ANCESTRY ESTIMATION OF BIRACIAL INDIVIDUALS

USING DENTAL MORPHOLOGICAL TRAITS

by

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DEDICATION

Dedicated to my PoBear and my Mo.
Sissy loves you.
You are my constant inspiration and motivation.
My love for you extends to the moon and back.

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I am immensely grateful to the group of Biracial individuals who volunteered to participate in this study. Thank you, without your contribution this research would not have been possible.

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LIST OF ABBREVIATIONS

Abbreviation	Description
3CNUM2	3-Cusped Maxillary Molar 2
4CNLM1	4-Cusped Mandibular Molar 1
4CNLM2	4-Cusped Mandibular Molar 2
AA	African American
BI	Biracial
C	Canine
CARAUM1	Carabelli's Cusp Maxillary Molar 1
C6LM1	Cusp 6 Mandibular Molar 1
C7LM1	Cusp 7 Mandibular Molar 1
DSHOVUI1	Double Shovel Maxillary Incisor 1
DWLM1	Deflecting Wrinkle Mandibular Molar 1
EA	European American
GPLM2	Groove Pattern Mandibular Molar 2
I	Incisor
L	Mandibular
M	Molar
MARUC	Mesial Accessory Ridge Maxillary Canine
OBS	Observation(s)
P	Premolar
PP	Posterior Probability
PROTOLM1	Protostylid Mandibular Molar 1
SHOVUI1	Shoveling Maxillary Incisor 1
U	Maxillary
UNK	Unknown
WINGUI1	Winging Maxillary Incisor 1

I. INTRODUCTION

Purpose and Problem

Ancestry estimation is a factor of the biological profile with the potential to aid in or hinder forensic identification (Spradley et al. 2008; Ousley et al. 2009; Bethard and DiGangi 2020). The estimation of ancestry is conducted by forensic anthropologists using metric and morphological traits of various skeletal elements (Spradley and Wiesensee 2013, Spradley and Jantz 2016, Hefner 2009; Cunha and Ubelaker 2019), including the dentition (Edgar 2013, Pilloud et al. 2014). However, regardless of the method used representative reference samples and appropriate group descriptions are necessary for accurate ancestry estimation. This is especially true for groups with multiple distinct ancestral histories such as American Biracial individuals. To date, skeletal samples of and research on non-European and non-White sample groups is limited. A sample of known U.S. American Biracial/mixed ancestry individuals has yet to be analyzed using modern standardized methodology. This study is an initial attempt to examine dental morphological trait presentation in a sample of living known self-identified Biracial individuals (defined below) for the purpose of ancestral estimation.

Research Statement

The main goal of this study is to understand the presentation of dental morphological traits in a Biracial sample (African/Black and European/White ancestry) and whether a Biracial sample group is more morphologically similar to an African American or European American group. Previous studies have established that African American (AA) and European American (EA) samples differ morphologically from their parental African and European populations and that while AA and EA groups differ from

each other they are more similar to each other when compared to other sample groups (Edgar 2002; 2005; 2009). Therefore, the hypotheses of this study (Table 1.1) seek to identify if the dental characteristics present in the Biracial sample are equally representative (intermediate[1]) of each of the parent samples or if they are dissimilar to the parent samples.

Table 1.1. Research question, null hypotheses, and alternative hypotheses.

QUESTION
Do Biracial individuals have dental characteristics more representative of African American or European American ancestry?
NULL A:
Biracial individuals' dental characteristics do not differ from African Americans.
NULL B:
Biracial individuals' dental characteristics do not differ from European Americans.
ALTERNATE A:
Biracial individuals' dental characteristics differ from both parent populations but is not intermediate.
ALTERNATE B:
Biracial individuals' dental characteristics are intermediate between the parent populations.

Background

Before discussing ancestry estimation using dental morphology in a Biracial sample, several important definitions are needed as well as a brief discussion of race, ancestry, and Biracialism in the United States and forensic anthropology. This is then followed by a discussion of the literature associated with using dental traits to estimate ancestry from human skeletal remains, especially methods for differentiating African Americans (Black) from European American (White).

[1] To mean amalgamated (Edgar 2009)

The topics of race and ancestry are appropriately long debated, and the debate continues at present. This work does not serve as an extensive historical breakdown of these topics. The following racial and ancestral information is presented in brief and should not be understood as a substitute for sociological or historical studies. These topics are presented for the purposes of understanding the background of the study sample and the importance of this works contribution to anthropology. This thesis is not a definitive final description of these topics. Further debate and education on and of these topics is required and ongoing by both the author and the discipline. As new information becomes available and further studies are completed the definitions described in this study may require updating.

Definitions

Race and ethnicity are social constructs that are used to categorize human groups. As Blakemore (2019) argues "race and ethnicity don't show up at the genetic level, but the concept of race still forms the human experience." Specifically, race is used as a way of categorizing humans based on shared physical traits such as skin color, while ethnicity tends to be used to classify groups based on cultural expression (e.g., religion linguistics, etc.) (Blakemore 2019). However, both are dynamic terms that differ by culture and time-period (Appendix A, Figure 1.1). The U.S. census currently includes five major racial categories (White, African American, Asian, American Indian and Alaskan Native, Native Hawaiian and Pacific Islander) and two ethnicities (Hispanic/Latino or Not Hispanic/Latino) (United States Census 2020). Likewise, ancestry is a term used to define an individual's line of decent and is population based on one's most likely place of geographical origin (Ousley et al. 2009; Relethford 1994; 2002; 2009).

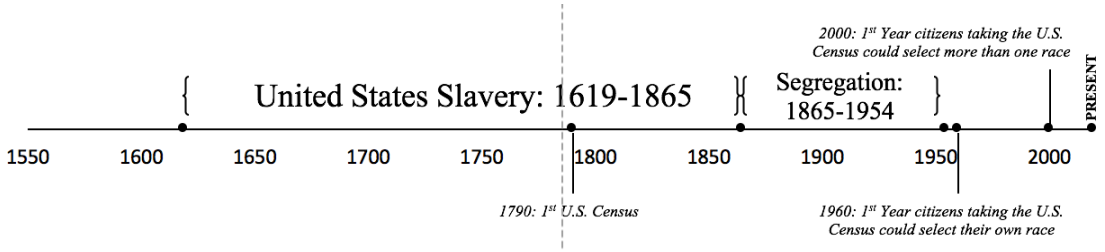

Figure 1.1. History of the census in the United States.

Admixture is another term that needs to be defined when discussing Biracial groups. Admixture is a state of being mixed. In genetics, admixture refers to the mixing of different ancestral or racial populations (Klimentidis et al. 2009). The admixed population often exhibits a mixture of ancestral traits. For example, the Hispanic population is admixed because it exhibits European, Native American, and African ancestries (Klimentidis et al. 2009, Bertoni et al., 2003). Other groups including African American (Parra et al. 2001), Native American (Klimentidis et al. 2009), and European American (Bryc et al. 2015) groups are also admixed. The admixture within these populations is a result of gene flow over an extended period. Admixture is a demonstration of within group variation. Studies attempting to understand human variation have found that most genetic variation in human populations is found within populations and not between them (Relethford 1994; Witherspoon et al. 2007).

The focus of many studies has been the admixture of African Americans with results showcasing that this group is admixed with varying degrees of European and Native American ancestries (Glass 1953; Glass 1955; Roberts 1955; Roberts and Hiorns 1962; Chakraborty 1992; Parra et al. 1998; Chima et al. 2000; Baharian et al. 2016). But, Hispanic and African populations are not the only admixed groups in America. Asian and European Americans are also admixed but to a lesser extent (Parra et al. 1998; Shriver et al. 2003; Parra 2007). However, the presence of admixture in a group does not dictate

that said group will identify as such. In a study on the genetic ancestry of African Americans, European Americans, and Latinos it was found that among self-reported European Americans in the United States at least 1.4% carries at least 2% of African ancestry (Bryc et al. 2015). Additionally, this study reported average proportions of ancestries for African Americans and showed that this group is admixed with 0.8% Native American, 73.2% African, and 24.0% European ancestry (Bryc et al. 2015).

Biracial populations are also admixed but over a shorter period and individuals can choose[2] to identify as such (Rockquemore and Brunsma 2008). Biracial individuals are the offspring of parents of two different races, usually due to an intended interrelationship between individuals of different ancestries (Donnella 2016). Social and cultural structures identify this group as being separate from the traditional race classifications (Parker et al. 2015). While there has been research examining the traits of admixed populations, very little work has focused on individuals classified as Biracial. This study will focus on dental trait frequencies of individuals that self-classify as Biracial, especially those who are the offspring of parents with African and European ancestries. For this study, biracial (BI) refers to individuals who are exclusively first or second generation of parents who are mixed African/African American/Black and European American/White races. In this thesis the term "Biracial" is capitalized to make it consistent with the other proper pronouns "African," "African American," "European," and "European American."

In 2000, the U.S. census began to allow individuals to select and self-identify as

[2] Identity is multifactorial and dynamic. Not all individuals with parents of two different races choose to identify as Biracial (Rockquemore and Brunsma 2002; Khanna and Johnson 2010; Swanson 2013; Parker et al. 2015 Albuja et al. 2018). This thesis focuses on Biracial individual's that identify as such and acknowledges that not all individuals do.

more than one racial category (Jones and Smith 2001; Allen and Turner 2001). Biracialism has become significant in the past few decades largely due to the rapid growth the population has experienced (United States Census 2010; Shih and Sanchez 2009). It is both one of the most salient racial issues of the twenty-first century and a new social phenomenon (Rockquemore 2002). While the existence of Biracial individuals is not new, the way in which Biracial individuals understand their identity, identify themselves, and are identified by others is new (Rockquemore and Brunsma 2002). In the past Biracial individuals, specifically those of White/Black interracial unions[3], may have self-identified as Biracial but were recognized by society as Black (Rockquemore and Brunsma 2002). This misclassification by society has led to this group being underrepresented in scientific analysis and not represented in forensic anthropology[4]. Sociologist Kerry Ann Rockquemore (2002, 499) notes that "there is no current, widely used lexicon in the social sciences to describe various combinations of racial mixture." A Biracial individual is one who exhibits a combination of two racial identities. For this research the term "Biracial" will be used to describe individuals with parents who have racial identities different from each other (i.e., one Black and one White parent, etc.). This research seeks to identify if the population that has a social race identification of Biracial also ancestrally identifies as such.

Race/Ancestry Estimation in Forensic Anthropology

"The price one pays for pursuing any profession, or calling, is an intimate

[3] Interracial marriage was not legal in the U.S. until 1967 when the U.S. Supreme Court ruled that laws that banned interracial marriage violated the Fourteenth Amendment.

[4] In 1932 anthropologist Caroline Bond Day's thesis, A History of some Negro-White Families, was published posthumously. This study used an anthropological lens to understand the hidden group of Negro-White (Biracial) individuals.

knowledge of its ugly side"

-James Baldwin[5]

It is well known and accepted by anthropologists that race does not have a biological basis (American Association of Physical Anthropology 2019), however, racial identity is a social phenomenon that affects the everyday life of all individuals (Chase 1977; Sauer 1992; Baker 1998; Lee 2000; Ousley et al. 2009; Sue 2010; Smedley 2012; Hartigan 2013; Chang 2013; Mukherjee 2016; Horowitz et al. 2019). In an effort to challenge and reject dominating racial worldviews and theories of biological and racial determinism, anthropologists renounced race in what Mukhopadhyay and Moses (1997) describe as an adoption of "a no-race position" (Harrison 1995). This refusal to acknowledge race has "prompted physical anthropologists implicitly to reassign discussions of race *as a social construct* to their cultural colleagues" (Mukhopadhyay and Moses 1997:521) and misconstrue the biological affects race has (Ifekwunigwe et al. 2017). For the forensic anthropologist, however, whose job it is to construct a biological profile from a set of remains in order to narrow down the possible identity of the individual, the no race position is problematic because forensic anthropologists work in a medical-legal sphere and with a public that operates under a socio-cultural race structure (Ousley et al. 2009).

Forensic anthropology has a long history of estimating race/ancestry from human skeletal remains. In the United States the population is politically and socially divided and described by racial categories (Perez and Hirschman 2009; Ousley et al. 2009; United States Census 2020; Balz 2020), and therefore the estimation of race or ancestry in forensic anthropological reports has long been considered an essential aspect of the

[5] "The Black Boy Looks at the White Boy" in *Esquire* (May 1961); republished in *Nobody Knows My Name: More Notes of a Native Son* (1961)

biological profile (Bethard and DeGangi 2020). The argument is that skeletal morphometric and morphological techniques can be used to estimate ancestry based on the heritability of traits (Carson 2006, Ta'Ala 2015). As Spradley and Weisensee (2017:167) note "In the United States, ancestry estimation is possible because there is a concordance (agreement) between social *race* categories *White* and *Black* and the population structure and population history of these groups."

The U.S. census includes five major racial categories: White, African American, Asian, American Indian and Alaskan Native, Native Hawaiian and Pacific Islander (United States Census 2020). Additionally, the options of 'Other' and 'Identified by two or more' can be chosen (United States Census 2020). The census also recognizes an ethnicity and allows individuals to choose 'Hispanic or Latino' or 'Non-Hispanic or "Latino" (United States Census 2020). Therefore, in the biological profile American forensic anthropologists tend to use these racial categories. For example, the computer program FORDISC (Jantz and Ousley 2005), which is used by anthropologists to assess the probabilities of ancestral affiliation, uses similarly named categories (White, Black, Hispanic, Native American, and several "Asian" categories) for the affiliation. Estimates of the factors of the biological profile are made in effort to understand what that individual would have looked like during life meaning that "an individual's self- or community-identified race could be a critical component that leads to identification of a set of unknown remains" (Pilloud et al. 2016: 121). Race can play a meaningful part in an individual's personal identity. Due to the complexities of race in social and cultural instances it can define how that individual is perceived in a society or culture, what spaces they are included in and excluded from, and the define the outcomes of certain

interactions (Poston 1990; Berry 2005; Berry and Candis 2013; Clemmons 2019). Understanding an individual's racial identity can aid in identifying the environments they were exposed to, their status in society, their relationships with others, and multiple other defining factors that can affect daily life. In a medico-legal/forensic context a social race category is typically assigned to an individual to identify them (Sauer 1992). Forensic anthropologists intend to apply human variation to the complex social and cultural norms of which it operates.

However, there are populations of individuals who do not fall perfectly within one of the five U.S. census racial categories. It is also clear that only one variable/classification is not able to directly explain differences between peoples (Ousley et al. 2009). Skin color, as discrete racial categories define populations, cannot be used as an indicator of the geographic populations because it is not likely to reflect ancestry or place of geographic origin (Relethford 2002). Meaning that the skin color of an individual cannot explicitly dictate that the individual is of a specific geographic origin. Yet, a geographic patterning to human variation has been shown in ancestral studies (Relethford 1994; 2009) and a difference between populations that is evident in skeletal morphology that does correlate with social race (Ousley et al. 2009, Spradley and Weisensee 2013).

Use of Dental Traits for Ancestral Estimation

Dental anthropology is a subdiscipline within the branch of biological anthropology and is focused on the origins, evolution, and development of dentitions to answer anthropological questions, including population and group relatedness and

affiliation[6] (Scott and Turner 1988). To understand affinity, dental anthropology utilizes morphological traits of the teeth which are the patterned bumps and grooves on the cusp and occlusal surfaces of dentition and the patterned root characteristics of human teeth (Turner et al. 1991). An early study on dental morphological traits, for example, focused on shovel shaped teeth and the higher frequency of this trait in north Asian and Native American groups in comparison to its lower frequency in African and European groups (Hrdlicka 1920). Following Hrdlicka's (1920) publication were more studies centered around human dental trait variation and the characterization of various geographical, racial, and ethnic groups. However, in some cases the reliance on single traits, such as shovel shaped incisors and Carabelli's cusp, was overused (Scott 1980, Hughes et al. 2011, Hefner et al. 2012). This criticism receives merit in Scott's (1980) study of the Carabelli's trait which found that while this trait is frequent in European populations, it is not as distinct from other geographic ancestral groups as previously thought.

An early study exploring gene heritability with dental morphological traits for group types was completed by Lasker in 1950, and one of the first studies to use dentition to estimate ancestry was completed by Lasker and Lee (1957). Dental anthropologists then began to use dental morphological traits more widely as a tool for assessing ancestry and examining population movement (Irish 1994, 1997, 1998; Edgar 2002, 2007; Scott 1973; Scott and Potter 1984; Turner 1987). But, modern dental anthropologists such as Dahlberg (1965, 1986), Alsoleihat (2013), Edgar (2005, 2013), Scott and Turner (1988, 1997), Irish (2013), Scott et al. (2013), Pilloud et al. (2014), Scott et al. (2016), and

[6] The group names used in this section are maintained from their respective publications. The author is not in agreement with out of date terms and group descriptions and only included them to present the history on this subject.

others, emphasize the relationship between ancestry and dental morphological traits in their studies using advanced methodologies and standardized scoring methods (e.g., Arizona State University Dental Anthropology System (ASUDAS); Turner et al. 1991). Dental morphological traits are inherently qualitative but current dental studies are approaching the study of these traits quantitatively in order to standardize trait observation and further how the data can be applied and understood. Statistical analysis is now used when applying dental morphological traits and can be applied to understand how groups relate to each other. The goal of the Alsoleihat (2013) study, for example, was to present a quantitative method that uses a variety of dental morphological traits to predict ancestry rather than individualized ones. The study showcased that the analysis of Mean Measure of Divergence (MMD) can be utilized to place individuals as members of ones of the following five ancestral groups: Australo-Melanesian, Western Eurasian, Sino-American, Sunda-Pacific, or Sub-Saharan African. The results of this method correctly place individuals into their ancestral group, but less successful in correctly identifying their regional group (related geographic areas combined to constitute a regional group). Importantly this study was able to identify how discriminant the traits used are for identifying individuals as members of particular ancestral groups. Edgar (2004) analyzed samples of African American (n=614) and European American (327) for comparison of two statistical methods MMD and pseudo-Mahalanobis D^2 to estimate biological affinity. The goal of the study was to understand whether or not both types of analysis yield similar results. The AA and EA samples were separated into temporal groups and analyzed for their bioaffinity. Results of this study indicated that it was difficult to preform a D^2 on a large quantity of traits indicating that this analysis is better

suited for small trait quantities. Edgar (2004) concluded that "there is very good agreement" between MMD and pseudo-Mahalanobis' D^2 statistical analysis (Edgar 2004; 61). While both types of statistical analysis have limitations these previous studies indicate that both can be applied for analysis to assess biological affinity using dental morphological traits.

Multiple studies have explored the biological affinity of African American/Black and European American/White groups. Understanding this relationship is pertinent to the current study because it will aid in further understanding the relatedness of a Biracial sample group. Edgars (2005) study discriminates between African American and European American individuals using logistic regression. Edgar (2005) demonstrated that eight traits are useful in estimating African American and European American ancestry. These traits include tuberculum dentale (on the upper canine), lower premolar cusp variation (on the lower 3rd and 4th premolars), deflecting wrinkle (on the lower 1st molar), trigonid crest (on the lower 1st molar), cusp 5 (on the lower 2nd and 3rd molars), and cusp 7 (on the lower 1st molar). These traits were selected because the trait frequencies for each of the traits exhibited significant diversity in the two considered groups (Edgar 2002). Edgar's (2005) study found that use of these traits to estimate ancestry accurately estimated ancestry in 90% of the test sample. Later Edgar (2007) used dental morphological characteristics as ancestry identifiers to understand the biological similarities of African Americans (AA) and the populations that contributed most to their gene pool, in other words, West Africans (WA) and Americans of western European (EA) descent. The relationship between AA and western Europeans (EU) was also analyzed. The results of the study conclude that African Americans have become more

divergent from their West African ancestors and morphologically more like their western European ancestors. These studies have answered important questions about the biological affinity of the studied populations.

Afridont and Eurodont Dental Complexes

Dental anthropologists Irish (Irish 1998, 2013) and Scott (Scott and Dorio 2010; Scott et al. 2013) have identified dental complexes for African/African derived and European/European derived individuals. These dental complexes are referred to as Afridont and Eurodont. The Afridont pattern is a combination of 11 traits (Bushman canine, 2-rooted UP1, UM1 Carabelli's, 3-rooted UM2, LM2 Y-groove, LM1 cusp 7, LP1 Tome's root, two-rooted LM2, UM3 presence, UI1 double shoveling and UM1 enamel extension) that characterizes sub-Saharan African populations (Irish 2013). The Eurodont refers to the dental morphological variation of Western Eurasian groups and the pattern includes the following dental traits: UI1 winging, UI1 shoveling, UI1 double shoveling, Bushman canine, UM1 enamel extensions, LM2 Y pattern, LM1 cusp 6, LM1 cusp 7, LM1 protostylid, LM1 deflecting wrinkle, 3-rooted lower first molars, UM1 Carabelli's cusp/tubercle forms, two or more lingual cusps on LP2, three-cusped UM2, four-cusped LM1 and LM2, two-rooted lower canines. These dental patterns establish that single traits are not significantly important in and of themselves (Irish 1998), but that the combination of particular traits can distinguish specific population groups (Irish 1998, 2013). A combination of Eurodont and Afridont traits were used to assess the bioaffinity Biracial, African American, and European samples. This combination of traits have been shown in multiple studies to separate groups derived from African and European populations (Irish 1993, 1997, 1998, 2000, 2006; Irish and Guatelli-Steinberg 2003;

Gregory 1922; Gregory and Hellman 1926; Weidenreich 1937; Dahlberg 1945, 1947, 1968; Robinson 1956; Le Gros Clark 1960; Swindler 1976; Wood and Abbott 1983; Wood et al. 1983, 1988; Hillson 1986; Wood and Engleman 1988; Aiello and Dean 1990; Tobias 1991; Turner and Hawkey 1991; Turner 1992; Brown and Walker 1993; Stringer 1997; Adler 2005; Aksianova 1979; Aksianova et al. 1977 ,1979; Alexandersen 1962, 1963; Bailey 2006; Brabant and Ketelbant 1975; Coppa et al. 1998, 2007; Cucina et al. 1999; Desideri and Besse 2010; Du Souich 2002; Gadzhiev 1979; Garcia Savoli 2009; Gauta et al. 2010; Guatelli-Steinberg et al. 2001; Hawkey 1998, 2002; Ismagulov and Sikhimbaeva 1989; Johnson and Lovell 1994 ; Kaczmarek 1992; Kaul and Prakash 1981; Khaldeeva 1979; Kirveskari 1974 ; Kochiev 1979; Laforest et al. 2011; Lipschultz 1997; Lukacs 1987 ; Pilloud 2009 ; Roler 1992; Rosenzweig and Zilberman 1967, 1969; Salo 2005; Scott and Alexandersen 1992; Senyurek 1952; Sofaer et al. 1986; Ullinger et al. 2005; Vargiu et al. 2009; Weets 2004; Zubov 1968). This study applies the traits in these dental complexes because of their success in separating African and European groups.

Modern Forensic Utility Methods

The use of dental morphological traits to estimate ancestry is advantageous because teeth are often still measurable even when other skeletal indicators are not. In recent years Edgar (2013) and Scott et al. (2016; 2018) have developed statistical methods for estimating ancestry of individual for forensic utility in the United States. The Edgar (2013) method distinguishes between African American (AA), European American (EA), South Floridian Hispanic (SFH), and New Mexico Hispanic (NMH) using discriminant function (DF) equations. Dental traits used in this method include tuberculum dentale (on the upper canine), lower premolar cusp variation (on the lower 3rd

and 4th premolars), deflecting wrinkle (on the lower 1st molar), trigonid crest (on the lower 1st molar), cusp 5 (on the lower 2nd and 3rd molars), and cusp 7 (on the lower 1st molar). The method is a multi-step application of equations to narrow the classification to AA/EA or SFH/NMH.

The rASUDAS (Scott et al. 2016; 2018) is an online system[7] that processes the dental scores of 15 possible dental morphological traits (winging, shoveling, interruption groove, hypocone, Carabelli's, cusp 5, enamel extensions, multiple lingual cusps, groove pattern, 4-cusped LM2, cusp 6, cusp 7, protostylid, deflecting wrinkle, 2-rooted upper premolars) of an individual. Then compares them against sample groups from seven categorized geographical regions (American Artic & Northeast Asia, Australo-Melanesia & Micronesia, East Asia, American Indian, Southeast Asia & Polynesia, Sub-Saharan Africa, and Western Eurasia). It uses a naïve Bayes classifier algorithm to output posterior probabilities for the "Expected bio-geographical origin." This system offers an efficient way to score dental morphological traits for individuals with a range of skill levels and is a useful tool for forensic application.

While the use of dental methods have improved in forensic anthropology with rASUDAS and other methods, Pilloud and Hefner (2016) have pointed out that forensic anthropology is in need of validated dental morphology methods which will result from the expansion of comparative data and advanced statistical methods. This study will contribute to this overall goal by examining the effect of short-term admixture on dental traits commonly used in forensic anthropology. Specifically, this study will examine the dental characteristics present in a small Biracial sample of living individuals and compare

[7] http://apps.osteomics.com/rASUDAS/

them to larger samples of African American and European American samples previously collected by Edgar 2013 and Scott et al. 2016; 2018). The study will specifically examine how self-identified Biracial individuals classify in a two-group comparison and then discuss how this can aid or hinder identification in forensic anthropological cases.

II. MATERIALS AND METHODS

As a skeletal collection of known or self-identified Biracial (Black/African American/White racial mix; African/African American and European/European American ancestral mixture) individuals does not exist in the United States, the sample for this research was obtained from living self-identified Biracial individuals. Approval for study on living individuals was granted by Texas State University Internal Review Board (2016S173) and dental impressions were taken by a licensed dentist to produce casts. The casts were then used to observe and score dental morphological traits to estimate biological affinity and individual ancestral affiliation (forensic utility). In this chapter, I will describe the samples and discuss the methodology used in this study. Samples groups that will be described include the African and European sample groups provided to me by Dr. Heather Edgar and the Biracial living sample. In addition, this chapter outlines the procedures to recruit living participants, taking dental impressions and making casts, collecting dental morphological data from the casts; and a description of the methodology used to assess ancestral affiliation (forensic utility) including the methods of Edgar (2013) and rASUDAS (Scott et al. 2016; 2018). The described methodology includes the statistical analyses performed for estimation of biological affinity using Mahalanobis D^2 and Mean Measure of Divergence (MMD) and forensic utility methods of Edgar (2013) and rASUDAS (Scott et al. 2016; 2018).

Sample Groups

The dental morphological data from African American (AA) and European American (EA) samples were collected by Dr. Heather Edgar[8]. The dental morphological

[8] Dr. Heather JH Edgar generously shared this data for the purpose of this research.

trait data from the sample of living self-identified Biracial individuals (BI) were collected as part of this study. The data collection for samples used in each respective forensic method are described in their respective publications (Edgar 2013; Scott et al. 2016; 2018). Data were collected from both binary sexes since there is little known sexual dimorphism in the frequencies of trait expression (Scott 1980; Nichol 1989; Hanihara 1992; Irish 1993; Scott and Turner 1997).

African American/Black and European American/White Samples

Dental morphological trait data on contemporary (post 1918) African Americans/Blacks (AA) and European Americans/Whites (EA) was provided by Dr. Heather JH Edgar[9]. The data from these samples were collected by Edgar at the University of Tennessee Health and Science Center, Case Western Reserve University, Arizona State University, and Ohio State University. Demographic characteristics of the AA and EA samples are in Table 2.1. All trait scores for these samples were provided by Edgar and further details on these samples can be explored in Edgar (Edgar 2002).

Table 2.1. African American/Black & European American/White Samples

Source	Group	Sex	N	Total *N*	Scorer
African American (AA)	AA	F	UNK	267	Edgar
		M	UNK		
European American (EA)	EA	F	UNK	137	
		M	UNK		

[9] Forensic Anthropologist; The University of New Mexico

Biracial Sample

The 13 individuals used in this research are self-identified[10] Biracial (BI) individuals and of either binary sex (male or female). At the time of data collection they were 18 years or older[11] and in good health[12]. Individuals that did not meet these criteria were not recruited. BI individuals are defined as first or second-generation individuals who are a mixture of both African American/African/Black and European American/European/White ancestral and racial classifications. First generation (Figure 2.1) individuals are those who have one biological parent that identifies as African American/African/Black and a second biological parent identifying as European American/European/White.

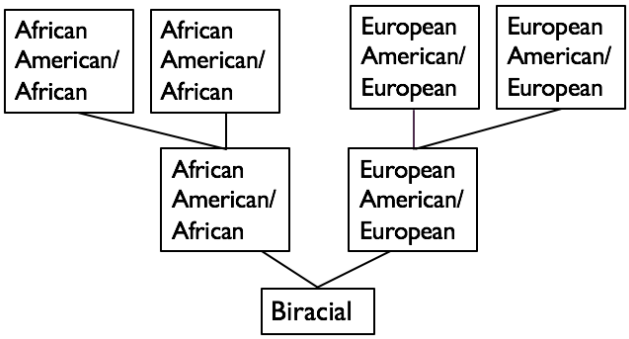

Figure 2.1. First generation Biracial individual depiction.

Second generation (Figure 2.2) individuals are those who have a biological parent, either mother or father, that identifies as Biracial with one grandparent that is African American/African/Black and one grandparent that is European

[10] Individuals were confirmed to meet the BI definition used in this study by the investigator via approved IRB methods (APPENDIX B).
[11] The age criteria were implemented to afford individuals the ability to provide their consent to participate and they had mostly erupted (3rd molars may have been un-erupted or congenitally absent and X-rays were of participants dentition were not collected) permanent dentition.
[12] This sample of living BI individuals was obtained by recruiting voluntary participants following Internal Review Board (IRB) approved procedures (APPENDIX B).

American/European/White. A second-generation BI individual is also a mixture of both African American/African and European American/European ancestry and therefore was included in this study.

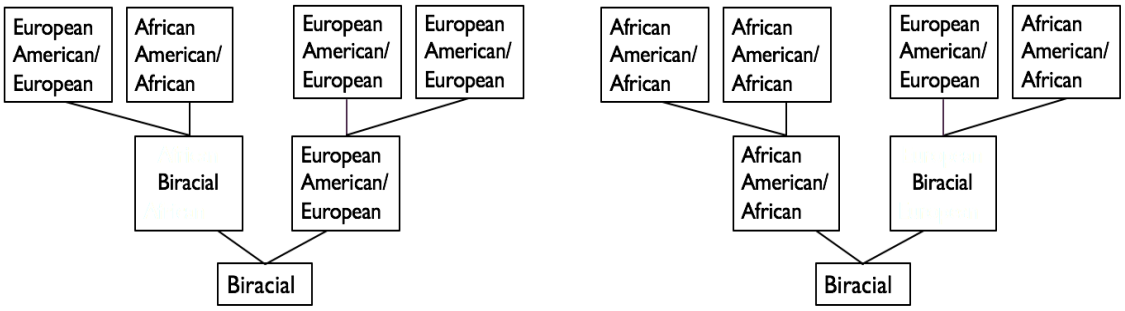

Figure 2.2. Second generation Biracial individual depiction.

The ancestral and racial background of the living subjects used in this research is exclusively those who self-identify as Biracial and were confirmed to meet (see Recruitment Procedures for Biracial Sample) the definition used in this study by the investigator. The general characteristics of the 13 BI individuals who volunteered to participate in this study is described in Table 2.2.

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Table 2.2. Characteristics of Biracial sample.

Sex	Mean Age Range	Sample Size	Erupted M3	1st Generation BI	2nd Generation BI
F	23.5	11	4	10	1
M	25	2	0	1	1

Table 2.3. details the ancestral information for the parents and grandparents of each of the 13 volunteers based on their completed survey and was used to confirm eligibility to participate. This table details how the parents, both the parents of the individuals and their grandparents, identify. Eleven individuals in this study are first generation Biracial individuals and two are second-generation Biracial individuals.

Almost all of the BI individual's identified their mother as White and their father as Black or African American. Two individuals identified their mother as Black or African American and their father as White and two individuals indicated they had a Biracial mother and a Black or African American father.

Table 2.3. Characteristics of Biracial sample detailing individuals ancestral/racial combination.

Individual #	Mother	Maternal Grandmother	Maternal Grandfather	Father	Paternal Grandmother	Paternal Grandfather
1	White	White	White	Black or African American	Black or African American	Black or African American
2	Black or African American	Black or African American	Black or African American	White	White	White
3	Black or African American	Black or African American	Black or African American	White	White	White
4	White	White	White	Black or African American	Black or African American	Black or African American
5	Biracial	Biracial	Biracial	Black or African American	Black or African American	Black or African American
6	Biracial	White	Black or African American	Black or African American	Black or African American	Black or African American
7	White	White	White	Black or African American	Black or African American	Black or African American
8	White	White	White	Black or African American	Black or African American	Black or African American
9	White	White	White	Black or African American	Black or African American	Black or African American
10	White	White	White	Black or African American	Black or African American	Black or African American
11	White	White	White	Black or African American	Black or African American	Black or African American
12	White	White	White	Black or African American	Black or African American	Black or African American
13	White	White	White	Black or African American	Black or African American	Black or African American

*The language used in this table is consistent with that used in the survey in order to stay true to each individuals self-I.D.

Recruitment Procedures for Biracial Sample

To recruit volunteers for the BI sample, requests were 1) sent via email to Texas State University affiliates (students, staff, and faculty), 2) advertised on social media (Facebook and Instagram), and 3) made through personal contact. The volunteers were asked to complete a 21-question survey (to self-report their racial/ancestral identity and indicate the racial/ancestral identities of their parents and grandparents) to determine inclusion/exclusion in the study (Appendix D). This survey was used to assure that a participant met the definition of Biracial used in this study. Respondents who met the requirements of this research were then contacted via email to participate in Part 2 of this research: the taking of dental impressions. A scheduled time was set for individuals who agreed to have their impressions taken by a licensed dentist.

Of the total sample of 13 self-identified Biracial individuals that were recruited, nine individuals were recruited though Texas State University affiliation, two individuals were recruited through social media advertisement, and two individuals were recruited through personal contact. Participants were allotted opportunities to consent[13] to participate at each stage[14] of the research and reserved the right to cease participation in this study at any time (Appendix B).

[13] Consent is required out of principle of respect for persons as outlined in the Belmont Report which describes the ethical principles that govern human subject research. Participants could provide their consent, if they: read the form and decided that they would participate in the described project; understood the general purposes of the research, the particulars of involvement, and the possible risks; and that they understood they were able withdraw at any time.

[14] In the survey at the onset and questions 19, 20 and 21; In-person during Part 2 of this research.

Dental Impressions and Casts / Safety of the Participants

Dental impressions[15] were taken by this study's participating licensed dentist[16] from a sample of 12 living self-identified Biracial individuals who voluntarily agreed to participate in this study. The thirteenth set was made by the participant's private dentist outside the state of Texas and mailed to the researcher by the participant.

Throughout this process the safety of the participants was insured in the following ways: 1) Impressions were taken by a licensed dentist[17]. 2) Participants had their impressions taken in a clean and secure lab location. 3) A combination consent and HIPAA form (Appendix C) allowed participants to provide their permission that they were voluntarily willing to participate in this research by allowing impressions of their dentition to be taken by a licensed dentist and insured the participant's privacy and protection of personal information that can be used to identify them. 4) A Health History form[18] (Appendix E) was completed by each participant to inform the dentist that the participant is in overall good health and able to participate in having their dental impression taken. 5) The practicing dentist reviewed the medical history with the patient

[15] An impression is used in dentistry to make an exact replica of the patients (participants in this research) teeth and surrounding tissues in the mouth (i.e. gums). The material used to take the impressions, Alginate (a detailed list of materials is defined further in this section), forms an imprint (i.e. a 'negative' mold) of the participant's teeth and gums. This impression can then be used to make a cast (i.e. a 'positive model) of the participant's dentition

[16] Outlined in the Dental Practice Act, Sec 251.003. Practice of Dentistry, a person is practicing dentistry if she/he "prescribes, makes, or causes to be made or offers to prescribe, make, or cause to be made by any means an impression of any portion of the human mouth, teeth, gums, or jaws;" (p 35) therefore, licensed dentist Dr. James Fancher acted as key personnel on this project and followed standard procedures for the practice of dentistry in the state of Texas as outlined in the Dental Practice Act (Texas Occupation Code, Chapters 251-267) and took the dental impressions of the participating individuals.

[17] Dr. James Fancher, DDS, PhD.

[18]The selected Part 2 participants were asked to complete a health history form to insure they were fit to participate. The Texas Dental Practice Act requires dentists to acquire a case history of individuals as outlined in Sec 258.052 (Texas Occupations Code, Chapter 251) which includes demographic information and the overall and dental health of the individual. The health history form that was used in this research is sanctioned by the American Dental Association and provided in Appendix D.

and performed a physical evaluation[19]. 6) The sensitive information[20] collected as per standard dentistry practice and IRB regulations is kept in a locked storage location[21] with accessibility offered solely to key personal (Drs. James Fancher and Daniel J Wescott) and I. For further definition of how the safety of participants was insured please refer to the IRB application (Appendix B; IRB Sections 6 &7).

Materials to acquire the impressions were prepared prior to taking the impressions and transported to the impression site (Appendix F). The steps used to take the dental impressions are provided in Appendix G and fulfil all requirements of the Internal Review Board approval and normal dentistry practices. Once the dental impressions were completed, casts of the teeth were made from the impressions. Casts were made at the same site the impressions were taken and trimmed in the Department of Dental Hygiene at Austin Community College[22]. The casts were then set to dry for a period of 24 to 48 hours before being analyzed.

Composing a Blind Sample

Once the casts were made, they were assigned a random number by Drs. Daniel

[19] As outlined in the Dental Practice Act Sec. 258.052 and the Texas Administrative Code, Title 22, Part 5, Chapter 108A, Rule 108.7, it is required that an initial medical history and a limited physical evaluation be performed for all dental patients (dental patients in this research are the participants who consented to proving their dental impressions). The dentist reserved the right to deny further participation to any individual who did not meet the criteria of being in good health. This precaution aided in assuring the safety of the participant. If the participant was in overall good health the practicing dentist proceeded to take his/her dental impressions.

[20] The permanent paper records for each participant include the survey, a combined Consent and HIPAA form, and a Health History form.

[21] The records collected on each participant are property of the researcher, Chaunesey MJ Clemmons, the licensed dentist, and Texas State University. They are stored securely at the Grady Early Forensic Anthropology Research Laboratory at Texas State University.

[22]The molded casts were set to dry for a minimum of 30 minutes and then removed from the impression tray. The casts were then trimmed by the dentist and I at the facilities located in the Department of Dental Hygiene at Austin Community College. To trim them the casts were softened by soaked in water prior to trimming them using the Whip Mix Trimmer machine. Once trimmed, excess stone material that had built up and formed bubbles, etc. on the dentition were carefully removed with fine tools by the dentist. No dental morphological traits were obstructed during this process and it was done to properly observe the traits without obstructions.

Wescott and James Fancher and marked on the cast. Throughout dental morphological analysis the casts were solely identified by this number and demographic information about the participant was withheld from me until the end of the study. Random categorization of the sample was completed in order to prevent bias by myself during data collection. When dental analysis was completed casts were reunited with their documents that included demographic information of the participants for further interpretation.

Storage of Materials: Records, Impressions and Casts

The records collected on each participant are property of the researcher, Chaunesey MJ Clemmons, the licensed dentist, and Texas State University. They are stored securely at the Grady Early Forensic Anthropology Research Laboratory (GEFARL) at Texas State University. The impressions, and completed casts made from the impressions are the sole property of the researcher, Chaunesey MJ Clemmons. While conducting this research access to the impressions and casts was exclusive to the researcher, Chaunesey MJ Clemmons, the supervisor of this research, Dr. Daniel Wescott, and the key personnel of this project, Dr. James Fancher. All materials were stored at GEFARL.

Dental Morphological Trait Data Collection

Observations of dental morphological traits on the maxillary and mandibular casts for the BI sample were completed in natural light with a 10X hand lens (Alsoleihat 2013) and using the expression count method (Turner 1985; Turner and Scott 1977). The expression count method considers that morphological traits are expressed on both antimeres and scores the trait on each but solely considers the highest expression of the

two antimeres in the final tabulation. The highest expression is used because the individual has the genetic potential to express a trait at that grade, therefore, reflecting their underlying genetic potential (Scott and Irish 2017). Details for how observations of dental morphological traits were made for the other samples included in this study can be found in their respective publications (Edgar 2002; 2013; Scott et al. 2016; 2018).

Dental samples were observed/scored for the 25 dental morphological traits listed in Appendix ~~H~~ following the Arizona State University Dental Anthropology System (ASUDAS). The data provided by Dr. Edgar contained data for each of the listed traits. Traits were assigned a trait code (i.e. Winging=WINGUI1) for ease of organizing data and are used throughout (Appendix B). The original ASUDAS scoring system is prescribed in Turner et al. (1991) and 21 of the 25 observed traits were scored according to the scoring scales described in this publication. Recent publications prescribe alternate scoring scales and four of the 25 observed traits were scored using these alternate scoring scales. The four traits include: winging, tuberculum dentale, premolar mesial and distal accessory ridges, and lower premolar cusp variation. The scoring scales for these four traits are described in the guidebook on ASUDAS by Scott and Irish (2017). Scores of the observed traits were based on each prescribed scoring scale.

All 25 crown traits were scored to ensure all existing estimation methods for dentition could be utilized. For bioaffinity analysis 13/25 of the traits were used, for Edgar (2013) analysis 13/25 were used, and for rASUDAS 13/25 traits were used. No root traits were scored for the BI sample because root traits are not accessible through casts.

Breakpoints and Dichotomization

Breakpoints (BP) are used for dental morphological trait data to dichotomize the data for ease of statistical analysis. A BP is a sectioning point used to distinguish between presence and absence of the trait. Scoring scales used to observe traits indicate absence and degrees of presence. For example, the 'Shoveling' trait can be scored 0 to 7, where 0 is none/flat lingual surface, 3 indicates semi-shovel with stronger ridging than in grade two, and 7 is a barrel shaped incisor (Turner et al. 1991). The BP's serve as thresholds that dictate whether a trait is scored as present (1) or absent (0).

Dichtomization is the process of formatting trait expression data from their rank-scale values to present and absent categories for the process of multivariate analysis. To dichotomize a zero, "0," represents absence, a one, "1," represents process, and nine, "9," serve as data that is missing, unobservable, or removed. This process is based on each trait's appraised morphological threshold (Haeussler et al. 1988), determined by Scott (1973), Nichol (1990), and others following standard procedure (Turner 1987). It is essential to complete this process for computational analysis.

For this study the breakpoints derived from Edgar (2002) and Scott and Irish (2017) were used. Edgar (2002) determined the breakpoints most appropriate for separating American Blacks and Whites. Breakpoints from Scott and Irish (2017) were used in correspondence with use of their prescribed scoring scale. The breakpoints used to determine the presence of trait frequencies are in Appendix J. Breakpoints for Edgar (2013) and rASUDAS (Scott et al. 2016; 2018) can be found in their respective publications.

Intra-rater and Inter-rater Reliability

Correctly identifying and scoring dental morphological traits requires training and practice to gain ample experience. These traits are qualitative and require an understanding of a wide range of variation for presence. Agreement on the degree of presence of a dental trait may differ both within a single observer's interpretations at various time intervals and between different observers at various time, which introduces the potential for error. Efforts in standardization for accessing these traits have been made (Turner et al. 1991, Scott and Irish 2017, Edgar 2017) nevertheless it is important to measure the reliability and accuracy of the data from this sample.

Dental traits were analyzed and recorded at two separate intervals for the purpose of intra-rater agreement analysis. For inter-rater agreement measurement photographs of the BI dental casts were sent to Dr. Richard Scott, an experienced colleague of dental anthropology, for scoring. A Cohen's Kappa (κ) was used to measure the amount of agreement between scorers. This analysis is meant for qualitative measurement of agreement and accounts for the possibility of agreement by chance.

The reliability analyses were completed to measure the consistency and reliability of measured scores. It is important that there is agreement between score intervals and scorers because when scoring each trait is given a raw score using a range, which will then be translated into a dichotomy for ease of statistical analysis. The dichotomy will deem the trait either present or absent. Assigning a trait an unreliable raw score would cause it to be dichotomized incorrectly and thus lead to an incorrect interpretation of presence and absence.

Results for the intra-reliability (Table 2.4) and inter-reliability (Table 2.5) test

show that there was majorly substantial to perfect agreement. Differences in scores may be attributed to experience and/or lack of trait presence evident in photographs.

Table 2.4. Results of Cohen's Kappa for Intra-rater reliability.

Trait	κ
WING	1
SHOV1	0.580645
INTGR2	1
HYP2	1
CARA1	0.682927
CUSP51	0

Table 2.5. Results of Cohen's Kappa for Inter-rater reliability.

Trait	κ
WING	0.628571429
SHOV1	0.628571429
INTGR2	0
HYP2	0.714285714
CARA1	0.638888889
CUSP51	0
MLCLP2	0.153488372
4CLM2	0.628571429
C61	1.008392511
C71	0.580645161
PROT1	1.209677419

Edgar (2013) Samples

This forensic method was developed using contemporary, 20th and 21st century, dental casts taken from living individuals. Dental casts of a total sample of 509 individuals affiliated with the groups African American (n=90), European American (n=145), South Florida Hispanic (n=191), and New Mexican Hispanic (n=83) from Case

Western Reserve University, University of Tennessee, Memphis, Health Science Center, Nova Southeastern University, and Economides Orthodontic Collection were used to develop this quantitative method (Edgar 2013, p2-3).

In Edgar (2013) the descriptions for each group are in Table 2.6. Group affiliation for individuals in each sample group was ascribed by the original researcher(s) or treating orthodontist and the individuals comprising these samples did not self-identify (Edgar 2013, p2-3).

Table 2.6. Characteristics of Edgar (2013) samples detailing the descriptions of each ancestral/racial group.	
Group	**Description from Edgar (2013)**
African American (AA)	Individuals with "at least part of their ancestry traceable to" (p2) West Africa as a result of the Trans-Atlantic slave trade.
European American	Individuals "thought to have European ancestry exclusively" (p2).
South Florida Hispanic (SFH)	Individuals have "ancestry primarily from Africa & Europe" (p2); SFH are "predominantly from Cuba, Puerto Rico, and the Caribbean" (p2).
New Mexico Hispanic	Individuals "ancestry is Native American and European, with only small contributions from Africa" (p2); NMH are "chiefly from Mexico or have long family histories in the territories of the United States (Bertoni et al. 2003)".

rASUDAS Samples

The rASUDAS method uses seven biogeographic population groups based on 17 world groups (Table 2.7.). Individuals comprising the sample size for the biogeographic groups are derived from the J.D. Irish and C.G. Turner II databases and include thousands of individuals. Further details on these databases can be found in Scott and Turner (1997) and Scott and Irish (2017) (Scott et al. 2018). To arrive at the seven biogeographic groups probabilities of association were calculated using trait frequencies for 21 regional groups, then pairwise distance was completed among 17 groups, and finally the distance matrix was used to produce a hierarchical cluster tree showcasing the final 7 groups and the linkage between them (Scott et al. 2018, p21). Scott et al. (2018) highlights that rASUDAS is based on a "large worldwide data set" and is "sufficient to evaluate the potential of dental morphology for ancestry estimation" (Scott et al. 2018; p22).

Table 2.7. Characteristics of Scott et al. (2016; 2018) samples detailing the descriptions of each ancestral/racial group.	
Bio-geographic Population Group	**Region Samples In Each Group**
American Artic & Northeast Siberia	Aleut, Inuit, Chukchi
Native American	North American, Mesoamerican, South American Indian
East Asia	China, Japan, Mongolia
Southeast Asia & Polynesia	Insular & Mainland Southeast Asia, Polynesia
Australo-Melanesia & Micronesia	Australia, New Guinea, big island Melanesia, Micronesia
Sub-Saharan Africa	West, East, & South Africa
Western Eurasia	Europe, North Africa, India

Analysis

Dental morphological trait data are qualitative and therefore must be edited prior to completing quantitative statistical analysis. The data were dichotomized based on the breakpoints (Edgar 2002, Scott and Irish 2017) and traits that are missing a significant amount of data were excluded from the analyses. This study used quantitative analyses to compare the BI, AA, and EA samples (see Mahalanobis D^2 and Mean Measure of Divergence (MMD). Then traits that were invariable in the analyzed populations or were unobservable in 50% of the BI population were excluded from analyses. In previous studies it has been shown that traits with a lack of expression variability have no discriminatory value and can therefore be removed from analysis (Harris and Sjøvold 2004). The ancestry of each individual was then estimated using current forensic methods: Edgar (2013) and rASUDAS (Scott et al. 2016; 2018) (see Forensic Utility).

Mahalanobis D^2 and Mean Measure of Divergence (MMD)

To assess the phenetic similarities of the multiple samples in the study, Mahalanobis D^2 and Mean Measure of Divergence (MMD) statistics were computed. Both statistics have been successfully used by dental anthropologists (Scott and Irish

2017). The D^2 was based on a tetrachoric correlation matrix and ran in Rx64 3.3.2 statistical computing software (R Core Team 2016). Mean measure of divergence was completed using the AnthropMMD package in Rx64 3.3.2 statistical computing software. Both statistical measures are done to measure the biological affinity among samples and were completed for the samples analyzed in this study.

The D^2 statistic uses dichotomous data for each individual and derives correlations that ‘‘are calculated within each group, and then pooled using sample size (for each pair of traits) to find the weighted average correlation’’ (Konigsberg, 1990:60). The advantage to this measure is that it adjusts for correlations between traits so it will not allow correlated traits to have more weight in the analysis (Irish 2010). To complete an MMD as many traits as possible should be used without including invariable traits into the analysis. This measure corrects for small sample sizes and low and high frequencies of traits (Irish 2010; Sjovold 1977). The dental traits used in these analyses include those of the Afridont and Eurodont dental complexes (see Introduction).

Following, that the BI sample group is derived from both parent groups African American and European American, which are derived from African and European populations this suite or combination of traits can be used for analysis the BI group distance. The dental complexes were combined for a total of 12 traits on six different teeth (Table 2.8) and those traits in the Afridont and Eurodont pattern that cannot be analyzed on casts were excluded.

Table 2.8. Dental Traits used from the Afridont and Eurodont Complexes.	
Trait	**Tooth**
Winging	UI1
Shoveling	UI1
Double Shoveling	UI1
Mesial Accessory Ridge	UC
Carabelli's Cusp	UM1
3-cusped	UM2
Groove Pattern	LM2
4-cusped	LM1
4-cusped	LM2
Cusp 6	LM1
Cusp 7	LM1
Deflecting Wrinkle	LM1
Protostylid	LM1
*U = maxillary, L = mandibular, I = incisor, C = canine, P = premolar, M = molar, Numbers=tooth number.	

Forensic Utility

To assess how the BI sample would classify using current forensic methods that utilize dental morphological analysis the methods described in Edgar (2013) and Scott et al. (2016; 2018) were completed. Analysis using these methods were completed as described by the authors in order to yield results indicative of each method.

Edgar Method

The method in Edgar (2013) uses discriminant function (DF) equations to distinguish between African American (AA), European American (EA), South Floridian Hispanic (SFH), and New Mexico Hispanic (NMH). First, an individual is classified into either of two categories: AA/EA or SFH/NMH (Hispanic American). Based on the result the method further narrows the classification, separating an individual into either AA or

EA or into either SFH or NMH. For ease of commutation the DF equations were input into excel and run simultaneously. Results for each DF equation were provided for each individual. The traits used in this analysis include: tuberculum dentale (on the upper canine), lower premolar cusp variation (on the lower 3rd and 4th premolars), deflecting wrinkle (on the lower 1st molar), trigonid crest (on the lower 1st molar), cusp 5 (on the lower 2nd and 3rd molars), and cusp 7 (on the lower 1st molar) (Table 2.9).

Table 2.9. Dental Traits used in Edgar (2013) analysis.	
Trait	**Tooth**
Double shovel	UI1
Shovel shape	UI2
Double shovel	UI2
Shovel shape	UC
Distal accessory ridge	UC
Tuberculum dentale	UC
Hypocone	UM1
Metacone	UM1
Metacone	UM2
Metaconule	UM2
Shovel shape	LI1
Shovel shape	LI2
Distal accessory ridge	LC
Lingual cusp complexity	LP3
Anterior fovea	LM1
Deflecting wrinkle	LM1
Protostylid	LM1
Cusp 7	LM1
Cusp 5	LM2
Cusp 7	LM2
*U = maxillary, L = mandibular, I = incisor, C = canine, P = premolar, M = molar, Numbers=tooth number.	

rASUDAS

An online system, rASUDAS by Scott et al. (2016; 2018), processes the dental scores of an individual and compares them against sample groups from 7 categorized geographical regions (American Artic & Northeast Asia, Australo-Melanesia & Micronesia, East Asia, American Indian, Southeast Asia & Polynesia, Sub-Saharan Africa, Western Eurasia) using a naïve Bayes classifier algorithm to output posterior probabilities for which bio-geographical origin the individual is expected to belong to.

The trait scores for each individual were input for all traits, excluding root traits and enamel extensions because these traits cannot be scored on casts, (winging, shoveling, interruption groove, hypocone, Carabelli's, cusp 5, enamel extensions, multiple lingual cusps, groove pattern, 4-cusped LM2, cusp 6, cusp 7, protostylid, deflecting wrinkle, 2-rooted upper premolars) (Table 2.10.) unless they were missing or unobservable. The input scores for the BI individuals were then analyzed against the Sub-Saharan Africa and Western Eurasia groups.

Table 2.10. Dental Traits used in rASUDAS analysis.	
Trait	Tooth
Winging	UI1
Shoveling	UI1
Interruption Grooves	UI2
Hypocone	UM2
Carabelli's Trait	UM1
Cusp 5	UM1
Multiple lingual Cusps	LP2
Groove Pattern	LM2
4-Cusped	LM2
Cusp 6	LM1
Cusp7	LM1
Protostylid	LM1
Deflecting Wrinkle	LM1
*U = maxillary, L = mandibular, I = incisor, C = canine, P = premolar, M = molar, Numbers=tooth number.	

III. RESULTS

Trait Frequencies

Trait frequencies provide information about which traits are more likely to belong to a certain group. The frequency of presence for the observed traits (Table 2.5.) for the samples of BI, AA, and EA individuals are provided in Table 3.1. Traits with the most diverse frequencies could be most useful in completing ancestry estimation in a forensic context, these traits are variable between the population groups and therefore should best discriminant between the groups in order to yield an ancestral classification.

The AA and EA groups express the same three highest frequency traits: Carabelli's cusp on the maxillary first molar, three cusps on the maxillary second molar, and groove pattern of the second mandibular molar. The BI group differs in that their highest frequency trait is a four cusped mandibular first molar followed by Carabelli's cusp on the maxillary first molar, then the three cusps on the maxillary second molar.

Table 3.1. Trait frequencies for AA, BI, EA.

Trait	# of OBS	AA	# of OBS	BI	# of OBS	EA
WINGUI1	235	0.021277	13	0.153846	129	0.007752
SHOVUI1	260	0.069231	12	0.166667	136	0.007353
DSHOVUI1	263	0.057034	13	0	137	0.036496
MARUC	226	0.234513	12	0	126	0.039683
CARAUM1	259	0.710425*	13	0.692308*	135	0.792593*
3CNUM2	207	0.89372*	12	0.583333*	130	0.853846*
GPLM2	193	0.777202*	13	0.230769	100	0.94*
4CNLM1	259	0.15444	12	0.833333*	131	0.122137
4CNLM2	186	0.602151	12	1	125	0.168
C6LM1	259	0.15444	12	0.083333	132	0.121212
C7LM1	250	0.472	12	0.166667	127	0.149606
DWLM1	224	0.357143	13	0.230769	118	0.20339
PROTOLM1	246	0.211382	12	0.333333	129	0.108527

*denotes one of the 3 highest frequency traits for that group

Mahalanobis D^2

A Mahalinobis D^2 was run to compare the African American (AA), European American (EA), and Biracial sample groups. The D^2 results in values that indicate phenetic similarity where lower values signify more similar groups and higher values signify dissimilar groups. Results show that the BI sample is dissimilar to EA (16.96467) and more similar to AA (10.38287). The groups AA and EA are most similar to each other (3.848024) (see Table 3.2, Figure 3.3). Principal coordinates (PC) 1 primarily separates the BI from EA and AA. On PC2 the BI group is intermediate between EA and AA.

Table 3.2. Mahalanobis D^2 distances of AA, BI, EA groups.

	AA	BI	EA
AA	0	10.38287	3.848024
BI	10.38287	0	16.96467
EA	3.848024	16.96467	0

Depicted in Figure 3.1. are the Mahalanobis D^2 distances of the three groups AA, BI, and EA visually depicting the dissimilarities of the three groups.

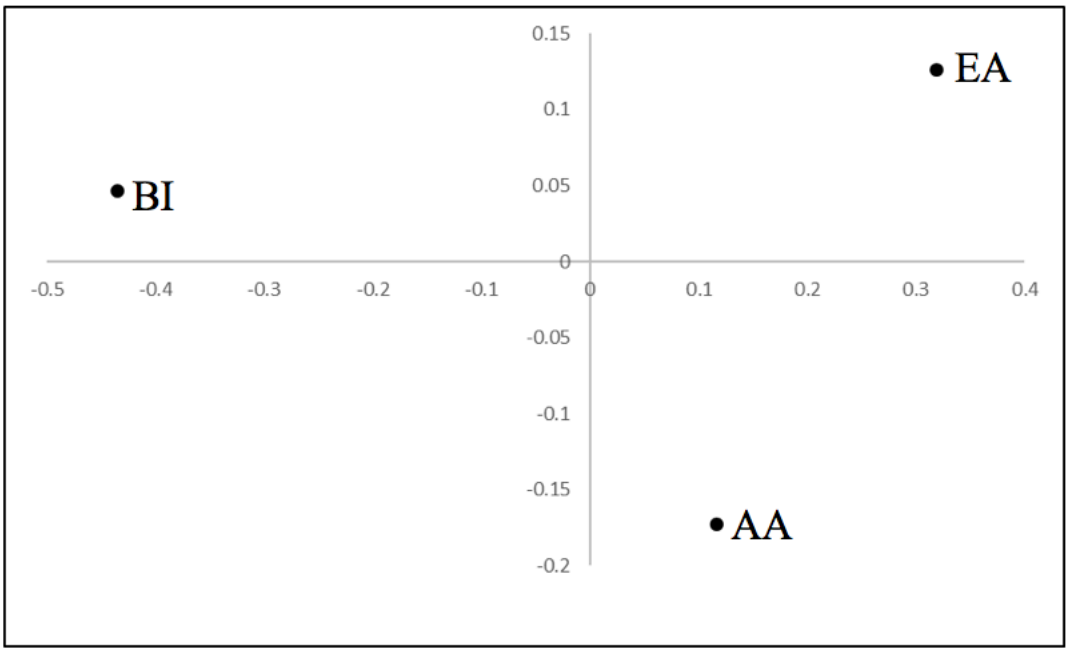


Figure 3.1. Scatterplot depicting the affinity of Biracial (BI), African American (AA), and European American (EA) sample groups. Plotted points are the principal components from Mahalanobis D^2.

Mean Measure of Divergence (MMD)

Mean measure of divergence was run to compare the African American (AA), European American (EA), and Biracial sample groups. The MMD results in values that indicate affinity where lower values signify closer affinity among groups and higher values signify further affinity among groups. Results show that BI is furthest in affinity to EA and closer in affinity to AA. The AA and EA groups are closer in affinity to each other (Table 3.2 and Figure 3.2).

Table 3.3. MMD distances of AA, BI, EA groups.			
	AA	BI	EA
AA	0	0.404125	0.178912
BI	0.404125	0	0.771433
EA	0.178912	0.771433	0

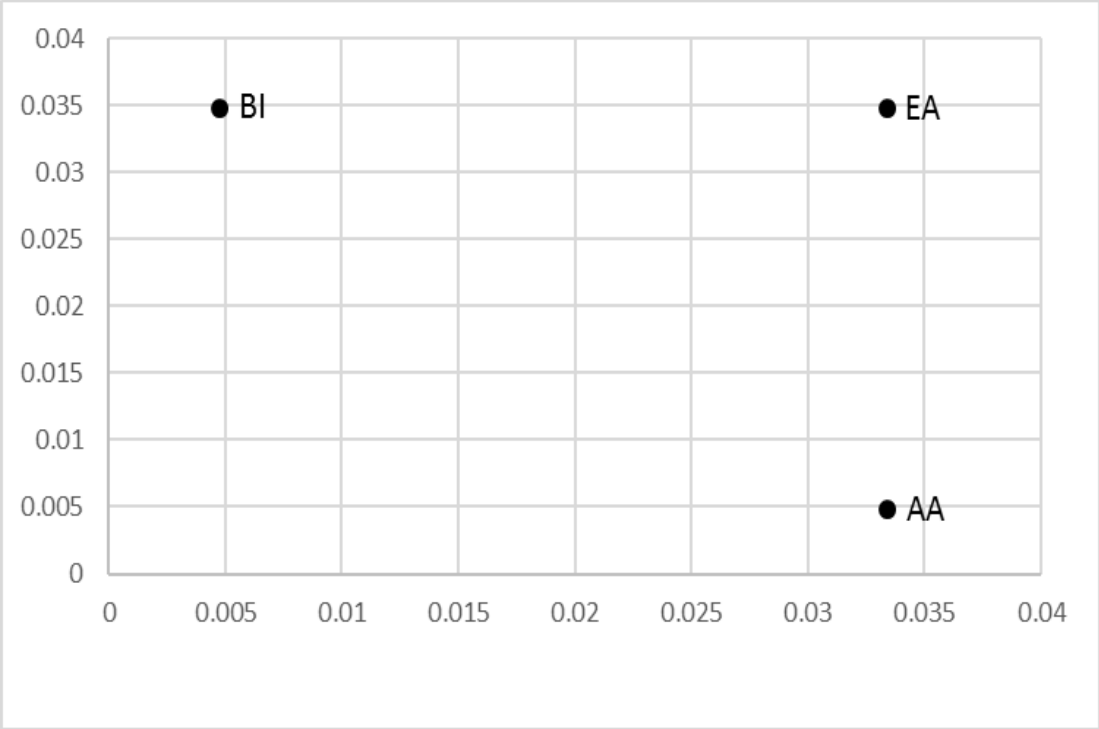


Figure 3.2. Scatterplot depicting the affinity of Biracial (BI), African American (AA), and European American (EA) sample groups. Plotted points are the standard deviations from Mean Measure of Divergence.

Posterior Probabilities

A two group Bayesian classifier was used to produce posterior probabilities for the Biracial sample group in relation to the two parent groups, African American and European American. Results are shown in Figures 3.3., 3.4. and Table 3.4., they echo the results of the D^2 and MMD analysis by showcasing that on an individual basis each Biracial individual is more similar to the African American group

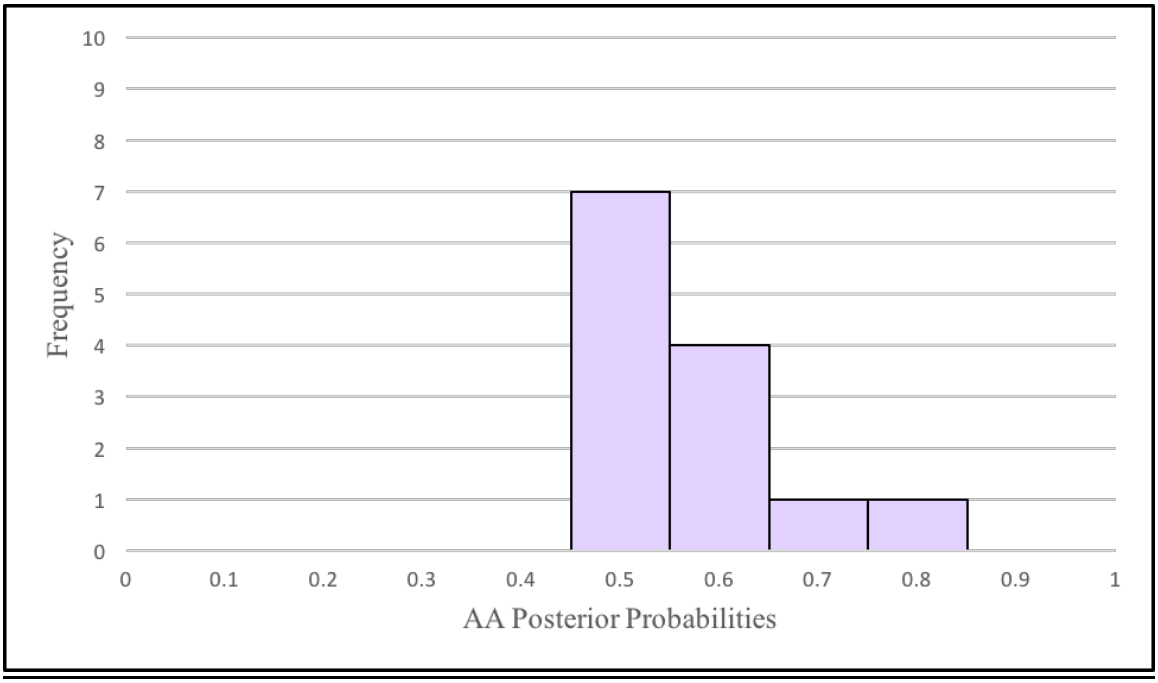


Figure 3.3. Frequency of posterior probabilities of the BI being classified as AA.

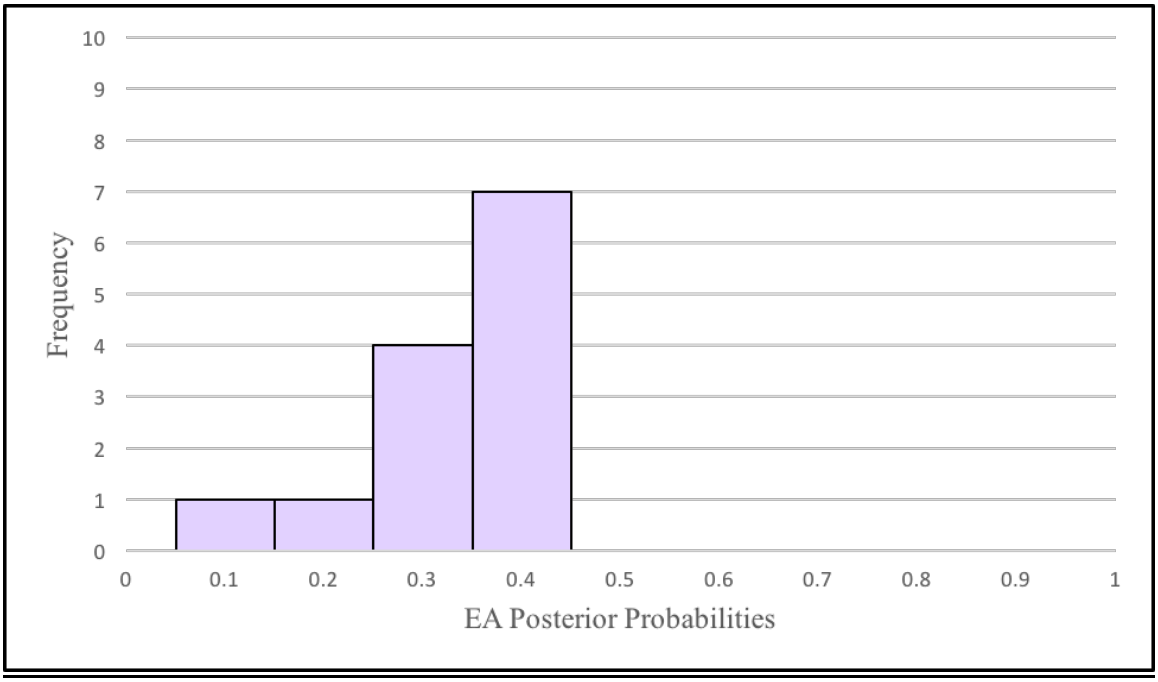


Figure 3.4. Frequency of posterior probabilities of the BI being classified as EA.

Table 3.4. Posterior probabilities (PP) of BI sample for AA and EA classification.

BI #	AA PP	EA PP
1	0.560118338	0.439881662
2	0.758126996	0.241873004
3	0.851273471	0.148726529
4	0.601066174	0.398933826
5	0.66419845	0.33580155
6	0.636967418	0.363032582
7	0.636967418	0.363032582
8	0.555566332	0.444433668
9	0.529727208	0.470272792
10	0.586079861	0.413920139
11	0.518898961	0.481101039
12	0.532792453	0.467207547
13	0.529727208	0.470272792

In Table 3.4. it is evident that some individuals are further from the European American group, Individual 3 represents this with a low PP of 0.148726529 for EA proximity and a high PP of 0.851273471 for AA proximity. Two individuals, six and seven, have the same reported PPs for proximity to both the AA and EA groups and this is likely due to sameness in traits, sameness in missing traits, and similarity in traits that drive the influence for group proximity calculation, or a combination of these events[23]. While all thirteen individuals are closer to the AA group the majority of individuals are also not far removed from the EA group. The PP results of individuals one, eight, nine, 10, 11, 12, and 13 PP results reflecting more close to an intermediate definition.

Individual Ancestral Affiliation Using Current Forensic Methods: Edgar (2013) and rASUDAS

This section will show the ancestral classification results of the sample [N=13] of self-identified Biracial individuals using current dental morphological quantitative methods: Edgar (2013) and rASUDAS (Scott et al. 2016; 2018).

[23] This thesis did not lend focus to which traits in a Biracial group influence overall proximity due to the small size of the Biracial sample.

Edgar (2013): Biracial Classifications

Of the 13 BI individuals, five classified into the AA/EA group and seven classified into the SFH/NMH group. One individual did not classify and was excluded from further analysis using this method. Figure 3.5. depicts the distribution of the BI individuals with the AA/EA affiliations clustered to the right and the SFH/NMH affiliations clustered to the left.

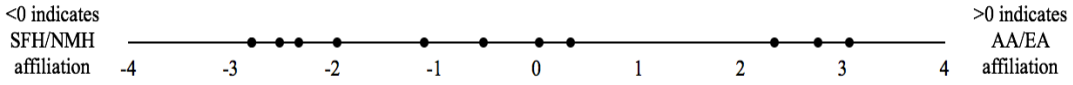


Figure 3.5. AA/EA or SFH/NMH Classification Results for BI Sample.

The classification of the five BI individuals in the AA/EA group was further narrowed. All five individuals classified with European American affiliation. Figure 3.6. depicts the distribution of the five individuals with results less than zero, all clustered to the left of zero, indicating their EA affiliation.

Figure 3.6. AA or EA Classification Results for BI Sample. >0 indicates AA affiliation.

In the SFH/NMH group classifications were further narrowed to results in all seven individuals classifying with New Mexico Hispanic affiliation. Figure 3.7. shows the distribution of the seven individuals with results greater than zero, all clustered to the right of zero, indicating their NMH affiliation.

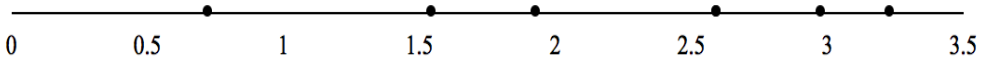


Figure 3.7. SFH or NMH Classification Results for BI Sample

Using the method described in Edgar (2013) the self-identified Biracial sample classified as either European American (N=5) or New Mexico Hispanic (N=7). Classification posterior probability results for each individual for African American, European American, and Hispanic (NMH/SFH) groups are provided in Table 3.5.

Table 3.5. Edgar (2013) Group Classification			
Individual	**African American**	**European American**	**Hispanic (NMH/SFH)**
1	0.00262	0.99578	0.00160
2	0.00056	0.30922	0.69022
3	0.21078	0.69577	0.09345
4	0.15272	0.84622	0.00106
5	0.00014	0.99116	0.00870
6	0.33919	0.62803	0.03278
7	0.72698	0.22236	0.05067
8	0.00610	0.99009	0.00381
9	0.03110	0.00552	0.96338
10	0.80343	0.13955	0.05702
11	0.00128	0.00067	0.99805
12	0.00020	0.00002	0.99978
13	0.00046	0.00010	0.99945

rASUDAS: Biracial Classifications

Classification results based on rASUDAS are presented in Figure 3.8 and Table 3.6. Figure 3.8. depicts the distribution of posterior probabilities from rASUDAS for the Biracial sample of 13. A posterior probability indicates the probability that the analyzed sample fits into that category. The closer the number is to 1 the greater the likelihood it belongs to that group. rASUDAS output a posterior probability for both the Sub Saharan African (SSA) and Western Eurasian (WE) groups. To create this figure, the posterior probabilities from the Western Eurasian output for all 13 individuals were used. Individuals to the left of the graph were classified into the Sub-Saharan Africa group and individuals to the right of the graph were classified into the Western Eurasian group.

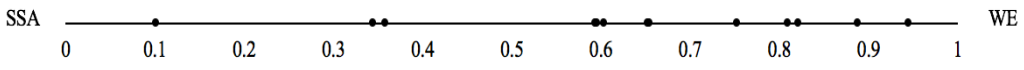


Figure 3.8. rASUDAS Posterior Probabilities for BI Sample

The rASUDAS program showed a total of ten BI individuals were classified into

the WE group and three were classified into the SSA group.

Table 3.6. rASUDAS Group Classification		
Individual	**Sub-Saharan Africa**	**Western Eurasia**
1	0.0414	0.9586
2	0.8813	0.1187
3	0.0244	0.9756
4	0.251	0.749
5	0.0943	0.9057
6	0.1177	0.8823
7	0.5169	0.4831
8	0.0893	0.9107
9	0.9203	0.0797
10	0.4383	0.5617
11	0.1056	0.8944
12	0.2022	0.7978
13	0.3234	0.6766

IV. DISCUSSION

Forensic anthropologists routinely develop a biological profile for unidentified individuals during medicolegal death investigations. One of the four components of the biological profile is the estimation of ancestry based on metric and morphological traits of the skull and dentition (Ousley et al. 2009; Spradley et al. 2008, Spradley and Wiesensee 2013, Spradley and Jantz 2016, Hefner 2009, Edgar 2013, Pilloud et al. 2018). While many of the current methods can reliably differentiate American Black and Whites, no major research has focused on classification of Biracial individuals (i.e., first or second-generation individuals who are a mixture of both African American/African/Black and European American/European/White ancestral and racial classifications). However, there is an increasing likelihood that individuals who self-identify as Biracial will become and are already part of medicolegal investigations. This study is an initial attempt to examine dental morphological trait presentation in a sample of living known self-identified Biracial individuals for the purpose of ancestral estimation. This research explored how a sample of self-identified Biracial individuals ancestrally classified using current dental morphological quantitative methods consisting of multivariate analysis and forensic application methods. The goal of this study was to understand if Biracial individuals are more similar to African Americans to European Americans based on dental morphological trait presentation.

Biodistance analyses show the Biracial group differs more from either parent group than the parent groups do from each other. The Biracial group differs in that their highest frequency trait is a four cusped mandibular first molar followed by Carabelli's cusp on the maxillary first molar, then the three cusps on the maxillary second molar.

Traits found in high frequencies among African populations (following the Afridont dental complex) (Irish 1998, 2013) include multiple traits that were excluded from analysis for various reasons. These reasons include: inability to observe/score due to inability to observe certain traits (root traits in Afridont complex: 2-rooted UP3, 3-rooted UM2, Tome's root, and 2-rooted LM2) on the dental casts; obliteration of occlusal surface on crowns due to dental work; and exclusion of maxillary third molar presence because without x-rays of the participants in the sample group it is unknown whether or not this tooth was absent congenitally or simply un-erupted. Because these are the high frequency traits these are the traits that perform best to characterize the group under analysis for agreement with an African group.

The results of this study suggest that accurate ancestral classification of Biracial individuals is complex. Using Edgar's (2013) method six Biracial individuals did not classify in the AA/EA group and the other five all classified as EA. This is despite the fact that bioaffinity statistical analysis (MMD and D^2) showed that for the Biracial sample used in this study more closely affiliated with the African American sample and less like the European American sample. Likewise, using the rASUDAS program only three individuals classified as Sub Saharan African. Considering the existing admixture of European ancestry in the African American population groups it is fair to follow the assumption that the Biracial group which is comprised of European American and the admixed African American ancestry would be more closely related to the European group (Bryc 2015). Only a few individuals from the Biracial sample classified as being more closely related to European American groups (see results). There is also no discernable pattern for how any of the Biracial individuals classified based on how their parents

identified. For example, of the two Biracial individuals who relayed that their mother is Biracial and their father is African American/Black did not classify similarly. One had a resulting rASUDAS classification of Western Eurasia and Edgar (2013) classification of European whereas the other had a rASUDAS result of Sub-Saharan Africa and Edgar (2013) result as New Mexico Hispanic. The remaining Biracial sample follows similar variance with no predictability for classification results. This thesis highlights that the dental patterns of Afridont and Eurodont (Irish 1998, 2013; Scott and Dorio 2010; Scott et al. 2013) and forensic methods (Edgar 2013 and Scott et al. 2016; 2018) may not be best suited to select for the Biracial group and additionally that we should reconsider how both parent groups are understood.

Results of this study accept that the frequency of dental traits observed in Biracial individuals differ from both parent populations, but is not intermediate. The geographic groups of the rASUDAS method are much more inclusive than the Edgar (2013) method (see Tables 2.3 and 2.4) which could help explain how and which trait frequencies are driving the classifications. Limitations of this study are acknowledged and expanded upon below as multiple confounding variables could be affecting results. First, the sample size in this study of the Biracial group is small and multiple confounding variables could be affecting results. The small sample is due to the need to analyze skeletal traits from living individuals because of the lacking skeletal collections of self-identified or known Biracial individuals (there are no such collections in the United States). Due to the lack of skeletal collections, this study relied on, and I am personally thankful for, volunteer participants who first self-identified as Biracial and then to allow their dental impressions to be taken. Given the time constraint of a graduate thesis program and the restrictions of

IRB for the main geographic location available to locate a sample (Texas State University, Central Texas, U.S.) it was impractical to presume acquiring a large or larger sample. A small sample limits the conclusions that can be drawn about which dental morphological trait are best able to characterize and classify a Biracial sample. It also limited the amount of data that could be accessed because dentition is subject to wear and alteration a small sample further narrowed how much applicable data was present.

The survey was implemented to establish confidence that each individual comprising the sample is Biracial. However, because these responses were self-reported there is not 100% certainty that they are accurate and reliable. This is due not to intentional falsification on the part of participants, but rather to the racial history of the United States and fluctuation of terminology that is used to described race and ancestry. As discussed in the introduction the qualifications for who constitutes as Black/African American and White/European American have differed throughout time in the U.S. Therefore, it is unknown how accurate and reliable ascribed group affiliations are regardless of if they are imposed by self or another. It should be noted that the skills of the anthropologists who collected data for the other samples in this study are not in question and the following is simply a statement expected in any study where data is not within complete control of study practitioners. The dental morphological trait scores for the African American and European American samples were not collected by the author and therefore subject to unknown. This study cannot be certain of any potential bias exhibited while the scorer collected data and thus these samples are subject to an unknown level of error.

Dental casts limited the the amount and type of traits that could be observed by

excluding observation of all root traits. Dental wear and alteration (such as fillings, dental wear, breakage, dental reconstructions) resulted in missing data for some of the traits. All of these can limit the data collected because the surfaces that contain the observable dental trait cannot be successfully analyzed. The break points and dichotomization for each dental trait was not consistent throughout each method and could have caused error when assessing the traits on the same field. Despite these limitations, this thesis showed how a sample of Biracial individuals classifies when using modern methodology for dental morphological traits.

Overall, this study indicates that current forensic estimation methods using dental traits failed to correctly sort Biracial individuals into the group corresponding to their bio-origin, self, and public identities. The bio-origin identity can be recognized within anthropology as ancestry estimates, a self-ancestral/race identity is how an individual views themselves in accordance with their society and culture, and a public ancestral-race identity is how an individual is perceived based on others' (the public) backgrounds, experiences, or implicit biases. These 3-components highlight that identity is multifaceted and dynamic. The results of the analysis completed here do not correspond to how the Biracial sample group views themselves nor with how the public would identify them. Due to this, providing the ancestry estimation could hinder identification of Biracial individuals given that it does not match with how they identified during life. If their biological profile states that they are singularly White/European descent or Black/African descent their profile could be excluded from the pool being narrowed down for identifying missing persons, because no one would be looking for them as singularly White/European descent or Black/African. How one self-identifies is also going to be

how that individual's family and friends identify them and further complicating cases of biracial individuals is that often the present as racially, ethnically, and ancestrally ambiguous so it is not guaranteed that someone in the public sphere (medical examiner other medico-legal workers) would appropriately associate them as being Biracial or any specific group in particular. This leads to individuals remaining unidentified. Further, this concept of 3-component identity is applicable to all cases involving the identification of non-White individuals given that some studies have shown existing forensic methods cannot correctly identify individuals belonging to non-White and non-Black groups.

Future research on Biracial groups would benefit from assessing a larger sample size and better comprehension of the histories behind the multiple facets that need to be considered when analyzing population infinity, including: the histories of geography, terminology, family, and methods. Further, considerations of the affects these components have on understanding classifications and how those classifications are relayed to the medico-legal environment. At present, the field of forensic anthropology should and is confronting its past and present issues rooted in systematic oppression (Bethard and DiGangi 2020) which is necessary in order to properly understand biological affinity of groups and especially for such a dynamic group as Biracial individuals.

V. CONCLUSION

In the year 2000 the U.S. census offered individuals the option to self-identify as more than one race for the first time. The last census, conducted in 2010, reported that 2.5% of the U.S. population self-identifies as two or more races (United States Census 2010). This group of individuals is referred to by the terms Biracial and/or Multiracial. In the United States a sample of self-identifying Biracial individuals had not been analyzed for the purposes of ancestral estimation prior to this study. Making the potential for ancestral misclassification of Biracial individuals is high when estimating the biological profile for the purposes of identification.

This study demonstrated the potential for misclassification of Biracial individuals using current dental morphological quantitative methods based on dental trait frequencies. The existing methods are not valid estimators/classifiers due to their misclassification of most Biracial individuals as European. These misclassifications can be highly attributed to the lack of reference samples necessary for comparative analysis. It should be acknowledged that this research uses a small sample size of thirteen, however, this sample is the first step to understanding how Biracial populations present among anthropological ancestral analysis.

Current anthropological ancestry estimation methods can not estimate ancestry for Biracial individuals and assign them to a Biracial group affiliation. Results showed that how a sample of Biracial individuals classifies is highly dependent on which method is being used. From a biological perspective, Biracial individual are most closely related to African American groups, which aligns with the socio-cultural history in the United

States and the legality behind the enforcement of positive assertive mating. However, if any of the individuals from the Biracial sample were reliant on current forensic methods to aid in their identification, their ancestry would be prevented from being accurately ascribed, especially since most classified as neither parent group or as European.

The results in this thesis can be broadly applied to the field of anthropology to reframe how the field thinks of identity. Language, context, socio-cultural frameworks, and biology all play a part in shaping group and individual identity. It is important for the anthropologists who practice estimating biological profiles to understand that multiple factors must be consider when reporting on these profiles. At this current time and from the results of this thesis it is clear that ancestry estimation can hinder identifications rather than positively narrow them down. This thesis raises necessary questions about how anthropologists approach race and ancestry and more so how do we think about the samples we are analyzing and how our analyses contribute to identification. To remedy the limitations of this study further research could be completed that assesses a larger sample sized of Biracial individuals.

According to the U.S. Census Bureau “the "two or more races" population is projected to be the fastest-growing” over the next several years. The Bureau predicts that in 2060 the Biracial and Multiracial populations will grow to represent 6.2 percent of the population, which is a significant increase (United States Census 2010). This increase can be contributed to more individuals embracing their mixed identity and identifying as such, and to increased gene flow provided by ease of global access. With the rise in these populations it has become a necessity to understand these admixed populations for the purposes of forensic identification. New methods are needed to accurately estimate

Biracial individuals and consideration should be allotted to exclude ancestry from the biological profile.

APPENDIX SECTION

APPENDIX A: HISTORY OF U.S. CENSUS RACIAL AND ETHNIC CATEGORIES

	1790	1800	1810	1820	1830	1840	1850	1860	1870	1880	1890	1900	1910	1920	1930	1940	1950	1960	1970	1980	1990	2000	2010
Slaves[1]	X	X	X	X	X	X																	
Free White Persons	X	X	X	X	X	X																	
Other Free Persons	X	X	X																				
Free Colored Persons				X	X	X																	
White							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Black							X	X	X	X	X		X	X									
Mulatto[2]							X	X	X	X	X		X	X									
Black Slaves							X	X			X												
Mulatto Slaves							X	X															
Indian								X	X	X	X	X	X	X	X	X							
Chinese									X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Quadroon[3]											X												
Octoroon[4]											X												
Japanese											X	X	X	X	X	X	X	X	X	X	X	X	X
Black (Negro[5])												X	X	X									
Other[6]															X	X	X						
Filipino														X	X	X	X	X	X	X	X	X	X
Korean														X	X	X			X	X	X	X	X
Hindu														X	X	X							
Negro															X	X	X	X					
Mexican															X				X				
American Indian																	X	X					
Aleut[7]																		X		X	X		
Eskimo																		X		X	X		
Hawaiian[8]																		X	X	X	X		
Part Hawaiian[9]																		X					
Negro, or Black																			X				
Other																			X	X			
(Indian) American																			X	X	X		
Central or South American																			X				
Puerto Rican																			X	X	X	X	X
Cuban																			X	X	X	X	X
Other Spanish																			X				
Black, or Negro																				X	X		
Asian Indian																				X	X	X	X
Vietnamese																				X	X	X	X
Samoan[10]																				X	X	X	X
Guamanian[11]																				X	X		
Mexican, Mexican American, Chicano[12]																				X	X	X	X
Other Spanish/Hispanic																				X	X		
Other race																					X		
Other Asian or Pacific Islander[13]																					X		
Black, African American or Negro																						X	X
Some other race																						X	X
American Indian or Alaska Native																							X
Other Asian																						X	X
Native Hawaiian																						X	X
Guamanian or Chamorro																						X	X
Other Pacific Islanders																						X	X
Other Spanish/Hispanic/Latino																						X	X

APPENDIX B: INTERNAL REVIEW BOARD APPLICATION: SYNOPSIS

SYNOPSIS

[Ancestry Estimation of Biracial Individuals Using Dental Morphological Traits]

1. Identify the sources of the potential subjects, derived materials or data. Describe the characteristics of the subject population, such as their anticipated number, age, sex, ethnic background, and state of health. Identify the criteria for inclusion or exclusion. Explain the rationale for the use of special classes of subjects, such as fetuses, pregnant women, children, institutionalized mentally disabled, prisoners, or others, especially those whose ability to give voluntary informed consent may be in question.

   The following response is a detailed description of the potential subjects to be used in Part 1 of this research.

   The potential subjects for this research are living individuals who are part of the population of Texas State University. These individuals are either students, faulty, or staff at Texas State University.

   All participants will be 18 years or older. These age criteria are necessary because it affords participants the ability to provide their consent. And specific to this research, individuals that are 18 years or older have fully erupted permanent dentition which is essential in observing ancestral traits in dentition.

   The biological sex of the participant's will be inclusive of both females and males. The inclusion of both sexes affords this research variability in trait expression and a wider view of overall biracial population.

   The ethnic background of the subjects can be any ethnic background.

   To be included in this study participants must be a part of the population of Texas State University, 18 years or older, and male or female. All individuals who do not meet the above listed criteria will not be included as subjects for Part 1 of this research.

   The following response is a detailed description of the potential subjects to be used in Part 2 of this research.

   The potential subjects for this research are living biracial individuals. The term biracial is inclusive of people who possess biological characteristics of two or more ancestries. Ancestry is socially and culturally correlated with race. The term race and terms known as races will be indicative of ancestry in this study. Therefore, individuals who self identify (provide responses) that they are two or more races will be considered eligible for participation in this research.

   All participants will be 18 years or older. These age criteria are necessary because it affords participants the ability to provide their consent. And specific to this research, individuals that are 18 years or older have fully erupted permanent dentition which is essential in observing ancestral traits in dentition.

   The biological sex of the participant's will be inclusive of both females and males. The inclusion of both sexes affords this research variability in trait expression and a wider view of overall biracial population.

The ethnic background of the subjects used in this research will exclusively those who can identify as biracial. Biracial individuals are those who posses a mixture of two ancestries.

The health of the individual must certify as the status of good. Please see the description below for the definition of what qualifies an individual to be in good health.

Good Health

The American Society of Anesthesiologists (ASA) Physical Status Classification System is commonly used to screen patients for their health status. Classifying patients as having good health will primarily be based on the completed health history followed by an oral interview conducted by the licensed dentist. Only ASA Class I and II patients will be accepted for this study following these guidelines. An exception to these rules is that pregnant persons (ASA Class II) will not be accepted.

-ASA I – A normal healthy patient; such as healthy, non-smoking, no or minimal alcohol use.

-ASA II – A patient with mild systemic disease; such as mild diseases only without substantive functional limitations. Examples include (but not limited to): current smoker, social alcohol drinker, pregnancy, obesity (30 < BMI < 40), well-controlled DM/HTN, mild lung disease.

-ASA III – A patient with severe systemic disease; Substantive functional limitations; One or more moderate to severe diseases. Examples include (but not limited to): poorly controlled DM or HTN, COPD, morbid obesity (BMI ≥40), active hepatitis, alcohol dependence or abuse, implanted pacemaker, moderate reduction of ejection fraction, ESRD undergoing regularly scheduled dialysis, premature infant PCA < 60 weeks, history (>3 months) of MI, CVA, TIA, or CAD/stents.

-ASA IV – A patient with severe systemic disease that is a constant threat to life; Examples include (but not limited to): recent (< 3 months) MI, CVA, TIA, or CAD/stents, ongoing cardiac ischemia or severe valve dysfunction, severe reduction of ejection fraction, sepsis, DIC, ARD or ESRD not undergoing regularly scheduled dialysis.

-ASA V – A moribund patient who is not expected to survive without operation.

-ASA VI – A declared brain-dead patient whose organs are removed for donor purposes.

To be included in this study participants must be 18 years or older, male or female, biracial (a racial and ancestral mixture of two or more races/ancestries), and in good health. All individuals who do not meet the above listed criteria will not be included as subjects for this research. The dentist, Dr. James Fancher reserves the right to deny any potential participant based on health criteria.

2. Describe the procedures for recruitment of subjects and the consent procedures to be followed. Include the circumstances under which consent will be solicited and obtained, who will seek it, the nature of information to be provided to prospective subjects, and the methods of documenting consent. (Include applicable **Consent**

Form (s) for review.) If written consent is not to be obtained, this should be clearly stated and justified.

3.

The following response outlines the procedures to be used to recruit potential subjects and the consent procedures that will be followed.

To recruit potential subject's emails will be sent to Texas State students, staff, and faculty who have an active Texas State email address with the intent to gather participants to take the survey. Recruitment events will be held at a central location on the Texas State University campus with the intent to recruit participants to take the survey. Additionally, all Texas State groups and organizations who affiliate themselves as multicultural will be contacted directly, through email, with the intent to gather participants to take the survey and provide details about this research with the intent to recruit participants who fall into the category of biracial and will voluntarily provide their dental impressions. A final recruitment procedure will be to offer incentives to participants.

Survey [Part 1]

The goal of the survey is to understand the diversity present at Texas State University. Understanding the demographics of the population at Texas State University is important to this research because it will indicate the possible sample size that is available. Currently, the Office of Institutional Research reports the student demographics of race/ethnicity for Fall 2015 to be: White-49.74%, Hispanic-33.21%, African-American-10.06%, Asian-2.50%, International-1.41%, Other-3.08%. Due to the restricted amount of options available (i.e. the option to self-identity as biracial is not available) for an individual to select the current reports offered by the University regarding the race/ethnicity of the student body is limited. This survey will allow participants a broader range of response options in order to fully report their self-identity by providing the option to select biracial and more than one race/ethnicity category. These responses will serve this research by permitting an understanding of the possible sample of potential subjects available at Texas State University. It will indicate approximately how many individuals are both willing to participate in research and how many of those willing participants fall into the category of biracial.

The survey will be built in a survey builder such as (but not limited to this application) SurveyMonkey. The Internal Review Board will be notified if the electronic distributor server, SurveyMonkey, is changed. The questions the survey will ask are included in the supporting documents section of this application (C_Clemmons_SurveyQuestions). The survey will request that participants provide their Texas State email address for future contact if that participant falls into the category of biracial.

The following disclosure statement will be included at the beginning of the survey prior to the participant responding to any questions:

"Your participation in this survey is completely voluntary and you may choose not to respond to any of the questions. Please be aware that if you consent to future contact the responses you provide may lead to future contact, by the researchers."

The following questions to request consent for future contact will be included in the survey:

19.	Can the Texas State Email address you provide be used to contact you if you have won one of the two $25 gift cards? (Please be aware that by clicking 'No' we will not be able to notify you of your winnings) -Yes -No
20.	Part 2 of this research Can the Texas State Email address you provide be used to contact you if you are eligible to participate in Part 2 of this research? (Please be aware that by clicking 'No' we will not be able to notify you if you are eligible for further research) -Yes -No
21.	What is your Texas State Email Address? (If you responded 'Yes' to the previous questions please provide your email address so you may be notified) [a fill-in box will be available for the response]

This disclosure statement is intended to make the participant aware that they may be contacted to participate in the research outlined in this application. This research would require participants to provide their dental impressions. The questions to request consent are intended to allow participants to either consent to or deny contact for Part 2 of the research outlined in this application. Participants will still have the opportunity to obtain the offered incentive (incentive is outlined in questions 2 and 8 of this synopsis).

Emails

Emails will be sent to Texas State students, staff, and faculty with the goal of recruiting participants to answer the questions provided in the survey. Access to the emails of students, staff, and faculty will be provided by the IT Assistance Center (ITAC) at Texas State University. Request for the email addresses will begin after approval from the IRB board to conduct this research has been given. The emails will not include personal identifiers, such as names, of the potential participants. The incentive of the opportunity to be entered into a drawing for one of two available $25 gift cards will be included in the emails sent out to recruit participants to take the survey. The emails are included in the supplemental documents: C_Clemmons_Specific_Email and C_Clemmons_General_Email. The general email is intended to be sent out to students, faculty, and staff who are not affiliated with a group or organization at Texas State University and the specific email is intended to address those who do.

Recruitment Events

The goal of the recruitment events is to peak the interest of students, staff, and faculty at Texas State University into the research that is being conducted. The

primary goal of these events is to gather participants to take the survey. As indicated above the survey will offer a better understanding of the diversity of the population attending Texas State University. A secondary goal of these events is the opportunity of public outreach with potential participants where I would be afforded the chance to discuss one-on-one the intent of the research and why it is important. At these events flyers will be handed out to participants of the event. The flyer will announce the research, the goals of the research and a link to and/or QR code for participants to access the survey. Included in the supplemental documents is a draft of what the flyer may look like (C_Clemmons_RecruitmentFlyer). Additionally, incentives will be offered to the participants of the events. These incentives include but are not limited to: candy, small trinkets, etc. Also, survey participants at these events will also be included in the email incentive to be entered in a drawing for the chance to win one of two $25 gift cards. Overall, the recruitment events may result in yielding higher voluntary participation among the students, staff, and faculty at Texas State University. These events will take place at a central location on campus such as, but not limited to, the Quad.

Access to Texas State University facilities for recruitment events will be provided by the Student Involvement department at Texas State University. Request for the Solicitation will begin after approval from the IRB board to conduct this research has been given. The term solicitation is defined by Texas State University as UPPS No. 07.04.03, "The purpose of this UPPS, or University Policy and Procedure Statement, is to set forth the University's policy regarding solicitation on both the San Marcos and Round Rock campuses. Solicitation on campus means the sale or offer for sale of any property, goods, products, or services, including the distribution of literature to promote a commercial message, including the receipt of or request for any gift or contribution."

Direct Contact

Direct contact, by email, to Texas State groups and organizations who affiliate themselves as multicultural will be made with the intent to recruit potential subjects. Potential groups and organizations include but are not limited to the graduate Student Color Alliance, the African Student Organization, the International Studies Club, the National Association for the Advancement of Colored People (NAACP), and the Pre-Medical/Pre-Dental Society. The limited list of groups here is meant to serve as an example for the extensive list of groups and organizations at Texas State University who identify as multicultural. All groups and organizations who affiliate themselves as multicultural will be included as targets for direct contact. The goal of directly contacting these groups is to gather participants to take the survey and provide details about this research directly to them with the intent to recruit participants who fall into the category of biracial and will voluntarily provide their dental impressions. Survey participants through direct contact will also be included in the email incentive to be entered in a drawing for the chance to win one of two $25 Visa gift cards.

Future Contact

All recruitment procedures are meant to recruit subjects who consent to participation in the survey and thus consent to being contacted if they identify as

biracial. Direct contact, through the email address provided by the participant, will be conducted by myself-the researcher. Only I will have access to the email addresses provided by participants. Only those who identify as biracial (please see the definition of biracial as indicated in question 1) will be contacted with the intent to have them consent to voluntary participate in this research and provide their dental impressions.

4. If your planned recruitment process involves emailing Texas State students, staff, faculty or other individuals using their active Texas State email address, provide details in the **Synopsis**. (In addition, the IRB will require a draft of your recruitment email, using the enclosed template and formatted as illustrated in the example in this document, submitted in addition to other required documents.

   The emails are included in the supplemental documents: C_Clemmons_Specific_Email and C_Clemmons_General_Email.

5. If you plan to distribute a survey to collect information directly from individuals who comprise a significant proportion of one or more Texas State affiliation groups, as defined in Section 04 of UPPS No. 04.01.02, Information Resources Identity and Access Management, you must follow the review and approval procedures outlined in UPPS No. 01.03.05, Administrative Surveys, and provide information in your **Synopsis** regarding review and approval. ?

   The survey is included in the supplemental documents: C_Clemmons_Survey.

6. Describe the project's methodology in detail. If applicable, detail the data collection procedures, the testing instruments, the intervention(s), etc. If using a survey, questionnaire, or interview, please provide a copy of the items or questions.

   The following response provides a description of this research's goals, methods and materials.

   Project Methodology

   This research focuses on the estimation of ancestry, which is critical to forming the biological profile of an individual. The biological profile allows forensic anthropologists to develop a picture of who an individual was when living with respect to age, sex, stature, and ancestry. The sample I plan to employ, through their voluntary participation, consists of biracial individuals. This population has yet to be explored in anthropological research. The questions this research sets out to answer are: Do biracial individuals have dental characteristics more representative of African American or European American ancestry? and If biracial individuals have dental characteristics more representative of either African American or European American ancestry is this due to the heritable ancestry of their biological fathers or mothers? (the ancestries defined in these questions are meant as an example and this research is not limited to the biracial identify of those ancestries alone). To answer these questions, I plan to analyze the dental morphological traits present on biracial individual's dentitions. The results of my research have interdisciplinary applications that will contribute to an understanding of population variation in a traditionally underrepresented sample.

Data Collection Procedures

Survey [Part 1]

To assess whether or an individual is a viable candidate for this study a survey will be employed which asks potential participants to answer questions about how they self-identify and how they would identify their parents and grandparents. Specifically, the survey asks individuals which race they classify themselves, parents, and grandparents as. Race is partially correlated with ancestry and used as a social and cultural tool by forensic anthropologists to infer on biological characteristics. This survey will assure that a potential participant is biracial and of mixed ancestry. The questions the survey will ask are included in the supporting documents section of this application (C_Clemmons_SurveyQuestions).

Dental Impressions [Part 2]

Dental impressions will be taken from a sample of living biracial individuals who voluntarily agree to participate in this study. Outlined in the Dental Practice Act, Sec 251.003. Practice of Dentistry, a person is practicing dentistry if she/he "prescribes, makes, or causes to be made or offers to prescribe, make, or cause to be made by any means an impression of any portion of the human mouth, teeth, gums, or jaws;" (p 35) therefore, licensed dentist Dr. James Fancher will act as key personnel on this project and will follow standard procedures for the practice of dentistry in the state of Texas as outlined in the Dental Practice Act and take the dental impressions of the participating individuals. Included in the attached documents is a copy of Dr. Fancher's license (C_Clemmons_Dr.FanchersLicense) and the Dental Practice Act (C_Clemmons_DentalPracticeAct).

Methods and Materials of Dental Impressions

Location

Participants will have their impressions taken at a clean and secure lab location. A possible location for the impression to be taken is in the lab located in building ELA on Texas State University Campus-San Marcos in room 226. This lab is in a central location on campus which will provide easy access for voluntary participants. Also, it contains all the necessities outlined but the Texas State Board of Dental Examiners for locations to conduct sessions. According to the Texas State Board of Dental Examiners before a session, in this instance a session to take dental impressions, begins at any location the location must have: access to properly functioning sterilization system, ready access to an adequate supply of potable water, and ready access to toilet facilities. The use of this location does require inspection by the Environmental Health, Safety, and Risk Management department at Texas State University. A statement from a representative of the Environmental Health, Safety, and Risk Management department at Texas State University is included in the attached documents of this application.

Records/Forms

The process involves participants filling out a Consent form, Health History form, and a HIPAA notice form. The Consent form and HIPAA notice will be combined to result in a single form for the participant to fill out (C_Clemmons_Consent_Form). All of the forms indicated in this section are

included in the attached documents.

Consent Form

The consent form allows participants to provide their permission that they are voluntarily willing to participate in this research by allowing impressions of their dentition to be taken by a licensed dentist. The HIPAA Notice form will insure the privacy and protection of participants given health information and their personal identifiers.

Health History Form

The Dental Practice Act requires dentists to acquire a case history of individuals as outlined in Sec 258.052. The case history includes an overall health history of the individual and requests the demographics, health history, and dental history of the individual. The health history form that will be used in this research is sanctioned by the American Dental Association and provided in the attached documents.

HIPAA Notice Form

The HIPAA Notice form will insure the privacy and protection of participants given health information and their personal identifiers. The HIPAA Notice is included within the Consent form.

These forms can be found in the attached documents of this application.

Physical Evaluation

Once the paperwork is completed the practicing dentist will perform a physical evaluation. As outlined in the Dental Practice Act Sec. 258.052 and the Texas Administrative Code, Title 22, Part 5, Chapter 108A, Rule 108.7, it is required that an initial medical history and a limited physical evaluation be performed for all dental patients (dental patients in this research are the participants who consented to proving their dental impressions). These rules define a medical history and limited physical examination as:
(3)(A) The medical history shall include, but shall not necessarily be limited to, known allergies to drugs, serious illness, current medications, previous hospitalizations an significant surgery, and a review of the physiologic systems obtained by patient history. A "check list," for consistency, may be utilized in obtaining initial information. The dentist shall review the medical history with the patient at any time a reasonable and prudent dentist would do so under the same or similar circumstances. (3)(B) The limited physical examination shall include, but shall not necessarily be limited to, measurement of blood pressure and pulse/heart rate. Blood pressure and pulse/heart rate measurements are not required to be taken on any patient twelve (12) years of age or younger, unless the patient's medical condition or history indicate such a need. If the participant is in overall good health (please refer to response one for the definition of good health) the practicing dentist will proceed to take their dental impressions.

Impressions

An impression is used in dentistry to make an exact replica of the patients (participants in this research) teeth and surrounding tissues in the mouth (i.e. gums). The impression material used to take the impression, Alginate (a detailed list of materials is defined further in this section), forms an imprint (i.e. a ‘negative’ mold) of the participant’s teeth and gums. This impression can then be

used to make a cast (i.e. a 'positive model) of the participant's dentition.

A minimum of two sets of impressions will be taken, one of the upper dentition and one of the lower dentition, each set will require the participant have an impression tray held in their mouth on their dentition for approximately 30 to 60 seconds. Potential risks and procedures for minimizing potential risks are outlined in the following sections 6 and 7.

Materials

Impression Materials

Dental impressions will be collected for biracial individuals. Jeltrate Alginate will be used as the impression material. Alginate Powder Scoop & Water Measure Set will be used to measure the appropriate amount of impression material to water ratio for proper mixing. Spatulas/Knifes-Alginate Spatula, Spatula Plaster 8R, Spatula Lab Rigid, Knife-Lab Plaster (to be used on impression material), Impression Tray Upper-Sizes S,M,L (used to hold the impression material and place on the dentition for impression), Impression Tray Lower-Sizes S,M,L (used to hold the impression material and place on the dentition for impression), and Mixing bowls (to mix impression materials), Dermacea gauze sponge (for use by the dentist), Disposable mouth mirrors (for use by the dentist to analyze patient dentition), Dri-gard towels (for the patient to wear during the impressions), Miratray adhesive spray (for stronger adherence of the impression material to the impression trays).

Sterilization and Disinfectant Materials

Requirements of sterilization and disinfectant materials will be further addressed by the by the representative of the Environmental Health, Safety, and Risk Management department at Texas State University at the inspection of the location. Below is a preliminary description of the materials that may be used to sterilize and disinfect the location.

The following is a description of the materials:

-Sodium hypochlorite at 5-6% (diluted household bleach) is a very adequate surface disinfectant that is cheap with few side effects. This is good for durable surfaces such as table tops and chairs. Easy to prepare, easy to wipe down. The only limitation is that it can be corrosive.

-Plastic barriers to cover table tops and other surfaces. This is simple to do with plastic backed towels and saran wrap. Everything is disposable between patient encounters. These are easy to use and also very effective.

-Glutaraldehyde can be used as a disinfectant (see the Steps to Taking an Impression). This is considered a high-level disinfectant that can also be used for surfaces. The problem is it may not be tolerated well by a small number of people, so care of use with this disinfectant will be practiced.

-Impressions will be placed in a sealable bag and with solution to sterilize them will be added.

If further explanation of how the location of the dental impressions will be sterilized and disinfected is required it will be addressed at the inspection of the location by the representative of the Environmental Health, Safety, and Risk Management department at Texas State University. Approval of this inspection will be submitted to the Internal Review Board once it is complete.

Steps to Taking an Impression

1. Arrange all impression materials and supplies in a unit dose and/or disposable manner so that no cross contamination can occur.
2. The impression taker (Dr. Fancher) will follow standard barrier technique (gloves and paper gown).
3. The proper size disposable impression trays will be selected for each patient.
4. Alginate impression material will be mixed according to the manufacturer's directions.
5. The alginate material will be placed in the lower tray.
6. The loaded impression tray will be inserted over the patient's lower teeth and comfortably seated.
7. When the alginate material has set (30 -90 seconds) the impression will be removed from the patients mouth.
8. Steps #4 – 7 will be repeated for the maxillary impression.
9. The impressions will be placed in zip lock bags, sprayed with sterilant/high level disinfectant (2.5-3.4% Glutaraldehyde solution).
10. After a minimum of 10 minutes the impressions will be removed from the sealed bag, rinsed in tap water, and poured with dental stone.
11. If at any time the patient may experience difficulty the impression procedure will be terminated.

Approximate total time participants will be participating is thirty minutes.

Storage of Materials and Records

Materials: Impressions and Casts

The impressions and completed casts made from the impressions will become property of the researcher, Chaunesey Clemmons, and Texas State University. Access to the impressions and casts will be exclusive to the researcher, Chaunesey Clemmons, the supervisor of this research, Dr. Daniel Wescott, and the key personnel of this project, Dr. James Fancher. All materials will be stored at the Grady Early Forensic Anthropology Research Laboratory which is a facility run by the Forensic Anthropology Center at Texas State. The casts from the impressions will not be kept locked for the purposes of continually examination. The impressions and casts will solely be identified by a number. This number will only be associated with the racial history of the participant but not with key identifiers of the participant. Only those with access to the records have the ability to trace the number affiliated with an impression or cast to a participants' key identifiers.

Records: Forms

The following forms which will be kept as records on each participant are the survey, a Consent form, a Health History form, and a HIPAA notice form. The Consent, Health History, and HIPAA notice forms are considered supplemental documents and will be submitted with the IRB application. The records collected on each participant will be property of both the Texas State University and the practicing dentist, Dr. James Fancher for five years. Access to the health records will be exclusive to the researcher, Chaunesey Clemmons, the supervisor of this

research, Dr. Daniel Wescott, and the key personnel on this project, Dr. James Fancher. All materials will be stored at the Grady Early Forensic Anthropology Research Laboratory which is a facility run by the Forensic Anthropology Center at Texas State. Records of participants will be kept in a locked cabinet in this facility.

Research to be Conducted from Materials

Goal of This Research

The main goal of this study is to understand the genetic admixture of dental traits in a biracial population. This research sets out to address questions about trait dominance and trait heritability. This research sets out to address questions about trait dominance and trait heritability, and which traits are expected to be present in higher frequencies in biracial individuals.

The first hypothesis seeks to recognize which traits from the parent populations are more frequent in biracial individuals. If the biracial individual has traits that are more dominant from a single ancestry this will inform on trait dominance. The second hypothesis seeks to answer questions about whether dominant traits are more likely to be inherited from the mother or father.

Data Collection

Dental morphological traits will be observed on the maxillae and mandible of a sample of European American, African American, and biracial (mixed ancestry) individuals. Observation of the traits will be done in natural light, with a 10X hand lens (Alsoleihat 2013). Dental impressions will be collected from the biracial sample groups and casts will be molded for analysis.

All dental samples will be observed/scored. For each sample a full suite of traits will be scored but only certain traits will be used in the study. Each trait list has been derived from previous research and is in accordance with the Arizona State University dental anthropology system (ASUDAS) (Turner et al. 1991) and the expression count method (Turner 1985). The traits in the list below are those that will be used in the study, however, not limited to these traits.\

Trait List

TRAIT	DESCRIPTION	TOOTH
Anterior Fovea	Located on the anterior occlusal surface.	Lower 1st molar.
Canine Distal Accessory Ridge	Located on the distolingual fossa between the distolingual marginal ridge and tooth apex.	Upper & lower canines.
Canine Mesial Ridge	Located on the mesiolingual marginal ridge.	Upper canine
Carabelli's Trait	Located on the lingual surface of the mesiolingual cusp.	Upper molars
Cusp 5 (Metaconcule)	5th cusp located in the distal fovea of the upper molars between metacone and hypocone.	Upper molars.
Cusp 5 (Hypoconulid)	Located on the distal occlusal aspect.	Lower molars.
Cusp 6	Located on the distal fovea of the lower molars lingual to cusp 5.	Lower molars.
Cusp 7 (Metaconulid)	Located between cusps 2 and 4 in the lingual groove.	Lower molars.
Distosagittal Ridge	An extension from the buccal cusp to the distal occlusal border at or near the sagittal sulcus.	Upper 1st premolar.
Distal Accessory Ridge	Located between the apex of the tooth and the distolingual marginal ridge in the distolingual fossa.	Upper and lower canines.
Deflecting Wrinkle	It is a medial ridge variation on cusp 2.	Lower first molar.
Double-Shoveling	The presence of labial marginal ridges.	Upper & lower incisors, canine, 1st premolar.
Distal Trigonid Crest	A bridge between cusps 1 & 2.	Lower molars.
Enamel Extensions	Located in an apical direction as projections of the enamel border.	Upper molars & premolars.
Groove Pattern	Pattern of the groove.	Lower molars.
Hypocone	Cusp 4, distolingual cusp.	Upper molars.
Interruption Groove	Grooves are located across the cingulum and sometimes continue down the root.	Upper incisors.
Labial Convexity	Located on the labial surface, a marked degree of convexity.	Upper incisors.
Lower Premolar Cusp Variation	Considers the size and number of lingual cusps.	Lower premolars.
Metacone	Cusp 3, distobuccal cusp.	Upper molars.
Odontome	Located on the premolar occlusal surface as any pin-sized, spike-shaped enamel and dentin projection.	Upper & lower premolars.
Parastyle	Located on the buccal surface	Upper molars.

	of the mesiobuccal cusp.	
Peg-Shaped Incisor	Tooth is reduced in size and peg-shaped.	Upper lateral incisor.
Peg-Shaped Molar	Tooth is small and is peg-shaped.	Upper third molar.
Premolar Lingual Cusp Variation	Variation of the number of lingual cusps and their size.	Lower premolars.
Premolar Mesial and Distal Accessory Cusps	Located at the mesial and/or distal ends of the sagittal grooves.	Upper Premolars.
Protostylid	Located on the buccal surface of cusp 1.	Lower molars.
Shoveling	It is the presence of lingual marginal ridges.	Upper & lower incisors, canine.
Tricusped Premolars	Three cusps.	Upper premolars.
Tuberculum dentale	Located on the lingual surface in the cingular region.	Upper incisors and canine.
Winging	Bilateral, Unilateral, Straight, Counter.	Upper central incisors.
(Turner et al. 1991, Edgar 2002)		

This study seeks to examine dental morphological trait frequencies in a sample of individuals of biracial/mixed ancestry.

7. Describe any potential risks — physical, psychological, social, legal or other — and state their likelihood and seriousness. Describe alternative methods, if any, that were considered and why they will not be used.

   The following response specifies the potential risks involved in collecting the data for this research.

   Potential risks to participating individuals and to the practicing dentist are minimal. The potential risk of filling out the survey used to both asses the diversity of Texas State University and to identify qualifying members for participation is the possibility that participants may take offense to the questions asked and possible misunderstandings of the purpose of the proposed questions may occur. The potential risks of having dental impressions taken to the participating individuals are: vomiting, nausea, allergic reaction, choking, acquiring infection, and the potential to swallow excess impression material. Participants may also experience slight discomfort while the impression material is in their mouth. The taking of dental impressions is invasive, however, when taken by a licensed dentist practicing under standard procedures for the practice of dentistry risks are improbable. The procedures for protecting against and minimizing any potential risks is outlined the below section.

8. Describe the procedures for protecting against or minimizing any potential risks and include an assessment of the likely effectiveness of those procedures. Include a discussion of confidentiality safeguards, where relevant, and arrangements for providing mental health or medical treatment, if needed.

   The following response detail the procedures for protecting and minimizing against the potential risks involved.

The potential risks involved in taking the dental impressions is minimized by having a licensed dentist take the impressions. Dr. James Fancher, a licensed dentist, has obtained the proper education and training to be a licensed dentist in the state if Texas thus he will follow standard procedures for the practice of dentistry in the state of Texas. A licensed dentist taking the impressions will help minimize the following risks: vomiting, the swallowing of excess impression material, and choking. Health histories of the participants will be collected in order to avoid the potential risks of the participant having an allergic reaction and/or becoming sick during the procedure, vomiting and nausea. To avoid the risk of infection disposable impression trays will be used to eliminate the possibility of cross contamination between patients and the practicing dentist will wear gloves when interacting with the participant as to maintain a constant barrier in order to prevent contamination.

There is no serious risk to the participant in taking the survey, but some of the questions may be personal. The questions asked by the survey are not outside the normal societal and cultural realm of American demographic inquiry. Participants will have to have answered the same or similar questions when filling out their demographics for normal applications that asses such information. These applications are inclusive of but not limited to university entrance exams, university applications, census, and medical and legal documents. Therefore, the potential risks of inquiring about the participant's demographics is minimized because the questions do not fall outside the normal range of inquiry requested by society.

9. Describe and assess the potential benefits to be gained by the subjects, as well as the benefits that may accrue to society in general as a result of the proposed study.

   The following response details the potential benefits to be gained by the participating subjects.

   Potential benefits for the participating subjects is their contribution to the scientific community. The dental impressions they provide will allow the opportunity for new research within the field of anthropology. This new research will analyze a population that has never been studied before. The analysis made on the dental morphological traits provided by the impressions will help to answer questions about human variation and genetic heritability. Incentives for the participants include an opportunity to win a $25 gift card (there will be two that are possible to obtain) by participating in the survey. Additionally, those who attend the recruitment events will be offered incentives that include but are not limited to: candy, small trinkets, etc. Furthermore, subjects will be participating in research that will encourage future studies on varying populations that have yet to be explored. The importance of including diverse populations in human studies is so that science, specifically the field of anthropology, can help to accurately answer questions about human evolution, human diversity, and genetic diversity.
10. Clearly describe any compensation to be offered/provided to the participants. If extra credit is provided as an incentive, include the percentage of extra credit in relation to the total points offered in the class. Also, if extra credit is provided, describe alternatives to participation in your research for earning extra credit.

The following response details the incentives that will be offered to participating subjects.

An incentive of the opportunity to be entered in for a chance to obtain one of two $25 Visa gift cards is being offered to participants of the survey used in this research. In addition, those who attend the recruitment events will be offered incentives that include but are not limited to: candy, small trinkets, etc.

11. Discuss the risks in relation to the anticipated benefits to the subjects and society.

    The following response is a discussion of the risks in relation to the benefits to the participants and society.

    The anticipated benefits of the participant's contributions to the field of anthropology outweigh the potential risks. Every precaution and procedure to minimize potential risks will be carried out so to effectively limit the risks that they are improbable to occur. The data provided by the participants will benefit overall society. The field of anthropology strives to understand humans and specifically, forensic anthropologists work to accurately identify and employ methods useful in legal settings for the identification of individuals. This research will explore the characteristics of the biracial population and, ultimately, provide results that will lend themselves to a better understanding of human variation. Differences found in human variation allow forensic anthropologists to narrow down prospects of possible identity with the ultimate goal of identifying an individual. Social applications of our forensic anthropology are found in a medico-legal setting.
12. Identify the specific sites/agencies to be used as well as approval status. Include copies of approval letters from agencies to be used (note: these are required for final approval). If they are not available at the time of IRB review, approval of the proposal will be contingent upon their receipt.

    Attached is an approval letter from the Chair of the Anthropology department stating that I am able to use room 226 which is located in the Evans Liberal Arts building at the Texas State University-San Marcos campus. The use of this location does require inspection by the Environmental Health, Safety, and Risk Management department at Texas State University. A statement from a representative of the Environmental Health, Safety, and Risk Management department at Texas State University is included in the attached documents of this application.
13. If you are a student, indicate the relationship of the proposal to your program of work and identify your supervising/sponsor faculty member.
    The following response indicates the relationship of the proposal to my program of work and identifies my supervising/sponsor faculty member.

    As a graduate student at Texas State University on track to complete a Master's thesis in Biological Anthropology this proposal is related to this work in that it will grant approval for me to begin collecting data from living subjects. The data collected, survey responses and dental impressions, will be used to analyze the ancestry of biracial individuals. The intent of this proposal is to gain approval

from the Internal Review Board for data collection from living individuals. The supervising/sponsor faculty member of this research is Dr. Daniel Wescott. The attached documents include a copy of his curriculum vitae which details his qualifications.

14. In the case of student projects, pilot studies, theses, or dissertations, evidence of approval of Supervising Professor or Faculty Sponsor should be included. Thesis and dissertation proposals must be approved by the student's committee before proceeding to the IRB for review.

    The research conducted on the data collected will result in a Masters Thesis in Biological Anthropology. The thesis proposal has not yet been approved by my committee. The defense date for my thesis proposal is set for April 19, 2016. In the attached documents is a statement from my thesis advisor/chair of my committee, Dr. Daniel Wescott, which states his support to go forward in conducting this research.
15. If the proposed study has been approved by another IRB, attach a copy of the letter verifying approval/disapproval and any related correspondence. If the proposed study has not been reviewed/approved by another IRB, please state this explicitly.

    The proposed study has not been reviewed or approved by another IRB.
16. Identify all individuals who will have access, during or after completion, to the results of this study, whether they be published or unpublished.

    Individuals who have access to the results of this study include myself, Dr. Daniel Wescott, Dr. James Fancher, Texas State University and all its departments, students, faculty, and staff, the scientific community, and the general public.
17. Provide date of completion of the required CITI training on the protection of human subjects. Applicants must provide training dates for themselves and for supervising faculty member. All training must be current and not expired.

    The supplemental documents include date of completion receipts of the CITI training for myself, Dr. Daniel Wescott, and Dr. James Fancher.

APPENDIX C: CONSENT

Participants were allotted opportunities to consent to participate at each stage of the research,and reserved the right to cease participation in this study at any time.

In the survey at the onset and questions 19, 20 and 21; In-person during Part 2 of this research

In the initial survey Question 20 asked individuals if they were willing to participate in Part 2 of this research. Part 2 of the research is defined for IRB purposes as being contacted to have their dental impressions taken. Biracial individuals who met all criteria of the survey were contacted via email for participation in Part 2 of the research and asked to come into a secure lab space during a previously arranged time to have their impressions taken. Upon arriving individuals were asked to complete a consent form which outlined the study and the requirements of the participant including any and all potential risks to them (Appendix C). The consent form included a Health Insurance Portability and Accountability Act of 1996 (HIPAA) notice detailing the regulations of HIPAA. This notice provided the participant with information regarding the protection of their personal health information and insured the privacy and protection of participants given health information and their personal identifiers. The conclusion of the form requests the participant to sign, providing their consent, if they: read the form and decided that they would participate in the described project; understood the general purposes of the research, the particulars of involvement, and the possible risks; and that they understood they were able withdraw at any time. Their signature of consent was accompanied by my signature, the researcher, who is obtaining consent.

CONSENT FORM

TEXAS STATE ®

Informed Consent Form

Study Title: Ancestry Estimation of Biracial Individuals Using Dental Morphological Traits

Principal Investigator: Chaunesey Clemmons

Co-Investigator/Faculty Advisor: Dr. Daniel Wescott

Sponsor: Dr. James Fancher

This consent form will give you the information you will need to understand why this research study is being done and why you are being invited to participate. It will also describe what you will need to do to participate as well as any known risks, inconveniences or discomforts that you may have while participating. We encourage you to ask questions at any time. If you decide to participate, you will be asked to sign this form and it will be a record of your agreement to participate. You will be given a copy of this form to keep.

Purpose and Background

You are being asked to be part of a research project. We are trying to learn more about the ancestral representation of biracial individuals. If you agree to be part of this research, we will ask you to provide answers to questions of your health history, allow a physical evaluation that will access your health to be conducted, and for a licensed dentist to take dental impressions from you. The entire process should take approximately 45 minutes to complete. The research is being conducted by Chaunesey Clemmons of Texas State University, cmc324@txstate.edu, (402-315-8368) and Dr. James Fancher, jpfancher@earthlink.net, (210-896-8578) .

Risks and Discomforts

We don't think that there are any serious risks to you. General risks of having your dental impressions taken include: vomiting, the swallowing of excess impression material, and choking. You may also feel slight discomfort while the impression material is in your mouth.

You may choose not to continue with the process for any reason at any time.

Benefits and Alternatives

There are no direct benefits to you for participating in this research. However, society may benefit from the results. You will not receive anything for participating.

Extent of Confidentiality

The impressions are confidential; we are recording your name solely for the use of an identifier on your health form. The impressions will only be associated with an assigned number. We will keep the health record in a locked file cabinet at Texas State University for five years and then we will destroy the records. Only the researcher, Chaunesey Clemmons, the supervisor of this research, Dr. Daniel Wescott, and the key personnel on this project, Dr. James Fancher will have access to the health records.

HIPPA

Will health information about you be created, used or shared with others during this study?

State and federal laws, including the Health Insurance Portability and Accountability Act (HIPAA), require researchers to protect your health information. This section of this form describes how researchers, with your authorization (permission), may use and release (disclose or share) your protected health information in this research study. By signing this form you are authorizing Chaunesey Clemmons, Dr. James Fancher, and Dr. Daniel Wescott to create, get, use, store, and share protected health information that identifies you for the purposes of this research.

The health information includes all information created and/or collected during the research as described within this consent form and/or any health information in your medical record that is needed for the research and that specifically includes [personal identifiers such as name, address, telephone number, social security number, and/or

medical record number; demographic information, e.g. age, race, gender; the results of physical exams, medical history, past medical conditions, or medications, or illnesses or hospitalizations that may occur during participation in the research].

During the conduct of the research, the researchers may use or share your health information:

- With each other and with other researchers involved with the study;
- With law enforcement or other agencies, when required by law;
- With representatives of government agencies (i.e., Food and Drug Administration), review boards including Texas State University Institutional Review Board and other persons who watch over the safety, effectiveness, and conduct of research; and

If all information that identifies you is removed from your health information, the remaining information is no longer subject to the limits of this Authorization or to the HIPAA privacy laws. Therefore, the de-identified information may be used and released by the researchers (as permitted by law) for other purposes, such as other research projects.

You will not have access to the health information related to this research study until the study is done. However, this information is available to your doctor in the case of an emergency. At the end of the study, you will again have access to health information that is normally within your medical records (treatment, insurance and billing information). However, the researcher may not give you access to the research records or information that is not usually kept in your medical record, as it is not required by HIPAA.

How will your health information be protected?

The researchers agree to protect your health information and will only share this information as described within this research consent/authorization form.

When your health information is given to people outside of the research study, those agencies that receive your health information may not be required by federal privacy laws (such as the Privacy Rule) to protect it. They may also share your information with others without your permission, if permitted by laws that they have to follow.

Can I withdraw or be removed from the study?

Your Authorization for release of health information for this research study [*expires at the end of the study*], but can be canceled sooner if you decide to withdraw your permission.

You may change your mind and cancel this Authorization at any time. To cancel this Authorization, you must write to: [*Chaunesey Clemmons: Department of Anthropology 601 University Drive San Marcos, TX 78666*].

If you cancel this Authorization, you may no longer be allowed to take part in the

research study. Even if you cancel this Authorization, the researchers may still use and disclose health information they have already obtained as necessary to maintain the integrity and reliability of the research and to report any adverse (bad) effects that may have happened to you.

The dentist, Dr. James Fancher, has authority to exclude any participants, based on health criteria, from this study.

What are my rights as a research subject?
If you have questions or concerns regarding your privacy rights under HIPAA, you should contact Texas State University IRB at Ph.: (512) 245-2334.

Right to Refuse to Sign this Authorization
You do not have to sign this Consent/Authorization. However, because your health information is required for research participation, you cannot be in this research study if you do not sign this form. If you decide not to sign this Consent/Authorization form, it will only mean you cannot take part in this research. Not signing this form will not affect your non-research related treatment, payment or enrollment in any health plans or your eligibility for other medical benefits.

Participation is Voluntary
Your participation is voluntary, and refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You may discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled.

Questions
If you have any questions or concerns about your participation in this study, you may contact the Principal Investigator, Chaunesey Clemmons: cmc324@txstate.edu, (402-315-8368) and Dr. James Fancher , jpfancher@earthlink.net, (210-896-8578) .

This project [IRB #2016S673] was approved by the Texas State IRB on [05-31-16]. Pertinent questions or concerns about the research, research participants' rights, and/or research-related injuries to participants should be directed to the IRB chair, Dr. Jon Lasser 512-245-3413 - (lasser@txstate.edu) or to Monica Gonzales, IRB Regulatory Manager 512-245-2334 – (mailto:MEG201@txstate.edu).

If requested, a summary of the findings will be provided to participants upon completion of the study. To access results of the study, please contact Chaunesey Clemmons through email cmc324@txstate.edu.

DOCUMENTATION OF CONSENT
I have read this form and decided that I will participate in the project described above. Its general purposes, the particulars of involvement and possible risks have been explained to my satisfaction. I understand I can withdraw at any time.

☐ By checking this box you agree to be photographed during your participation in Part 2 of this research. The photographs obtained may be used in a comprehensive body of work that will advocate for the value of work done by the Forensic Anthropology Center at Texas State University.

Printed Name of Study Participant	**Signature** of Study Participant	Date

Signature of Person Obtaining Consent	Date

APPENDIX D: SURVEY TO ASSESS CANDIDACY

The survey was used to assure that a potential participant complied with the definition of Biracial used in this study. It was dispersed via email using the software system *Qualtrics* to the entirety of the student, staff, and faculty population at Texas State University. Individuals recruited via social media and personal contact completed the survey via email or in person. The total number of individuals who were sent the survey via email was 90,722. The survey was completely voluntary and participants could choose not to answer the questions and those who chose to participate remain confidential. The questions in the survey ask individuals which social race they classify themselves, parents, and grandparents. Additionally, it asks demographic questions about the individuals including their country of origin, age, and year of education. The survey was accessed electronically unless taken in person in which case the participant received a hard copy. A total of 6,775 surveys were started with a completion of recorded responses to the survey being 6,151. Responses were then filtered through a series of steps to determine eligibility of the respondent. The first step was to select individuals who answered Question 5 of the survey ("What is your race/ethnicity?") with the response "Biracial/Biethnic (I am two races)." A total of 561 individuals answered that they were Biracial. These respondents were further filtered to agree with the parameters of this study to solely include Biracial individuals of a Black/White racial mix (i.e. African/African American and European/European American ancestral mixture). This narrowed down the possible participant list to 59 individuals. The participants were filtered again to exclude all individuals who answered "No" or did not respond to Question 20 ("Can the Texas State Email address you provide be used to contact you if you are eligible to participate in Part 2 of this research?"). This step narrowed down the potential participant pool to 51 individuals. Finally, the survey responses of the 51 individuals were further reviewed to insure that the respondent indicated that one parent was Black/African American and one parent was White/European American.

SURVEY

Your participation in this survey is completely voluntary and you may choose not to respond to any of the questions. Please be aware that if you consent to future contact the responses you provide may lead to future contact, by the researchers.
1. What country were you born in? [a fill-in box was available for the response]
2. If you were born in the United States which state were you born in? [a fill-in box was available for the response]
3. In what age category do you fall into? -18-22 -23-27 -28-32 -33-37 -38-42 -43-47 -48 +
4. What is your year of education?

-Freshman -Sophomore -Junior -Senior -Graduate -Not a student, I am faculty or staff
5. What is your race/ethnicity? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -Biracial/Biethnic (I am two races) -Multiracial/Multiethnic (I am more than two races) -A race/ethnicity not indicated above
6. If you selected biracial/biethnic or multiracial/multiethnic, what are your races/ethnicities? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -A race/ethnicity not indicated above *-I did not select these categories*
7. What is the race/ ethnicity of your biological mother? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -Biracial (they are two races) -Multiracial (they are more than two races) -A race/ethnicity not indicated above
8. If you selected biracial/biethnic or multiracial/multiethnic, what is their races/ethnicities? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -A race/ethnicity not indicated above *-I did not select these categories*
9. What is the race/ethnicity of your biological father? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -Biracial (they are two races) -Multiracial (they are more than two races) -A race/ethnicity not indicated above
10. If you selected biracial/biethnic or multiracial/multiethnic, what is their races/ethnicities? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -A race/ethnicity not indicated above

-*I did not select these categories*
11. What is the race/ ethnicity of your biological maternal grandmother? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -Biracial (they are two races) -Multiracial (they are more than two races) -A race/ethnicity not indicated above
12. If you selected biracial/biethnic or multiracial/multiethnic, what is their races/ethnicities? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -A race/ethnicity not indicated above -*I did not select these categories*
13. What is the race/ ethnicity of your biological maternal grandfather? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -Biracial (they are two races) -Multiracial (they are more than two races) -A race/ethnicity not indicated above
14. If you selected biracial/biethnic or multiracial/multiethnic, what is their races/ethnicities? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -A race/ethnicity not indicated above -*I did not select these categories*
15. What is the race/ ethnicity of your biological paternal grandmother? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -Biracial (they are two races) -Multiracial (they are more than two races) -A race/ethnicity not indicated above
16. If you selected biracial/biethnic or multiracial/multiethnic, what is their races/ethnicities? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -A race/ethnicity not indicated above -*I did not select these categories*
17. What is the race/ ethnicity of your biological paternal grandfather? -White -African American

-Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -Biracial (they are two races) -Multiracial (they are more than two races) -A race/ethnicity not indicated above
18. If you selected biracial/biethnic or multiracial/multiethnic, what is their races/ethnicities? -White -African American -Asian -American Indian and Alaskan Native -Native Hawaiian and Pacific Islander -A race/ethnicity not indicated above -*I did not select these categories*
19. Can the Texas State Email address you provide be used to contact you if you have won one of the two $25 gift cards? (Please be aware that by clicking 'No' we will not be able to notify you of your winnings) -Yes -No
Part 2 of this research 20. Can the Texas State Email address you provide be used to contact you if you are eligible to participate in Part 2 of this research? (Please be aware that by clicking 'No' we will not be able to notify you if you are eligible for further research) -Yes -No
21. What is your Texas State Email Address? (If you responded 'Yes' to the previous questions please provide your email address so you may be notified) [a fill-in box was available for the response]
Thank you for your participation.

APPENDIX E: HEALTH HISTORY

The selected Part 2 participants were asked to complete a health history form to insure they were fit to participate. The Texas Dental Practice Act requires dentists to acquire a case history of individuals as outlined in Sec 258.052 (Texas Occupations Code, Chapter 251) which includes demographic information and the overall and dental health of the individual. The health history form that was used in this research is sanctioned by the American Dental Association and provided below.

Health History Form

ADA
American Dental Association
www.ada.org

E-mail: Today's Date:

As required by law, our office adheres to written policies and procedures to protect the privacy of information about you that we create, receive or maintain. Your answers are for our records only and will be kept confidential subject to applicable laws. Please note that you will be asked some questions about your responses to this questionnaire and there may be additional questions concerning your health. This information is vital to allow us to provide appropriate care for you. This office does not use this information to discriminate.

Name: (Last, First, Middle) | Home Phone: *Include area code* ( ) | Business/Cell Phone: *Include area code* ( )

Address: (Mailing address) | City: | State: | Zip:

Occupation: | Height: | Weight: | Date of birth: | Sex: M F

SS# or Patient ID: | Emergency Contact: | Relationship: | Home Phone: ( ) | Cell Phone: ( ) *Include area codes*

If you are completing this form for another person, what is your relationship to that person?

Your Name | Relationship

Do you have any of the following diseases or problems: *(Check DK if you Don't Know the answer to the question)*	Yes	No	DK
Active Tuberculosis	☐	☐	☐
Persistent cough greater than a 3 week duration	☐	☐	☐
Cough that produces blood	☐	☐	☐
Been exposed to anyone with tuberculosis	☐	☐	☐

If you answer yes to any of the 4 items above, please stop and return this form to the receptionist.

Dental Information *For the following questions, please mark (X) your responses to the following questions.*

	Yes	No	DK
Do your gums bleed when you brush or floss?	☐	☐	☐
Are your teeth sensitive to cold, hot, sweets or pressure?	☐	☐	☐
Does food or floss catch between your teeth?	☐	☐	☐
Is your mouth dry?	☐	☐	☐
Have you had any periodontal (gum) treatments?	☐	☐	☐
Have you ever had orthodontic (braces) treatment?	☐	☐	☐
Have you had any problems associated with previous dental treatment?	☐	☐	☐
Is your home water supply fluoridated?	☐	☐	☐
Do you drink bottled or filtered water?	☐	☐	☐
If yes, how often? Circle one: DAILY / WEEKLY / OCCASIONALLY			
Are you currently experiencing dental pain or discomfort?	☐	☐	☐

	Yes	No	DK
Do you have earaches or neck pains?	☐	☐	☐
Do you have any clicking, popping or discomfort in the jaw?	☐	☐	☐
Do you brux or grind your teeth?	☐	☐	☐
Do you have sores or ulcers in your mouth?	☐	☐	☐
Do you wear dentures or partials?	☐	☐	☐
Do you participate in active recreational activities?	☐	☐	☐
Have you ever had a serious injury to your head or mouth?	☐	☐	☐

Date of your last dental exam:
What was done at that time?

Date of last dental x-rays:

What is the reason for your dental visit today?

How do you feel about your smile?

Medical Information *Please mark (X) your response to indicate if you have or have not had any of the following diseases or problems.*

	Yes	No	DK
Are you now under the care of a physician?	☐	☐	☐

Physician Name: Phone: *Include area code* ( )

Address/City/State/Zip:

Are you in good health?	☐	☐	☐
Has there been any change in your general health within the past year?	☐	☐	☐

If yes, what condition is being treated?

Date of last physical exam:

	Yes	No	DK
Have you had a serious illness, operation or been hospitalized in the past 5 years?	☐	☐	☐

If yes, what was the illness or problem?

Are you taking or have you recently taken any prescription or over the counter medicine(s)?	☐	☐	☐

If so, please list all, including vitamins, natural or herbal preparations and/or diet supplements:

_____

_____

_____

_____

Form S500

Medical Information

Please mark (X) your response to indicate if you have or have not had any of the following diseases or problems.

(Check DK if you Don't Know the answer to the question)	Yes	No	DK
Do you wear contact lenses?	☐	☐	☐
Joint Replacement. Have you had an orthopedic total joint (hip, knee, elbow, finger) replacement?	☐	☐	☐
Date: _____ If yes, have you had any complications?_____			
Are you taking or scheduled to begin taking either of the medications, alendronate (Fosamax®) or risedronate (Actonel®) for osteoporosis or Paget's disease?	☐	☐	☐
Since 2001, were you treated or are you presently scheduled to begin treatment with the intravenous bisphosphonates (Aredia® or Zometa®) for bone pain, hypercalcemia or skeletal complications resulting from Paget's disease, multiple myeloma or metastatic cancer?	☐	☐	☐
Date Treatment began: _____			

	Yes	No	DK
Do you use controlled substances (drugs)?	☐	☐	☐
Do you use tobacco (smoking, snuff, chew, bidis)?	☐	☐	☐
If so, how interested are you in stopping? (Circle one) VERY / SOMEWHAT / NOT INTERESTED			
Do you drink alcoholic beverages?	☐	☐	☐
If yes, how much alcohol did you drink in the last 24 hours? _____			
If yes, how much do you typically drink In a week? _____			
WOMEN ONLY Are you:			
Pregnant?	☐	☐	☐
Number of weeks: _____			
Taking birth control pills or hormonal replacement?	☐	☐	☐
Nursing?	☐	☐	☐

Allergies - Are you allergic to or have you had a reaction to: To all **yes** responses, specify type of reaction.	Yes	No	DK
Local anesthetics_____	☐	☐	☐
Aspirin _____	☐	☐	☐
Penicillin or other antibiotics_____	☐	☐	☐
Barbiturates, sedatives, or sleeping pills _____	☐	☐	☐
Sulfa drugs _____	☐	☐	☐
Codeine or other narcotics _____	☐	☐	☐

	Yes	No	DK
Metals_____	☐	☐	☐
Latex (rubber) _____	☐	☐	☐
Iodine _____	☐	☐	☐
Hay fever/seasonal _____	☐	☐	☐
Animals_____	☐	☐	☐
Food _____	☐	☐	☐
Other _____	☐	☐	☐

Please mark (X) your response to indicate if you have or have not had any of the following diseases or problems.

	Yes	No	DK
Artificial (prosthetic) heart valve	☐	☐	☐
Previous infective endocarditis	☐	☐	☐
Damaged valves in transplanted heart	☐	☐	☐
Congenital heart disease (CHD)			
Unrepaired, cyanotic CHD	☐	☐	☐
Repaired (completely) in last 6 months	☐	☐	☐
Repaired CHD with residual defects	☐	☐	☐

Except for the conditions listed above, antibiotic prophylaxis is no longer recommended for any other form of CHD.

	Yes	No	DK
Cardiovascular disease.	☐	☐	☐
Angina	☐	☐	☐
Arteriosclerosis	☐	☐	☐
Congestive heart failure	☐	☐	☐
Damaged heart valves	☐	☐	☐
Heart attack	☐	☐	☐
Heart murmur	☐	☐	☐
Low blood pressure	☐	☐	☐
High blood pressure	☐	☐	☐
Other congenital heart defects	☐	☐	☐

	Yes	No	DK
Mitral valve prolapse	☐	☐	☐
Pacemaker	☐	☐	☐
Rheumatic fever	☐	☐	☐
Rheumatic heart disease	☐	☐	☐
Abnormal bleeding	☐	☐	☐
Anemia	☐	☐	☐
Blood transfusion	☐	☐	☐
If yes, date:_____			
Hemophilia	☐	☐	☐
AIDS or HIV infection	☐	☐	☐
Arthritis	☐	☐	☐

	Yes	No	DK
Autoimmune disease	☐	☐	☐
Rheumatoid arthritis	☐	☐	☐
Systemic lupus erythematosus.	☐	☐	☐
Asthma	☐	☐	☐
Bronchitis	☐	☐	☐
Emphysema	☐	☐	☐
Sinus trouble	☐	☐	☐
Tuberculosis	☐	☐	☐
Cancer/Chemotherapy/ Radiation Treatment	☐	☐	☐
Chest pain upon exertion	☐	☐	☐
Chronic pain	☐	☐	☐
Diabetes Type I or II	☐	☐	☐
Eating disorder	☐	☐	☐
Malnutrition	☐	☐	☐
Gastrointestinal disease	☐	☐	☐
G.E. Reflux/persistent heartburn	☐	☐	☐
Ulcers	☐	☐	☐
Thyroid problems	☐	☐	☐
Stroke	☐	☐	☐
Glaucoma	☐	☐	☐

	Yes	No	DK
Hepatitis, jaundice or liver disease	☐	☐	☐
Epilepsy	☐	☐	☐
Fainting spells or seizures	☐	☐	☐
Neurological disorders	☐	☐	☐
If yes, specify:_____			
Sleep disorder	☐	☐	☐
Mental health disorders	☐	☐	☐
Specify:_____			
Recurrent Infections	☐	☐	☐
Type of infection:_____			
Kidney problems	☐	☐	☐
Night sweats	☐	☐	☐
Osteoporosis	☐	☐	☐
Persistent swollen glands in neck	☐	☐	☐
Severe headaches/ migraines	☐	☐	☐
Severe or rapid weight loss	☐	☐	☐
Sexually transmitted disease	☐	☐	☐
Excessive urination	☐	☐	☐

	Yes	No	DK
Has a physician or previous dentist recommended that you take antibiotics prior to your dental treatment?	☐	☐	☐

Name of physician or dentist making recommendation:	Phone:

	Yes	No	DK
Do you have any disease, condition, or problem not listed above that you think I should know about?	☐	☐	☐
Please explain:			

NOTE: Both Doctor and patient are encouraged to discuss any and all relevant patient health issues prior to treatment.

I certify that I have read and understand the above and that the information given on this form is accurate. I understand the importance of a truthful health history and that my dentist and his/her staff will rely on this information for treating me. I acknowledge that my questions, if any, about inquiries set forth above have been answered to my satisfaction. I will not hold my dentist, or any other member of his/her staff, responsible for any action they take or do not take because of errors or omissions that I may have made in the completion of this form.

Signature of Patient/Legal Guardian:	Date:

FOR COMPLETION BY DENTIST

Comments:_____

_____

_____

_____

APPENDIX F: DENTAL IMPRESSION MATERIALS

Materials used by each patient includes a soft toothbrush, Crest toothpaste, and ACT mouthwash to brush their teeth prior to having their impression taken. Each participant wore a Dri-gard towel during the impression to prevent the contact of fluids. Disposable mouth mirrors were used by the dentist to analyze patient dentition. Jeltrate Alginate was used as the impression material. Alginate Powder Scoop & Water Measure Set was used to measure the appropriate amount of impression material to water ratio for proper mixing. An Alginate Spatula was used to mix the Alginate and water mixture in a mixing bowl. A Miratray adhesive spray was sprayed on the impression trays for stronger adherence of the impression material to the impression trays. The mixed impression material was placed into Impression trays which were used to hold the Alginate in place on the dentition to create the impression. Sizes of the impression trays included upper and lower sizes of small, medium, and large. Dermacea gauze sponges were used by the dentist to clean excess materials from the participant's facial area. Cidex cleanser was sprayed on each impression after it was taken to disinfect the impressions. Buffstone Buff stone material was used to create the casts of the impressions. The stone was mixed in a mixing bowl with a Spatula Plaster 8R spatula on a General-Purpose Vibrator. Once the stone set the impression a Knife-Lab Plaster knife was used to remove impressions from impression trays and excess stone used to create the cast. The dental impression materials were purchased through certified dental supply distributor: Henry Schein, Inc.

Materials to acquire the impressions were prepared prior to taking the impressions. The materials and equipment were ordered in bulk from dental supplier Henry Schein and stored at the Grady Early Forensic Anthropology Research Laboratory and then transported to a lab in Evan's Liberal Arts (ELA) building on Texas State University-San Marcos campus where impressions were taken. The required materials for taking the dental impressions were premeasured and consolidated for ease of transport between the lab locations.

The Alginate dental impression material was measured according to the manufacturing standards with an Alginate standard measuring scoop. Three scoops of Alginate powder were measured and put into a sealable plastic bag. One bag of the Alginate powder equaled one impression. One jar of 454g of Alginate impression material made bags for approximately 12 impressions equaling 6 sets of impressions per jar of impression material.

Casts were made at the same site the impressions were taken, therefore, the Buffstone model stone material for making the casts was premeasured according to the manufacturing standards. A 100g of stone was measured and placed into a sealable plastic bag. Approximately three bags of stone equaled one set of impressions. Casts were made at the same site the impressions were taken, therefore, the Buffstone model stone material for making the casts was premeasured according to the manufacturing standards. A 100g of stone was measured and placed into a sealable plastic bag. Approximately three bags of stone equaled one set of impressions. The stone material was mixed with water at a ratio of 30ml of water to 100g of stone in a bowl on a general purpose vibrator (used to remove excess air bubbles)

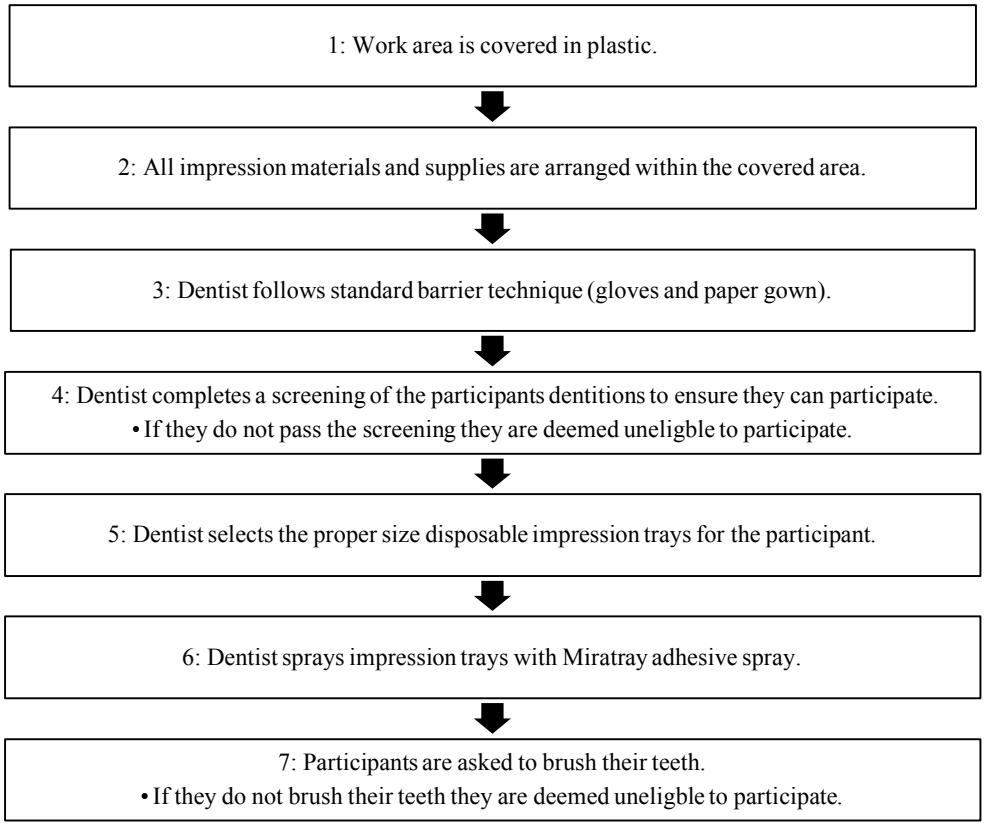
Additional materials for the impressions (listed in the materials section) were consolidated based on the number of impressions that were being taken that day and

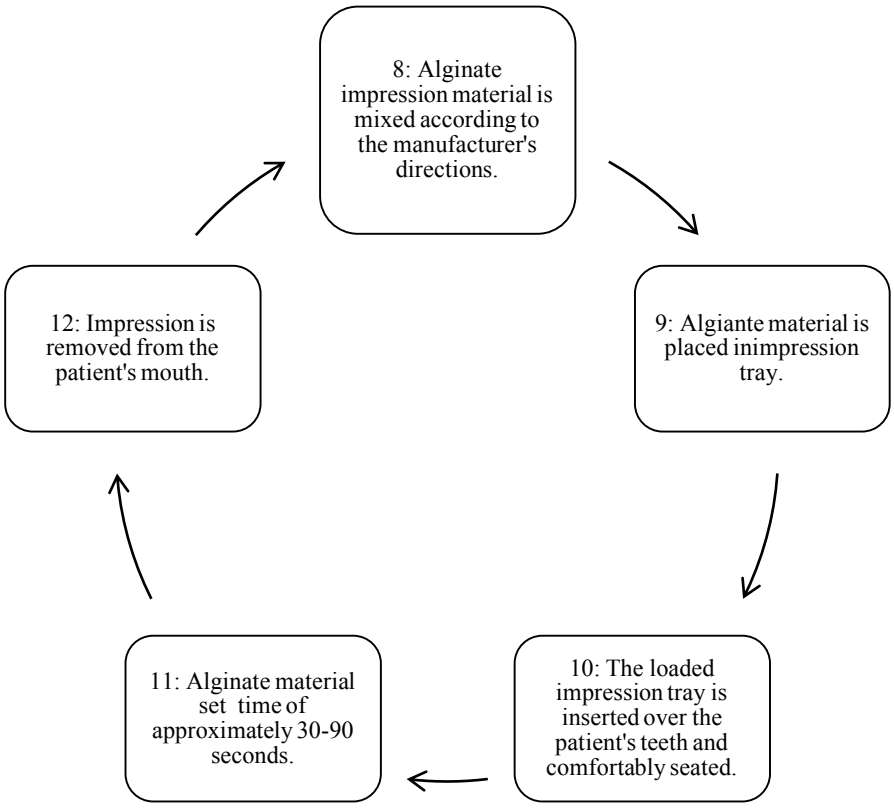
included spare items incase more materials were required (i.e. if two impressions were being taken on a given date then four tooth brushes were transported to the impression site).

APPENDIX G: STEPS TO TAKING A DENTAL IMPRESSION

The flow chart below provides the steps used to take the impressions. These steps fulfil all requirements of the Internal Review Board approval and normal dentistry practices. All steps that required contact with the participant were carried out solely by the dentist. All other tasks were completed in combination by myself and the dentist. If at any time the patient experienced difficulties the impression procedure was terminated. However, this did not occur during the data collection for this research.

FLOW CHART DEPICTING THE STEPS TO TAKE A DENTAL IMPRESSION





13: Steps #8-12 were repeated for the maxillary impression.

14: The impressions were placed in Ziploc (R) bags, sprayed with sterilant / high level disinfectant (2.5-3.4% Glutaraldehyde solution).

15: After a minium of 10 minutes, the impressions were removed from the sealed bag and rinsed in tap water.

16: The impressions were poured with dental stone to mold a cast of the impression.

APPENDIX H: DENTAL MORPHOLOGICAL TRAIT LIST

TRAIT	DESCRIPTION	SCORING SCALE	TOOTH/TEETH
Winging	Bilateral rotation with three degrees of expression.	ASUDAS (Scott and Irish 2017)	Upper central incisors.
Labial Convexity	Located on the labial surface, a marked degree of convexity.	ASUDAS (Turner et al. 1991)	Upper incisors.
Shoveling	It is the presence of lingual marginal ridges.	ASUDAS (Turner et al. 1991)	Upper & lower incisors, canine.
Double-Shoveling	The presence of labial marginal ridges.	ASUDAS (Turner et al. 1991)	Upper & lower incisors, canine.
Interruption Groove	Grooves are located across the cingulum and sometimes continue down the root.	ASUDAS (Turner et al. 1991)	Upper incisors.
Tuberculum dentale	Located on the lingual surface in the cingular region.	ASUDAS (Scott and Irish 2017)	Upper incisors and canine.
Distal Accessory Ridge	Located between the apex of the tooth and the distolingual marginal ridge in the distolingual fossa.	ASUDAS (Turner et al. 1991)	Upper and lower canines.
Mesial Accessory Ridge (Bushman Canine)	The size of the mesiolingual ridge on the canine.	ASUDAS (Turner et al. 1991)	Upper canine.
Peg-Shaped Incisor	Tooth is reduced in size and peg-shaped.	ASUDAS (Turner et al. 1991)	Upper lateral incisor.
Premolar Mesial and Distal Accessory Ridges	Ridges expressed on the mesial and distal lobe sections.	ASUDAS (Scott and Irish 2017)	Upper and lower premolars.
Tricusped Premolars	Three cusps.	ASUDAS (Turner et al. 1991)	Upper premolars.
Metacone	Cusp 3, distobuccal cusp.	ASUDAS (Turner et al. 1991)	Upper molars.
Hypocone	Cusp 4, distolingual cusp.	ASUDAS (Turner et al. 1991)	Upper molars.
Cusp 5 (Metaconcule)	5^{th} cusp located in the distal fovea of the upper molars between metacone and hypocone.	ASUDAS (Turner et al. 1991)	Upper molars.
Carabelli's Trait	Located on the lingual surface of the mesiolingual cusp.	ASUDAS (Turner et al. 1991)	Upper molars

Parastyle	Located on the buccal surface of the mesiobuccal cusp.	ASUDAS (Turner et al. 1991)	Upper molars.
Lower Premolar Cusp Variation	Considers the number of lingual cusps.	ASUDAS (Scott and Irish 2017)	Lower premolars.
Anterior Fovea	Located on the anterior occlusal surface.	ASUDAS (Turner et al. 1991)	Lower 1st molar.
Deflecting Wrinkle	It is a medial ridge variation on cusp 2.	ASUDAS (Turner et al. 1991)	Lower first molar.
Distal Trigonid Crest	A bridge between cusps 1 & 2.	ASUDAS (Turner et al. 1991)	Lower molars.
Protostylid	Located on the buccal surface of cusp 1.	ASUDAS (Turner et al. 1991)	Lower molars.
Cusp 5 (Hypoconulid)	Located on the distal occlusal aspect.	ASUDAS (Turner et al. 1991)	Lower molars.
Cusp 6	Located on the distal fovea of the lower molars lingual to cusp 5.	ASUDAS (Turner et al. 1991)	Lower molars.
Cusp 7 (Metaconulid)	Located between cusps 2 and 4 in the lingual groove.	ASUDAS (Turner et al. 1991)	Lower molars.
Cusp Number	A count of the number of cusps.	ASUDAS (Turner et al. 1991)	Lower molars.
Groove Pattern	Pattern of the groove.	ASUDAS (Turner et al. 1991)	Lower molars.

APPENDIX I: DENTAL MORPHOLGOICAL TRAITS AND TRAIT CODES

TRAIT	TRAIT CODE	TRAIT	TRAIT CODE
Winging	WINGUI1	Cusp 5 (Metaconcule)	C5UM1 C5UM2
Labial Convexity	LABCURVUI1	Carabelli's Trait	CARAUM1 CARAUM2
Shoveling	SHOVUI1; SHOVLI1 SHOVUI2; SHOVLI2 SHOVUC	Parastyle	PARAUM1 PARAUM2
Double-Shoveling	DSHOVUI1 DSHOVUI2	Lower Premolar Cusp Variation	LINGVARLP3 LINGVARLP4
Interruption Groove	INTGRUI1 INTGRUI2	Anterior Fovea	AFOVEALM1
Tuberculum dentale	TDUI1 TDUI2 TDUC	Deflecting Wrinkle	DEFWRINLM1
Distal Accessory Ridge	DARUC DARLC	Distal Trigonid Crest	DTCLM1
Mesial Accessory Ridge (Bushman Canine)	MESACCRIDGEUC	Protostylid	PROTOLM1 PROTOLM2
Peg-Shaped Incisor	PEGUI2	Cusp 5 (Hypoconulid)	C5UM1 C5UM2
Premolar Mesial and Distal Accessory Ridges	PMRP3 DACRP3 PMRP4 DACRP4	Cusp 6	C6LM1 C6LM2
Tricusped Premolars	TRICUP3 TRICUP4	Cusp 7 (Metaconulid)	C7LM1 C7LM2
Metacone	METAUM1 METAUM2	Cusp Number	CNUMLM1 CNUMLM2
Hypocone	HYPOUM1 HYPOUM2	Groove Pattern	GPLM1 GPM2
*U = maxillary, L = mandibular, I = incisor, C = canine, P = premolar, M = molar, Numbers=tooth number			

APPENDIX J: DENTAL MORPHOLGOICAL TRAIT BREAKPOINTS

Trait	Breakpoint (BP) of Presence	BP Scale
WINGUI1	1+	Scott and Irish 2017
LABCURVUI1	2+	Edgar 2002
SHOVUI1	3+	Edgar 2002
SHOVUI1, SHOVUI2 SHOVLI1; SHOVLI2	1+	Edgar 2002
SHOVUC	2+	Edgar 2002
DSHOVUI1, DSHOVUI2	1+	Edgar 2002
PEGUI2	1	Edgar 2002
PMRP3, PMRP4 DACRP3, DACRP4	2+	Scott and Irish 2017
INTGRUI1	1+	Edgar 2002
INTGRUI2	1+	Edgar 2002
TDUI1	2+	Scott and Irish 2017
TDUI2	2+	Scott and Irish 2017
TDUC	2+	Scott and Irish 2017
MESACCRIDGEUC	1+	Edgar 2002
DARUC	2+	Edgar 2002
TRICUP3	1	Edgar 2002
TRICUP4	1	Edgar 2002
METAUM1	5+	Edgar 2002
METAUM2	5+	Edgar 2002
HYPOUM1	5+	Edgar 2002
HYPOUM2	2+	Edgar 2002
C5UM1	1+	Edgar 2002
C5UM2	1+	Edgar 2002
CARAUM1	1+	Edgar 2002
CARAUM2	1+	Edgar 2002
PARAUM1	1+	Edgar 2002
PARAUM2	1+	Edgar 2002
SHOVLI1	1+	Edgar 2002
SHOVLI2	1+	Edgar 2002
DARLC	1+	Edgar 2002
LINGVARLP3	2+	Scott and Irish 2017
LINGVARLP4	2+	Scott and Irish 2017
AFOVEALM1	2+	Edgar 2002
CNUMLM1	5 and 6	Edgar 2002
CNUMLM2	4-6	Edgar 2002
DEFWRINLM1	1+	Edgar 2002
DTCLM1	1	Edgar 2002
PROTOLM1	1+	Edgar 2002
PROTOLM2	1+	Edgar 2002
C5LM1	1+	Edgar 2002
C5LM2	1+	Edgar 2002
C6LM1	1+	Edgar 2002
C6LM2	1+	Edgar 2002
C7LM1	1+	Edgar 2002
C7LM2	1+	Edgar 2002
GPLM1	1	Edgar 2002
GPLM2	1	Edgar 2002

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