16.94**	8.41**	3.84**	---

*P<0.05 ** P<0.001

In Figure 4.4, the scatter plot can be used to visualise the following for the nasal dimensions:

- white groups are different from coloured and black population groups;
- whites and coloured males have the greatest within group variation; and
- black males and black females have the least within group variation.

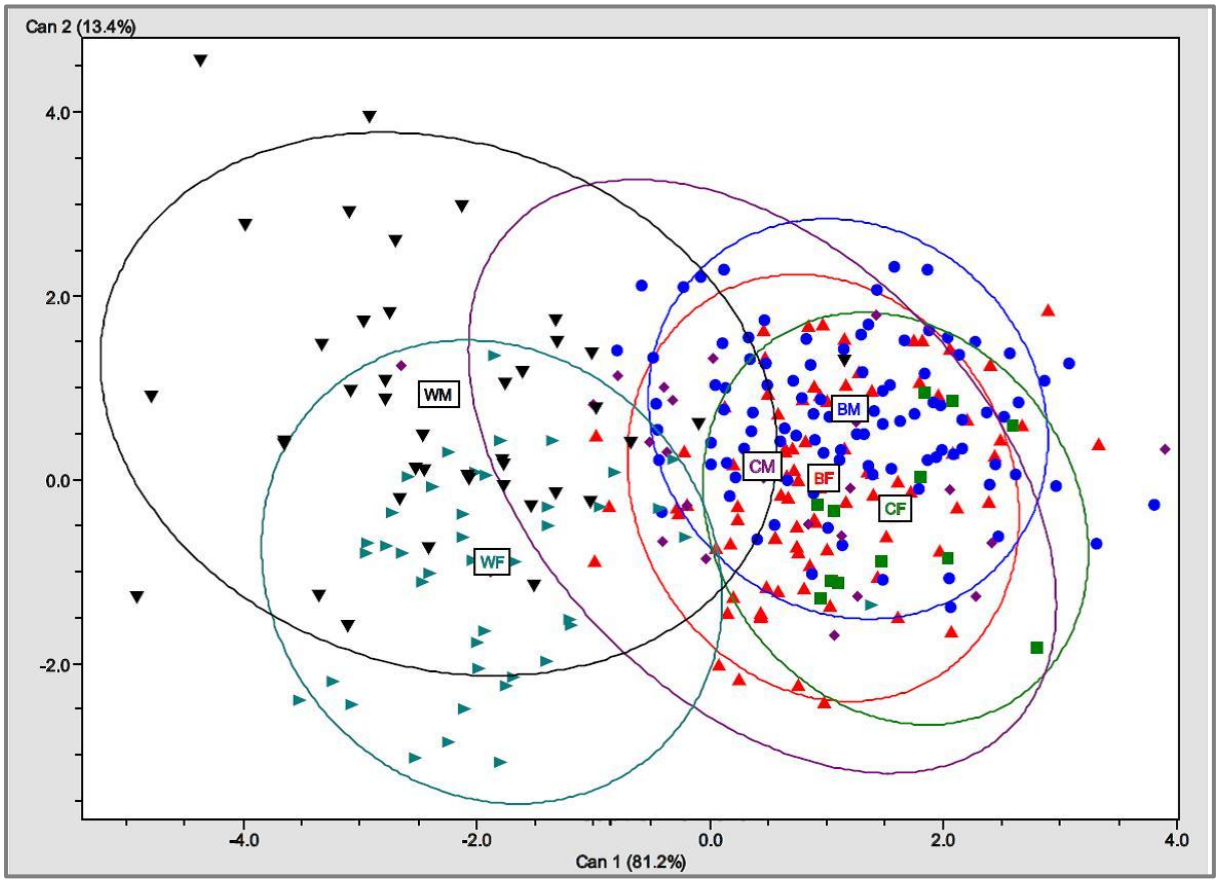

Figure 4.4. *A canonical variate scatter plot for PCA, accounting for 95% of the variation of the six-way sex and ancestry DFA using five PC variables: PC1, PC2, PC3, PC4 and PC5.*

4.2 Geometric Morphometrics

GPA used the 13 digitised landmarks to create a mid-facial shape consisting of the nasal aperture and nasal bones. Additional data from the Pretoria and Raymond A. Dart bone collections, digitised for other research projects, were used in this analysis to increase the total sample size to 332.

4.2.1 Generalised Procrustes analysis

4.2.1.1 Ancestry analysis

The three-way DFA of population groups, using seven variables, ProCoord12, ProCoord14, ProCoord17, ProCoord19, ProCoord22, ProCoord32 & ProCoord41, resulted in an overall 74 % total correct classification (Table 4.12). All groups D^2 were statistically significant at $p<0.001$.

Black South Africans classified 70% correctly, coloureds classified 60% correctly and whites classified 90% correctly. Blacks and coloureds frequently misclassified as each other, while white South Africans infrequently misclassified as black or coloured (Table 4.12).

Table 4.12. Three-way ancestry DFA results using seven procrustes coordinates: ProCoord12, ProCoord14, ProCoord17, ProCoord19, ProCoord22 and ProCoord32.

From Group	Total Number	Into Group			% correct
		B	**C**	**W**	
B	199	140	52	7	70.5
C	38	11	23	4	60.5
W	95	4	5	86	90.5

Total Correct: 246 out of 332 (74.1%) cross-validated

From the scatter plot in Figure 4.5, the following variation can be visualised:

- greater variation is noted between white and non-white groups than between non-white (black and coloured) South Africans;
- coloured South Africans (illustrated with the blue dots) are more variable than either black or white groups;
- these results are consistent with those observed for the above-mentioned linear measures.

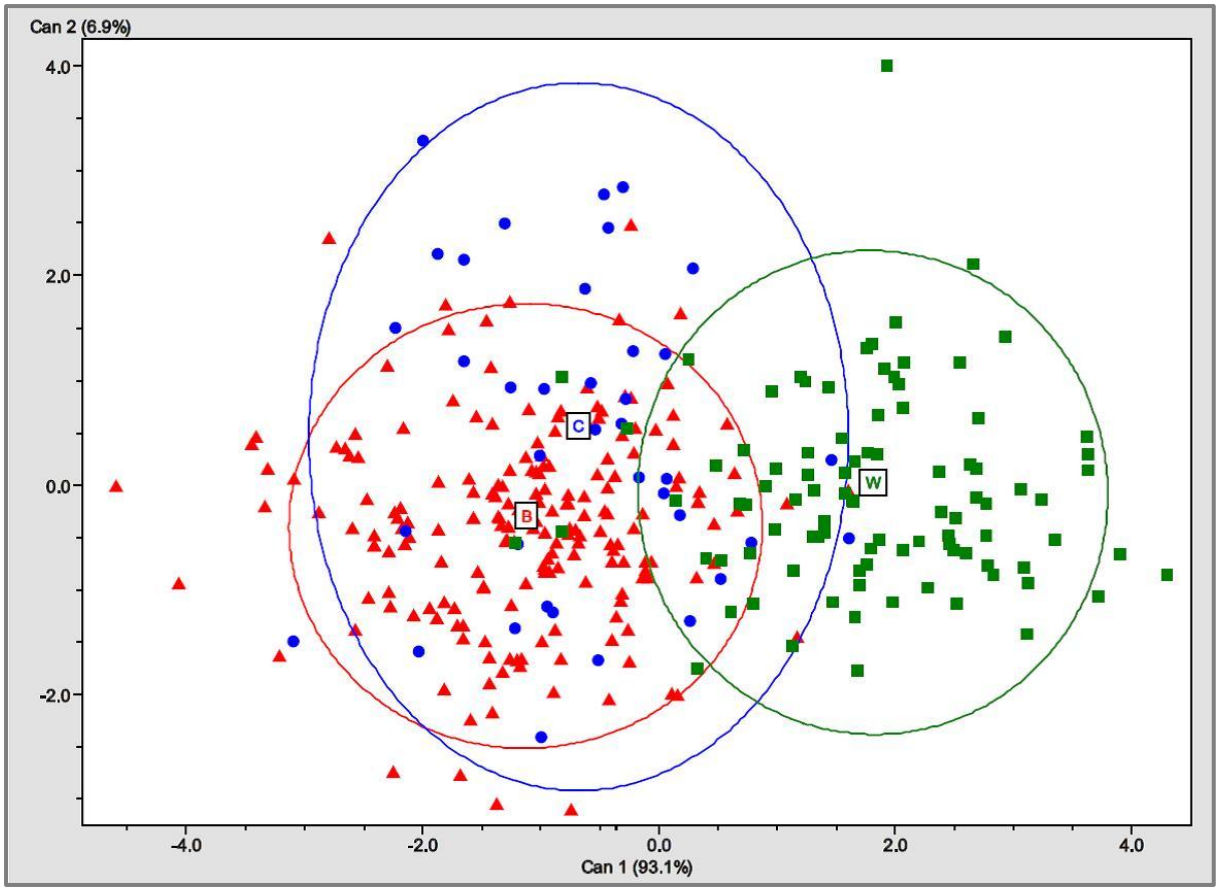

Figure 4.5. *A canonical variate scatter plot accounting for 100% of the variation, showing the results of the three-way ancestry DFA, using seven variables: ProCoord12, ProCoord14, ProCoord17, ProCoord19, ProCoord22 and ProCoord32.*

4.2.1.2 Sex and ancestry analysis

Using a six-way DFA, five variables were selected, ProCoord3, ProCoord9, ProCoord13, ProCoord14 and ProCoord31, and resulted in an overall correct classification of 54% (Table 4.13). Except for coloured females and black females (p=0.218), all groups' D^2 were statistically significant at p<0.001, (Table 4.14).

Black females classified 51% correctly and often misclassified as coloured female but never as white male. Black males classified 52% correctly and often misclassified as coloured male. They never misclassified as either white female or white male (Table 4.13).

Coloured females classified 23% correctly and frequently misclassified as black female. They never misclassified as coloured male or white female/male. Coloured males classified 24% correctly and often misclassified as black males (Table 4.13).

White females classified 67% correctly and often misclassified as white males. White females never misclassified as black males or coloured females. White males classified 74% correctly and often misclassified as white females but never as black female/males or coloured females (Table 4.13).

Table 4.13. DFA results for a six-way sex and ancestry DFA using five procrustes coordinates: ProCoord3, ProCoord9, ProCoord13, ProCoord14 and ProCoord31.

From Group	Total Number	Into Group						% correct
		BF	**BM**	**CF**	**CM**	**WF**	**WM**	
BF	103	52	11	24	15	1	0	50.5
BM	96	14	50	12	20	0	0	52.1
CF	13	6	4	3	0	0	0	23.1
CM	25	3	8	2	6	4	2	24.0
WF	49	2	0	0	1	33	13	67.3
WM	46	0	0	0	4	8	34	73.9

Total Correct: 178 out of 332 (53.6%) Cross-validated

Table 4.14. Mahalanobis D^2 distance and statistical significance from a six-way sex and ancestry DFA, using five procrustes coordinates: ProCoord3, ProCoord9, ProCoord13, ProCoord14 and ProCoord31.

Group	**BF**	**BM**	**CF**	**CM**	**WF**	**WM**
BF	---	2.11**	0.62	2.31**	10.74**	15.74**
BM	2.11**	---	1.44*	1.21**	13.76**	14.15**
CF	0.62	1.44*	---	2.66**	12.30**	15.79**
CM	2.31**	1.21**	2.66**	---	7.68**	8.50**
WF	10.74**	13.76**	12.30**	7.68**	---	3.06**
WM	15.74**	14.15**	15.79**	8.50**	3.06**	---

*p<0.05 ** p<0.001

From the scatter plot in Figure 4.6, the following differences can be visualised:

- larger differences in variation for ancestry than sex for all groups;
- greatest within group variation for ancestry was in coloured males and females; and
- largest between groups variation for ancestry was between white and non-white groups.

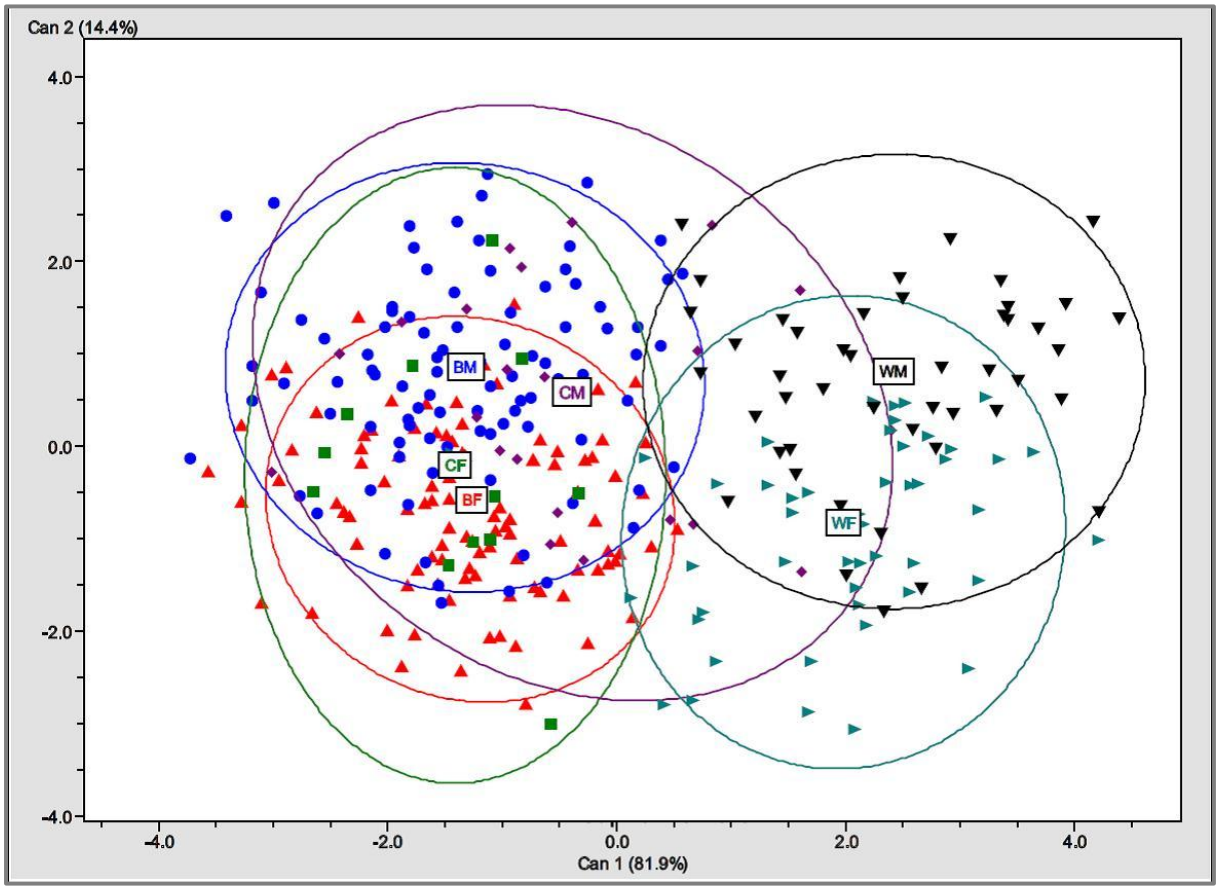

Figure 4.6. *A canonical variate scatter plot accounting for 96.3% of the variation using five procrustes coordinates: ProCoord3, ProCoord9, ProCoord13, ProCoord14 and ProCoord31, in a six-way DFA.*

4.2.2 Principal component analysis of Procrustes coordinates

Thirteen PCs from the procrustes coordinates were selected, PC1, PC2, PC3, PC4, PC5, PC6, PC8, PC9, PC12, PC17, PC19, PC22 and PC32, and account for 74% of the overall nasal variation among black, white and coloured South Africans (Table 4.15).

Table 4.15. The 13 PC scores selected using Forward Wilks Stepwise selection with their individual and cumulative weights (%).

Principal Component (PC)	Percentage Variance (%)	Cumulative Percentage (%)
1	17.292	17.292
2	14.275	31.567
3	8.914	40.481
4	7.195	47.676
5	6.806	54.482
6	6.045	60.526
8	4.081	64.607
9	3.347	67.954
12	2.355	70.309
17	1.318	71.627
19	1.153	72.780
22	0.953	73.733
32	0.274	74.007

4.2.2.1 Principal component analysis for ancestry

The 13 selected variables (as mentioned above) were used in a three-way DFA (sexes pooled). Overall cross-validated classification was 76% (Table 4.16). All groups D^2 were statistically significant at $p<0.001$.

Blacks classified 71% correctly and frequently misclassified as coloured. Coloured classified 53% correctly and often misclassified as black. Whites classified 95% correctly and often misclassified as coloured but never as black (Table 4.16).

Table 4.16. DFA for PCA using 13 variables: PC1, PC2, PC3, PC4, PC5, PC6, PC8, PC9, PC12, PC17, PC19, PC22 and PC32.

From Group	Total Number	Into Group B	C	W	% correct
B	199	141	55	3	70.9
C	38	15	20	3	52.6
W	95	0	5	90	94.7

Total Correct: 251 out of 332 (75.6%) cross-validated

In the scatter plot in Figure 4.7, the nasal shapes show:

- whites were distinct from black and coloured South Africans, and
- the greatest within group variation was observed among coloured South Africans.

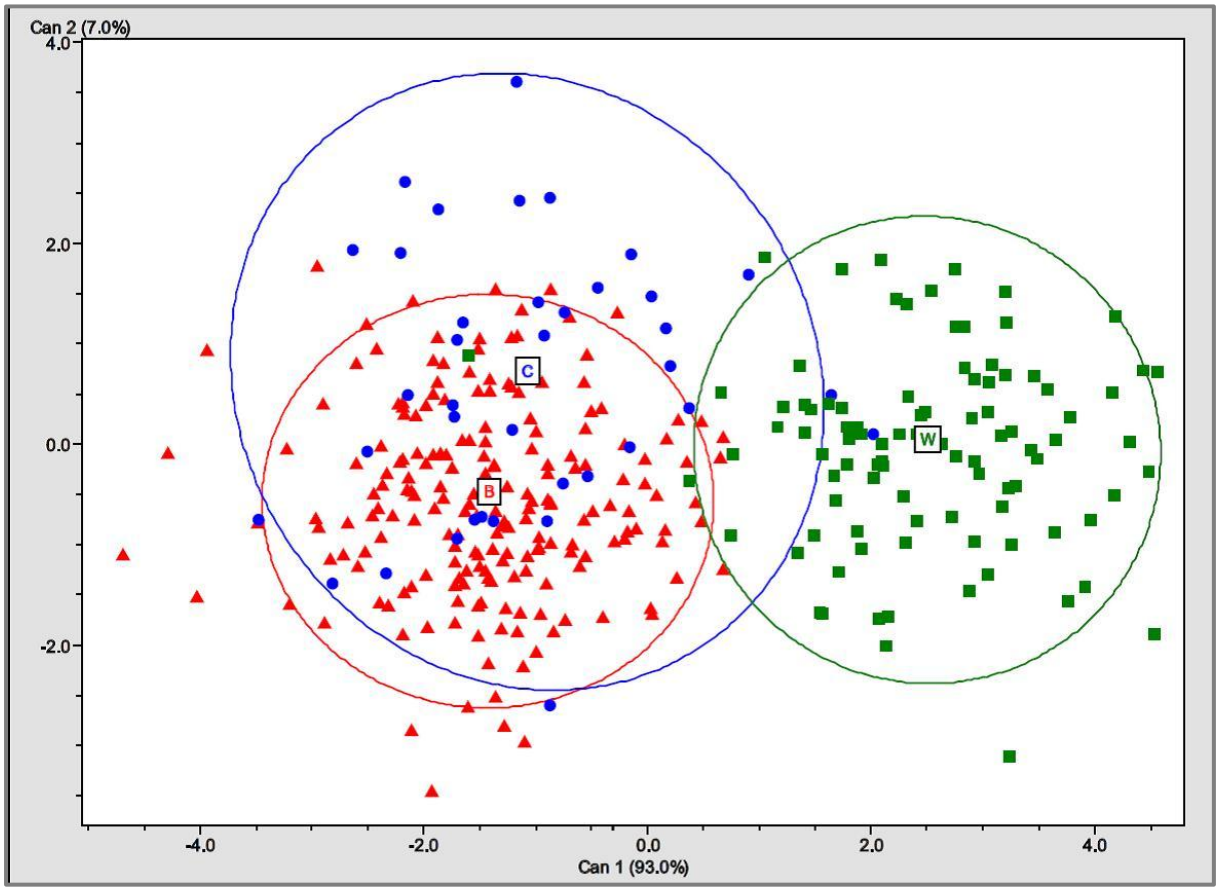

Figure 4.7. *A canonical variate scatter plot for GPA, accounting for 100% of the variation, showing the results of the three-way ancestry DFA using 13 PCs.*

In Figure 4.8, nasal shape is illustrated with PC1, which accounts for 17% of the total variation in the sample. The nasal shape of blacks and coloureds is wider and shorter than white South Africans. When PC1 was plotted against PC2 (Figure 4.9), a clear separation from white and black South African groups was observed, with a wide dispersion for coloureds.

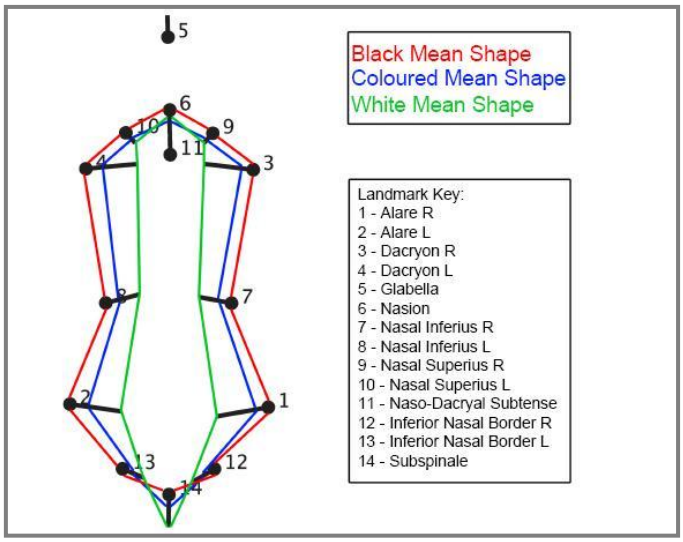

Figure 4.8. *Shape changes between black, coloured and white South Africans based on the 14 digitised landmarks. PC1 is based on canonical variate mean scores and accounts for 17% of the total variation.*

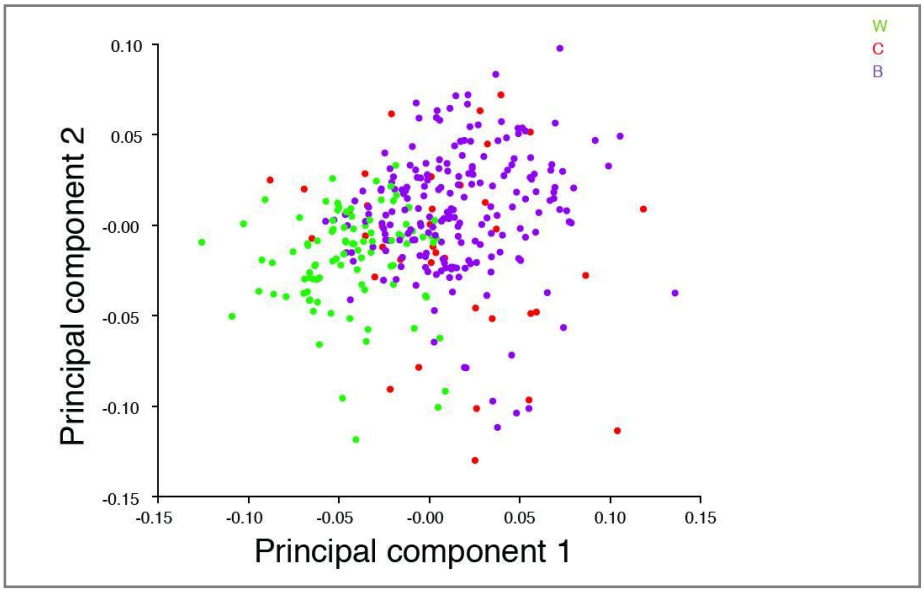

Figure 4.9. *A scatter plot of the PCs for GPA; showing a distinct separation between the white and black groups, while the coloureds are highly variable.*

4.2.2.2Principal component analysis for sex and ancestry

The six-way DFA of sex and ancestry selected variables, PC1, PC2, PC3, PC5, and PC9, resulting in an overall correct classification of 52% (Table 4.17). All groups' D^2 were statistically significant ($p<0.001$), except for coloured females and black females ($p=0.139$) and coloured females and black males ($p=0.07$), (Table 4.18).

Black females classified 50% correctly and often misclassified as coloured females. They never misclassified as white females or males. Black males classified 52% correctly and misclassified frequently as coloured females and never as white males (Table 4.17).

Coloured females classified 23% correctly and misclassified often as black female but never as white females or males. Coloured males classified 44% correctly and misclassified frequently as black females (Table 4.17).

White females classified 57% correctly and misclassified often as white males and never as coloured females. White males classified 63% correctly and often misclassified as white females and coloured males (Table 4.17).

Table 4.17. Six-way sex and ancestry DFA results using five PC variables: PC1, PC2, PC3, PC5 and PC9.

From Group	Total Number	Into Group						Percent Correct (%)
		BF	**BM**	**CF**	**CM**	**WF**	**WM**	
BF	103	51	14	20	18	o	0	49.5
BM	96	13	50	19	13	1	0	52.1
CF	13	5	2	3	3	0	0	23.1
CM	25	6	4	1	11	2	1	44.0
WF	49	1	1	0	3	28	16	57.1
WM	46	0	0	0	3	14	329	63.0

Total Correct: 172 out of 332 (51.8%) Cross-validated

Table 4.18. Mahalanobis D^2 distance and statistical significance from a six-way PCA of five variables: PC1, PC2, PC3, PC5 and PC9.

Group	BF	BM	CF	CM	WF	WM
BF	---	1.97**	0.74	1.89**	10.71**	13.68**
BM	1.97**	---	0.91	2.30**	13.11**	13.41**
CF	0.74	0.91	---	2.54**	14.35**	16.42**
CM	1.89**	2.30**	2.54**	---	7.02**	8.40**
WF	10.71**	13.11**	14.35**	7.02**	---	1.07**
WM	13.68**	13.41**	16.42**	8.40**	1.07**	---

**p<0.001

The scatter plot in Figure 4.10 is used to visualise the following:

- differences between ancestry groups is more pronounced than differences between sex groups;
- greatest between group variation is between white and non-white groups; and
- greatest within group variation is in coloured females.

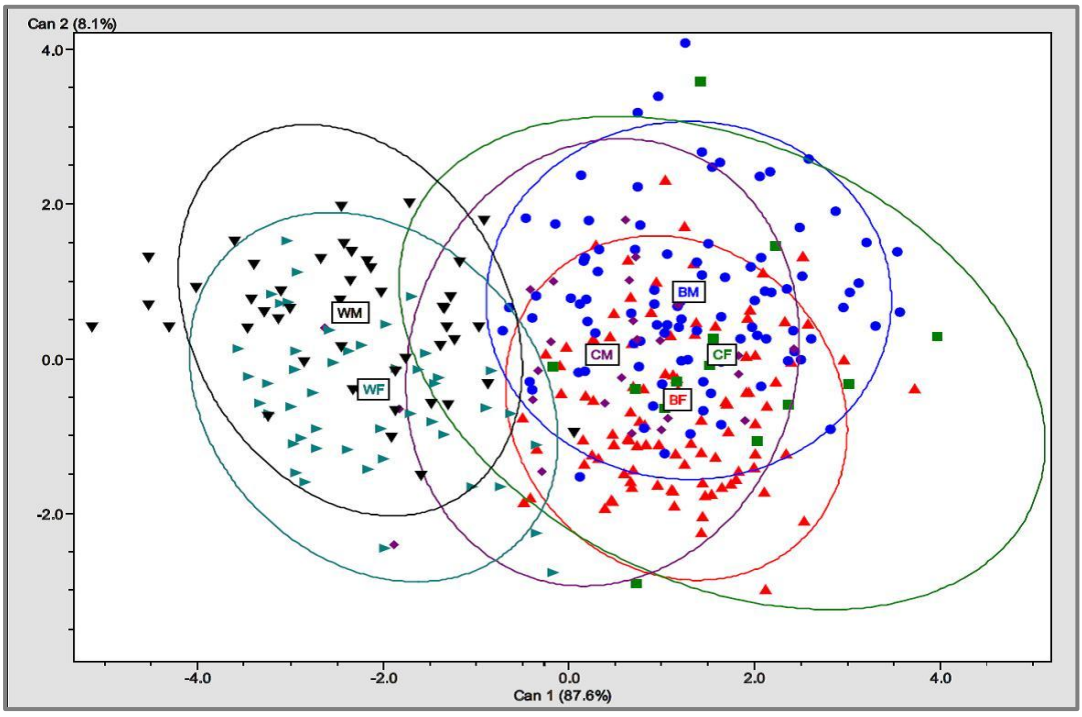

Figure 4.10. *A canonical variate scatter plot accounting for 96% of the variation using PCA, shows the results of the six-way sex and ancestry DFA.*

4.3 Elliptical Fourier analysis

4.3.1 Principal component analysis

Ten effective[11] PCs are derived through the EFA and account for 94% of the total variation. In Table 4.19, the PC scores, as well as individual and cumulative weights, are summarised.

Table 4.19 The 10 PC scores derived through EFA, with the individual and cumulative weights (%).

Principal Component (PC)	Percentage Variance (%)	Cumulative Percentage (%)
1	41.3	41.3
2	18.7	60.0
3	10.6	70.6
4	6.0	76.6
5	4.5	81.1
6	3.7	84.8
7	2.8	87.6
8	2.6	90.2
9	2.0	92.2
10	1.4	93.6

In Figure 4.11, mean shape derived through EFA and its two standard deviations for PC1, which accounts for 41% of the total variation, represent differences in ancestry (sexes pooled).

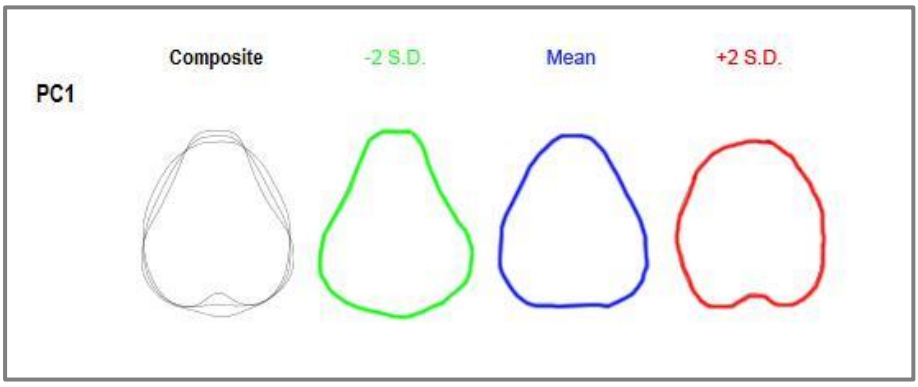

Figure 4.11. *Output from Shape 1.3, of variation in PC1. Mean shape is depicted in the centre (blue) with the shape of two standard deviations above and below the mean on right and left, respectively.*

[11] PC scores greater than 1/total number of principal components derived

In Figure 4.12, the mean shape distribution of the nasal aperture for PC1 against ancestry is shown. The nasal aperture shapes for blacks appear to be more rounded with a slightly superior nasal spine compared to the inferior nasal border. The coloured group falls close to the sectioning point but still appear more similar in shape to black South Africans than whites. The nasal aperture shape for white South Africans is the most distinct and farthest from the sectioning point; with a pear-shaped aperture and a more inferior nasal spine compared to inferior nasal border. Additional ancestry effect graphs for other PCs are available in Appendix B

Figure 4.13 shows the same PC shape distribution but for sex and ancestry. Interestingly, the nasal aperture shape for coloured females is most similar to those of blacks South Africans, whereas coloured males are more distinct in shape to blacks and whites. Additional sex and ancestry effect graphs are available in Appendix B.

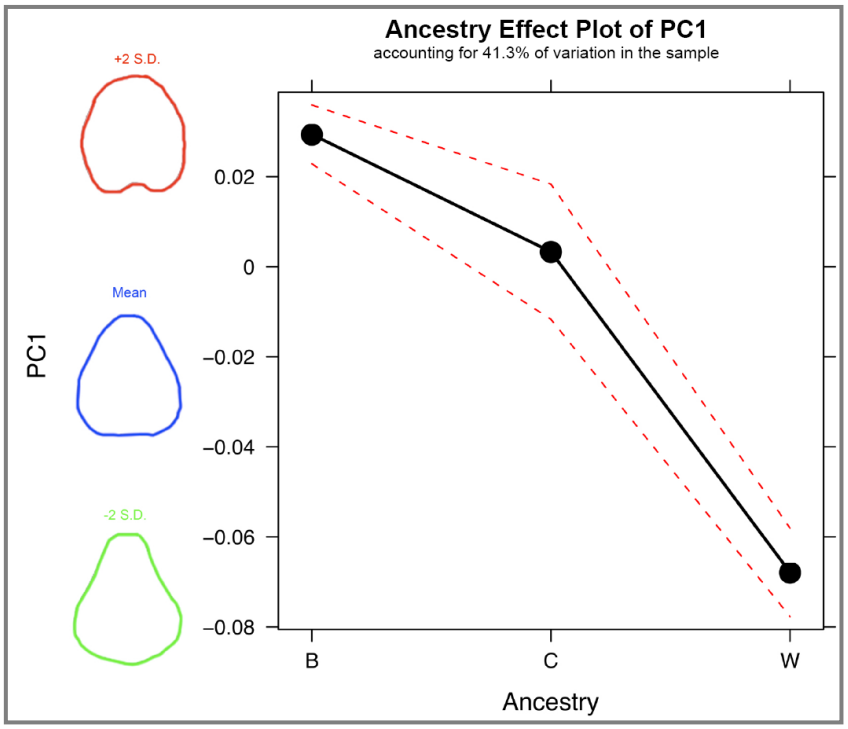

Figure 4.12. *Ancestry effect plot of PC1 accounting for 41.3% of sample variation.*

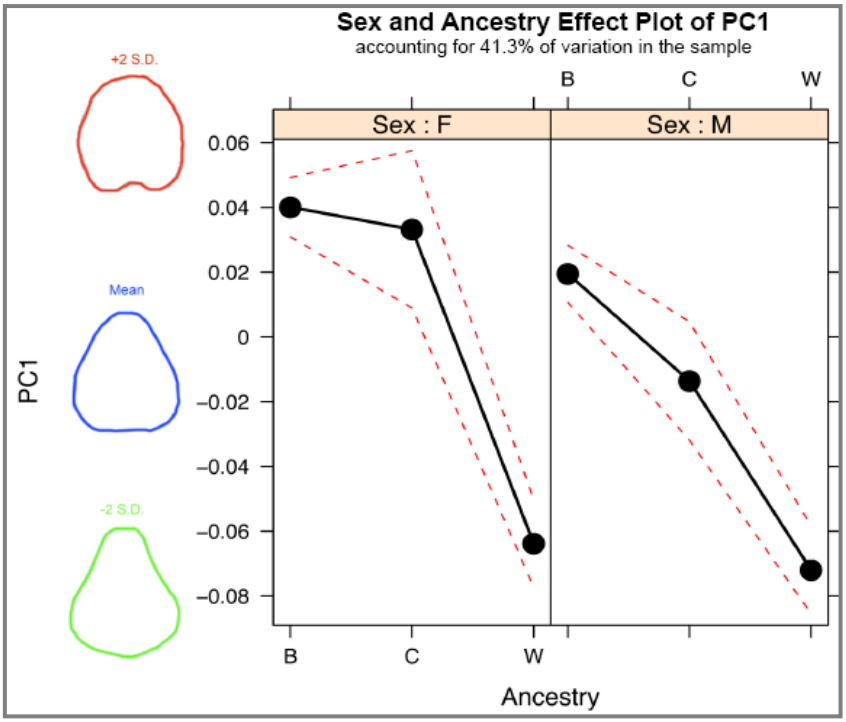

Figure 4.13. *Sex and ancestry effect plot of PC1 accounting for 41.3% of sample variation.*

4.3.1.1 Principal component analysis for ancestry

A three-way DFA (sexes pooled) was performed using seven variables, PC1, PC2, PC3, PC4, PC5, PC8, and PC10, and resulted in an overall correct cross-validated classification of 65% (Table 4.20). All groups D^2 were statistically significant at $p<0.05$ (Table 4.21).

Blacks classified 62% correctly and often misclassified as coloured. Coloureds classified 39% correctly and often misclassified as black. Whites classified 85% correctly and occasionally misclassified as coloured (Table 4.20).

Table 4.20. Three-way ancestry DFA results using seven PC variables: PC1, PC2, PC3, PC4, PC5, PC8, and PC10.

From Group	Total Number	Into Group B	C	W	Percent Correct (%)
B	190	117	59	14	61.6
C	36	14	14	8	38.9
W	84	5	8	71	84.5

Total Correct: 202 out of 310 (65.2%) Cross-validated

Table 4.21. Mahalanobis D^2 distance and statistical significance from three-way ancestry analysis of seven variables: PC1, PC2, PC3, PC4, PC5, PC8 and PC10.

Group	**Black**	**Coloured**	**White**
Black	---	0.70 *	6.06 **
Coloured	0.70 *	---	4.97 **
White	6.06 **	4.97 **	---

*p<0.05 **p<0.001

The scatter plot in Figure 4.14 shows the following:

- the largest between group variation in nasal shape was for blacks and whites South Africans;
- whereas the greatest within group variation was found in coloured South Africans.

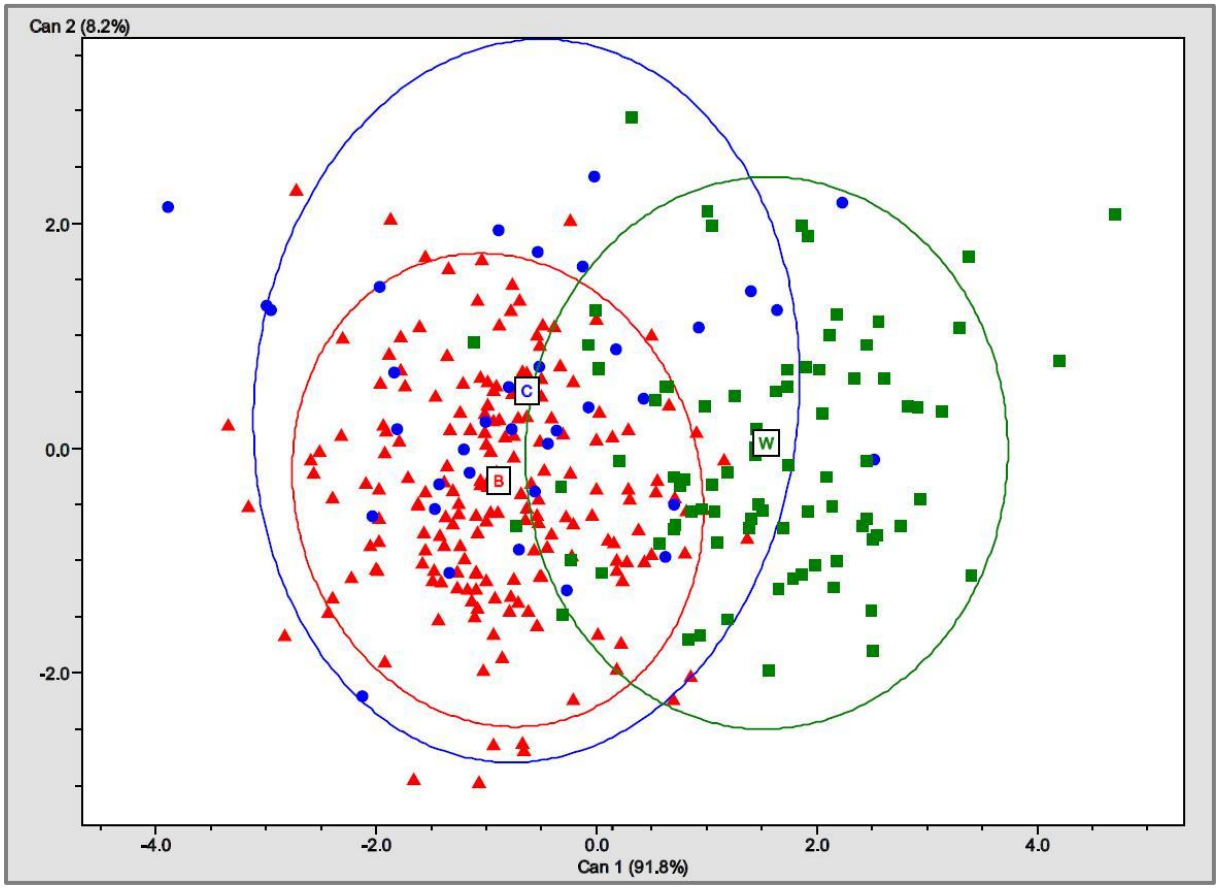

Figure 4.14. *A canonical variate scatter plot accounting for 100% of the variation showing the results of the three-way ancestry DFA.*

4.3.1.2 Principal component analysis for sex and ancestry

A six-way DFA of sex and ancestry, using five variables, PC1, PC2, PC5, PC6, and PC9, and resulted in an overall correct cross-validated classification of 52% (Table 4.22). All groups' D^2 were statistically significant at $p<0.05$ (Table 4.23).

Black females classified 48% correctly and frequently misclassified as black males. Black males classified 32% correctly and often misclassified as black females (Table 4.22).

Coloured females classified 31% correctly and often misclassified as black females and never misclassified as white females. Coloured males classified 26% correctly and frequently misclassified as black females (Table 4.22).

White females classified 50% correctly and often misclassified as white males and never as black females. White males classified 57% correctly and frequently misclassified as white females (Table 4.22).

Table 4.22. DFA results using five PC variables: PC1, PC2, PC5, PC6 and PC9.

From Group	Total Number	Into Group						% correct
		BF	**BM**	**CF**	**CM**	**WF**	**WM**	
BF	91	44	17	13	13	1	3	48.4
BM	99	23	32	12	18	5	9	32.3
CF	13	6	1	4	1	0	1	30.8
CM	23	5	4	1	6	4	3	26.1
WF	42	0	3	1	3	21	14	50
WM	42	1	1	2	1	13	24	57.1

Total Correct: 131 out of 310 (42.3%) cross-validated

Table 4.23. Mahalanobis D^2 distance and statistical significance from a six-way DFA of five PC variables: PC1, PC2, PC5, PC6 and PC9.

Group	**BF**	**BM**	**CF**	**CM**	**WF**	**WM**
BF	---	0.43*	1.65*	1.74**	6.66**	7.60**
BM	0.43*	---	1.46*	1.05*	4.23**	5.33**
CF	1.65*	1.46*	---	2.87**	8.39**	8.40**
CM	1.74**	1.05*	2.87**	---	3.22**	4.32**
WF	6.66**	4.23**	8.39**	3.22**	---	1.06**
WM	7.60**	5.33**	8.40**	4.32**	1.06**	---

*p<0.05 **p<0.001

In the scatter plot in Figure 16, the following points can be visualised:

- differences between ancestry groups are larger than differences between the sexes in each group;
- largest within-group variation is in coloured males and females and white males and females; and
- least within-group variation is in black males and females.

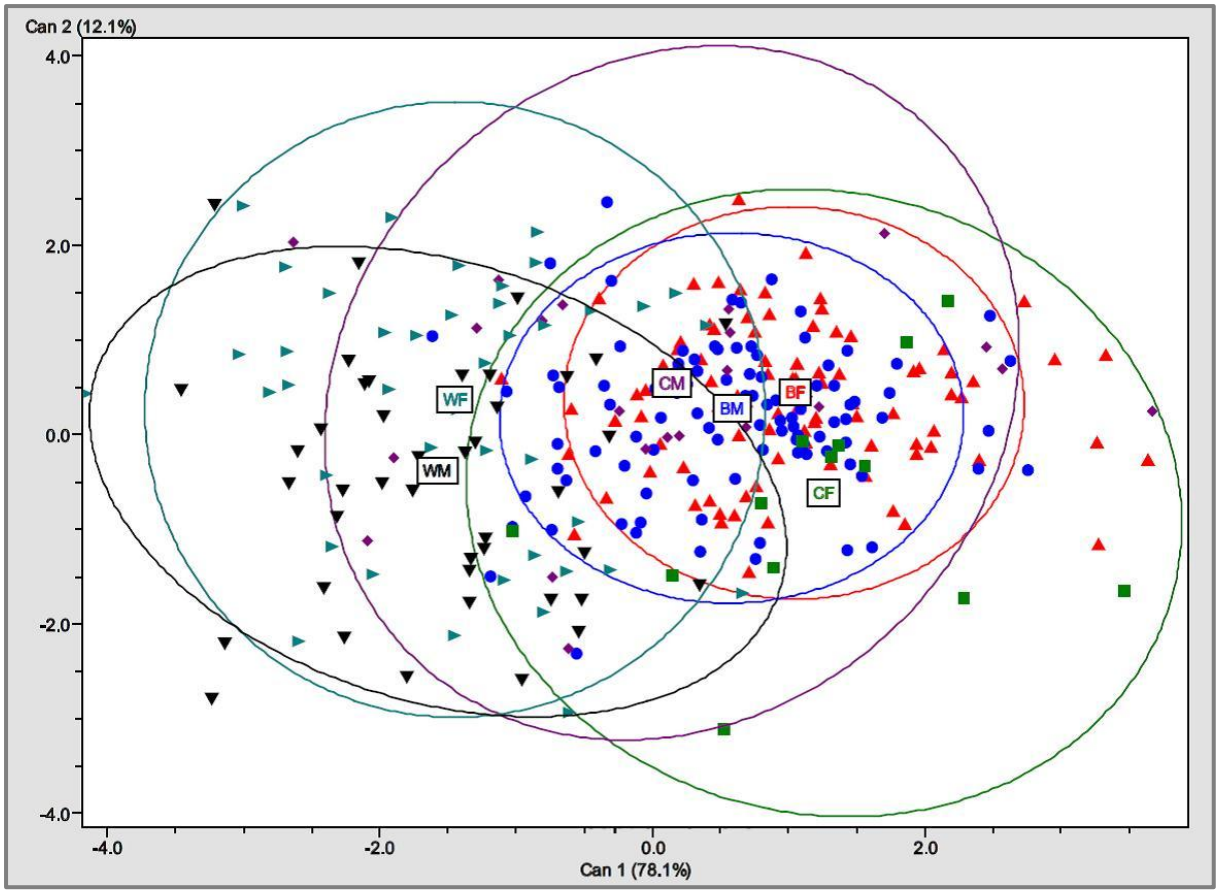

Figure 4.15. *A canonical variate scatter plot accounting for 90.2% of the variation, showing the results of the six-way sex and ancestry DFA.*

CHAPTER FIVE: DISCUSSION

This study examined morphological variation of black, white and coloured South Africans using multivariate techniques. Morphological variation exists in the mid-facial[12] region of all groups with coloureds being the most variable and white South Africans the most distinct. However, none of the three groups analysed displays extreme trait expression previously representing different ancestral groups (Rhine, 1990). Sexual dimorphism was found for all groups but was shown to be less significant than ancestral variation.

Previous studies from Franklin et al. (2007); Patriquin et al. (2002) and İşcan & Steyn (1999), found South African groups to be morphologically distinct from each other as well as other European and African populations. However, none of these studies looked specifically at the mid-facial region using morphometric techniques. L'Abbé et al. (2011) studied mid-facial variation of South African populations using discrete trait analysis and noted that current techniques do not adequately describe observable morphology. Furthermore, while L'Abbé et al. (2011) found a relationship between mid-facial morphology and ancestry, black and white South Africans did not display extreme trait expression as previously noted for black and white North Americans in Rhine (1990). The purpose of this study was to use GM, including GPA and EFA, and standard craniometric analysis to evaluate mid-facial shape among three South African groups. DFA and PCA were used to establish classification accuracies and to interpret differences between the groups.

The main aim of this discussion is to:

- interpret observed variation in ancestry among South African groups within the context of the country's history, and
- compare accuracy of linear measures and GMs in evaluating shape and size variations in the mid-facial region.

[12] As stated in Chapter Three, p. 30; mid-facial refers to the nasal aperture and nasal bones, including inter-orbital breadth.

5.1 Interpretation of observed variation among ancestral groups

5.1.1 The influence of history on morphological variation

Historical and recent South African history provides a possible explanation as to the results of this study. In South Africa, social race and political segregation greatly influenced socialisation and marriage (c.f. Jacobson et al., 2004; Washington Post, 2004). Although abolished in 1994, the social and physical effects of Apartheid remain. Past segregation laws, such as the Group Areas Act (1950), prohibited inter-racial marriage and, in turn, affected inter-racial socialisation of South Africans (Jacobson et al., 2004). While marital segregation was initially between white and non-white South Africans, marriage between black and coloured South Africans was also made illegal at a later period. These behavioural trends could explain morphological differences as reflected in the study, where whites South Africans rarely misclassified into black or coloured but coloured South Africans often misclassified as black.

Coloured South Africans are a heterogeneous group that comprise largely of descendants of slaves brought in from other African countries, indigenous Khoi and San, Asian and European people. These groups had assimilated into the late nineteenth century Cape colonial society (Adhikari, 2005; Carstens, 1996). Therefore, the fact that they are the most morphologically varied population group in this study is not surprising. The coloured sample demonstrated the highest within group variation and the lowest classification accuracies (39-61%).

White South Africans classified 91-95% correctly. White South Africans have the highest classification accuracies of all the groups (Table 5.1) which suggest that whites are the most distinct of the three populations. In a previous pilot study, mid-facial variation between black and white South Africans was assessed and found classification accuracies of 91-96% in both groups. These results are corroborated in this study in that few misclassifications occurred between blacks and whites, with most misclassifications found amongst coloured South Africans.

The results are not unexpected, considering the country's history and the difference in the ancestral geography of white and black South Africans- Europe and Africa, respectively. Forced separation of white, black and coloured groups also led to reducing gene flow and to maintaining morphological differences. However, with the end of apartheid, the current

morphological differences between South African groups are set to change in the future, as cultural ideals towards racial definitions and mate selection change.

Edgar & Hunley (2009) explain biological variation as an inter-play between biology and culture, which leads to continual biological changes in the constituency of races. Racial variation is a feedback loop between cultural and biological factors such that cultural views of race create an incomplete barrier to mate selection (Edgar & Hunley, 2009). This contributes to the distribution of observable biological variation but because cultural definitions of race change, any biological variation is only specific to a particular time and place (Edgar & Hunley, 2009).

Edgar & Hunley (2009) explain this concept through a description of the history of European Americans. Traditionally, European culture, or the geographical area that one was from, influenced mating selection in the United States. For example, South European Americans were considered a different nationality/race to East European Americans. Over a short period of 150 years there was a change in the cultural definition of "European" in America which saw a greater commingling of different White American groups until they were culturally recognised as a single European-derived group.

In some countries, morphological variation is becoming increasingly irrelevant. For example in New Zealand, which has never had state imposed socialising restrictions, the inter-racial marriage rate is much higher than in South Africa. European New Zealander's are 354 times more likely to marry a person from a different cultural group than white South Africans (Jacobson & Heaton, 2006). In New Zealand, 1 in 3 marriages of Maori's (New Zealand's indigenous people) are to individuals of different ancestry (Jacobson & Heaton, 2006). Whereas, in South Africa, 685 same-race marriages occur for every black inter-racial marriage. The high level of inter-racial marriage in New Zealand is reflected in their changing cultural definitions of race. At the last census, in 2006, 10% of people declared their race as "New Zealander", rather than selecting a specific "European", "Maori" or "other" option (Census, 2006). This presents a clear example of altering racial definitions to fit a changing social perception.

5.1.2 Observed ancestral variation

Nasal height and breadth demonstrated significant variation in size among black, white and coloured South Africans (İşcan & Steyn, 1999), as well as black and white North Americans (Gill et al., 1986). In a craniometric study, İşcan and Steyn (1999) showed that white South

Africans had greater nasal heights and smaller nasal breadths than black South Africans. When these variables were used to create a discriminant function formula, ancestry was classified 86-92%, correctly.

Unlike İşcan & Steyn (1999), L'Abbé et al. (2011) used discrete trait analysis and found that the majority of white, black and coloured South Africans had rounded nasal apertures which implied an intermediate index. Furthermore, they noted that over 50% of all three groups had intermediate inter-orbital breadths. Ultimately, L'Abbé et al. (2011) suggested that nasal breadth and inter-orbital breadth are less reliable traits for distinguishing ancestry, unless the sex of the individual is known or can be reliably estimated. Hefner (2009) also found the majority of Europeans to have intermediate inter-orbital breadths but narrow nasal breadths. In contrast, this study demonstrated significant between group variability for nasal breadth, nasal height and inter-orbital breadth, irrelevant of sex.

In this study inter-orbital breadth was shown to be an important variable in ancestry variation, with the most significant variation observed between black and white South Africans. In the PCA, inter-orbital breadth was found to consistently rank second or third in the loading of all five PCs. Other variables, such as nasion-dacryon angle, were found to have greater variation between groups but their loadings varied significantly between PC1 to PC5. The results from the PC loadings show that the variable nasion-dacryon angle (not analysed in other studies) was the most variable measure within and between the three groups. As a result, mid-facial shape was better defined when this measure was added. The results of the PC loadings suggest that while inter-orbital breadth is not the most variable measure, it is a key measure in accurately assessing mid-facial shape. If removed from the analysis, the overall classification accuracy, using the mid-facial region, would likely decrease.

The difference in findings on general nasal aperture shape and sexual dimorphism among this study, L'Abbé et al. (2011) and Hefner (2009) is likely due to the differences in the methodology used. Discrete visual analysis looks at traits individually, not collectively and it is not as sensitive to minute variation as quantitative multivariate analysis.

As with L'Abbé et al. (2011) this study found nasal breadth across all groups to be relatively intermediate but nasion-dacryon angle differed tremendously between the groups. The combination of unique differences in the nasal bone area and subtle nasal aperture variations provided a greater distinction among the three groups. While this study also showed sexual

dimorphism, predominantly in relation to size, it was much less influential on shape than ancestry.

5.1.2.1 White South Africans

White South Africans had the most acute nasal-dacryon angle, smallest inter-orbital breadth, longest nasal height and the narrowest nasal breadth when compared to black and coloured South Africans. This combination resulted in a unique shape that presents with narrow, long nasal bones and a narrow nasal aperture when compared to the other South African groups.

Shape analysis corresponds to these linear findings, indicating that white South Africans have the narrowest mid-facial region of all three groups. White South Africans also have a distinct nasal spine and subspinale in relation to the inferior nasal border when compared to black and coloured South Africans. This combination of traits creates a distinct long, pear shaped mid-facial region, with narrow nasal bones, long and narrow to intermediate nasal apertures. These findings contradict those of L'Abbé et al. (2011) who found 56% of white South Africans to have rounded nasal apertures and only 43% had long nasal apertures. A long/narrow nasal aperture was traditionally considered a defining trait of European ancestry (Rhine, 1990).

Along with distinct nasal bone dimensions, white South Africans also have a distinct nasal aperture shape, with the nasal aperture being concave (Figure 4.12). This shape is visibly different to the convex nasal aperture shape most commonly observed in black South Africans.

5.1.2.2 Black South Africans

Shape analysis showed black South Africans to have a similar nasal aperture shape to coloured South Africans, with similar inferior nasal borders but a higher nasal spine; creating a more heart-shaped nasal aperture when outlined with EFA. In the nasal bone region, white South Africans have relatively straight, parallel boarders, created from similar distances between the left and right nasal superius and inferius and dacryon landmarks. Black South Africans, on the other hand, have a greater distance between the nasal inferius than the nasal superius, thus creating a wider top to the nasal aperture than seen in white and coloured South Africans.

In Figure 4.12, blacks South Africans have a very convex, to the point of being almost round, nasal aperture shape with a nasal spine superior to the inferior nasal border. Along with a

wide and rounded nasal aperture, black South Africans have the widest inter-orbital breadth and nasal bone width along with the shortest nasal height; this creates an overall larger mid-facial region when compared to other groups. İşcan & Steyn (1999) and L'Abbé et al. (2011) also found black South Africans to have wider and rounder nasal apertures than white South Africans. However while L'Abbé et al. (2011) found the majority (87%) of black South Africans to have rounded nasal apertures, they also noted that only 12% had wide apertures, which has traditionally been considered the typical nasal shape of African groups (Rhine 1990). This study found black South Africans to have the roundest and the widest nasal apertures out of three South African groups, but additional research is needed to determine whether this trait is applicable to other Africans groups.

5.1.2.3 Coloured South Africans

Coloured South Africans have a similar mid-facial region to black South Africans with the mean linear differences of most variables to be only 1-2 mm. The overall shape analysis of coloured South Africans indicates a similar but slightly narrower match to the mean shape found in blacks. One main variation between black and coloured is a more inferior subspinale in the coloured group.

Coloured South Africans have a wider inter-orbital breadth and nasale inferius width compared to whites but a similar nasal superius width. This combination creates a wide nasal bone region and nasal bone base, making the superior region of the nasal aperture more rounded (convex) than that of white South Africans.

Based on more traditional theories, African groups should have wide nasal apertures and European groups long/narrow nasal apertures, with groups like coloured South Africans falling into the rounded category (Hooten, 1947; Rhine, 1990). However, L'Abbé et al. (2011) found significant overlap in shape variation between black and coloured groups, with 92% of coloured South Africans having rounded nasal apertures as well as 87% of blacks. This study also found shape variation for black South Africans fell into the range of variation seen in coloureds (Figure 5.1). This suggests that the distinct shape differences among populations, as suggested by de Villiers (1968); Hooten (1947) and Rhine (1990), do not accurately represent within group variation in modern South Africans.

5.1.3 Observed sexual dimorphism

İşcan & Steyn (1999) found enough significant sexual dimorphism among black and white South African groups to create sex specific discriminant functions for estimating ancestry.

However, L'Abbé et al. (2011) found one trait, inter-orbital breadth, to have significant sexual dimorphism. This study found that nasal height exhibited the most sexual dimorphism, with the variable being more than 3mm longer in males. Most sexual dimorphism expressed in this study resulted from size differences between males and females in each group, with males being generally larger than females. An exception was in coloured South Africans, where inter-orbital breadth and nasal breadth, in particular, were similar in size between the sexes. White South Africans exhibited the most significant sexual dimorphism. The reasons that contribute to the high levels of sexual dimorphism within the white South African group could not be determined from this research. Although males were generally larger in all groups, females had a larger/less acute nasal-dacryon angle, which translates into a flatter nasal bone region.

While size differences contributed to sexual dimorphism within the groups, the overall shape of the mid-facial region did not change significantly. In Figure 5.1, the influence of sex on ancestry in mid-facial morphology is demonstrated. The greatest variation is shown along the x-axis, which clearly shows significant variation between white and non-white groups. Except for coloured South Africans, the groups vary by sex along the y-axis. These results indicate that while sexual dimorphism exists, ancestral variations have a greater impact on mid-facial morphology. The significance of this finding is that mid-facial morphology can be used to distinguish between ancestry groups without knowledge of the person's sex.

In figure 5.1, coloured females and males are positioned very close to the y-axis sectioning point, showing that coloured South Africans are the least sexually dimorphic. Coloured South Africans differ from the other groups in that they are the only population which has greater within group morphological variation related to ancestry than sexual dimorphism.

Comparing the sexes between groups generally shows that the greatest similarities are between the common sexes. Black and coloured females have similar measurements across the board, except for nasal height which is much smaller in coloureds. With one of the largest nasal breadths and the smallest nasal height, this would create a more rounded nasal aperture shape in coloured females. In comparison, white females have longer, narrower nasal apertures than the other female groups and the smallest and most angled nasal bones, creating a thin, narrow and, in comparison to width, long mid-facial region.

White and coloured males have a similar shape to white and coloured females respectively, but in white South Africans the males are significantly larger. While this contributes to

separating a group by sex it does not significantly change the overall shape variation within the respective group. This finding can be demonstrated by individuals most often classifying as the correct ancestral group but the wrong sex.

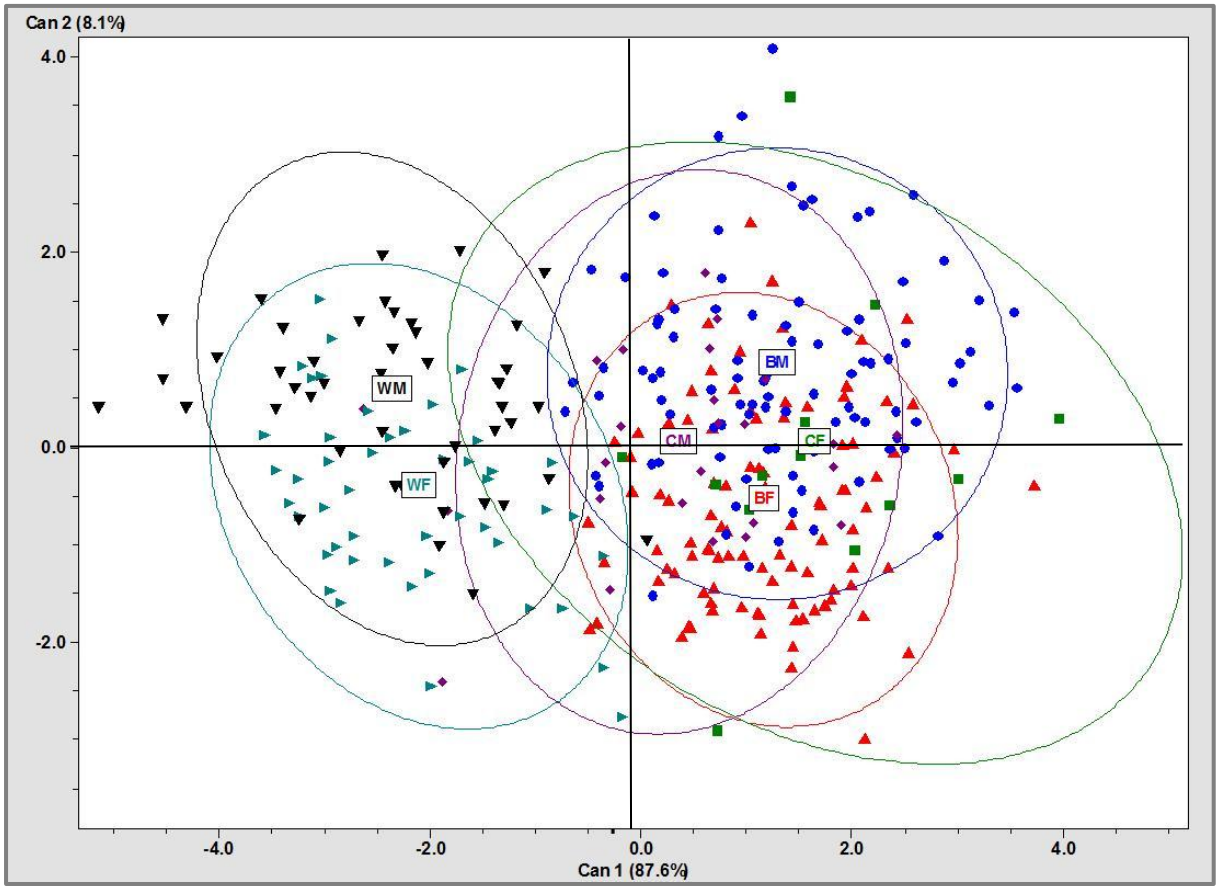

Figure 5.1. *A canonical variate scatter plot for six-way sex and ancestry DFA, demonstrating the shape variation between and within each population group.*

5.2 Accuracy and applicability of various methods

The methods used in this study are split into two broad headings: GM, which include GPA and EFA, and linear measures. PCA and DFA were used in all analyses to identify the most dimorphic variables among the South African groups and to evaluate practical significance through classification accuracy. All analyses found between group variations were statistically significant, except when using uncorrelated linear measures with DFA.

5.2.1 Linear measures

Nasal bone and aperture shape were analysed through multivariate analysis of craniometric measurements and are influenced by size. White South Africans were found to differ significantly from black and coloured South Africans. Using uncorrelated linear data analysis, no significant variation was found between black and coloured South Africans. However, differences between coloured and black groups were found significant when using PCA. The high variability in the coloured South African group had little impact on the overall results, with classification accuracies only changing 0–2% among the three groups, between standard linear measures and the PCA.

The fact that morphological differences between black and coloured groups only become apparent when using PCA indicate that while variation between these two groups exists, the variation is small and may not be suitable for making accurate ancestry estimates. This is supported by the classification accuracies (Table 5.1) which show that while black and coloured South Africans correctly classified above 50%, they correctly classified at much lower rates than white South Africans and most often misclassified as each. The results from L'Abbé et al. (2011) support this finding, and note no significant variation between black and coloured South Africans when using discrete morphological traits.

In six-way sex and ancestry analysis, white females and males classified with the highest accuracy of 71% and 66% respectively. This is not surprising as all analysis showed the greatest variation to be among white and non-white South African groups. The lowest classification accuracy was seen in black females who classified 38% correctly. However, black females misclassified most frequently as black males suggesting that there is greater variation between the ancestral groups than between the sexes. No statistical significance was found between black females and coloured males and females. This result differs from other methods, but might be explained by the different designs of the techniques used. The linear analysis showed that while some sexual dimorphism exists, the greatest variation lies between white and non-white South African ancestral groups.

5.2.2 Geometric morphometrics

5.2.2.1 Generalised Procrustes analysis

GPA used the 13 digitised landmarks to simulate nasal aperture and bone shape. All three ancestral groups were found to be statistically significant. The shape analysis confirms much of the linear findings with white South Africans having the longest nasal height and smallest inter-orbital breadth and nasal breadth. Even though both linear measures and GPA used the same digitised landmarks more detailed shape analysis is available using GPA.

White South African subspinale's were found to be lower than the inferior nasal border, whereas black South African subspinale's are almost on the same plane as the inferior nasal border; creating a flatter base to the nasal aperture.

GPA gave greatest ancestry classification accuracies of all the methods used (Table5.1), but much of the same conclusions from the linear measures are confirmed with these results. Higher classification accuracy is not surprising considering that GPA was able to collect and analyse the most complete shape of the mid-facial region of all the methods used.

Six-way analysis of sex and ancestry found, as with the linear measures, the lowest classification accuracy with coloured females at 23%, and the highest classification accuracy with white males at 74%. As with the linear measures most groups misclassified as the opposite sex of the same group, except for black South Africans. Interestingly black South Africans misclassified most commonly as coloured South African of the same sex, but coloured South Africans did not misclassify in the same way. This may be a result of black and coloured South Africans having similar mid-facial shapes but blacks being more sexually dimorphic.

The benefit of using GPA is that the digitised landmarks are analysed together rather than as separate measurements. The improvement in accuracy compared to the linear measures suggests that size is playing a significant role in analysing group variation and classifying individuals.

5.2.2.2 Elliptical Fourier analysis

EFA used outlines of the nasal aperture only, from photographs, and found all three ancestral groups to be statistically significant. Because EFA used outlines, the shapes produced are more fluid than those produced with GPA. This allows more minute variations in shape to be analysed and to be used to distinguish variations. EFA was better able to show the concave

nasal aperture shape of white South Africans when compared to the convex nasal apertures of coloured and black South Africans. Similarly to the GPA, EFA found white South Africans had a much narrower superior region of the nasal aperture and lower nasal spine, than black and coloured South Africans. Despite the increased detail observed in the nasal aperture shape this method produced the lowest classification accuracies in both ancestry and sex and ancestry analysis (Table 5.1).

White South Africans had the highest classification accuracy, but it was dramatically reduced from 95% to 85%. The accuracy of correctly classifying coloured South Africans also dropped dramatically from 61% to 39%. These results indicate that while unique ancestral variations exist in nasal aperture shape, nasal bone shape in relation to the nasal aperture is equally as important when analysing ancestral variation. Linear measures support this conclusion, in which nasal-dacryon angle from the nasal bone region was found to be the most variable measure between groups. Therefore the use of EFA, alone, was less valuable as it left out the nasal bone region.

The accuracy of correctly classifying black South Africans differs a little to the other groups, in that it was more accurate than the linear measures but less than GPA. This may indicate that the majority of mid-facial variation in black South Africans is in the inferior nasal bone region and superior area of the nasal aperture. Linear measures did not analyse this area and EFA only analyses the latter.

EFA was able to provide greater visual analysis of shape among different ancestral groups, than the other methods used. However, this study found the technique was limited in the ability to use those shapes to produce high classification accuracies. This increase in detail but decrease in accuracy demonstrates the importance of using appropriate methodology for analysis of any data, as EFA showed; increasing the detail of one area for analysis while removing another may not produce the most accurate results.

Table 5.1. Classification accuracies of three-way ancestry estimation for each method used in this study.

Group	Correct classification (%)			Most commonly misclassified as (n)		
	Linear	**GPA**	**EFA**	**Linear**	**GPA**	**EFA**
Black	55.3 -56.9	70.5-70.9	61.6	Coloured (11-13)	Coloured (52-55)	Coloured (59)
Coloured	54.1-56.8	52.5-60.5	38.9	Black (78-79)	Black (11-15)	Black (14)
White	92.8	90.5-94.7	84.5	Coloured (4)	Coloured (5)	Coloured (8)

CHAPTER SIX: CONCLUSION

South Africa has a large number of unidentified persons with the Gauteng region alone reporting 1 445 bodies left unidentified in 2009 (SAPA, 2010). South Africa has a need for accurate and reliable methods of personal identification from skeletal remains and this research is a step in better understanding the variation among three of the major population groups of the country.

Past segregation, laws and social behaviour have affected the actions and ideals of South Africans. Segregation resulted in race groups being forced into designated areas where people could live, work and attend school (Jacobson et al., 2004). Inter-racial marriage was illegal and remained so until 1989. Thus, political, social and linguistic barriers inhibited any significant gene flow between the major ancestral populations in South Africa.

This study has demonstrated significant mid-facial variation among South African groups and this variation may be useful in estimating ancestry, particularly between white and non-white South African groups. EFA presented with the lowest classification rates, indicating that nasal aperture shape must be considered in context with the nasal bones when analysing ancestral variation.

Black South Africans have wide but short mid-facial regions with the nasal aperture appearing convex. White South Africans have a mean shape defined by a narrow nasal bone and nasal aperture region, in relation to their length. This creates and a concave appearance of the nasal aperture. Coloured South Africans were found to be the most similar to black South Africans but have slightly narrower mid-facial regions. Coloured South Africans were also found to be the least sexually dimorphic but the most variant of all groups.

An interesting finding from this study was that while sexual dimorphism existed within the groups, it had no practical effect on correctly classifying ancestral groups. This is a critical find, as many papers are being published which show ancestry to have an effect on the estimation accuracy of other biological information, such as, sex and age at death.

Using the mid-facial region would not be able to give a confident classification of coloured or black South African. However, it would enable a fast and highly reliable estimation of white or non-white South African, which could be used to narrow the possibilities of identification.

Importantly, the linear measures have shown to be just as accurate as the modern GM techniques for sorting groups based on mid-facial morphology. The benefit of being able to confidently use craniometric analysis, in replacement of other methods, is that it is a fast and cost effective tool. This study used a digitiser to obtain the raw data but, with the exception of nasal-dacryon angle, all the measurements can be obtained using callipers.

Due to limits in sample availability, only a small number of coloured crania were able to be digitised. Coloured South Africans are clearly a highly variable group but the small sample size may have affected our understanding of the true variation for this population. Considerations for similar research need to consider other South African populations, such as Indian and Asian groups; however, this may be limited to the availability of an adequate sample. Digitising a greater number of variables in the mid-facial region and including the nasal bones in the EFA analysis may present greater group variation.

Future research should focus on creating a South African database for input into FORDISC™ or creating new discriminant function formula; which will enable fast accurate ancestry estimations. Current formulae do not take into account the full scope of mid-facial variation and rely on a known or accurately estimated sex evaluation first (İşcan & Steyn, 1999).

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Appendix A

Digitised crania from the Pretoria Bone collection

White South African		*Black South African*			
Accession #	**Sex**	**Accession #**	**Sex**	**Accession #**	**Sex**
5056	F	3428	F	5175	M
5373	F	4436	F	5202	M
5464	F	4437	F	5293	M
5472	F	4516	F	5370	M
5576	F	4540	F	5428	M
5577	F	4578	F	5449	M
5594	F	4727	F	5508	M
5644	F	4752	F	5569	M
5693	F	4944	F	5603	M
5895	F	4956	F	5614	M
6226	F	4998	F	5615	M
6259	F	5767	F	5680	M
6322	F	5797	F	5691	M
6338	F	5932	F	5794	M
6339	F	5957	F	5796	M
6432	F	6177	F	5798	M
6449	F	6234	F	5862	M
6461	F	6290	F	5863	M
6473	F	6370	F	5912	M
6512	F	6388	F	6141	M
4966	M	6470	F	6188	M
5347	M	3177	M	6251	M
5387	M	3178	M	6300	M
5587	M	4212	M	6312	M
5683	M	4361	M	6353	M
5817	M	4380	M	6371	M
5848	M	4413	M	6393	M
5866	M	4425	M	6394	M
5875	M	4429	M	6401	M
6031	M	4583	M	6403	M
6196	M	4592	M	6417	M
6232	M	4609	M	6423	M
6381	M	4885	M	6426	M
6398	M	4896	M	6440	M
6407	M	4955	M	6459	M
6437	M	5132	M		

Digitised crania from the Raymond A. Dart collection

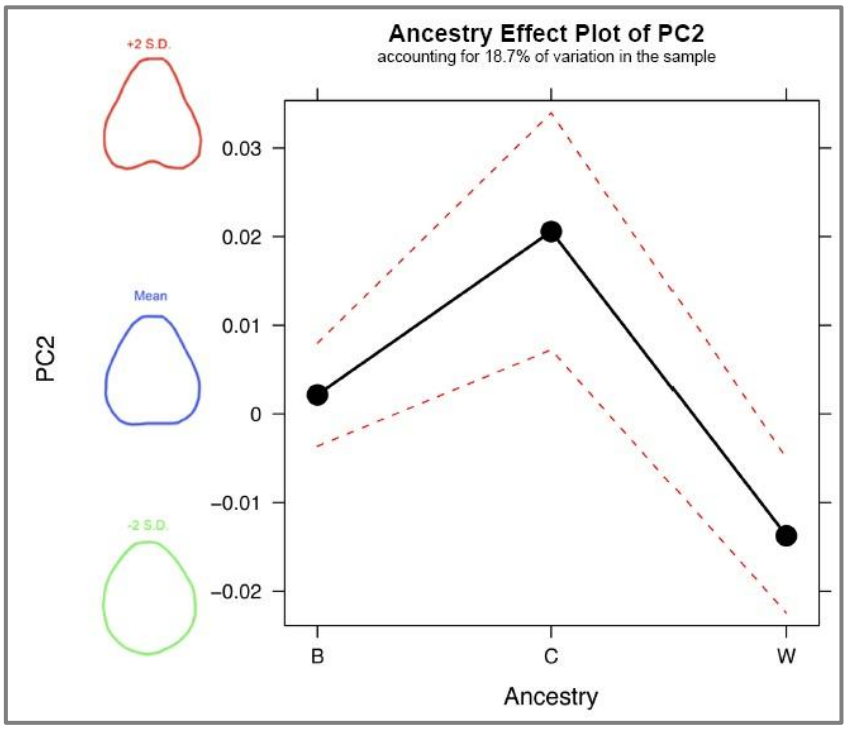
Black South African						*White South African*			
Accession #	**Sex**	**Accession #**	**Sex**	**Accession #**	**Sex**	**Accession #**	**Sex**	**Accession #**	**Sex**
A218	F	A2848	F	A3256	M	A2228	F	A4021	M
A644	F	A2849	F	A3273	M	A2424	F	A4028	M
A828	F	A2850	F	A3277	M	A2481	F	A4081	M
A866	F	A3057	F	A3304	M	A2946	F	A4091	M
A892	F	A3060	F	A3364	M	A3029	F	A4092	M
A1389	F	A3124	F	A3383	M	A3086	F	A4279	M
A1490	F	A3170	F	A3395	M	A3129	F		
A1499	F	A3180	F	A3400	M	A3221	F		
A1534	F	A3195	F	A3404	M	A3347	F		
A1536	F	A3196	F	A3409	M	A3476	F		
A1549	F	A3282	F	A3422	M	A3496	F		
A1557	F	A3292	F	A3438	M	A3858	F		
A1630	F	A3348	F	A3447	M	A3859	F		
A1631	F	A3419	F	A3458	M	A3895	F		
A1666	F	A3450	F	A3462	M	A3938	F		
A1791	F	A3459	F	A3483	M	A3951	F		
A1811	F	A3487	F	A3491	M	A3972	F		
A1867	F	A3498	F	A3500	M	A4015	F		
A1895	F	A3503	F	A3518	M	A4084	F		
A1925	F	A3517	F	A3538	M	A4108	F		
A1951	F	A3563	F	A3565	M	A1334	M		
A1965	F	A3590	F	A3582	M	A2010	M		
A2012	F	A3614	F	A3642	M	A2186	M		
A2020	F	A3617	F	A3644	M	A2187	M		
A2067	F	A3643	F	A3670	M	A2395	M		
A2075	F	A3653	F	A3672	M	A2500	M		
A2254	F	A3689	F	A3684	M	A2647	M		
A2258	F	A3691	F	A3687	M	A2665	M		
A2279	F	A3741	F	A3695	M	A2700	M		
A2314	F	A3791	F	A3697	M	A2710	M		
A2320	F	A3080	M	A3711	M	A3046	M		
A2348	F	A3093	M	A3736	M	A3542	M		
A2349	F	A3120	M	A3784	M	A3648	M		
A2354	F	A3128	M	A3796	M	A3650	M		
A2367	F	A3139	M	A3798	M	A3700	M		
A2404	F	A3145	M	A3828	M	A3882	M		
A2419	F	A3159	M	A3836	M	A3902	M		
A2430	F	A3192	M	A4280	M	A3921	M		
A2464	F	A3218	M	A4282	M	A3948	M		
A2741	F	A3219	M			A3981	M		

Digitised crania from the Kirsten collection

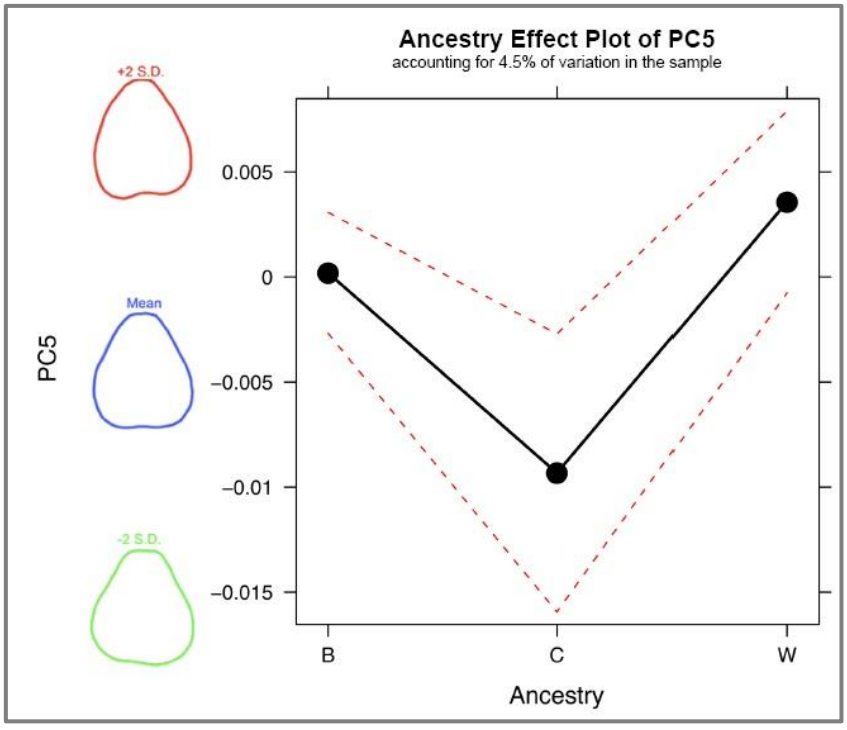
Coloured South African		*White South African*	
Accession #	**Sex**	**Accession #**	**Sex**
AN295	F	AN401	F
AN344	F	AN800	F
AN377	F		
AN379	F		
AN399	F		
AN428	F		
AN469	F		
AN490	F		
AN693	F		
AN753	F		
AN797	F		
AN864	F		
AN865	F		
AN202	M		
AN208	M		
AN349	M		
AN384	M		
AN391	M		
AN411	M		
AN414	M		
AN447	M		
AN521	M		
AN623	M		
AN694	M		
AN721	M		
AN772	M		
AN778	M		
AN790	M		
AN807	M		
AN808	M		
AN827	M		
AN867	M		
AN869	M		
AN875	M		
AN900	M		
AN913	M		

Appendix B

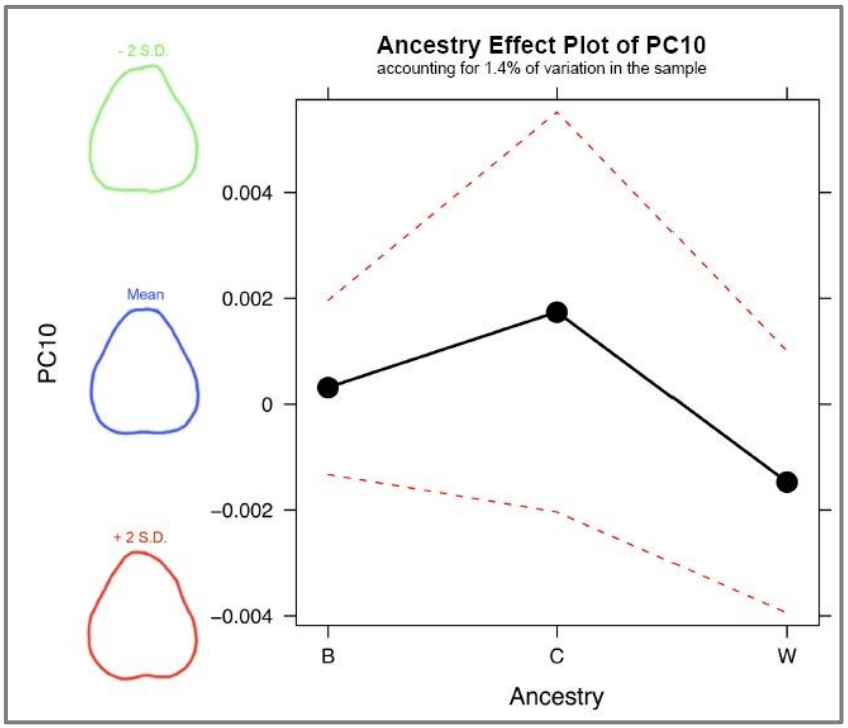
Ancestry effect plots for EFA



Ancestry effect plot of PC2 accounting for 18.7% of sample variation.

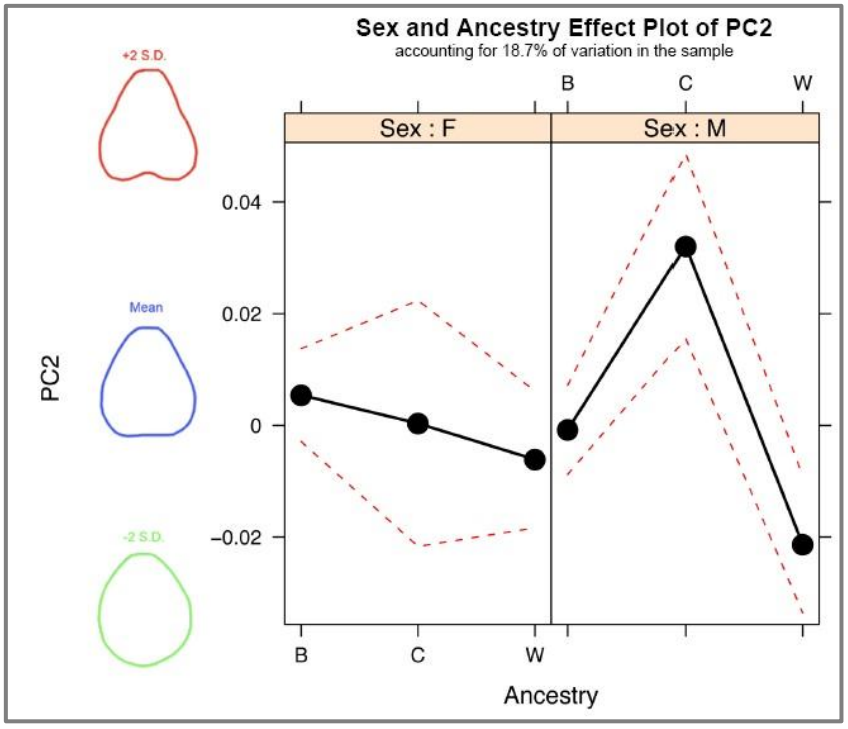


Ancestry effect plot of PC5 accounting for 4.5% of sample variation.

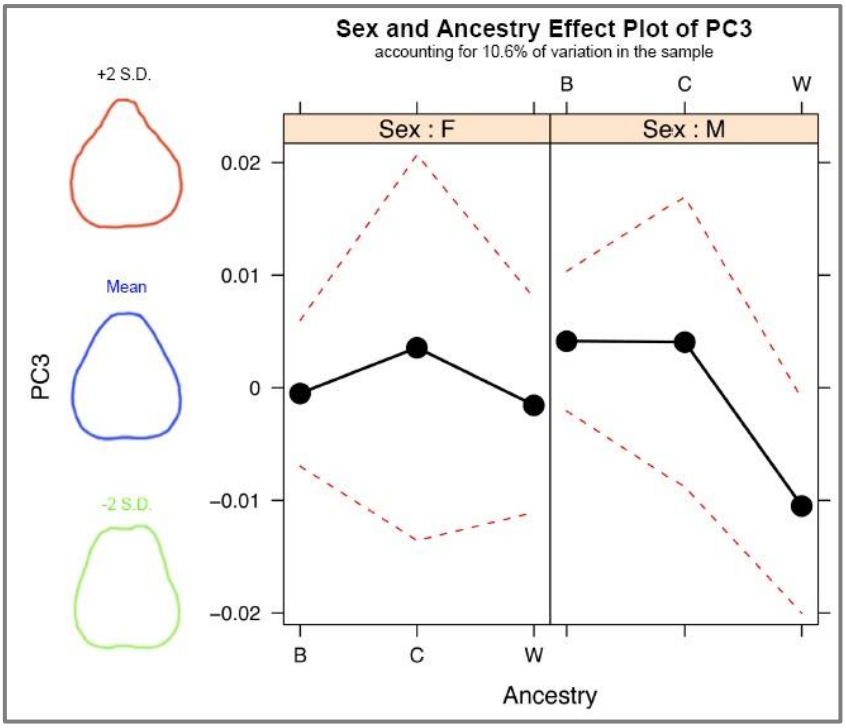


Ancestry effect plot of PC10 accounting for 1.4% of sample variation.

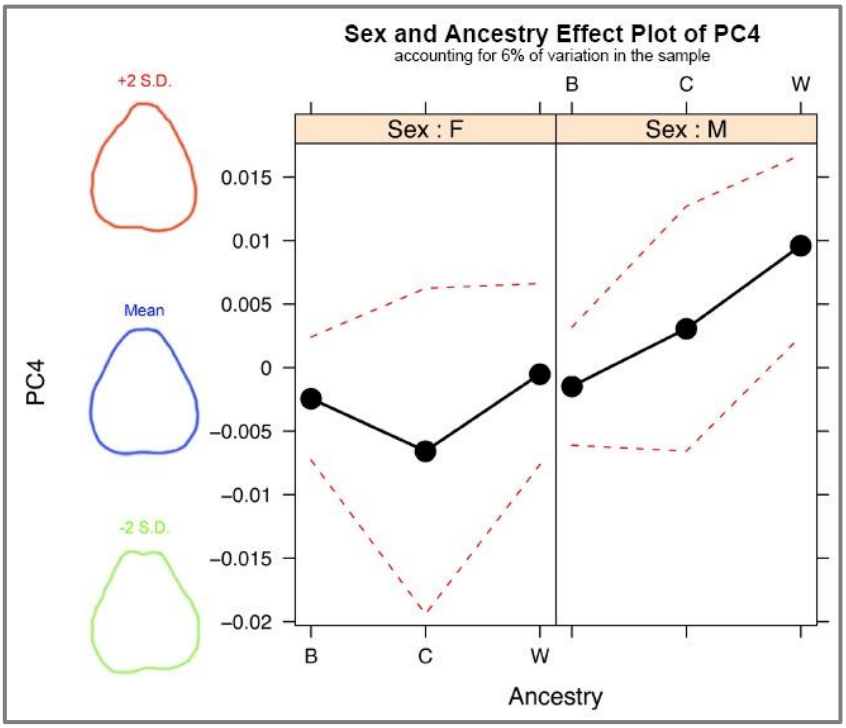
Sex and ancestry effect plots for EFA



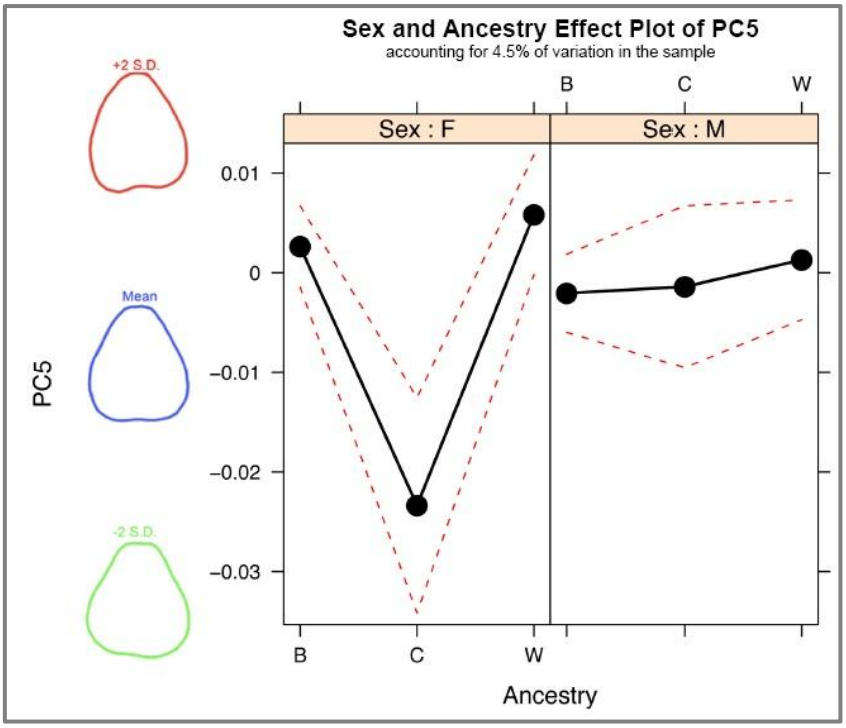
Sex and Ancestry Effect plot of PC2which accounts for 18.7% of variation.



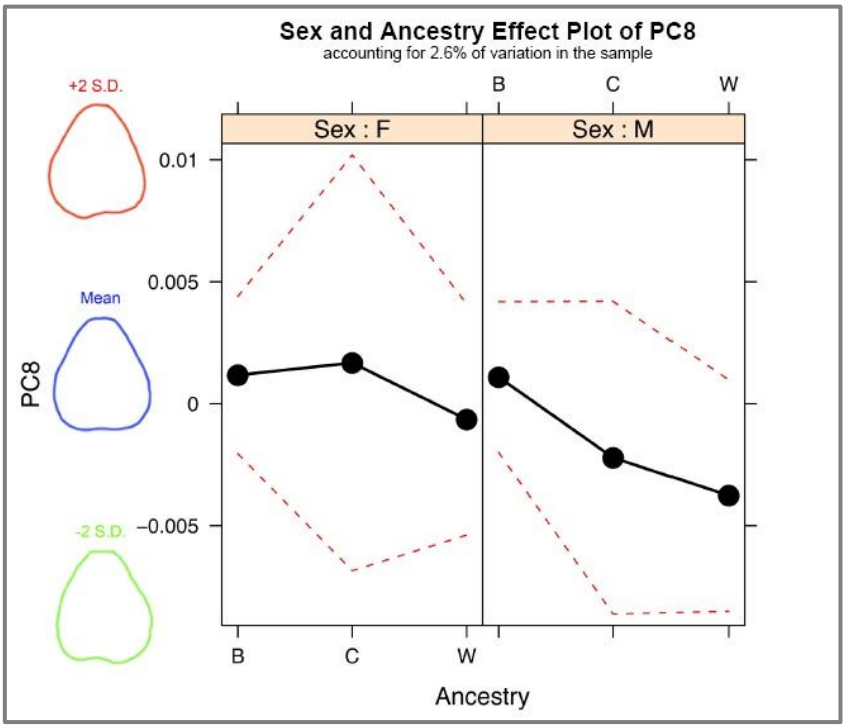
Sex and Ancestry Effect plot of PC3 which accounts for 10.6% of variation.



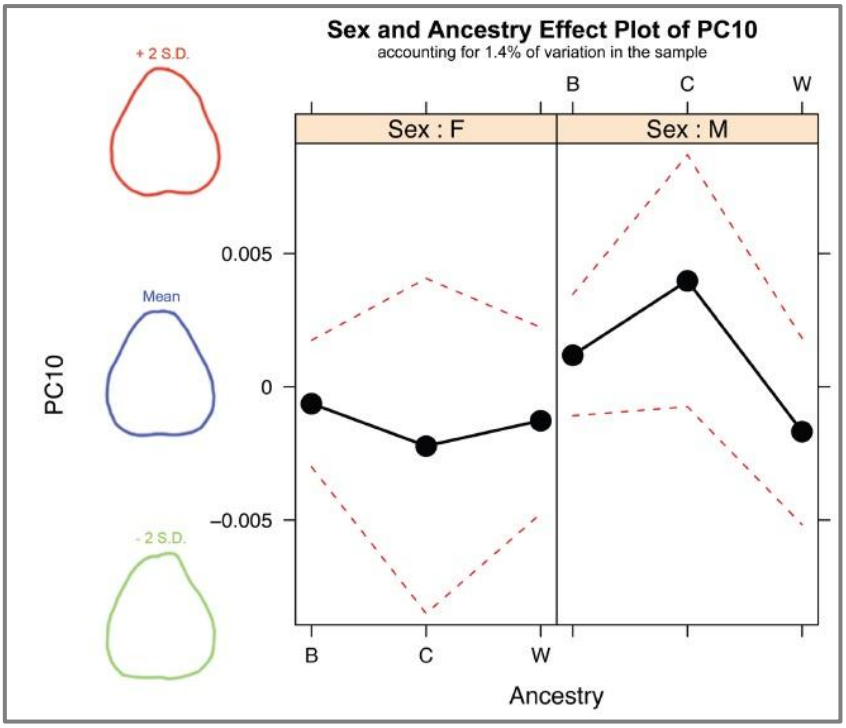
Sex and Ancestry Effect plot of PC4 which accounts for 6% of variation.



Sex and Ancestry Effect plot of PC5 which accounts for 4.5% of variation.



Sex and Ancestry Effect plot of PC8 which accounts for 2.6% of variation.



Sex and Ancestry Effect plot of PC10 which accounts for 1.4% of variation.