THESIS

# SHOVEL-SHAPED INCISORS AND THE MORPHOLOGY OF THE ENAMEL-DENTIN JUNCTION: AN ANALYSIS OF HUMAN UPPER INCISORS IN THREE DIMENSIONS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Arts

Colorado State University

Fort Collins, Colorado

Spring 2011

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# ABSTRACT

SHOVEL-SHAPED INCISORS AND THE MORPHOLOGY OF THE ENAMEL-DENTIN JUNCTION: AN ANALYSIS OF HUMAN UPPER INCISORS IN THREE DIMENSIONS

One of the most common morphologies associated with human upper incisors is that of shovel-shaping. An ordinal framework has been developed to score the expression of shovel-shaping in the central and lateral upper incisors, from absent (0) to extremely shoveled (7). Changes in the distribution of incisor enamel related to shoveling are likely the product of the growth process and is genetically determined. The present study provides a window on this process by examining the morphological correspondence between the incisor crown and the enamel-dentin junction (EDJ). The EDJ will be visualized and analyzed using the non-destructive three-dimensional method of micro-computed tomography (μCT) with the Amira software package. The sample consists of 10 upper incisors ($I^1$ or $I^2$) from 10 individuals in collections housed at Colorado State University. Seven teeth were chosen due to their variation in degree of shoveling, and three teeth chosen due to their variation in degree of non-shoveling and are used as an out group.

Due to the genetics involved with dental initiation and shape patterning, studies on modern human populations concerning shovel-shaped incisors have suggested shoveling as a highly heritable trait likely due to genetic influence. It is not surprising that shoveling is population specific in living humans, more predominantly seen in Asian and Native American populations, and less frequent in European and African populations. Therefore, a connection of external and internal morphology might support a genetically driven morphology that appears early in the development trajectory of the anterior teeth. Shoveling has also been used to assess and diagnose ancient human groups. For example, australopithecines, Asian *H.erectus* and Neanderthals commonly express shoveling, although of different forms. The present study will help contextualize the use of shoveling as potential autapomorphy in fossil hominins.

ACKNOWLEDGEMENTS

I would first and foremost like to thank my father for bestowing encouragement and advice throughout the course of my graduate work. My admiration for you and your guidance throughout my life has brought me to follow in your footsteps and obtain a graduate degree in anthropology. Without the endless hours we've spent discussing my courses, research ideas, and obstacles, I would not have been able to complete my Masters. I would also like to sincerely thank my husband for his enduring support and love; he is and will always be my pillar.

I am grateful to my advisor Dr. Michelle Glantz, for giving me the confidence to explore my research interests and guidance throughout this process. Sharing the same passion for teeth as I have has made this research enjoyable. Thank you to Dr. Jason LaBelle for his motivation, enthusiasm for archaeology, and expressing an interest in my graduate work. Thank you to Dr. Christian Puttlitz for making my research idea come to life and granting me access to your wonderful associates. I am indebted to Cecily Broomfield for her patience and scanning my sample; and to fellow graduate student, Ben Gadomski, for his assistantship on this project and sense of humor.

Lastly, thank you to Dr. Jon Glenn, D.D.S. for his mentorship and introducing me to the field of dentistry. Without your inspiration, I would have not found dental anthropology.

To my family:

My father, Thomas Pridham,

Aunt, Marjorie Thomas,

and Husband, William Denton

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# CHAPTER I

# INTRODUCTION

The human dentition develops with great regularity under a strict set of genetic controls that determine tooth size and morphology (Kaczmarek, 1991). Crown morphological analyses represent an important research focus of biological anthropologists who study recent modern human skeletal samples from the archaeological record as well as fossil hominins. In recent human samples, crown traits often express population affinities, the evolution of subsistence patterns and demographic structure (Scott and Turner II, 1997). In fossil hominin research, crown traits are often used to reconstruct evolutionary relationships through qualitative and quantitative methods, among genera, species, and sub-species of fossil hominins (i.e., Bailey, 2002; Scott and Turner, 1997). However, the vast majority of this work pays little attention to the internal morphology of the dental tissues, such as the enamel-dentin junction.

The present study is interested in the internal morphology of the dental tissues and their relationship to external crown traits. This study seeks to examine the morphology of the enamel-dentin junction (EDJ) with the non-destructive three-dimensional method of micro-computed tomography (μCT) in a small sample (n=10) of modern human upper incisors, in order to explore the relationship between shovel-shaping of the incisor crown and the EDJ. The incisor morphology that will be examined here, shovel-shaping, has been observed in both recent modern humans and fossil hominins in varying degrees and frequencies (Crummett, 1995; Robinson, 1956). The μCT method used here has previously been used to explore the correspondence between external and internal morphologies on a sample of post-canine hominin teeth (Skinner et al., 2008; Skinner et al., 2009a; Skinner et al., 2009b) and produced positive results. By establishing a relationship between external and internal morphology, the present study may provide the justification for relying on examinations of the EDJ in order to reconstruct the crown morphology in specimens that have high degrees of dental wear so that assessments of trait expression and population affinity may be made. In addition, traditional methods of histological sectioning are highly destructive and thus are typically avoided in fossil hominin research (Olejniczak et al., 2007; Schwartz et al., 1998; Smith and Tafforeau, 2008). Other benefits of my proposed method in the present research are that it is non-destructive and no loss of morphological information occurs; once scanned, the tooth is able to be three-dimensionally reconstructed in the appropriate software with very little dimensional data inferred (Olejniczak et al., 2008).

Before this method for analyzing crown traits is accepted, however, it must be tested with other tooth types with a variety of different discrete traits expressed. This present study will offer nondestructive means of visualizing the internal and external morphology of incisors. The study intends to provide a methodology that is easily reproducible, while providing valuable information about the EDJ of incisors that have not yet been published. In this regard, the present study also may illuminate developmental trajectories in the formation of external morphology and therefore support a close relationship between the genotype and phenotype with regard to discrete dental traits.

# HYPOTHESIS

The null hypothesis to be tested is that no significant relationship exists between the EDJ morphology and the characteristics of shoveling in incisor occlusal morphology. The alternative hypothesis is that the characteristics of shoveling observed in the occlusal morphology mirrors that of the EDJ morphology.

In the projected hypothesis, if the null is upheld, then one could argue that the development of the internal and external morphology is not linked. Thus, the internal morphology would not be a good proxy for external expression. In a general sense, this may indicate that environmental factors, rather than genetic ones, have a greater impact on external enamel folding.  For example, studies have suggested traits that add to the tooth material (enamel and/or dentin), such as shoveling, would increase the overall strength of the incisor. This morphology might be selected on to protect the tooth from being worn down and finally lost in populations who use their front teeth more frequently for tool use (Hrdlička, 1920; Kimura et al., 2009; Scott and Turner 1997).

However, based on previous research outlined in the background, I anticipate the null to be overturn, and that there is a strong correlation in the expression of shoveling between the occlusal and EDJ morphology. Thus, in well-developed shovel-shaped incisors, I anticipate to see a well-developed degree of shoveling characteristics in the EDJ.  If expectations are correct, this research will project that discrete trait recognition in the EDJ of incisors can be used to make population distinctions among modern humans. This research will also provide further insight on the development of the shoveling trait that is seen in modern humans, which can be applied toward studying the variation of shoveling characteristics in the EDJ of fossil hominins.

# CHAPTER SYNOPSES

Chapter II reviews the current literature concerning the enamel-dentin junction. This chapter first outlines the basics of dental histology and the genetics behind the dentition. The Neanderthal dentition is reviewed in regards to the ramifications of this study, the incidence of shovel-shaped incisors among fossil hominins and modern humans, as well as the current literature concerning three-dimensional methods and recent 3D hominin studies of the enamel-dentin junction. Chapter III describes the sample and a detailed discussion of the methods used to collect data. This chapter also provides a detailed outline of the statistical methods performed. Chapter IV summarizes the results of the statistical analyses found for shovel-shaped incisors and non-shovel-shaped incisors, and discusses the applicability of the results to the present study's hypothesis. Chapter V is a synopsis of the results of this investigation and recommendations for future research.

# CHAPTER II

# LITERATURE REVIEW

This literature review encompasses dental anatomy, histology and touches on the EDJ's role in dental development. The genetics behind dental initiation and patterning will follow. The debate of the uniqueness of Neanderthal dentition and dental development will be presented in regards modern humans. This discussion will lead into the incidence and characteristics of shovel-shaped incisors observed among fossil hominins and modern humans. Lastly, this chapter will discuss previous studies concerning three-dimensional methods applied to hominin and dental studies, including recent 3D hominin studies that have focused on the enamel-dentin junction.

## THE HUMAN DENTITION

### *Dental anatomy and histology*

The main tissues of a tooth are 1) enamel, 2) cementum, 3) dentin, and 4) pulp. The basic structure and tissues of a human incisor is illustrated in *Figure 2.1*. Cementum is a thin tissue that lines the outer surface of the dentin in the root area, which aids in the anchoring of the tooth to the alveolar bone. The pulp is the tissue that keeps the tooth alive. For the purposes of this study, these two tissues are not considered. This present study is focused on the junction where the enamel and dentin meet. Enamel is the hardest known substance in our bodies (97% mineralized), and functions to protect the inner part of the tooth (Hillson 2007). This enamel covering may be described as the crown of the tooth. Because enamel does not remodel, the crown of the tooth wears over the lifetime of an individual.

The thickness of human enamel varies from 2 to 2.5mm; enamel thickness reduces gradually toward the neck of the tooth, while its density diminishes from the occlusal surface toward the EDJ (Konjević et al., 2003). Dentin forms the core of the tooth and encapsulates the pulp chamber. Dentin is supported by the vascular system, and is only visible when a tooth exhibits extreme wear (Hillson 2007).

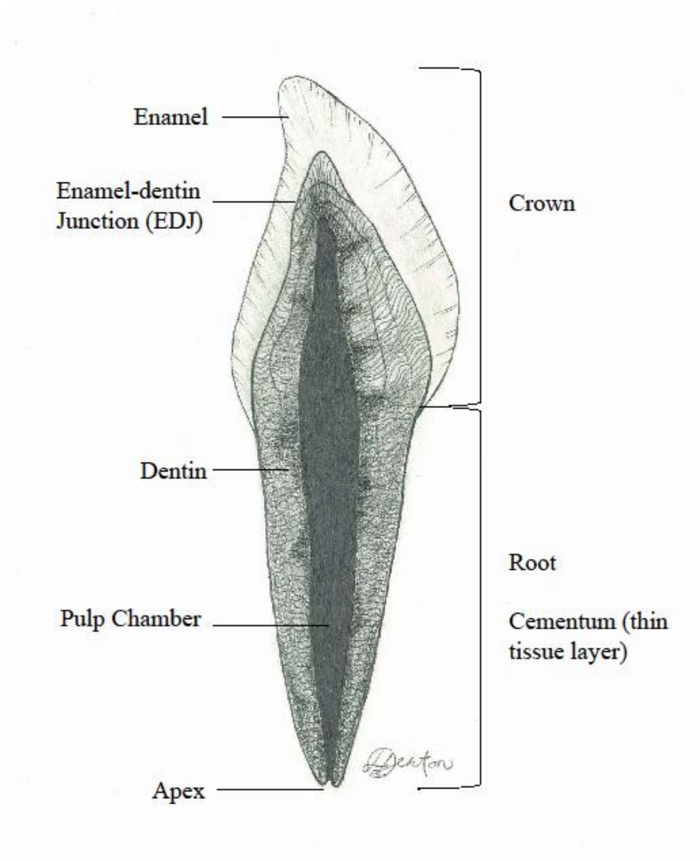



*Figure 2.1.* The anatomy of a human incisor.

The EDJ is the boundary between the enamel cap and the underlying dentin. Crown morphology and, subsequently, discrete trait expression, are likely driven by the development of the EDJ as this marks the separation of dentin formation cells, odontoblasts, and enamel formation cells, ameloblasts. In this regard, the development of the EDJ and crown structures have been studied and presented valuable information concerning discrete trait expression in primates and hominins (Skinner et al., 2009).

*The role of EDJ in dental development*

The EDJ is the initial location for cells that are responsible for the secretion of enamel and dentin (Smith and Tafforeau, 2008). It is thought that the EDJ is greatly influenced by enamel knots that form before the mineralization of the tissue secret (Smith and Tafforeau, 2008). The role of enamel knots will further be discussed with the genetics of the dentition. Lucas (2004) describes that the first enamel formed, at the EDJ, has not been well studied, and appears to have a random orientation under light microscope. Yet, EDJ angles formed with Striae of Retzius, internal enamel growth layers, occurs with a regular periodicity providing insight to enamel development (Bromage et al., 2007; Guatelli-Steinberg and Reid, 2007). This is the area, as stated above, where thin bundles of enamel crystals begin to grow outward to the surface, guided by the secreted protein matrix (Mann et al., 1990; Smith and Tafforeau, 2008). Correspondingly, the dentin begins to secrete its collageneous matrix starting at the dentin horn (tip of EDJ) underlying the future cusp tip, and downward until it reaches the apex of the root (Smith and Tafforeau, 2008). While the EDJ in particular has not been well researched, dental development, enamel formation patterns and timing have been well studied. The further enamel that is laid down is highly organized with a definite pattern (Lucas, 2004; Mann et al., 1990). Thus, for this reason, enamel pattern and timing relate to external crown morphology.

As described above, the final crown shape and thickness of enamel is largely determined by ameloblast secretory activity (Beynon et al., 1991). Accordingly, tooth size is governed by the amount of enamel, dentin, and size of the pulp cavity. Therefore the changes in tooth size and crown morphology are the result of variation in enamel and dentin volume, as well as pattern distribution (Gantt et al., 2007). Further discussion on modern human formation patterns and rates will be reviewed and compared with trends observed in Neanderthals, in the Neanderthal dentition.

## GENETICS OF THE DENTITION

Sharpe has suggested that the dentition of any species is as unique as its DNA and because patterns are inherited, the developmental processes that direct pattern formation must be genetically controlled (Sharpe, 2000:7). Epithelial-mesenchymal interactions control the development of individual teeth, thus it is likely the case for the patterning of the dentition as a whole (Zhao et al., 2000:165). Msx-2 gene expression localized in the enamel knot (EK) provides a molecular link between tooth initiation and shape (Sharpe, 2000:9). EK is a clump of cells in the center of the tooth germ, which form at the late bud stage, marking the beginning of tooth shape development (Jernvall and Thesleff, 2000:14). EK is associated with signaling factors (SHH, BMP2, BMP4, BMP7, FGF4, and FGF9) and therefore is thought to be a potential organizing center for regulation of crown patterns (Zhao et al., 2000:165). If this is the case, the entire cusp pattern may be a consequence of the dynamic process of interaction between these various signaling factors, rather than specific genes programming specific cusps (Zhao et al., 2000:165). Furthermore, the 'field theory' proposed as early as 1939 by Butler states that genes are overall responsible for size, patterning and morphology, as in each morphologic group (i.e., incisors, canines, premolars, molars), the more anterior the tooth is in the dental arcade the more genetically stable. Thus, central incisor, $I^1$, is more stable while the lateral incisor, $I^2$, is genetically more variable (Krogman, 1967). Understanding the genetics involved with dental patterning and development is important for biological anthropologists since there exists an inherited and molecular basis of tooth development and shape; however, there has yet to be resolution in a specific gene or if genes in general predict discrete traits on specific teeth.

With regard to shoveling, hereditable studies concerning shoveling have been done on twins, siblings, and parent offspring pairs to suggest that shoveling is a result of genetics. Blanco and Chakraborty (1976) examined shoveling in parent-offspring and sibling pairs; their statistical analysis concluded 68% of total variability is explained by the additive effect of genes. Similarly, sibling and twin studies (i.e., Portin and Alvesalo, 1974; Hanihara and Tanaka, 1970) compared shovel-shaped frequency to a random sample from the overall study population to conclude that shovel-shaped incisors are indeed hereditable and thus controlled by genes. Contrary to Abrahams (1949), Portin and Alvesalo (1974) overruled shoveling as being a Mendelian trait and consider shoveling to be a complex polygenic trait. Yet, there still remains the possibility of an environmental agent causing the trait to be more prevalent in affected families than in a random population sample. Until genetic studies on populations with shoveling are conducted, there will still be uncertainty whether how much influence the genotype has on this discrete expression.

Consideration of phenotypic factors that might play a role in shovel-shaped incisors would include cultural practices where teeth are involved in the process of hide softening, or other means of using teeth excessively for tool use (Hrdlička, 1920; Kimura et al., 2009). As previously stated in Chapter I, extra enamel folding which creates the shoveling trait, is thought to consequently strengthen the overall tooth (Hrdlička, 1920; Kimura et al., 2009). This would be extremely beneficial for protecting the inner tooth's structure from heavy enamel wear. Examples of modern human populations who exhibit shoveling and have been linked to possible tooth tool use are circumpolar peoples, Mongolians, and other Native Americans groups. Yet, most of the literature leans towards the genotype influencing shovel-shaped incisors; thus future studies in search for the gene or genes responsible will determine how much effect the genotype truly has on the expression of this discrete trait.

Recently, Kimura et al. (2009) examined DNA from modern Japanese populations to claim that in *ectodysplasin A receptor* (EDAR), there is a nonsynonymous-derived variant, allele 1540C, that is associated with Asian specific hair thickness and also associated with shovel shaped incisors. The study showed that shovel shaping grades are positively correlated with the mesiodistal diameter of $I^1$, which is plausible since it is expected as shoveling increases, so will the enamel volume and consequently the length of the tooth. Kimura et al. (2009) notes that the discovered allele, 1540C, is absent in European and African populations, thus it is thought that selection may be more involved than genetic drift.  Thus, a functional adaptation may be an overgrowth of upper incisors involved in shoveling that is seen in *Homo erectus* and Neanderthals (Kimura et al., 2009). Since EDAR affects ectodermal organs (i.e., enamel, hair), selection could have acted on hair structure, or sweat and mammary glands, where tooth shoveling is but a by-product (Kimura et al., 2009).

Overall, Kimura and colleagues presented a genetic determinant for shovel-shaped incisors, yet caution that EDAR cannot solely explain heritability and suggest other genetic factors should be sought out. This study has paved the way to understanding the genetic basis of shovel-shaped incisors in Asian modern human populations, which reveals to biological anthropologists the importance of revisiting shoveling characteristics seen in fossil hominins. If the null of this present study is overturned, this research will contribute support to the genetic basis of shovel-shaped incisors reported by Kimura et al. (2009). The overarching goal of this research is to provide a context and a methodological approach to understanding the expression of shovel-shaping and the role of the EDJ in fossil hominin studies. Like recent modern humans of Asian descent (this would include Native Americans), shoveling albeit a different form, appears in ancient humans.

## THE NEANDERTHAL DENTITION

Shoveling characteristics are an important feature in the Neanderthal dentition. As there also exist a high frequency of shovel-shaped incisors in modern human populations, this brief introduction to the Neanderthals is meant to provide a summary of their characteristic dental morphological features and introduce the debate concerning the uniqueness of those features in comparison to modern humans. The results of this study will provide context and methodology for prospective research concerning the comparison of discrete traits in the EDJ morphology of Neanderthals to modern humans.

Much debate has centered on the uniqueness of Neanderthal biology and behavior, specifically in the context of the origin of modern humans. Neanderthals first appeared in Europe at the start of the Late Pleistocene, and persisted into the early Upper Paleolithic (Wolpoff, 1999). Some scholars argue that Neanderthals are a sub-species of *Homo sapiens* (i.e., Cartmill and Smith, 2009; Guatelli-Steinberg, 2009 and reference w/in Bräuer; Mayr, 1963), where others refute this argument in favor of species distinctiveness, *Homo neanderthalensis* (i.e., Bailey, 2006; Tattersal, 1986; Tyrell and Chamberlain, 1998; Smith, 2008; Stringer, 1992). In addition to these views, Neanderthals may also be understood as either an archaic group of *Homo sapiens* (Howell, 1994; Howells 1976), or a geographically defined race of *Homo sapiens*, in which their morphological features are no longer exhibited in a high frequency in any living cline (Wolpoff, 1999). If Neanderthals are a geographical variant of *Homo sapiens* and contributed to the modern human gene pool, we would expect to see evidence of gene flow (Bailey, 2006; Guatelli-Steinberg and Reid, 2008). On the other hand, if Neanderthals were a distinct species, we would expect to see different evolutionary trajectories between *Homo sapiens* and *Homo neanderthalensis* (Bailey, 2006:11). The evolutionary problem is significant for our understanding of Neanderthals and the origin of modern humans.

Dental features can be utilized to help solve this taxonomic problem. *Table 2.1* presents a list of discrete dental traits considered by some to be autopomorphies of

Neanderthals, but some traits are also present at low and high frequency among modern human populations.

Neanderthal Dental Features

| High Frequency | Low Frequency |
|---|---|
| Incisor Shoveling ($I^1$, $I^2$) | Double Shoveling ($I^1$) |
| Labial Convexity ($I^1$, $I^2$) | Four cusped ($M_2$) |
| Tubercle ($I^1$, $I^2$) | Three cusped ($M^2$) |
| Canine mesial ridge | Enamel extension ($M^1$) |
| Cusp 5 ($M^1$, $M^2$) | Deflecting wrinkle ($M_2$) |
| Carabelli's cusp ($M^1$, $M^2$) | Distal trigonid crest ($M_2$) |
| Mesial lingual groove ($P_3$) | Mesial lingual groove ($P_4$) |
| Transverse Crest ($P_3$, $P_4$) | |
| Asymmetry ($P_3$, $P_4$) | |
| Multiple lingual cusps ($P_4$) | |
| Mesially placed metaconid ($P_4$) | |
| Distal accessory ridge ($P_3$, $P_4$) | |
| Cusp 6 ($M_2$) | |
| Mid-trigonid crest ($M_1$, $M_2$) | |
| Large anterior fovea ($M_1$, $M_2$) | |
| Y groove pattern ($M_2$) | |

*Table 2.1*. Neanderthal dental features. Adapted from Bailey (2006). I=incisor, P=premolar, M=molar. Superscript denotes maxillary teeth, subscript denotes mandibular teeth.

Researchers disagree over the area of the dentition, either post-canine or anterior, that is the most diagnostic of Neanderthals. Bailey (2002, 2006) argues that Neanderthal post-canine morphology is distinctive due to the combination of the following discrete traits: asymmetrical lower premolar crowns, a mid-trigonid crest (MTC) on lower molars, and smaller metacones and larger hypocones on upper molars when compared to modern humans. The MTC, a crest that connects the protoconid and metaconid, is a crucial derived trait for Bailey, as she notes it is found on more than 90% of Neanderthals and only 33% in early modern humans (2002).

Olejniczak et al. (2008) regards Neanderthal molars as possessing a significantly larger EDJ surface area than modern humans. Neanderthal molar enamel is thinner than modern humans; however it is deposited over a larger volume of coronal dentin where the absolute enamel volume is similar to modern humans (Olejniczak et al., 2008:12). Some scholars argue that the shape of Neanderthal's post-canine teeth generally fall within the range of variation in modern humans, making it hard to distinguish between the two groups (Cartmill and Smith, 2009:372).Carabelli's trait is an example of a post-canine trait that is also present in high frequency of Caucasian modern humans as well as Neanderthals. However, Bailey (2006) notes that it is the combination of dental traits expressed in Neanderthal teeth, not just the presence of one or another, that makes their dentition different from that of modern humans.

Trinkaus (1987) noted that anterior teeth dimensions strongly scale with body mass. In this regard, the large anterior teeth of the Neanderthals are a consequence of an elevated lean body mass (Cartmill and Smith, 2009; Trinkaus, 1987 and references w/in). The crown morphology of Neanderthal anterior teeth can be difficult to study as they exhibit paramasticatory behavior (Cartmill and Smith, 2009), which can lead to extremely worn, or completely worn enamel. As seen in *Table 2.1*, the anterior dental traits are related to shoveling seen in incisors, where the rest of the discrete traits are in the post-canine dentition. Bailey (2006) and Crummett (1995) are both in agreement that Neanderthal shovel-shaped incisors exhibit a high frequency of labial convexity and a well-developed tubercle, which is different from the shoveling seen in other fossil hominins and modern humans. The differences in shoveling expressed in Neanderthals, other fossil hominins, and modern humans will further be discussed in this chapter.

*Growth and development of Neanderthal teeth*

The one thing that makes modern humans different from other hominins is our increased length in child dependency. This child dependency allows us to compare and contrast the growth and development among and between hominin populations (Mann et al., 1990). The consensus is that there is accelerated maturation of Neanderthal children. Based on dental calcification and eruption time, there is also accelerated dental development (Tiller, 1995). Neanderthal crown and root formation matches that of modern humans, while a late peak in root extension and a more complex EDJ sets Neanderthals apart (Bayle et al., 2009:66). Neanderthal anterior teeth show a more uniform perikymata distribution from the cusp to the cervix, similar to *Australopithecus* and *Paranthropus*, rather than modern humans (Smith, 2008; Guatelli-Steinberg and Reid, 2008). Neanderthals have an enamel formation time of 7-8 days, suggesting that the lateral enamel formation falls in the modern human range which is 8-9 days (Guatelli-Steinberg and Reid, 2007:237). Similarly, the total enamel formation of the anterior teeth in Neanderthals is within the modern human range of population variation (Reid et al., 2008:226).

The findings of Reid and Dean (2006) on the range of variation in enamel formation of northern European and south African modern humans provides a comparative for understanding and predicting Neanderthal development. Striae periodicities are consistent between S. African and N. Europeans, and cuspal enamel formation of the anterior teeth is almost identical (Reid and Dean, 2006:337). The first molars of S. Africans form faster than of N. Europeans, whereas M2 and M3 are not very different (Reid and Dean, 2006:388); this is probably due to the fact M1 is the first molar to erupt during growth and development. The anterior teeth do form faster in S. Africans and is possibly due to the documented low perikymata count (Reid and Dean, 2006:339). Neanderthal premolar enamel formation times may be intermediate between these populations, as their hypothetical periodicities (since we cannot actually measure these in Neanderthal populations) are higher (Reid et al., 2008:231). In contrast, total enamel formation times of molars of Neanderthals are similar to the previously stated populations. Understanding the growth and development of Neanderthal dentition and the similarities to modern humans helps to provide insight to the evolutionary relationships that exist. Again, the goal of this study is to help provide understanding though the dentition, the relationship between fossil hominins, and other ancient and modern human populations.

Reid and colleagues suggest that perhaps molar enamel formation times are constrained within a species and are closely linked to somatic growth; however variation of enamel formation and somatic growth among species or populations within a species remains unknown (Reid et al., 2008:233). While lateral enamel formation can account for 60-95% of the total enamel formation time, Guatelli-Steinberg and Reid remark that it should not be assumed to reflect the total enamel formation when compared to modern humans; the data is also not sufficiently informative for inferring aspects of Neanderthal life history (2008:246). Similarly, Tiller (1995) and Mann (1990) demonstrate that assumptions made of Neanderthal development rate, based on dental enamel histology and single specimens are suspect as it does not incorporate the range in geographical variation that exists in all populations of Neanderthals (Tiller, 1995:65).

While advances in technology have led us to achieve more accurate data on dental development in Neanderthals, we should consider crown morphological traits as well when comparing Neanderthals with modern humans. The distinctiveness of Neanderthal dental traits still comes into question as recent research suggests that we can better answer evolutionary questions in ancient human (fossil) populations if we look at the structure that underlies crown morphology and its variation of expression. It would be significant if multiple distinctive traits in crown morphology of Neanderthals were examined in relation to the internal morphology of teeth to see if one could distinguish late Pleistocene hominins in the same manner as *Australopithecus* and *Pan* species. The results of this present research can be applied to future research concerning incisor EDJ morphology and trait development in Neanderthals in comparison to modern humans.

## INCIDENCE OF SHOVEL-SHAPED INCISORS

The most common occlusal morphology exhibited in late Pleistocene hominin anterior teeth is shoveling on the incisors, yet the variation is different from the shoveling seen in high frequency among Asian *Homo erectus*, and modern human Asian and Native American populations (Crummett, 1995). Shoveling is a trait expressed in the crown, or occlusal morphology, and is described as an expansion of this dental tissue as there is an increase amount of enamel visible. Incisors that express shoveling are characterized by marginal ridges (Crummett, 1995) and resemble a coal shovel, resulting in the term 'shovel-shaped' incisors (Hrdlička, 1920) (*Figure 2.2*). Central and lateral incisors are of average to over-average in size, where the cutting edge is generally thicker and broader than non-shoveled teeth  (Hrdlička, 1920).Yet, Crummett (1995) describes three aspect of shoveling, which we should take in account when analyzing this trait among hominins: 1. marginal ridges of the incisor (original definition), 2. development of lingual tubercle on the base of tooth, from a small lump to independent cusp, and 3. mesiodistal curvature, aka labial convexity. The last two characteristics are commonly seen in Neanderthals, where marginal ridges are more common in modern humans. For the purpose of this study, shoveling will be broken down to the three characteristics described by Crummett (1995), marginal ridges, lingual tubercle, and labial convexity, to quantitatively assess their significance in the external and internal morphology.

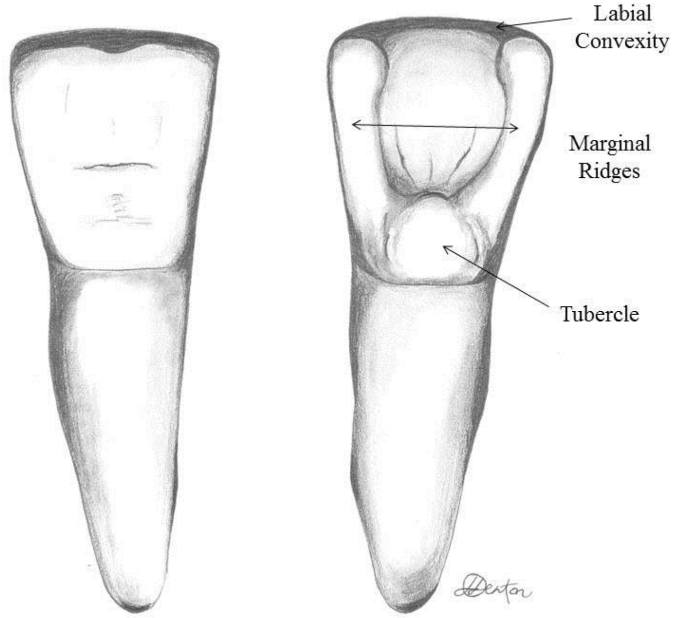



*Figure 2.2.* Lingual view of a non-shovel-shaped incisor (left) and a shovel-shaped incisor (right).

*Shovel-shaped incisors in hominins*

Research has noted the presence of shoveling in hominoids, such as baboons, gibbons, macaques, and even other mammals (Robinson, 1957; Weidenreich, 1937).

Weidenreich (1937) argues shoveling is a primitive characteristic from pongids. Yet, Robinson (1956) points out shoveling is not directly derived from *pongid* as we see an increase in expression in hominins. Robinson (1956) states that shovel-shaped incisors and the presence of moderate to well-developed tubercles are a common hominin attribute. *Table 2.2* identifies those hominin fossils that express shoveling. The earliest of hominins that express shoveling are the genus *Australopithecus*. According to Robinson (1956), australopithecines are characterized by moderate shoveling with the presence of a lingual tubercle similar to Neanderthals and *H.erectus*. *H. erectus* and Neanderthal shoveling differs. Neanderthals display more developed marginal ridges, and a lingual tubercle, sometimes in the presence of a lingual cusp (Crummett, 1995; Robinson, 1956). It could be argued that Neanderthal shoveling is not directly comparable to that of modern humans, and cannot be scored using the ASUDAS plaques as the plaques are based off of modern human variation. These plaques will be discussed further in the methodology.  Yet, while marginal riding may be observed in Neanderthal teeth, it is not considered to be in high frequency, thus the presence of a lingual tubercle and labial convexity take precedence when discussing shoveling in Neanderthals. It is important to note that shoveling exhibited in Neanderthal teeth are on the extreme high end in shoveling degree, where modern humans can be categorized as moderately shoveled in their presence.

*Figure 2.3* illustrates the difference in shoveling morphology between Asian *H. erectus* and Neanderthals, as well as a comparison with a modern human non-shoveled incisor to visualize the characteristics of shoveling. As seen in *figure 2.3*, *H. erectus* shoveled incisors characteristically appear to have thick marginal ridging with no labial convexity, whereas Neanderthals have an overall larger occlusal surface with the presence of a tubercle and labial convexity. Additionally, shovel-shaped incisors have been found in numerous modern human populations ranging from a low to high frequency.

| Species | Site, Specimen | Author(s) |
|---|---|---|
| Neanderthal | Krapina | Carbonell, 1963; Coppa et al., 2005; Weidenreich, 1937 |
| | Skhul I, V | Carbonell, 1963; Coppa et al., 2005 |
| | Tabun I, III | Carbonell, 1963; Coppa et al., 2005 |
| | Ehringsdorf | Carbonell, 1963; Weidenreich, 1937 |
| | Le Moustier | Carbonell, 1963; Coppa et al., 2005; Weidenreich, 1937 |
| | Gibraltar 2 | Coppa et al., 2005 |
| | Hortus VII, VIII, IX | Coppa et al., 2005 |
| | La Quina 18 | Carbonell, 1963; Coppa et al., 2005 |
| | Montsempron | Coppa et al., 2005 |
| | Saint-Césaire | Coppa et al., 2005 |
| | Amud I | Coppa et al., 2005 |
| | Shanidar 2 | Coppa et al., 2005 |
| | Sima de los Huesos | Coppa et al., 2005 |
| | Spy 2 | Coppa et al., 2005 |
| *Homo erectus* | Zhoukoudian | Martinón-Torres et al., 2008 |
| | Sangiran | Martinón-Torres et al., 2008 |
| | Dmanisi | Martinón-Torres et al., 2008 |
| *Australopithecus* | Swartkrans | Robinson, 1956 |
| | Sterkfontein | Robinson, 1956 |
| | Makapan | Robinson, 1956 |

*Table 2.2*. Hominin fossils exhibiting maxillary shovel-shaped incisors

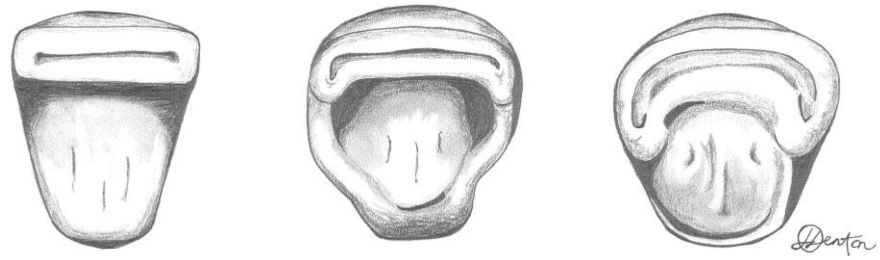



*Figure 2.3*. Occlusal view of morphological variation in shovel-shaped incisors. Left: modern human central incisor exhibiting no shoveling, Center: central incisor from Zhoukoudian, China (*H. erectus*) expressing marginal ridges and a straight margin, Right: central incisor from Krapina, Croatia (Neanderthal) expressing a lingual tubercle and a distinctive mesial distal curvature. Adapted from Cartmill and Smith, 2009.

*Shovel shaped incisors in modern humans*

Many human populations express shovel shaping, with the highest frequency and associated traits occurring in Asian and Native American groups (*Table 2.3*). The association of a lingual tubercle with shoveling in modern humans is more infrequent than seen in Neanderthals (Carbonnell, 1963). As stated earlier, modern human shoveling is marked by the presence of marginal ridging. There is geographical variation of the frequency of shovel-shaped incisors among populations exhibiting shoveling, i.e., Japanese, Native Americans, Chinese, Circumpolar people (Hrdlička, 1920; Kimura et al., 2009). Thus, Kimura and colleagues (2009) show that shovel-shaped incisors are good indicators of large ethnic groups in Asia (i.e., Chinese, Japanese, Mongolian), as well as smaller groups within the population. There are variation and frequency differences for instance in Northern Japanese versus Southern Japanese populations.

Hrdlička (1920) suggested that sexual dimorphism is linked with the degree in shoveling, as he found that shoveling is more pronounced in Chinese females than males. On the other hand, Hanihara and Tanaka (1970) observed only a small difference in sexes of the mean depth of the lingual fossa in Japanese and Pima populations; therefore, they concluded that it is safe to state shoveling is not sexually dimorphic. Additionally, Portin and Alvessalo (1974) ruled out the possibility of shovel-shaping being sex-linked in their sibling pair study, as the frequency of the trait in the two sexes was not significantly different.

However, the depth of the lingual fossa that was measured by Hanihara and Tanaka (1970) has not been measured on other studies concerning shoveling. The most common means of measuring the degree of shoveling were plaques modeled by Dahlberg in 1949 which categorized shoveling in terms of semi-shoveling, shoveling, and double shoveling. These plaques were updated by Turner et al. (1991), and provide an ordinal degree of expression. These plaques will be used in the present study and will further be discussed in the methods.

Consistent with Hrdlička's initial conclusion that shoveling may be a sexually dimorphic trait, Kimura et al. (2009) found that Japanese females have overall smaller incisors and a larger expression of shoveling and double shoveling than Japanese males. While it is unclear why females exhibit a more pronounced degree of shoveling than males, Kimura and colleagues suggest this might be due to mechanisms of morphogenesis, genes on sex chromosomes, levels of sex hormones, or the time and duration of development (Kimura et al., 2009).Overall, shovel-shaped incisor research has shown that modern human populations that could be Mongolian in descent (i.e., Asians, Eskimos, Native Americans, and South Pacific Islanders) have a higher frequency of shoveling characteristics than do Caucasian and African descendants (Carbonell, 1963; Davies, 1976).

| Group | Frequency (%) | | Author(s) |
|---|---|---|---|
| | Central | Lateral | |
| Chinese | 66.2 | 56.9 | Hrdlička, 1920 |
| | 80 | | Davies, 1976 |
| Mongolian | 24 | 75 | Hrdlička, 1920 |
| Eskimo | 40 | 57 | Hrdlička, 1920 |
| | 6 | | Davies, 1976 |
| Japanese | 77.9 | 72.7 | Hrdlička, 1920 |
| Finn | 80 | | Davies, 1976 |
| Native Americans | 67 | 76 | Hrdlička, 1920 |
| Pima Indians | 99 | 81 | Dahlberg, 1951 |
| Pueblo Indians | 81 | 81 | Dahlberg, 1951 |
| Afghans | 40 | | Davies, 1976 |
| Maasai | 31 | | Davies, 1976 |
| Polynesian | 4 | | Davies, 1976 |
| New Guineans | 9 | | Davies, 1976 |
| Melanesian | 6 | 6.7 | Hrdlička, 1920 |
| Australian Aborigines | 28 | | Davies, 1976 |
| African American | 4.9 | 3.8 | Hrdlička, 1920 |
| Caucasian American | 2.6 | 1 | Hrdlička, 1920 |

*Table 2.3.* Modern human populations exhibiting shovel-shaped incisors. Adapted from Hrdlička (1920).

The use of a modern human sample in this present study helps us to understand the expression of shoveling and the variation in frequency that be related to other fossil hominins that express shoveling. The conjecture this study makes is that if a connection of the EDJ morphology is made with the occlusal morphology in one group with one expression of the characteristics of the shoveling trait, then it is possible to connect it in another sample population of modern humans or hominin fossils. To try and encompass the wide spectrum of variation that exists in modern humans, it is imperative to obtain large sample sizes and from a variety of existing populations. Due to time and cost constraints, this present study has a relatively small sample (n=10) and should be regarded as a pilot study that supports not only the feasibility of the method for examining shovel-shaped incisors, but also the significance of the EDJ in crown trait determination.

## PREVIOUS 3D METHODOLOGY STUDIES ON THE EDJ

*Three dimensional methods*

There are multiple computer tomography scanning systems that are used to visualize the internal and external anatomy of bones and teeth, including medical CT, μCT, industrial CT, and synchrotron X-ray CT systems (Gantt et al., 2007:119). The majority of hominin studies that use three-dimensional techniques focus on the permanent dentition (Smith, 2008:207). By assessing the multiple studies using three-dimensional techniques within paleoanthropology, we can understand the applicability and limits of each technique as it is applied to hominin studies. This review also underscores the suitability of μCT in the present study and for other research that investigates discrete trait expression in the EDJ of incisors.

Portable confocal scanning optical microscope (PCSOM) was developed for non-contact and non-destructive means of imaging early hominin hard tissues (Bromage et al., 2007). It can be used for high-resolution views of external microstructures (perikymata) and internal microstructures (cross striations and Striae of Retzius) from naturally fractured or worn enamel surfaces (Bromage et al., 2007:194). This method is advantageous compared to conventional light microscopy, because thin sections do not need to be produced. It also allows for circular polarized light to be projected on the image, and provides a Z-axis where one can see the Striae of Retzius and cross striations in the tooth enamel (Bromage et al., 2007:194).

Smith and Tafforeau (2008) consider that incremental features of enamel microstructures are ideally visualized in SR-μCT. This method provides results similar to light and confocal microscopy, as it can reveal structures smaller than traditional histological sections, periods of developmental stress, calculate crown formation time, age at tooth eruption, and age at death (Smith and Tafforeau, 2008:276). Gantt et al. (2007) also use a type of ST-μCT method, a high-resolution X-ray CT system (HRXCT) to enamel thickness. This proved to be an effective quantitative method for visualizing the EDJ, obtaining volumetric data, and 3D reconstruction of both extant and extinct hominid dentition (Gantt et al., 2007:117). Smith, Gannett and colleagues view the X-ray CT system as superior over medical and micro-CT systems, as it produces accurate models of tooth growth and allows for comparison to actual thin section data of the same specimens and (Gantt et al., 2007; Smith, 2008). While X-ray CT systems are useful for comparing thin sections, again thin sections do not allow for adequate sample size as it is destructive to fossil hominins.

The most used method with regard to the examination of hominin dental morphology is micro-computed tomography (μCT). This technique provides non-destructive high-resolution visualizations that can be measured accurately (Olejniczak et al., 2007:104-5), and in the appropriate software environment, dental tissues can be separated and the EDJ visualized (Schwartz et al., 1998:527). This is due to the difference in enamel and dentin degree of mineralization, making their densities allow for straightforward tissue segmentation (Skinner et al., 2009a). Recent studies have proven that micro-computed tomography is indeed a more accurate and precise method than direct measurements. By using this method we can standardize how we take dental measurements. Smith found that µCT can be used for assessing linear enamel thickness, extension rates, and allowing for periodicity assessment, as it is most difficult to visualize (2008:219). Similarly, Kim et al. (2006) evaluated the accuracy of micro-computed tomography in tooth measurement, to provide support that this new methodology is a more reliable way to produce linear measurements for incisors and molars than 2D photographs for the internal and external structures.

*Limitations and accuracy of micro-computed tomography*

Olejniczak et al. (2007) demonstrated the ideal resolution and slice thickness parameters, as well as disadvantages for µCT, which is informative for future studies. One disadvantage of the µCT is the negative relationship between specimen size and scanning resolutions, where isolated teeth or small mandible fragments are more easily scanned at high-resolution than are crania and mandibles (Olejniczak et al., 2007:113; Skinner et al., 2009b:78). This limitation positively affects the present study, as the sample consists of only isolated teeth. A potential disadvantage with µCT scans is the inability to clearly depict tissues as it can show a blurred boundary (Schwartz et al., 1998), thus the importance lies in the software program that is employed.

The accuracy and reproducibility of visualizing internal and external dental structures, as well as measuring dental and craniofacial morphology using computed tomography, has been assessed and proven a reliable method ( i.e., Christiansen et al., 1986; Kim et al. (2006); and Waitzman et al., 1992). Christiansen et al. (1986) studied tempormandibular joint (TMJ) measurements and established that there was no significant difference between CT and macroscopic measurements, with an accuracy of CT linear measurements (0.4-0.8mm). The authors also tested for intra- and inter-observer error, and found that reproducibility of CT measurements was little affected by experience with CT. There was greater angular error than linear among observers. However, the error of intra- and inter-observer error was within accepted limits, (0.4-0.9mm) and (0.5-0.8mm) respectively. Kim et al. (2006) concluded that CT linear measurements are in line with direct measurements. The volume obtained in Kim et al. was underestimated. Although some researchers have had trouble estimating volume, this study's methodology will show that the volume can accurately be attained using the Amira µCT software.

Similarly, Waitzman et al. (1992) observed excellent agreement of linear measurements derived from CT software, with direct skull measurements. Contrary to Christinsen et al., Waitzman and colleagues found the angular measurements to be acceptable, error under 5%. The ability to produce non-destructive measurable models for modern humans and fossil hominins, promises to increase sample size and open new research questions about internal dental morphology that will improve our understanding of hominid dental evolution. Viewing internal dental morphology was not previously possible without the use of such three-dimensional methods of computed tomography, and now we have comprehensive studies of enamel and dentin that could perhaps be more telling about questions of dental morphology and development in human evolution than ever before.

## PREVIOUS HOMININ EDJ STUDIES

The handful of three-dimensional research on the EDJ surface in hominin teeth have been directed toward dental development. These provide information on fossil hominins and modern humans that aid our understanding of evolutionary relationships in dental development and morphology.

Bromage et al. (2007) used a portable confocal scanning optical microscope on naturally fractured molars of *A. africanus* to study the periodicity and EDJ angles with the Straie of Retzius for information on enamel growth rates. Boyde, (1964) previously found that acute angles indicate a higher ameloblast differentiation rate. The EDJ angle was more obtuse in molars of *A. africanus* than *Paranthropus*, where *A.africanus* molars increase from cusp to cervix more than *Paranthropus*. Molar periodicity had a high variation of 6-7 days, falling in the range of modern humans (6-12) and chimpanzees (6-8). Where the crown formation time was estimated to be 3-3.2 years, similar to *P.boisei.* Their study concluded there may be differences in enamel growth mechanism between *A. africanus* and *Paranthropus*.

Tafforeau and Smith (2008) examined applicability of SR-µCT using a moderate slice thickness of 30µm for further studies on Neanderthal dental development. However, no results have been published yet concerning dental development and the EDJ. The available three-dimensional studies on Neanderthals have been more concerned with enamel thickness differences among modern humans. Olejniczak et al. (2008) used µCT to determine that the EDJ of Neanderthal molars exhibit a significantly larger surface area than modern humans. As the authors note, this clearly corresponds to the larger occlusal surface area that Neanderthals exhibit.

Variation in the EDJ shape, and the relationship between discrete molar traits and the EDJ, has been shown to be useful in distinguishing primate taxa and early hominins. Olejniczak et al. (2007) showed the shape of the EDJ in molars is different among anthropoid primates, where Skinner et al. (2009a) added that the EDJ shape of molars can discriminate *Pan* and sub-species. Skinner et al. (2008, 2009b) has significantly shown that discrete traits in mandibular molars are associated with the EDJ in hominin fossils, and can appropriately distinguish *Australopithecus* species. The µCT methodology employed by Skinner and colleagues present the most current accurate scanning and visualizing methods, and will be discussed further in terms of the methodology of this present study. For example, Carabelli's cusp in australopithecines has been visualized on the EDJ and can be used to distinguish one group from the other.

Schwartz et al. (1998) examined the EDJ in relation to Carabelli's cusp of robust and gracile australopithecines from high-resolution CT scans in order to investigate the degree this trait influences enamel thickness. These authors discovered that there is a position difference among hominins of the Carabelli feature at the EDJ and occlusal surface which may explain the functional role of this extra cusp. This feature in turn affects the linear thickness of enamel at the protoconal dentin horn of the molar, as *A. africanus* has thinner enamel than *P.robustus*. More recently, Hunter et al. (2010) demonstrated that Carabelli's cusp in modern humans is also visible in the corresponding area of the EDJ. They also claim that it may be genetically produced. Carabelli's cusp is a trait that is also present in Neanderthals, thus the variation of expression at the EDJ in Neanderthals should also be further examined for a fuller understanding of the development of Carabelli's cusp among a variety of hominins and modern humans.

Most of these studies have focused on australopithecines, and more recently biological anthropologists are using three-dimensional technology for Neanderthal studies. Further research on relationships between the internal and external morphology of the Neanderthal dentition in comparison to modern humans is the next logical path for Late Pleistocene studies, and will be discussed in the conclusion with the results of this present study. This present study corresponds to the studies carried out by Schwartz et al. (1998) and Hunter et al. (2010), in the fact that a similar discrete trait found in ancient human populations as well as modern human populations will be examined at the EDJ level and assessed for significance.

## SUMMARY

The basic structure of an incisor and dental histology has been discussed, with the enamel-dentin junction as the focus of this current study. The molecular mechanisms involved with dental development, along with heritable studies on shovel-shaping introduce the important genotypic influence on this trait. The Neanderthal dentition in comparison to modern humans was discussed for the purpose of understanding dental traits that are seen in hominin fossils and modern humans. The occurrence of shovel shaped incisors in hominins and modern humans has been summarized, as well as the differences in shoveling morphology. A review of the available three-dimensional methodologies and EDJ studies on hominins has been provided for this current study's research implications and possible future applications.

# CHAPTER III

# MATERIALS AND METHODS

This chapter will present the sample used in this study, along with the exclusion criteria in choosing the sample and limitations of the study. The methods of scoring procedures for shoveling and wear, along with measurements taken in the Amira software will be explicated. The statistical tests that are used to evaluate the hypothesis outlined in Chapter I as well as the methodology are explained.

## MATERIALS

### *Sample*

The sample used in this study consists of 10 isolated permanent incisors ($I^1$ or $I^2$) from 10 modern humans in collections housed in the human remains repository at Colorado State University (*Table 3.1*). The seven shoveled incisors consist of the pre-contact Gallina population of northern New Mexico, dated 1200 A.D (n=4), as well as a more contemporary Asian sample (n=3). Three non-shoveled teeth in the sample belong to individuals from the comparative osteology collection (n=1) and a late 19$^{th}$ century Colorado cemetery collection (n=2) that are predominantly European in descent. They were chosen due to their expression of non-shoveling and are used as an out-group. Due to limitations of the µCT, teeth could not be intact to the maxilla, thus isolated teeth could only be selected. The second limitation to this study's sample size is the availability of shoveled teeth from Native American and Asian populations, because the osteological collections at Colorado State University are predominately Caucasians, and therefore generally lack shoveling. While the sample size is small, three populations allow for some of the variation of shoveling to be observed in modern humans.

| Tooth | Specimen | Province | Shovel | Wear |
|---|---|---|---|---|
| L. $I^1$ | 19B | $19^{th}$ C. CO cemetery | 0 | 4 |
| $I^1$ | 5PE527.6 00-16 | $19^{th}$ C. CO cemetery | 1 | 4 |
| $I^2$ | Tooth 4 | Osteology | 1 | 2 |
| $I^1$ | Tooth 3 | Asian/Osteology | 2 | 2 |
| R. $I^2$ | 84.2.0 | Native American/Gallina | 3 | 4 |
| $I^2$ | Tooth 1 | Asian/Osteology | 4 | 3 |
| L.$I^1$ | 83.76.1 | Native American/Gallina | 4 | 2 |
| R.$I^2$ | 83.1.1.3 | Native American/Gallina | 4 | 3 |
| R.$I^2$ | 84.1.1 | Native American/Gallina | 4 | 2 |
| $I^1$ | Tooth 2 | Asian/Osteology | 5 | 3 |

*Table 3.1*. Description of sample used in this present study. L=left, R=right, $^1$ central incisor, $^2$ lateral incisor. Shovel is based on a scale from 0-7, and wear 0-8.

*Exclusion criteria*

The ideal sample would consist of a large sample size that would equally encompass all grade levels of shoveling from a variety of modern human populations to be able to see the full range of the morphological variation in the occlusal morphology and the EDJ.

Although deciduous teeth as well as lower incisors also express shoveling, the trait is not as pronounced as it is in permanent teeth (Bailey, 2006; Hrdlička, 1920). Also, permanent teeth are more commonly used in previous studies utilizing µCT. Upper incisors were chosen due to their variation in shoveling expression. Because advanced dental wear changes the morphology of the crown as well as the secretory patterns of odontoblasts, the upper incisors that are part of this sample could not express much attrition. Attrition was scored according to Buikstra and Ubelaker’s (1994) scoring procedure for incisors (*Figure 3.1*). The scale ranges from no wear (1) to a significant amount of wear as dentin is completely exposed (8). This study only included incisors that had less than a level 4 degree of attrition in order to insure visibility of crown features and minimize measurement error (*Table 3.1*).

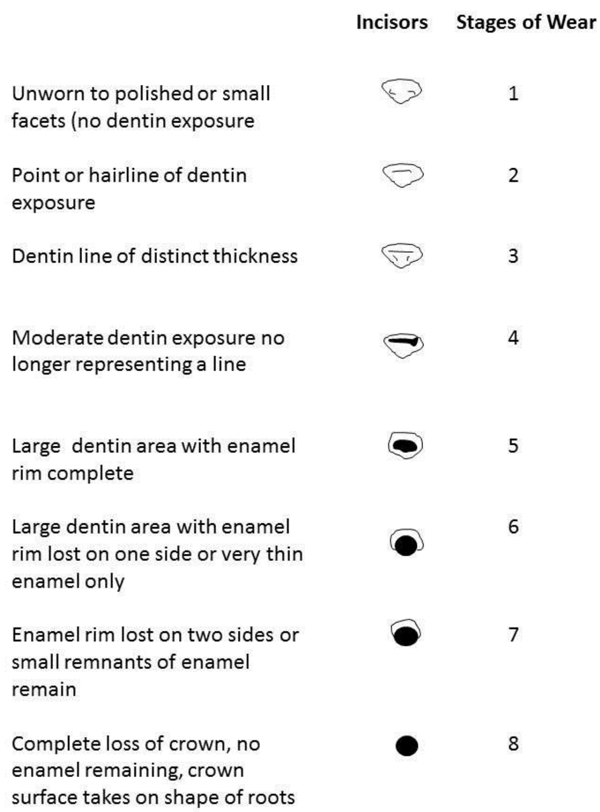



*Figure 3.1.* Surface wear scoring chart for incisors and canines. Adapted from Buikstra and Ubelaker 1994.

## METHODS

*Micro-computed tomography and EDJ Reconstruction with Amira*

A µCT scanner housed and maintained by Dr. Puttlitz's lab at Colorado State University's orthopedic bioengineering research center, was used to image the sample of 10 teeth. Under the supervision of laboratory director Dr. Puttlitz, Cecily Broomfield, a research associate, scanned the sample at high resolution, with the scan parameters: 70 Kvp, 111 µA, 2 frame average, 10 µm slice thickness (1000 slices per millimeter), with an aluminum filter. The scan parameters are similar to the recent EDJ studies by Skinner et al. (2008, 2009a, 2009b), with the slight difference that Skinner and colleagues used 14µm, 100 Kvp, and 94 mA. This present study allows for a comparative methodology and a state of the art scan.

The following method of segmentation follow those outlined by Skinner et al. (2008, 2009a, 2009b). Homogenous tissue segmentation of enamel and dentin is produced by examining the 3D-voxel-value histogram and the grey-scale value distribution of tissues (*Figure 3.2*). The set attenuation values based on the peak distribution of the incisors for enamel are 7,000-12,000, and for dentin are 3,400-6,800. Alveolar bone that was still attached to two of the teeth in the sample was considered to be the same density as the dentin and thus had to undergo further segmentation as so not to affect the dentin volume. The orthoslices were examined, and the alveolar bone was hand selected and removed from the dentin layer. Once tissues are segmented, the outer surface and EDJ can be reconstructed as a triangle-based surface model with the aid of Amira software (**v5.3.2**, www.amira.com, San Diego, CA). In this software environment, the tooth can be rotated and enlarged for evaluation of trait expression.

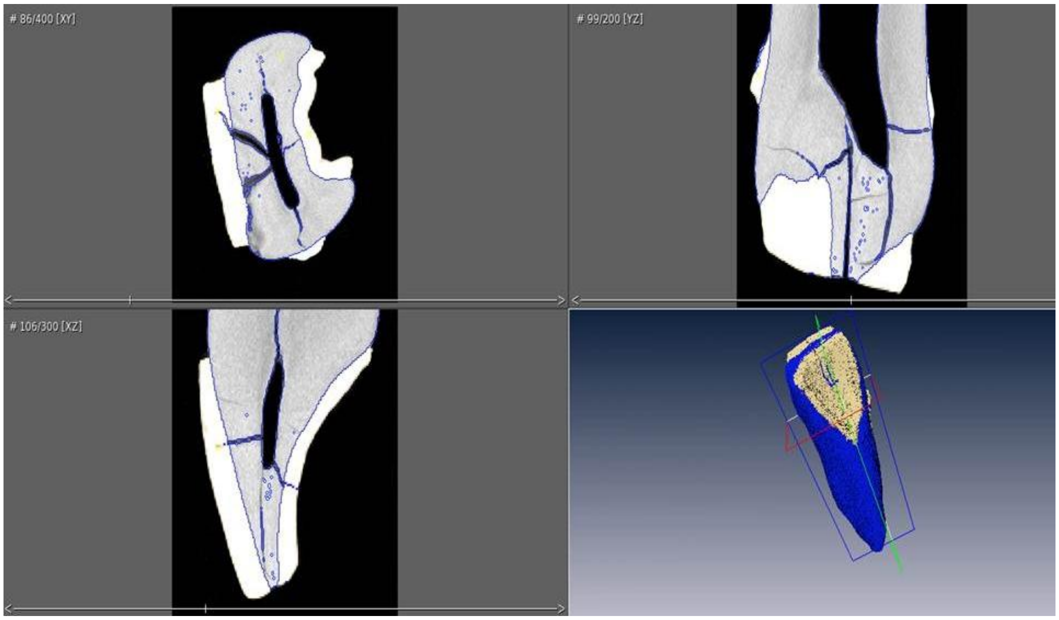



*Figure 3.2*. Representation of the grey-scale value distributions and resulting in tissue segmentation (Tooth 1) in Amira software. Top left: occlusal view, top right: mesial view, bottom left: distal view, and bottom right: 3D reconstruction.

*Quantification of discrete traits*

Each tooth was scored to determine the degree of trait expression according to the Arizona State University Dental Anthropology System (ASUDAS) dental plaques. The scale for shoveling ranges from 0 (not present) to 7 (trait fully expressed) (*Figure 3.3)*. The ASUDAS allows for observation and a three-dimensional appreciation of traits and establishes important relationships between populations (Turner II et al. 1991). While significantly important for standardizing the 58 most easily and reliably observed traits into plaques, the ASUDAS permits observation beyond the presence/absence dichotomy of cladistics to provide an ordinal system which helps to qualify a truly continuous feature (Turner II et al., 1991). The visual differences in the degree of shoveling can be somewhat subjective and have a tendency to produce inter-observer error. The ASUDAS shoveling plaques address this issue and promote replication between observers. The sample ranges from 0-5 in degree of shoveling, expressing the range from no shoveling to relatively large in shoveling (*Table 3.1*). Non-shovel-shaped incisors will be categorized as teeth that scored a 0 or a 1, where shovel-shaped incisors will consist of 2 or higher. The shoveling plaques only assess marginal ridge expression, thus the presence of labial convexity and/or tubercles will be measured in Amira.


*Figure 3.3*. Arizona State University Dental Anthropology System plaque 12, shoveling in permanent upper incisors. Plaque key: 0, none – flat lingual surface; 1, faint – very slight mesial and distal elevations seen and felt; 2, trace – elevations easily seen (minimum extension for most observers); 3 and 4, semi-shovel – stronger ridges tending to converge at the cingulum; 5, shovel – ridges almost contact at cingulum; 6, marked shovel – ridges sometimes coalesce at cingulum; 7, barrel (upper second incisors only). (Adapted from Hillson, 1996).

Amira software was used to establish the 3D segmentation of the occlusal surface and the EDJ.  Standard measurements of mesiodistal diameter, labial-lingual diameter, crown height, and enamel and dentin volume were collected from these surfaces to the nearest 0.01mm (*Figure 3.4*). Characteristic measurements of shoveling include 1.the lingual base height, 2. the average marginal ridge thickness, and lastly 3. the labial convexity (*Figure 3.4*). In the presence of a tubercle, the lingual base height was measured on the occlusal and EDJ surface from the cingulum to the top of the tubercle. Marginal ridge thickness of both surfaces was measured by taking 10 arbitrary points on each side of the tooth and the average was calculated. The labial convexity was measured on the EDJ surface using the 3D angle option starting at the most posterior point of the EDJ, with the angle measured to be taken at the most labial point of the EDJ, with the last point at the midline of the tooth. The average will then be taken from the angles taken on the left and right side of the tooth. *Table 3.2* lists the variables measured in Amira with their abbreviations and definitions.

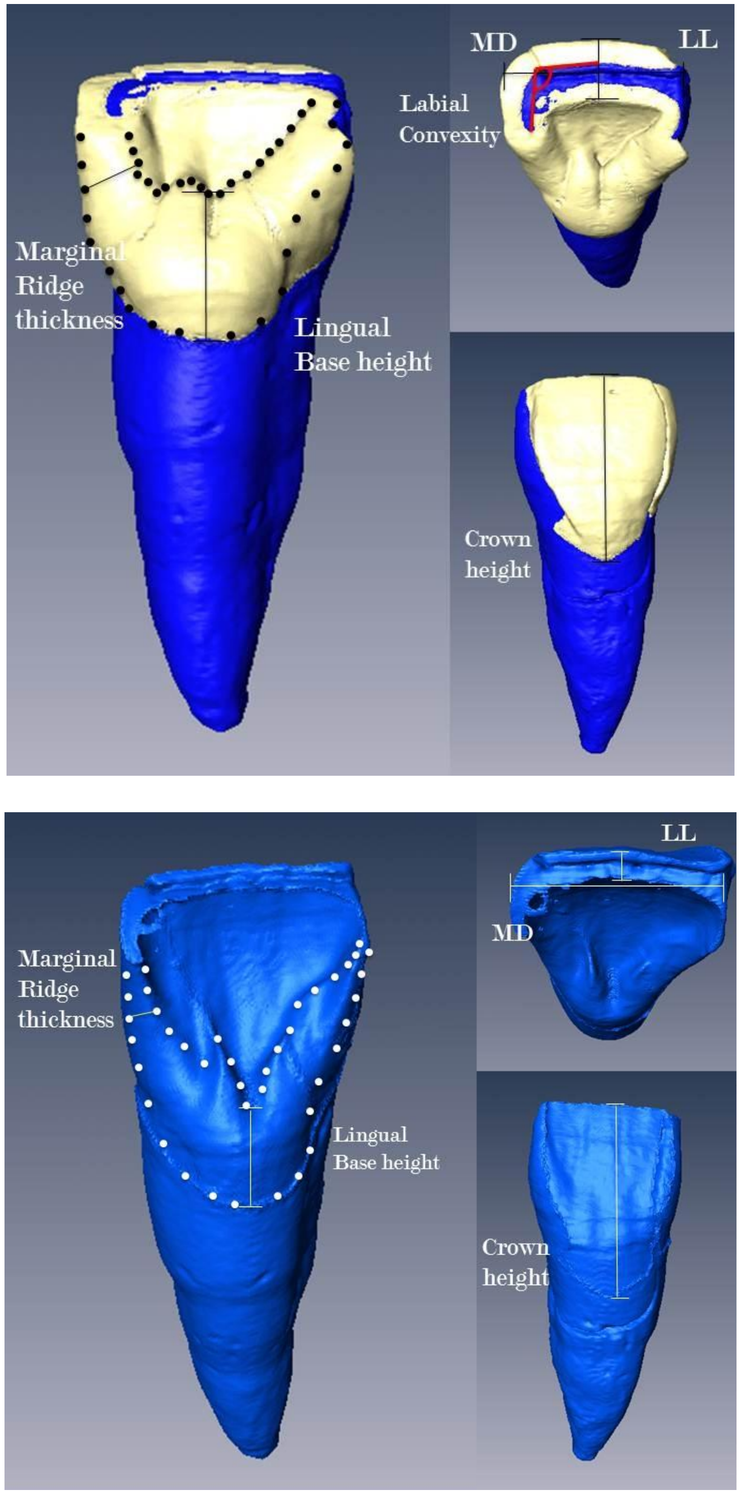



*Figure 3.4*. Reconstruction of a shovel-shaped incisor (Tooth 2, shovel:5), with the following measurements taken on the occlusal surface (top) and EDJ surface (bottom). MD, Mesiodistal length, LL, Labial-lingual length. White, enamel tissue, blue, dentin tissue.

| Variable | Abbreviation | Description |
|---|---|---|
| Mesiodistal diameter: occlusal | MD-O | Maximum length of the crown* |
| Labial-lingual diameter: occlusal | LL-O | Maximum width of the crown* |
| Crown height: occlusal | CH-O | Cemento-enamel junction to occlusal surface* |
| Marginal ridge thickness(Avg): occlusal | Mrt-O | Avg. of ridge thickness* |
| Lingual base height: occlusal | Lbh-O | Lingual cingulum to tubercle spine * |
| Mesiodistal diameter: EDJ | MD-E | Maximum length of the EDJ* |
| Labial-lingual diameter: EDJ | LL-E | Maximum width of the EDJ* |
| Crow height: EDJ | CH-E | Cemento-enamel junction to EDJ* |
| Labial Convexity (Avg): EDJ | LC-E | Angle of mesial-distal curve* |
| Marginal ridge thickness (Avg): EDJ | Mrt-E | Avg. of ridge thickness* |
| Lingual base height: EDJ | Lbh-E | Lingual cingulum to tubercle spine* |

*Table 3.2*. Variable Descriptions. EDJ= Enamel-dentin junction, *Determined instrumentally

While the sample does not exhibit extreme wear, there are a handful of measurements that can be affected by the amount of wear present on some of the teeth and must be addressed. The labial-lingual diameter is not affected by occlusal attrition. Yet, the mesiodistal diameter is affected by attrition as it can reduce the original mesiodistal diameter (Hillson, 1996). In the event of broken enamel on the mesial or distal side, the most mesial/distal point on the EDJ will be used in substitution, as wear patterns differ on the mesial and distal surfaces (*Figure 3.4*). While this reduces the mesiodistal diameter, it does not fabricate a point where the author perceives the missing enamel to be. This prevents overestimation, and will make the measurements tighter. Crown height is a variable that can be used to discuss total attrition using the difference between occlusal and EDJ crown height. Enamel volume can also be affected by occlusal attrition and broken enamel, as the original amount of enamel present is reduced. Consequently, if there is extreme wear or broken enamel on the mesial or distal side, this could affect the average marginal ridge thickness. As stated previously, in the event of missing enamel mesially or distally, the point will be taken on the outer most EDJ surface.

*Statistics*

The statistical methods were performed using SAS (v.9.2, www.sas.com, Cary, NC), and were aimed at refuting the null hypothesis that there is no significant relationship between the EDJ morphology and the characteristics of shoveling in incisors that is seen in the crown morphology. Pearson and Spearman correlations were utilized to determine whether the characteristics of shoveling seen in the occlusal morphology mirror that in the EDJ. Lastly, principal component analysis was used to identify the variables that explain the most amounts of variation, and aim at distinguishing shoveled and non-shoveled morphology.

The reliability and reproducibility of the study was investigated by testing 1. the amount of error between direct measurements taken using a digital caliper and µCT measurements for the entire sample, and 2. the amount of inter- and intra-observer variability using four randomly selected teeth from the sample. Intra-observer variability was considered for two observers, where the second observer is a graduate student with experience in µCT measurements in the Amira software. This data set was subjected to a paired t-test to test for variance equality by comparing the means of two small samples.

A Pearson's correlation coefficient was calculated for each set of occlusal and EDJ variables, along with the degree of shoveling and wear. Pearson's correlation coefficient (r) is a bivariate analysis that measures the strength of the linear relationship between the two variables (Ott and Longnecker, 2010). The bounds of r are (-1, 1), representing the positive or negative directional relationship where the numerical value measures the degree in strength. When r=0, there is no linear relationship between x and y. The coefficient of determination is $r^2$, which is how we can determine the percentage of variability in y that is explained by x (i.e., $r^2 = (0.606)^2 = 37\%$). Spearman's correlation was also run using volume and labial convexity against all linear measurements to see if there is a linear relationship. The volume was transformed by taking the cubic root value. The angle for labial convexity was transformed into radians and then arc length by taking a fourth of the tooth's EDJ mesiodistal diameter as the radius of the circle the angle is from. Spearman's correlation differs from Pearson's in that it is a non-parametric alternative where one of the variables (i.e., enamel volume, dentin volume, and labial convexity angle) are not assumed to be normally distributed and interval, thus are converted to ranks and then correlated (Ott and Longnecker, 2010).

Principal component analysis (PCA) was performed on the 13 dental measurements taken in Amira, as an exploratory way to examine the data and look at relationships among variables. The purpose of PCA is to reduce the large number of variables, 13 in this study, to a smaller number of variables, called principal components, whilst retaining as much of the variation that exists in the original 13 variables (Jolliffe, 1986). The test is run under the assumption that the dental measurements are correlated, and will derive factors that explain the most amount of variation in the sample. Ideally, three or four components will be retained in the analysis, accounting for at least 80% of total variation (Jolliffe, 1986). Following PCA protocol for small sample sizes, each tooth's observations were multiplied by 10, making an adequate PCA sample size of n=100.

Due to the study's limitation of having a small sample size of n=10, the results of the statistical analyses must be interpreted and applied cautiously. With a small sample, there requires a larger difference between measurements/groups to be claimed statistically significant. Thus, p-values from the correlation and t-test analyses must be considerably smaller than the alpha value of 0.05, to be concluded as statistically significant.

## SUMMARY

This chapter first discussed the sample used in the present investigation. Next, this chapter discussed the methods that will be used to collect data, including the review of analytical to test the significance of the proposed hypothesis. Attrition and shoveling morphology will be scored on a visual inspection and in comparison to the standard wear score by Buikstra and Ubelaker, and the ASUDAS shoveling plaques. Standard measurements and variables will be taken on the tooth reconstruction in Amira. Pearson's and Spearman's correlations will be used to express the degree to which the variable that describes the EDJ and the occlusal surface are correlated and to assess the statistical power of these correlations among the patterns of variation for the occlusal and EDJ morphology. Principal component analysis will be used to reduce the large number of variables to only a few that explained the most variation, and to aim at distinguishing the non-shovel-shaped incisors from the shovel-shaped incisors. Lastly, inter- and intra-observer error will test for determining the validity and replication of the employed methods. The results of this analysis will be presented in the subsequent chapter.

# CHAPTER IV

# RESULTS OF INVESTIGATION

The objective of this study is to assess the correlation between the occlusal and EDJ morphology in a sample of teeth exhibiting varying degrees of shovel shaping, from none to 7. The expected result is that there is a strong correlation between the two surfaces, thus the degree of shoveling on the occlusal surface mirrors that of the EDJ. This result would effectively overturn the null hypothesis. While incisors are highly variable in terms of developmental rates and crown morphology among geographical populations of modern humans, I expect the results of this analysis to not only provide a deeper understanding of shovel-shaped incisors, but also show that the EDJ morphology of incisors should be something to further investigate. *Table 4.1* presents the reconstruction of all shoveled and non-shoveled teeth in the sample and serves for visual reference concerning the study's results.

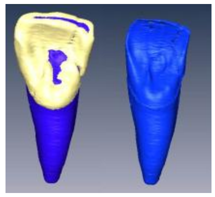
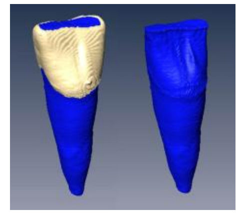
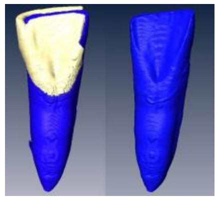
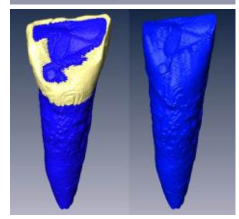
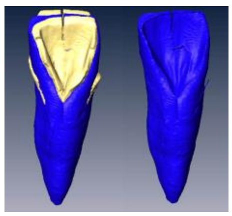
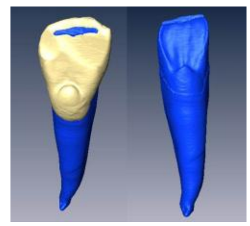
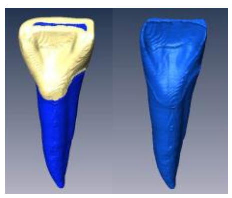
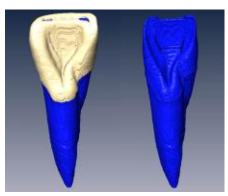
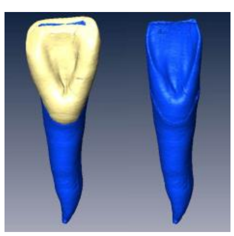
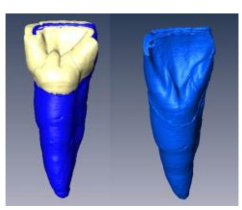
| Shovel-shaped incisors | | Non-shovel-shaped incisors | |
|---|---|---|---|
| Tooth, Shovel | Occlusal EDJ | Tooth, Shovel | Occlusal EDJ |
| Tooth 3 ($I^1$), 2 |  | 19B($I^1$), 0 |  |
| 84.2.0 ($I^2$), 3 |  | 5PE527.6 00-16 ($I^1$),1 |  |
| Tooth 1($I^2$), 4 |  | Tooth 4 ($I^2$), 1 |  |
| 83.1.76.1 ($I^1$),4 |  | | |
| 83.1.1.3 ($I^2$), 4 |  | | |
| 84.1.1 ($I^2$), 4 |  | | |
| Tooth 2 ($I^1$), 5 |  | | |

*Table 4.1.*A 3D reconstruction of the occlusal and EDJ surfaces in the sample.

# RESULTS

## *Descriptive Statistics*

Descriptive statistics are provided for the incisors examined in the study and can be found in *Table 4.2*. The Asian descent teeth vary in degree of shoveling from a 2-4, whereas the Gallina teeth express shoveling at a degree of 3-5. Three shoveled teeth had the presence of a lingual tubercle, as did one non-shoveled tooth. All scored shoveled teeth exhibited marginal ridges. Two non-shovel-shaped incisors expressed slight marginal ridging, thus were scored a 1. All shovel-shaped incisors have the classic straight margin, characterized by Asian *H. erectus* and modern Asians. However, on average the entire sample exhibits over a 90º angle, and can be described as exhibiting slight labial convexity. Upon closer examination, non-shoveled teeth have an average of 100.5º, whereas shoveled teeth have a considerable higher average of 116.6º.  It appears that shoveling in degree from 2-3 have larger obtuse angles, whereas shoveling continues to increase, the angle decreases back closer to 90˚. The angle of labial convexity has not been measured by previous studies, yet suggests that as shoveling increases, the tooth's angles are due to the variation in size. This variable will further be discussed in inter- and intra-observer error.

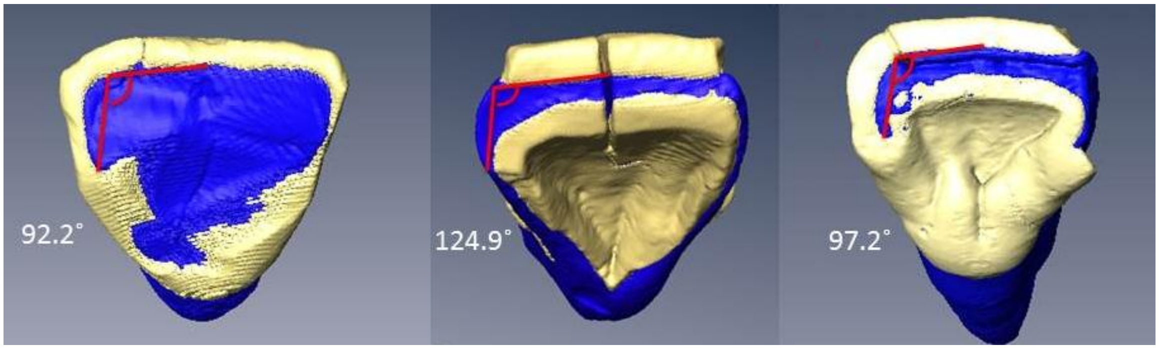



*Figure 4.1* Comparison of the average labial convexity in a non-shoveled incisor (left, score 1), moderate shoveled incisor (center, score 4), and developed shoveled incisor (right, score 5).

As expected, the shovel-shaped incisors express a higher mean of all linear measurements; with the exception of the EDJ labial-lingual diameter. The occlusal labial-lingual diameter of shoveled teeth (mean 2.2mm) is not much larger than in non-shoveled teeth (mean 2.0mm). This is an interesting outcome, in that the labial-lingual diameter of the EDJ surface is statistically significantly smaller in shoveled teeth. This dimension of the tooth is perhaps a result of conservation of tooth volume, and will further be discussed in the PCA results. The variables that showed the highest difference were crown height for both surfaces, including enamel and dentin volume.

*Descriptive statistics for maxillary shovel-shaped incisors*

| Variable | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| MD-O | 7 | 7.4657 | 0.9489 | 6.12 | 8.82 |
| LL-O | 7 | 2.2114 | 0.2290 | 1.91 | 2.66 |
| CH-O | 7 | 9.5000 | 0.8073 | 8.66 | 10.92 |
| Mrt-O | 7 | 1.8214 | 0.7014 | 0.60 | 2.94 |
| Lbh-O | 7 | 1.6714 | 1.7714 | 0.00 | 4.51 |
| MD-E | 7 | 6.1143 | 0.8141 | 4.61 | 7.14 |
| LL-E | 7 | 0.6043 | 0.2423 | 0.23 | 0.88 |
| CH-E | 7 | 9.168 | 0.9197 | 8.15 | 10.62 |
| Mrt-E | 7 | 1.0571 | 0.5617 | 0.23 | 1.99 |
| Lbh-E | 7 | 1.3828 | 1.3969 | 0.00 | 3.13 |
| LC-E | 7 | 116.67 | 14.201 | 97.20 | 132.20 |
| Dentin Vol. | 7 | 359.79 | 44.972 | 299.21 | 425.71 |
| Enamel Vol. | 7 | 90.947 | 27.400 | 51.36 | 122.37 |

*Descriptive statistics for maxillary non-shovel-shaped incisors*

| Variable | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| MD-O | 3 | 7.2733 | 1.2604 | 5.93 | 8.43 |
| LL-O | 3 | 2.0333 | 0.7649 | 1.37 | 2.87 |
| CH-O | 3 | 7.6600 | 1.0751 | 6.68 | 8.81 |
| Mrt-O | 3 | 0.0800 | 0.1385 | 0.00 | 0.24 |
| Lbh-O | 3 | 1.2700 | 2.1997 | 0.00 | 3.81 |
| MD-E | 3 | 5.7167 | 1.8943 | 3.55 | 7.06 |
| LL-E | 3 | 1.4567 | 0.5967 | 0.77 | 1.85 |
| CH-E | 3 | 7.4767 | 0.8156 | 6.68 | 8.31 |
| Mrt-E | 3 | 0.0733 | 0.1270 | 0.00 | 0.22 |
| Lbh-E | 3 | 0.9867 | 1.7089 | 0.00 | 2.96 |
| LC-E | 3 | 100.50 | 9.5252 | 92.20 | 110.90 |
| Dentin Vol. | 3 | 331.75 | 87.455 | 248.28 | 422.71 |
| Enamel Vol. | 3 | 57.363 | 3.0310 | 53.95 | 59.74 |

*Table 4.2 Descriptive* statistics for shoveled and non-shoveled incisors. Variable abbreviations found in Chapter 3, *Table 3.1*.

*Correlation Analyses*

The coefficient will be high if the two variables approximate the regression line. The variables used in the correlation analyses and abbreviations are found in Chapter 3, *Table 3.1*.

As expected, Pearson's correlation analysis reveals that four out of the five linear measurement pairs are significantly correlated at the $p<0.05$ level (*Table 4.3*). Generally, as one dimension of the EDJ structure increases, occlusal dimensions increase as well. The strongest correlations, where the dimensions in EDJ surface accounts for approximately 90% or higher of the variability observed in the same dimensions in the occlusal surface are, crown height (r =0.97), marginal ridge thickness (r =0.95), and labial base height (r =0.98). While three teeth were missing enamel on the mesial and/or distal surface, nevertheless, the mesiodistal diameter of the occlusal and EDJ are highly and significantly correlated (r=0.79). Interestingly, the one paired dimension that is not statistically correlated, labial-lingual diameter, only explains 7% variation (r =0.26).

| Variable | N | Pearson's Correlation (r) | $r^2$ | p |
|---|---|---|---|---|
| Mesial-distal diameter | 10 | 0.79730 | 0.63568 | 0.0057* |
| Labial-lingual diameter | 10 | 0.26902 | 0.07237 | 0.4523 |
| Crown height | 10 | 0.97493 | 0.95048 | <0.0001* |
| Marginal ridge thickness | 10 | 0.95542 | 0.91283 | <0.0001* |
| Labial-base height | 10 | 0.98421 | 0.96867 | <0.0001* |

*Table 4.3.* Pearson's Correlation coefficients for the five paired occlusal and EDJ morphology, * significant at the 0.05 level (2-tailed).

The Pearson's correlation matrix for 10 of the observed variables (non-transformed) is present in *Table 4.4.* Besides the paired linear measurements, there exist three other significant correlations that are revealing in terms of shoveling morphology. The labial-lingual diameter of the EDJ is negatively correlated with the occlusal surface's crown height and marginal ridge thickness. As the labial-lingual diameter of the EDJ increases, the occlusal crown height and occlusal marginal ridge thickness decreases. This is present in descriptive statistics, as the most shoveled teeth exhibit a smaller labial-lingual diameter than the non-shoveled incisors (*Figure 4.2*). The mesiodistal diameter of the EDJ is negatively correlated with the EDJ labial base height. This correlation is a bit more difficult to interpret, as only 40% of the sample exhibited tubercles, thus the significance of this correlation is questioned, as it is only r=0.642, where r≥±0.64 is significant at the 0.05 level. The height of the EDJ does not seem to influence the mesiodistal diameter of the occulsal and EDJ surface (r=-0.043, r=0.035 respectively). Interestingly, the weakest correlation is between the mesiodistal diameter of the EDJ and the occlusal marginal ridge thickness (r=-0.0003). These two variables would seem to be correlated in shovel-shaped incisors, yet they appear to not influence the other.

Pearson's Correlation Coefficient (r)

| | LL-O | LL-E | MD-O | MD-E | CH-O | CH-E | Mrt-O | Mrt-E | Lbh-O | Lbh-E |
|---|---|---|---|---|---|---|---|---|---|---|
| LL-O | 1.000 | . | . | . | . | . | . | . | . | . |
| LL-E | 0.269 | 1.000 | . | . | . | . | . | . | . | . |
| MD-O | 0.209 | 0.236 | 1.000 | . | . | . | . | . | . | . |
| MD-E | 0.519 | 0.397 | **0.797** | 1.000 | . | . | . | . | . | . |
| CH-O | -0.286 | **-0.678** | -0.044 | -0.021 | 1.000 | . | . | . | . | . |
| CH-E | -0.223 | -0.606 | -0.043 | 0.035 | **0.975** | 1.000 | . | . | . | . |
| Mrt-O | 0.344 | **-0.677** | -0.093 | -0.003 | 0.473 | 0.442 | 1.000 | . | . | . |
| Mrt-E | 0.352 | -0.559 | -0.202 | -0.083 | 0.422 | 0.422 | **0.955** | 1.000 | . | . |
| Lbh-O | -0.091 | -0.321 | -0.390 | -0.569 | 0.125 | 0.083 | 0.452 | 0.618 | 1.000 | . |
| Lbh-E | -0.128 | -0.386 | -0.433 | **-0.642** | 0.120 | 0.085 | 0.451 | 0.615 | **0.984** | 1.000 |

*Table 4.4.*Pearson's Correlation matrix for 10 of the observed variables. r ≥ ±0.64 is significant at the 0.05 level for a two-tailed test (bold r values).

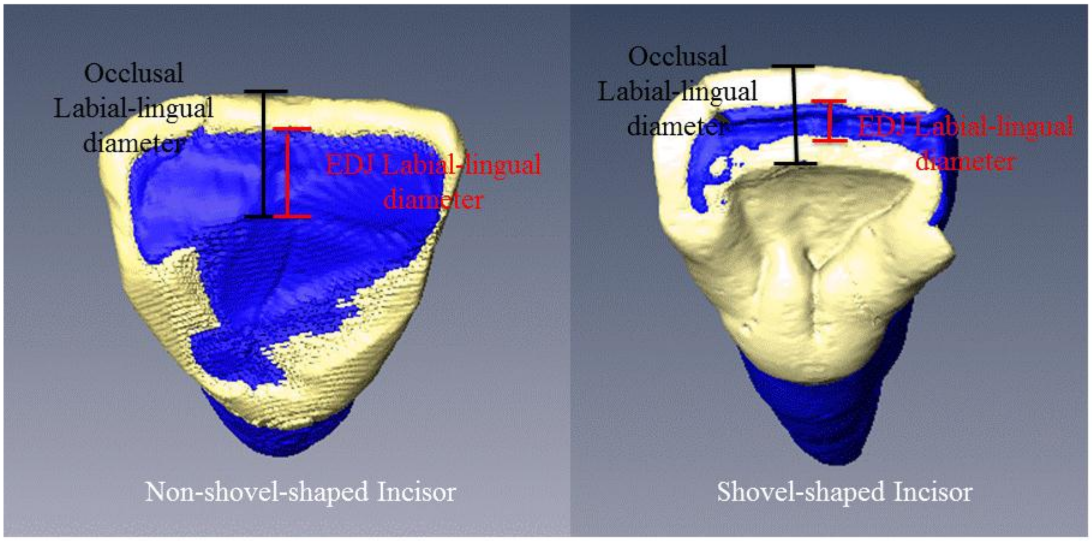



*Figure 4.2*. EDJ surface labial-lingual diameter comparison for non-shoveled incisor with diameter 1.75mm (left) and shoveled incisor with diameter 0.88mm (right).

The Spearman's correlation analysis for the transformed volume and labial convexity angle against all measurements only produced one significant correlation; enamel volume is positively correlated with the occlusal surface mesiodistal diameter (*Table 4.3*). Nevertheless, it is conceivable that as enamel volume increases, the mesiodistal diameter (length) of the crown surface will as well. Labial convexity and dentin volume were not found to be significantly correlated with the other measurements observed. As the shoveled teeth exhibited slight labial convexity, measurement of this angle should further be investigated to determine the variation in angle degree seen in modern humans as this is not typically seen in modern human shoveling.

| Variable | N | Spearman's Correlation ($r_s$) | $r^2$ | p |
|---|---|---|---|---|
| MD-O, 3√EV | 10 | 0.66061 | 0.43640 | 0.0376* |

*Table 4.5*. Significant Spearman's Correlation. * significant at the 0.05 level (two-tailed test). See *Appendix B* for variable descriptions.

In terms of overall shovel-shaped incisor variability, the correlation results indicate that regardless of the degree of shoveling, there exists a very strong relationship between the EDJ and occlusal morphology.

*Principal components analysis*

The 13 dental measurements taken in Amira were subjected to a principal components analysis using ones as prior communality estimates, where each variable is set to contribute 1% of the total variation. The principal axis method was used for the extraction of components, and was followed by a varimax rotation (orthogonal).

Four components displayed eigenvalues greater than 1, and the results of a scree test suggested that only the first three components were meaningful, thus retained for rotation and further analysis. The total amount of variance explained by the first three components is 90.84%. The varimax rotation offers a simpler interpretation, as the amount of variance each component explains is distributed more equally between the three components (Jolliffe, 1986). The corresponding factor loadings for the 13 measurements, eigenvalues, and percentage of total variance in the unrotated and varimax rotated analysis are presented in *Table 4.6*. In interpreting the rotated factor pattern, a variable was said to load on a given component if the factor loading was .40 or greater for that component, and was less than .40 for the other two. Using these criteria, two variables were found to load on the first component, which was subsequently denoted as the size contributing component. The second and third component had four variables and two variables loaded respectfully, and are denoted as shape components.

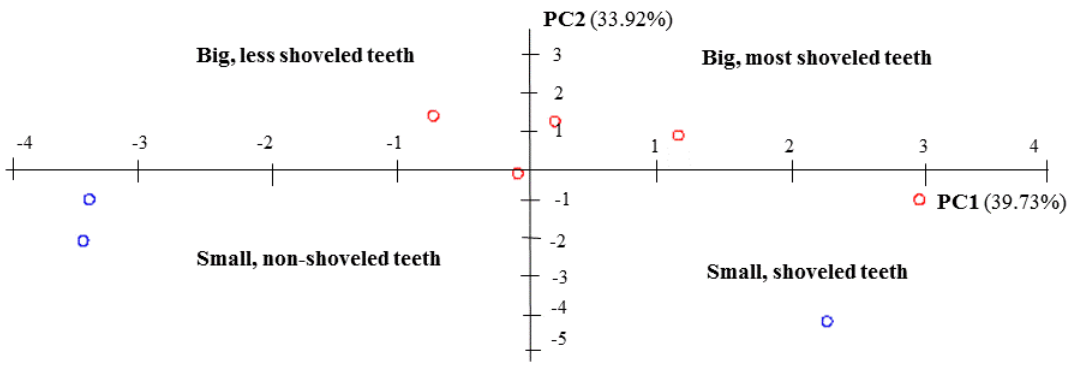
<u>Principal Components Analysis</u>

| | Unrotated | | | Rotation | | |
|---|---|---|---|---|---|---|
| Variable | PC 1 | PC 2 | PC 3 | PC 1 | PC 2 | PC 3 |
| LL-O | -.26 | .37 | **.75** | .08 | .29 | **.84** |
| LL-E | **-.75** | -.37 | .31 | .31 | **-.84** | .01 |
| MD-O | **-.54** | **.56** | 0 | .38 | -.13 | .14 |
| MD-E | **-.68** | **.67** | .16 | **.66** | -.12 | **.50** |
| CH-O | **.53** | **.58** | **-.64** | .04 | **.96** | -.05 |
| CH-E | **.48** | **.59** | **-.50** | .08 | **.92** | .01 |
| Mrt-O | **.64** | **.62** | .37 | .39 | **.59** | **.67** |
| Mrt-E | **.71** | **.49** | **.47** | **.53** | **.50** | **.67** |
| Lbh-O | **.82** | -.21 | .38 | **.95** | .12 | .08 |
| Lbh-E | **.85** | -.24 | .34 | **.97** | .15 | .03 |
| LC | **-.46** | **.74** | -.11 | **-.73** | .25 | **.43** |
| EV | .29 | **.67** | -.15 | .16 | **.52** | .08 |
| DV | -.32 | **.70** | .33 | .37 | .05 | **.68** |
| Eigenvalue | 4.6118 | 3.9368 | 1.9959 | 3.6509 | 3.5575 | 2.5498 |
| Total var. | 39.73% | 33.92% | 17.19% | 31.45% | 30.65% | 21.97% |

*Table 4.6*. Factor loadings, eigenvalues, and total variance. Marked loadings are >0.40 and in bold. The total variance explained by PCA is equal to 100%, the first three components explain 90.84%, 84.07% in rotation. For variable descriptions see Chapter 3, *Table 3.1*.

Overall in terms of the present study, each of the 13 variables highly loads onto at least one of the three factors between 0.50-0.80, determining its dependence on the size and shape of the incisor. The PCA confirms the findings of the correlation analyses, of the strong relationship between the EDJ and occlusal morphology. *Figure 4.3* illustrates that PC2 vs. PC1 is distinguishing the non-shoveled and shoveled incisors by size, whereas the second graph PC3 vs. PC1 clearly is depicting the shape of the incisor. The one non-shoveled tooth outlier can be explained, as it not only expressed slight marginal ridging, but the presence of a well-defined tubercle, rightly placing it in the shoveling size and shape component quadrants.

A)




B)

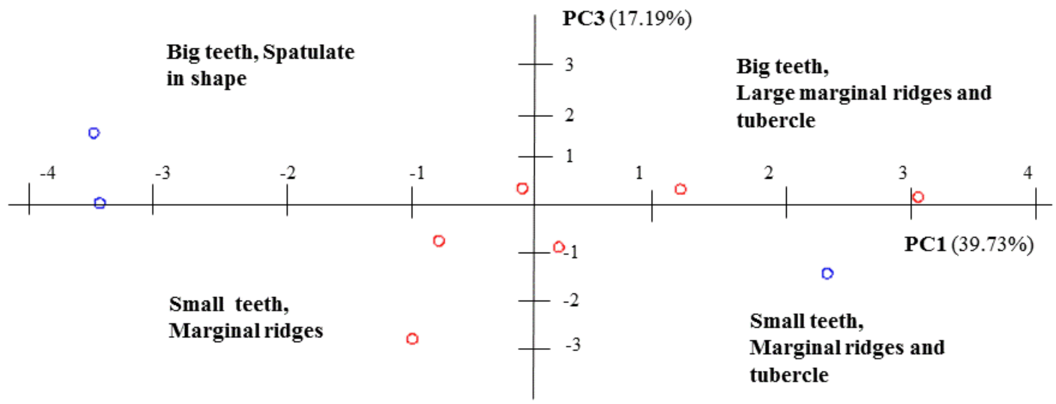



*Figure 4.3.* A) PC1 vs. PC2 explains 73.65% of the variation for size and incisor shape, B) PC1 vs. PC3 explains 56.92% of the variation for shoveling shape (unrotated). Blue= non-shovel-shaped incisors, red= shovel-shaped incisors.

The rotated components were then computed into factor scores, which provide linear composites of the variables that demonstrated meaningful loadings for each component (Jolliffe, 1986). It appears that Factor 2 is describing overall incisor shape, as the EDJ crown height and labial-lingual diameter influence the enamel volume and occlusal crown height. This is reiterated with the correlation analysis, the smaller the EDJ labial-lingual diameter, the larger amount of volume, and as it appears crown height are expressed in shoveling teeth. Factor 3 is more specifically describing shoveling shape, as the shoveling characteristics of EDJ and occlusal marginal ridge thickness and labial-base height are related to the occlusal labial-lingual diameter.

| Variable | Factor 1 (Size) | Factor 2 (Shape) | Factor 3 (Shape) | Communality Estimate |
|---|---|---|---|---|
| LL-O | **0.56** | -0.40 | **0.54** | 0.76 |
| LL-E | 0.25 | **-0.80** | -0.31 | 0.80 |
| MD-O | **0.77** | 0.03 | -0.13 | 0.61 |
| MD-E | **0.97** | -0.08 | -0.05 | 0.95 |
| CH-O | 0.01 | **0.95** | 0.07 | 0.91 |
| CH-E | 0.06 | **0.91** | 0.08 | 0.84 |
| Mrt-O | 0.12 | **0.46** | **0.84** | 0.93 |
| Mrt-E | -0.01 | 0.35 | **0.92** | 0.97 |
| Lbh-O | **-0.62** | 0.06 | **0.68** | 0.85 |
| Lbh-E | **-0.67** | 0.09 | **0.67** | 0.90 |
| LC-E | **0.84** | 0.25 | -0.11 | 0.77 |
| EV | 0.30 | **0.63** | 0.27 | 0.56 |
| DV | **0.79** | 0.02 | 0.29 | 0.70 |

*Table 4.7*. Rotated factor pattern and final communality estimates from PCA. Marked loadings are >0.40 and in bold. Note: n=100. Variable abbreviations and descriptions in Chapter 3, *Table 3.1*.

*Direct vs. µCT measurements*

The direct occlusal measurements taken using calipers were paired with the occlusal measurements taken in Amira in a t-test to determine the accuracy of the measurements used in the study (*Table 4.8*). While in Amira the tooth can be enlarged for a more accurate view to take measurements, examining the raw data (*Appendix A and B*), the CT measurements seem to underestimate at most by 0.3mm. The variables that expressed the most difference in measurement were the mesiodistal diameter, labial-lingual diameter, and marginal ridge thickness with p-values 0.07-0.08. The variable with the least amount of variance was the labial-base height, most likely due to the visible distinction of a tubercle on the tooth. However, the μCT measurements are in line with the direct measurements, as the error produced is within the acceptable 5%. This result supports previous research that has stated reliable linear measurements on post-canine teeth (i.e., Christiansen et al., 1986; Kim et al., (2006); and Waitzman et al., 1992) by adding that linear measurements in μCT are also reliable on incisors.

<u>Student's t Test Values for Direct vs. μCT Variability</u>

| Variable | Mean | SD | SEM | 95% CI (mean) | p |
|---|---|---|---|---|---|
| LL-O (mm) | 0.0580 | 0.0964 | 0.0305 | -0.01-0.12 | 0.0894 |
| MD-O (mm) | 0.0380 | 0.0590 | 0.0187 | -0.004-0.08 | 0.0723 |
| CH-O (mm) | 0.1870 | 0.4368 | 0.1381 | -0.12-0.49 | 0.2088 |
| Mrt-O (mm) | 0.0300 | 0.0467 | 0.0148 | -0.003-0.06 | 0.0726 |
| Lbh-O (mm) | -0.0220 | 0.1424 | 0.0450 | -0.12-0.07 | 0.6369 |

*Table 4.8.* Direct vs. μCT measurements. SEM= standard error of the mean, CI= confidence interval. '*'significant at the 0.05 level (two-tailed). See *Table 3.2* for variable descriptions.

*Intra-observer and inter-observer error*

In order to test for intra- and inter-observer error, the segmentation of the tissues was the first step to complete before taking measurements. With the set attenuation values on the grey scale value distributions, the enamel and dentin tissues were identifiable and able to seamlessly separate. Four teeth were measured twice by the author, and by another observer. This data set was then subjected to a paired t-test to test the equality of the means.

Intra-observer error shows the present study produces accurate measurements within an acceptable rate of 5% error (*Table 4.9*). Surprisingly, the variable with the most error is the dentin volume. However, when examining the actual dentin measurements, the error lies in a maximum of 0.10mm. The occlusal marginal ridge thickness had the next greatest amount of error, and will be discussed further with inter-observer error. The variables that produce the least amount of error were the occlusal and EDJ crown height.

Student's t Test Values for Intra-observer Variability

| Variable | Mean | SD | SEM | 95% CI (mean) | p |
|---|---|---|---|---|---|
| LL-O (mm) | 0.0450 | 0.0420 | 0.0210 | -0.02-0.11 | 0.1217 |
| LL-E (mm) | 0.0450 | 0.0614 | 0.0307 | -0.05-0.14 | 0.2388 |
| MD-O (mm) | -0.0375 | 0.0842 | 0.0421 | -0.17-0.09 | 0.4388 |
| MD-E (mm) | -0.0550 | 0.0900 | 0.0450 | -0.19-0.08 | 0.3089 |
| CH-O (mm) | 0.0175 | 0.1193 | 0.0596 | -0.17-0.20 | 0.7883 |
| CH-E (mm) | 0.0050 | 0.1308 | 0.0654 | -0.20-0.21 | 0.9439 |
| Mrt-O (mm) | -0.0400 | 0.0294 | 0.0147 | -0.08-0.006 | 0.0727 |
| Mrt-E (mm) | -0.0950 | 0.1933 | 0.0967 | -0.40-0.21 | 0.3981 |
| Lbh-O (mm) | 0.1050 | 0.1969 | 0.0984 | -0.20-0.41 | 0.3644 |
| Lbh-E (mm) | 0.2075 | 0.2399 | 0.1200 | -0.17-0.58 | 0.1821 |
| LC (°) | 4.8250 | 8.9417 | 4.4709 | -9.40-19.05 | 0.3595 |
| EV ($mm^3$) | 0.0125 | 0.0250 | 0.0125 | -0.02-0.05 | 0.3910 |
| DV ($mm^3$) | -0.0775 | 0.0544 | 0.0272 | -0.164-0.009 | 0.0651 |

*Table 4.9.* Intra-observer error. SEM= standard error of the mean, CI= confidence interval. '*'significant at the 0.05 level (two-tailed). See *Table 3.2* for variable descriptions.

Comparable to intra-observer error, inter-observer error for all measurements are within the reasonably accepted 5% error, denoting that the measurements used in this present study are easily reproduced by other observers *(Table 4.10)*. The highest variability was seen in the EDJ marginal ridge thickness as a set of arbitrary points of the two observers were taken; not only does there exist error overall, but in each arbitrary length taken. The next variable that had considerably higher error than others was similarly the marginal ridge thickness of the occlusal morphology for the same reasons. The EDJ marginal ridge thickness resulted in a higher error rate due to the smaller surface the measurements were taken from. However, as stated previously, the error is well within the acceptable rate of 5%. The variables that had the least amount of inter-observer error were the labial convexity angle, and the occlusal crown height. Dentin volume was not seen as producing a considerable amount of inter-observer error; this result concludes that the dentin volume is in fact reproducible, and that the error was to the author's alone.

<u>Student's t Test Values for Inter-observer Variability</u>

| Variable | Mean | SD | SEM | 95% CI (mean) | p |
|---|---|---|---|---|---|
| LL-O (mm) | 0.2525 | 0.4092 | 0.2046 | -0.39-0.90 | 0.3050 |
| LL-E (mm) | -0.0775 | 0.2427 | 0.1213 | -0.46-0.30 | 0.5684 |
| MD-O (mm) | 0.2400 | 0.3089 | 0.1544 | -0.25-0.73 | 0.2180 |
| MD-E (mm) | 0.0825 | 0.1584 | 0.0792 | -0.16-0.33 | 0.3741 |
| CH-O (mm) | -0.1425 | 0.6969 | 0.3484 | -1.25-0.96 | 0.7100 |
| CH-E (mm) | -0.1675 | 0.2269 | 0.1135 | -0.52-0.19 | 0.2363 |
| Mrt-O (mm) | 0.6000 | 0.5111 | 0.2556 | -0.21-1.41 | 0.1005 |
| Mrt-E (mm) | 0.4300 | 0.3360 | 0.1680 | -0.10-0.96 | 0.0832 |
| Lbh-O (mm) | 0.5225 | 0.7798 | 0.3899 | -0.71-1.76 | 0.2727 |
| Lbh-E (mm) | -0.1625 | 0.4679 | 0.2340 | -0.90-0.58 | 0.5373 |
| LC ($^{o}$) | 0.0750 | 7.9596 | 3.9798 | -12.59-12.74 | 0.9861 |
| EV ($mm^3$) | 0.9375 | 1.8750 | 0.9375 | -2.04-3.92 | 0.3910 |
| DV ($mm^3$) | -11.0800 | 22.2155 | 11.1078 | -46.42-24.26 | 0.3920 |

*Table 4.10.* Inter-observer error. SEM= standard error of the mean, CI= confidence interval. '*'significant at the 0.05 level (two-tailed). See *Table 3.2* for variable descriptions.

## SUMMARY

This chapter summarized the results of the present study. The Pearson's correlation results indicate that the null hypothesis can be rejected in favor of the alternative that there is significant correlation between the occlusal and EDJ morphology in upper incisors. The PCA results are more difficult to interpret, but clearly depict the factors size and shape distinguishes non-shovel-shaped incisors from shovel-shaped incisors. Inter- and intra-observer error demonstrates that the innovative methodology designed for measuring the occlusal and EDJ surfaces of upper incisors is accurate and easily replicated. This investigation of the EDJ provides further information concerning the development of shoveling, as well as the complete expression of this feature in the occlusal morphology. These findings have interesting implications for continued studies of shovel-shaped incisors in modern human populations, as well as future research concerning this trait in Neanderthals, and will be discussed in the conclusion.

# CHAPTER V

# DISCUSSION AND CONCLUSION

*Origins of the present study*

The morphology of the EDJ and its relationship to the overall morphology of the tooth crown is a relatively new research trajectory in dental anthropology. An examination of the EDJ provides insights into 1. discrete trait variation in degree of expression at the EDJ in modern human populations, 2. a possible developmental relationship of discrete traits at the EDJ that are visible in the occlusal surface, and finally 3. applications toward hominin taxonomy. This research requires the use of techniques that are non-destructive and allow for the visualization of the internal and external structure. The purpose of the present research was to examine previously unexplored crown morphology, shoveling of the incisors, to determine if there is a level of correspondence concerning shoveling characteristics between the underlying EDJ and the crown surface.

This research brought about ideas of examining the EDJ of incisors that express a distinctive trait, shoveling, that is seen not only in modern humans, but in Neanderthals and other fossil hominins. The goal has been to investigate if the morphology of the EDJ mirrors that of the occlusal surface. The results can shed light to factors of possible genetic influence of shovel-shaped incisors and evolutionary relationships that may exist among and between hominins.

Since the EDJ of incisors have not been previously studied using µCT, this pilot study achieved the aim to assess the level of correspondence of shoveling, between the EDJ and occlusal surface in modern humans, and to create a reproducible and accurate methodology for visualizing, and measuring characteristics seen in incisors.

Recent studies have suggested the positive applicability of µCT in terms of its non-destructive and accurate method to visualize the internal and external morphology of hominin teeth (Schwartz et al., 1998; Skinner et al., 2008; Skinner et al., 2009a, Skinner et al., 2009b). Previous early hominin EDJ studies on post-canine morphology have expressed variation in the shape of the EDJ as well as specific trait expression, i.e. Carabelli's cusp, visible at the EDJ (Hunter et al., 2010; Schwartz et al., 1998). Thus the studies are instrumental in understanding the development of tooth shape, discrete traits, as well as the genetic undertones that may be involved during dental development.

*Summary of results*

A small sample (n=10) of modern human upper incisors that express different degrees of shoveling were examined. In most variables compared in this study, a correspondence between external and internal morphology exists. In other words, no matter what the degree of shoveling, expressed, the morphology of the EDJ was a strong reflection of the morphology of the crown.

Morphological differences between non-shovel-shaped and shovel-shaped incisors include overall size, where shoveled incisors have a relatively greater amount of enamel and dentin volume. The study has identified that the EDJ may be specifically responsible for the shoveling expression observed in the occlusal morphology; as there are statistically significant correlations between the dimensions of the tooth and shoveling characteristics of the occlusal and EDJ surface. The exception to this was the labial-lingual diameter, as the correlation between the two surfaces was weak. This variable was a surprising result as the EDJ labial-lingual diameter was smaller in shoveled teeth. This appears to be a volume conservation mechanism. The volume is extended mesiodistally and lingually as shoveling increases. Perhaps this may also be related to the amount of space available in the maxilla.

Since the angle of labial convexity has not been previously measured, this variable requires further investigation, as to what constitutes full expression of labial convexity vs. a straight margin. All incisors, shoveled and non-shoveled, visually expressed the typical straight margin characteristic of modern human shoveling, yet expressed over a 90$^{o}$ angle on the mesial-distal surface. This appears to relate again to the overall size of the tooth, as the moderately shoveled teeth seemed to have a more obtuse angle, where the more developed shoveling decreased back closer to 90˚. This is most likely due to the fact that the volume is extending into the marginal ridges, as the tooth cannot just continue to increase laterally, but must conserve the space it has in the maxilla. While there were large angular differences among inter- and intra-observer error, it was within the acceptable rate of error. This variable is critical for examining shoveling seen in Neanderthals, and thus creates a framework to further understanding the increase in expression of certain shoveling characteristics in a population.

One of the major results of this work is to highlight the necessity of developing plaques to ordinally score the degree of shoveling seen in the EDJ. Based on observations, slight to moderate, to developed shoveling can positively be identified. Therefore, a similar system to the ASUDAS plaques for shovel expression in the occlusal surface should be made for the EDJ surface. Importantly, this would allow for the comparison of degree of shoveling expressed at the EDJ surface and what is observed at the occlusal surface. This would also allow for future population comparison of the degree of shoveling in the EDJ.

The methodology of this study has proven its accuracy and replicability. However, the limitations of a small sample size have produced statistics that are significant, but should be interpreted and used cautiously. The direct linear measurements taken on the occlusal surface using a digital caliper are in line with the CT linear measurements taken in Amira. While Amira allows to the rotation and enlargement of the sample to get a closer and more accurate reading, compared to direct measurements, it seems to slightly underestimate the true size of the tooth at most by 0.3mm. The intra-observer and inter-observer error both concluded that the measurements taken in Amira produce an error rate no higher than 5% and is easily reproducible. Overall, these results provide evidence for the use of μCT in this study and for future studies concerning the examination of the EDJ surface in upper incisors.

The results of the study support the idea that the morphology of the EDJ controls the overall morphology of the crown. The correlations as mentioned previously strongly relate the EDJ morphology to that seen in the crown. Additionally, the PCA results confirmed that most of the variables measured could be reduced to the factors of tooth size, overall incisor shape, and shoveling shape. Although this study is not a direct genetic test, it does not refute but supports what others have suggested about the phenotype, shoveling morphology, being under strict genetic control (Blanco and Chakraborty, 1976; Hanihara and Tanaka, 1970; Kimura et al., 2009; Portin and Alvesalo 1974). This is based on the growth and development of the tooth, where the EDJ plays a crucial role in the determination of the overall occlusal morphology (Beynon et al. 1991; Gannet et al., 2007; Smith and Tafforeau, 2008), previous heredity studies on shoveling seen in modern human populations (Blanco and Chakraborty, 1976; Hanihara and Tanaka, 1970; Portin and Alvesalo, 1974), and finally, Kimura and colleagues (2009) EDAR genetic find linking shoveling to a specific allele.

Although this study is not a direct test of the distribution of character states of hominin teeth, it may in the future be applied to such studies. As previous research has noted, information at the EDJ may be present that is not observed in the occlusal morphology of hominin fossils due to attrition. Thus, the EDJ of incisors should be able to facilitate comparisons of discrete trait expression with other hominins. Specifically, the results of this study can be applied to future research of shovel-shaped characteristics in the EDJ morphology of Neanderthals.

*Future Directions*

Neanderthal and modern human shoveling is different, and can be seen as a multi-allelic system. This research has already demonstrated that slight, moderate, to developed shoveling can be identified in the EDJ. Additionally, correlation coefficients establish that there are statistically strong relationships between the EDJ and occlusal morphology of upper incisors. Thus, there is potential that the extreme shoveling and double shoveling expressed by Neanderthals can also be visualized and positively identified using non-destructive µCT. This would allow for the comparison of variability in shoveling characteristics expressed by Neanderthals in relation to modern humans.

Future directions also potentially lead to using the EDJ morphology of incisors for taxonomic assessments among hominins. It would be helpful to investigate incisor EDJ surface of primates to determine if modern human incisors can positively be distinguished from other species. An investigation on how shoveling seen in primates is similar and different from modern humans will be essential for this direction. This examination would be significant in aiming to answer the larger taxonomic affiliation of Neanderthals.

*Final conclusions*

The purpose of this study was to assess the relationship between the occlusal and EDJ morphology of shoveling in upper incisors using µCT. The expectation was that the shoveling characteristics observed in modern humans were hereditable traits that are due to genetics, or a combination of genetics and environmental factors.

Given the current conclusions of Kimura et al. (2009), with the Allele 1540C associated with shoveling in incisors, this study gives considerable support for a genetically driven morphology that appears early in the developmental trajectory of the anterior teeth. The results indicated that shoveling can be seen in the EDJ surface, and that there exists a level of variation at the EDJ depending on the occlusal degree of shoveling. The results also identified that shoveling characteristics seen in the EDJ are linked to the overall size and shape of the incisor, and importantly the shape of shoveling. Thus, lending support for shoveling morphology to be a genetically driven trait. This study is an original contribution to knowledge concerning discrete trait expression in the

EDJ of upper incisors in modern humans. The results of this study contributes to our understanding of the development of shoveling characteristics, as well as the application of μCT in anterior teeth research for a non-destructive and three dimensional method that is reproducible and accurate.

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# APPENDIX A: μCT MEASUREMENTS

| Specimen # | Tooth | Shovel (0-7) | Wear (0-8) | LL-O | LL-E | MD-O | MD-E | CH-O | CH-E | Enamel Volume | Dentin Volume | Mrt-O | Mrt-E | Lbh-O | Lbh-E | LC- E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **19B**[a] | L. $I^1$ | 0 | 4 | 2.87 | 1.85 | 7.46 | 6.54 | 6.68 | 6.68 | 53.95 | 324.28 | 0.00 | 0.00 | 0.00 | 0.00 | 98.4º |
| **5PE527.6 00-16**[a] | $I^1$ | 1 | 4 | 1.86 | 1.75 | 8.43 | 7.06 | 7.49 | 7.44 | 59.74 | 422.71 | 0.00 | 0.00 | 0.00 | 0.00 | 92.2º |
| **Tooth 4**[a] | $I^2$ | 1 | 2 | 1.37 | 0.77 | 5.93 | 3.55 | 8.81 | 8.31 | 58.40 | 248.28 | 0.24 | 0.22 | 3.81 | 2.96 | 110.9º |
| **Tooth 3**[b] | $I^1$ | 2 | 2 | 1.91 | 0.76 | 8.82 | 7.14 | 10.92 | 10.62 | 122.37 | 345.92 | 0.60 | 0.23 | 0.00 | 0.00 | 111.3º |
| **Tooth 1**[b] | $I^2$ | 4 | 3 | 2.21 | 0.65 | 6.12 | 6.12 | 10.21 | 10.16 | 56.25 | 405.88 | 1.86 | 1.08 | 0.00 | 0.00 | 124.9º |
| **83.1.76.1**[c] | L. $I^1$ | 4 | 2 | 2.13 | 0.39 | 8.12 | 6.26 | 8.96 | 8.15 | 107.70 | 346.59 | 1.87 | 0.64 | 0.00 | 0.00 | 132.2º |
| **84.2.0**[c] | R. $I^2$ | 3 | 4 | 2.27 | 0.83 | 7.15 | 6.23 | 8.88 | 8.34 | 51.36 | 321.81 | 1.46 | 0.91 | 1.99 | 1.56 | 131.1º |
| **Tooth 2**[b] | $I^2$ | 5 | 3 | 2.66 | 0.88 | 7.95 | 6.77 | 9.43 | 9.13 | 107.63 | 425.71 | 2.94 | 1.99 | 4.51 | 3.13 | 97.2º |
| **83.1.1.3**[c] | R. $I^2$ | 4 | 3 | 2.20 | 0.23 | 7.62 | 5.67 | 8.66 | 8.66 | 104.11 | 373.44 | 1.97 | 1.12 | 2.07 | 2.04 | 119.9º |
| **84.1.1**[c] | R. $I^2$ | 4 | 2 | 2.10 | 0.49 | 6.48 | 4.61 | 9.44 | 9.12 | 87.21 | 299.21 | 2.05 | 1.43 | 3.13 | 2.95 | 100.1º |

[a] European descent, [b] Asian descent, [c] Gallina descent. $I^1$= Central Incisor, $I^2$= Lateral Incisor, L= Left, R= Right. Measurements are in (mm), and volume in ($mm^3$).

APPENIX B: DIRECT OCCLUSAL MEASUREMENTS

| Specimen # | Tooth | Shovel (0-7) | Wear (1-8) | MD-O | LL-O | CH-O | Mrt-O | Lbh-O |
|---|---|---|---|---|---|---|---|---|
| 19B[a] | L. $I^1$ | 0 | 4 | 7.48 | 2.74 | 6.73 | 0 | 0 |
| 5PE527.6 00-16[a] | $I^1$ | 1 | 4 | 8.40 | 1.88 | 7.52 | 0 | 0 |
| Tooth 4[a] | $I^2$ | 1 | 2 | 5.97 | 1.36 | 8.87 | 0.25 | 3.43 |
| Tooth 3[b] | $I^1$ | 2 | 2 | 8.87 | 1.93 | 10.98 | 0.55 | 0 |
| 84.2.0[c] | R. $I^2$ | 3 | 4 | 7.14 | 2.28 | 8.86 | 1.50 | 2.19 |
| Tooth 1[b] | $I^2$ | 4 | 3 | 6.10 | 2.25 | 10.40 | 1.90 | 0 |
| 83.1.76.1[c] | L. $I^1$ | 4 | 2 | 8.14 | 2.16 | 8.87 | 1.92 | 0 |
| 83.1.1.3[c] | R. $I^2$ | 4 | 3 | 7.71 | 2.19 | 10.07 | 2.10 | 2.02 |
| 84.1.1[c] | R. $I^2$ | 4 | 2 | 6.53 | 2.09 | 9.47 | 2.10 | 3.15 |
| Tooth 2[b] | $I^2$ | 5 | 3 | 8.12 | 2.90 | 9.58 | 2.97 | 4.50 |

[a]European descent, [b]Asian descent, [c]Gallina descent. $I^1$= Central Incisor, $I^2$= Lateral Incisor, L=Left, R=Right. Measurements are in (mm).