SEXUAL DIMORPHISM OF MODERN DECIDUOUS TOOTH CROWN TISSUES

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By

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ABSTRACT

This study presents data on the extent of the sexual dimorphism observed in tissue measurements of the deciduous tooth crowns for a heterogeneous modern human sample. A series of linear and area measurements were taken from crowns sectioned in a buccolingual plane. The buccal enamel and perpendicular buccal enamel, situated towards the incisal surface, are significantly thicker in female deciduous upper canines than male upper canines ($p = 0.002$ and $p = 0.001$, respectively). Furthermore, there is a distinct trend for the female enamel to be thicker than male enamel in the lower first incisor, and upper second incisor, and more so in the buccal enamel rather than lingual enamel. No differences in dentine-pulp thickness were observed between the sexes. Area measurements did not exhibit significant levels of sexual dimorphism, although the enamel cap area relative to both the total crown area and cervical diameter tend to be larger in the female lower canines, while the male lower canine dentine-pulp area relative to total crown area tends to be larger.

These results support conclusions of previous research that a sexually dimorphic distribution of enamel exists within the tooth crown, and that dimorphism centers on the canine. Unlike the plethora of permanent crown data which demonstrate that dentine is the main contributor to crown dimorphism—for the deciduous anterior crowns, this preliminary data suggests otherwise. The present study hypothesizes that tissue dimorphism of the deciduous anterior crowns arises during amelogenesis rather than during the mitotic phase of crown development. Sexual dimorphism, therefore, is suggested to be a result of the interplay between differential ameloblast secretion rates, extension rates and geometric factors affecting the deposition of enamel, rather than the result of mitotic embryonic tissue growth proposed for the permanent crowns.

This study provides an important preliminary step towards understanding the mechanisms which dictate deciduous crown tissue dimorphic characteristics. Furthermore, it offers evidence which suggests that deciduous canine buccal enamel is

sufficiently dimorphic to provide a basis for subadult sex determination of modern humans.

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Chapter 1

INTRODUCTION

Studies of modern human and extinct hominid sexual dimorphism traditionally classify sex based on the degree of morphological variation within the skeleton and dentition. The study of sexual dimorphism has aided in the interpretation of phylogenetic relationships amongst hominids by means of elucidating mating behaviour and sexual selective pressures from phenotypic changes over time (Reno et al., 2003; Frayer and Wolpoff, 1985). Fossil evidence demonstrates that the extent of hominid sexual dimorphism has decreased substantially from the Plio-Pleistocene Australopithecines and early *Homo* species to the recent human lineage. Despite this decrease, modern adult skeletons do still show consistent significant sex differences, while levels of subadult dimorphism are generally considered negligible (Frayer and Wolpoff, 1985).

Nevertheless, the determination of subadult sex from the human skeleton represents demographic data vital for research in the fields of forensic anthropology and bioarchaeology. While much research has been devoted to the development of both metric and morphological sex determination methods for the adult skeleton, few reliable and standardized methods exist for the subadult skeleton (Byers, 2002; Saunders, 2000). This lack, however, can be partially attributed to the fact that most striking sexual dimorphic differences are produced from secondary sex characteristics that arise at the time of puberty. Furthermore, issues of differential burial practices for infants, differential preservation, excavation, and recovery of immature skeletal elements can attribute to an under-representation of subadults in skeletal samples (Saunders, 1992).

Hunt and Gleiser (1955) examined the extent of dimorphism between the developmental rates of the skeletal and dental elements as a possible means for subadult sex determination. Based on the premise that the male skeleton matures more slowly than

the female skeleton, and that dental formation is more similar between the sexes, they hypothesized that males would exhibit closer dental and skeletal ages. Although accuracy levels ranged from 73-81%, radiographic assessment of skeletal age based on hand maturation is not applicable to interred or fragmentary remains, nor is the effect of pathology on development considered (Saunders, 2000). Choi and Trotter (1970) performed factor and discriminant analysis on various length and width ratios of fetal long bones to classify sex, but failed to produce accuracy rates above the minimum 75% standard (DeVito and Saunders, 1990). Weaver (1980) demonstrated that qualitative scoring of the elevation of the fetal auricular surface was 91% accurate for sexing fetal males, but was inaccurate for sexing females. More recent studies of juvenile mandible body shape and symphyseal base shape showed promise as sexing criteria, producing accuracy rates averaging 81% (Loth and Henneberg, 2001). Scheuer's (2002) blind test of this method, however, revealed inconsistent and low accuracy rates particularly for females. Despite researchers' efforts, juvenile skeletal elements have failed to provide an accurate measure of sex. It was stated by the Workshop of European Anthropologists therefore, that "for children…the deciduous teeth represent the only factor useful for sex diagnosis" (1980:525).

According to Saunders (1992), teeth should be good indicators of sexual dimorphism because their size remains constant after development. Because deciduous tooth crown completion occurs only a few months after birth, they are not *as* affected during childhood as are other skeletal elements by hormonal, nutritional and environmental factors (Alvesalo and Portin, 1980). Furthermore, it has been established that significant dimorphism in crown size exists in the permanent dentition of contemporary human populations (Schwartz and Dean, 2005; Garn et al., 1967b), and analysis of deciduous tooth crown measures show a significant, yet lesser degree of dimorphism (DeVito and Saunders, 1990; Black, 1978). As literature suggests, the potential for teeth as indicators of sex in subadults is evident; nevertheless, research pertaining to the nature of this dimorphism is needed. Since "primary teeth are…not

simply scaled-down versions of their longer-performing successors" (Harris and Lease, 2005:2), the extent of sex contribution to coronal variation is still in question.

To date, only a few studies have examined the extent of sexual dimorphism in the measures of the deciduous crown of contemporaneous and archaeological populations. Neither standard dimorphic measurements nor applicable cross-population methods for such measurements have been established. Presently, only Black (1978) and DeVito and Saunders (1990) have proposed methods based on discriminant function analyses of external deciduous tooth crown dimensions to separate the sexes.

1.1 Research Goals and Hypotheses

The establishment of normal deciduous dental variation in size and shape is a complex process involving the interplay of both intrinsic and extrinsic factors. Furthermore, determining the contribution of dimorphism to this normal variation requires a basic understanding of the effect of these factors on the timing and rates of coronal development. Therefore, the goals of this research are three-fold. The first goal is to provide a justification for the presence of sexual dimorphism in the deciduous dentition by outlining and acknowledging the genetic, hormonal and intra-uterine environmental agents which potentially contribute to growth variation between the sexes. Through an analysis of the tissues comprising the deciduous crowns, the second goal is to establish the presence or absence of sexual dimorphism in the dentition of two modern human populations by using a number of area and linear measurements. This research also examines which tissue(s) is the most significant contributor to dimorphism of the deciduous tooth crown, and which tooth type is the best predictor of sex. Lastly, the third goal is contingent upon the presence of dimorphism. Given a dimorphic finding, this research will provide a set of standardized deciduous coronal measurements for the prediction of subadult sex.

Upon the review and comparison of the many studies pertaining to the presence of dimorphism in the adult dental and skeletal systems, as well as studies concerning the

extent of sexual dimorphism in the subadult skeleton, a simple hypothesis is tested in this study. Given that the permanent crown tissues have been found to reflect differences between the sexes, and the subadult skeleton bears dimorphic features to a much lesser extent than the adult skeleton, *it is hypothesized that the human deciduous coronal tissues will exhibit sex differences to a lesser extent than the permanent coronal tissues.*

The tooth crown is composed of enamel, dentine and pulp. It is accepted that these tissues are 1) regulated independently during embryonic development (Dean, 2000; Harris and Hicks, 1998); 2) develop from different primordial tissues (Ten Cate, 1998; Tonge, 1989; Slavkin, 1974); and 3) develop during different critical phases of tooth formation (Harris et al., 2001). Since their relative contributions together ultimately determine crown size, measures of each tissue may in fact contribute further insight into the nature of the sexual dimorphism displayed by deciduous crown diameters. Upon consideration of past research on both permanent and deciduous crown dimorphism, *it is hypothesized that there is a disproportionate contribution of enamel and dentine to overall tooth size between males and females. In particular, a greater amount of enamel is expected in females, while a greater, and more significant, amount of dentine and pulp is expected in males.*

The following chapters will provide a summary of the contributions of past literature to the present thesis topic; an outline of the methods used; the results obtained; and a detailed discussion of these results in the context of previous research.

Chapter 2

REVIEW OF THE LITERATURE

2.1 Dental Development

2.1.1 Deciduous dental anatomy

The deciduous dentition comprises 20 teeth, 10 teeth in the lower (L) mandibular arcade and 10 in the upper (U) maxillary arcade. Each arcade consists of two quadrants in which five teeth erupt: the central incisor (i1); lateral incisor (i2); canine (c); first molar (m1); and the second molar (m2). Unlike the permanent dentition which contains four morphogenetic classes (incisors, canines, premolars and molars), the deciduous dentition consists of only three, the incisors (i1, i2), canines (c), and molars (m1, m2). The two deciduous molars are analogs of the permanent premolars; however, they are referred to as molars in clinical dental practice.

In comparison to their permanent analogs, deciduous teeth are generally smaller, more bunodont, and have disproportionately thin enamel and large pulp chambers (Harris and Lease, 2005). The crowns are shorter relative to overall tooth length, and appear squat relative to their permanent successors (Kraus and Jordan, 1965). Their thinner enamel is more porous and less resistant than the enamel of permanent dentition; these size and material differences may reflect the conservation of biological energy since they are replaced by permanent dentition about four years after their eruption (Harris and Lease, 2005; Kraus and Jordan, 1965).

The tooth crown is composed of three tissue types: the enamel, the dentine and the pulp. The enamel is the hard, non-cellular tissue that covers the outside of the tooth crown. Ninety-six percent to 98% (by weight) or 89-91% (by volume) of permanent enamel tissue consists of inorganic mineral of which approximately 90% is composed of hydroxyapatite crystallites. The remaining components are organic materials and water

(Hillson, 1996; Boyde, 1989; Kraus et al., 1969). The mineral content of the deciduous enamel is slightly lower than that of the permanent enamel, ranging from 76% to 85% by volume (Wilson and Beynon, 1989), while the chemical composition remains similar (Naujoks et al., 1967). The dentine constitutes the majority of the crown interior. It is separated from the enamel by the dentino-enamel junction (DEJ), and its inner aspect forms the walls of the pulp cavity. Mature dentine is composed of 75% inorganic material and 25% organic materials and water (Linde and Goldberg, 1993; Kraus et al., 1969), while research suggests that deciduous dentine contains even less mineral content (Hosoya and Marshall, 2005). Therefore, it is less mineralized than enamel. The coronal pulp is a vascularized and innervated tissue found at the crown center, surrounded by the dentine. The pulpal horns project from the pulp extending upwards towards the cusps and incisal edges; they are indicative of the cusp center (Kraus et al., 1969).

2.1.2 Embryonic and fetal odontogenesis

The tooth is a skin organ, deriving from the same basic germ layers as feathers, scales, hair and skin glands (Scott and Turner, 1997). Odontogenesis, or tooth development, occurs during the early stages of craniofacial development (Linde, 1984). During the third week of embryonic development, the embryo assumes a triploblastic, or three-layered form comprised of the ectoderm, mesoderm and endoderm; dental tissues develop from the primordial cells of the ectoderm and mesoderm (Ten Cate, 1998). The neural crest cells of the ectoderm-derived neural tube migrate throughout the head region and organize into a loose embryonic connective tissue called the ectomesenchyme (Moore, 1974; Slavkin, 1974). All dental tissues, except the enamel, have neural crest origin (Ten Cate, 1998). The oral epithelial cells, progenitors of enamel-forming cells, are derived from the surface ectoderm and cover the ectomesenchyme connective tissue (Ten Cate, 1998). The two cell types are separated by a basement membrane (Slavkin, 1974).

Prior to mineralization, five basic morphological stages have been identified: 1) dental lamina; 2) bud stage; 3) cap stage; 4) bell stage; and 5) enamel and dentine matrix formation (Figure 2-1). These stages are not discrete, but mark distinct morphological

transitions. Cellular proliferation and morphodifferentiation occurs throughout the earlier stages while histodifferentiation occurs throughout the later stages (Scott and Turner, 1997).

At five weeks (37 days) in utero, proliferative mitotic activity causes the oral epithelium to thicken into two bands around the presumptive oral region, which will ultimately form the lower and upper jaws (Ten Cate, 1998; Kollar, 1975). At six weeks after fertilization, each thickened band becomes two cellularly distinct regions: the vestibular lamina, which corresponds to the vestibule between the cheek and the teeth; and the dental lamina, which corresponds to the region of tooth development (Ten Cate, 1998; Hillson, 1996).

The bud stage occurs during the seventh and eighth fetal weeks. Localized cellular divisions occur within the epithelium and the ectomesenchyme of the dental lamina, causing the epithelium to bud downwards and be invaginated by the rapidly proliferating ectomesenchyme (Kollar, 1975). Cell activity appears to be greater within the basal region of the cervical loop than at the more distal epithelial regions and corresponding ectomesenchymal regions (Slavkin, 1974). Each epithelial bud corresponds to the location of its presumptive deciduous tooth (Miletich and Sharpe, 2003; Ten Cate, 1998; Partanen, 1990), with anterior tooth buds appearing before the posterior tooth buds (Lahdesmaki, 2006).

The cap stage is a proliferative histogenetic stage in which the epithelial bud lengthens and invaginates the mass of underlying condensed ectomesenchyme cells called the dental papilla (Ten Cate, 1998; Moore, 1974; Kraus and Jordan, 1965). The dental papilla will eventually become the dentine and pulp, whereas the epithelial cap-shaped enamel organ covering the papilla will produce the enamel. The enamel organ differentiates into four cellular types at this stage: the outer enamel epithelium, which is the outer cuboidal cell layer of the enamel organ; the inner enamel epithelium, which corresponds to the inner columnar cell layer; the stratum intermedium which overlies the inner enamel epithelium and interacts with this cell layer to produce enamel (Scott and Turner, 1997); and the stellate reticulum which separates the inner and outer layers and

maintains tooth germ shape, composed of polyhedral cells and intercellular fluid (Tonge, 1989; Linde, 1984). Concurrently during the cap stage, a series of intra-cellular changes occur in the ectomesenchymal cells lining the undersurface of the basement membrane. These cells transform from stellate-shaped to cuboidal, columnar preodontoblasts (Slavkin, 1974). Throughout the cap stage, mitotic activity is occurring within the inner enamel epithelium (Kraus and Jordan, 1965). The concentration of the cellular activity within the epithelium and ectomesenchyme, however, unlike during the bud stage, has shifted to the distal regions of the tooth rudiments (Slavkin, 1974). This gradient in cellular activity acts to rapidly increase cell size and shape.

The following stage, occurring at 14 weeks (Linde, 1984), is called the bell stage. The enamel organ assumes a 'bell' shape as its inner enamel epithelium deepens. At this stage, histodifferentiation and morphodifferention occur as a result of a series of reciprocal epithelio-mesenchymal interactions initiated by the ectomesenchyme (Ten Cate, 1998; Tonge, 1989; Slavkin, 1974). During the early bell stage, rapid growth occurs within the inner enamel epithelium and dental papilla. The differential mitotic rates within the inner enamel epithelium itself, accompanied by the pressure exertion from the enveloping dental follicle, the cervical loop and the dental papilla, and the slower rate of growth in the germ diameter are all causal factors in epithelial folding. The folding of the inner enamel epithelium establishes the future crown pattern (Ten Cate, 1998; Scott and Turner, 1997).

During the late bell stage, the epithelium signals the rapid enlargement and nuclear polarization of the cells of the papilla, initiating the terminal differentiation of the papillar cells into pre-odontoblasts and then odontoblasts (Ten Cate, 1998; Scott and Turner, 1997). The odontoblasts begin to secrete dentine matrix, proceeding towards the pulp from the basement membrane away from the acellular basement membrane. As the cell moves forward, a cellular extension, the odontoblast's process, trails behind. It is around this process in which hydroxyapatite crystals are deposited, mineralizing the matrix. Because a lag exists between the time of matrix deposition and mineral

deposition, a layer of predentine always exists between the odontoblast and the mineralized dentine (Ten Cate, 1998).

The initial deposition of dentine matrix then signals the elongation and nuclear polarization of the inner enamel epithelial cells, inducing the terminal differentiation of inner enamel epithelium into pre-ameloblasts and then ameloblasts—exemplifying the need for reciprocal cellular interactions of these tissues to direct odontogenesis (Ten Cate, 1998; Slavkin, 1974). The maturation zone of terminal differentiation begins at the distal aspect of the inner enamel epithelium and spreads downwards, causing mitotic cessation in this locale (Linde, 1984; Kraus and Jordan, 1965). The concurrent growth in zones adjacent to the maturation zone is bound by the cervical loop. This exerts pressure on the matured future cuspal area, thrusting it upwards, and therefore controlling the size and shape of the crown (Ten Cate, 1998). The bell stages ends only when all the inner enamel epithelial cells have ceased mitotic activity and terminally differentiated into ameloblasts and have acquired a secretory function (Ten Cate, 1998).

Upon the enlargement of the cells of the papilla and subsequent terminal differentiation of the cells of the papilla and inner enamel epithelium, there is a gradual elimination of the acellular zone of the basement membrane, establishing the dentino-enamel junction (DEJ) (Ten Cate, 1998).

By the end of the bell stage, the majority of the final tooth size has been determined by cellular proliferation of the inner enamel epithelium and papilla (Ten Cate, 1998). Dentine thickness, therefore, is the product of mitotic action in the growing tooth germ, whereas enamel thickness is the product of post-mitotic secretory action of ameloblasts; only subsequent enamel appositional growth contributes to the final crown size and shape (Alvesalo, 1997).

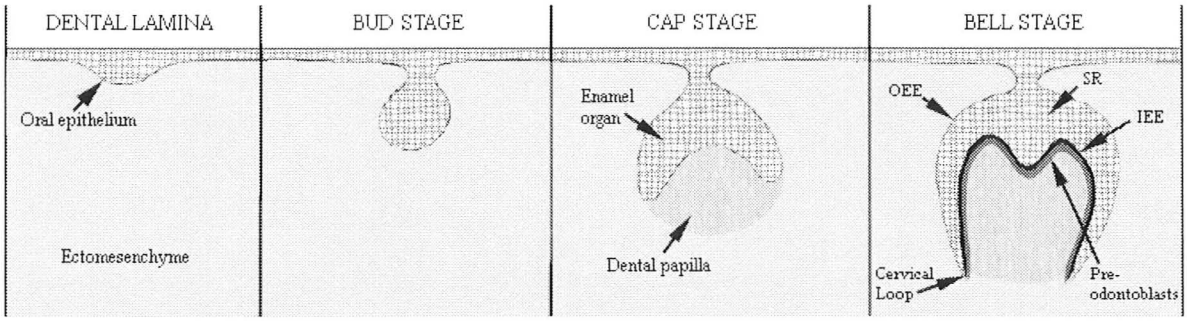

Figure 2-1. Stages of dental development (OEE = outer enamel epithelium; IEE = inner enamel epithelium; SR = stellate reticulum). Adapted from Miletich and Sharpe (2003:R70).

2.1.3 Amelogenesis and dentinogenesis

Each ameloblast begins its non-vital cellular secretions at the DEJ, and moves outwards distally to the presumptive crown surface, depositing a sinusoidal ribbon pattern of organic protein matrix composed of 90% amelogenin and 10% enamelin, tuftelin and amelin behind (Ten Cate, 1998). Almost immediately after initial matrix deposition, apatite crystallites are formed within the enamel matrix (Brookes et al., 1995). This is an initial layer of soft, partially-mineralized enamel which is laid down along the matrix ribbon pathway as ameloblasts are differentiating from the inner enamel epithelium. The maturation phase follows in which ameloblasts undergo structural changes by phagocytosing their own organelles associated with matrix synthesis, and assume a ruffled or smooth border at the cell surface. The ruffled-end ameloblasts deposit inorganic mineral, while the smooth ended ameloblasts remove water and enamel proteins (Ten Cate, 1998). The enamel at this time, therefore, undergoes substantial matrix protein resorption and the hydroxyapatite crystallites grow rapidly in length to almost completely mineralize the tissue (Ten Cate, 1998; Brookes et al., 1995; Shellis, 1984; Deutsch and Pe'er, 1982). These crystallites are arranged in rods (or prisms) along the length of each ameloblast pathway (Paine et al., 2001).

Enamel is characterized by its incremental nature; a series of long- (striae of Retzius) and short-period (cross-striations) incremental lines are formed across the long

axis with regular periodicity within each rod. This incremental phenomenon is hypothesized to be the result of periodic changes in the variation of rod width which allows for changes in the density and packing arrangement of the crystallites (FitzGerald and Rose, 2000; Ten Cate, 1998; Boyde, 1989). Modern humans show a periodicity of 6-12 days for the striae of Retzius (Smith et al., 2003). The first enamel to be laid down appears in longitudinal tooth sections as successive striae layers of "domes" covering the dentine horn, while later striae layers form discontinuously down the side of the crown like "sleeves", effectively burying the "dome" layers (FitzGerald and Rose, 2000). The enamel "dome" layers are referred to as cuspal enamel and the enamel "sleeves" as lateral or imbricational enamel (Figure 2-2). Short-period cross-striations are indicative of a circadian 24-hour rhythm of ameloblast activity along a prism (FitzGerald, 1998; Dean, 1987). Matrix deposition and subsequent mineral formation occurs until the extent of the enamel has been laid down.

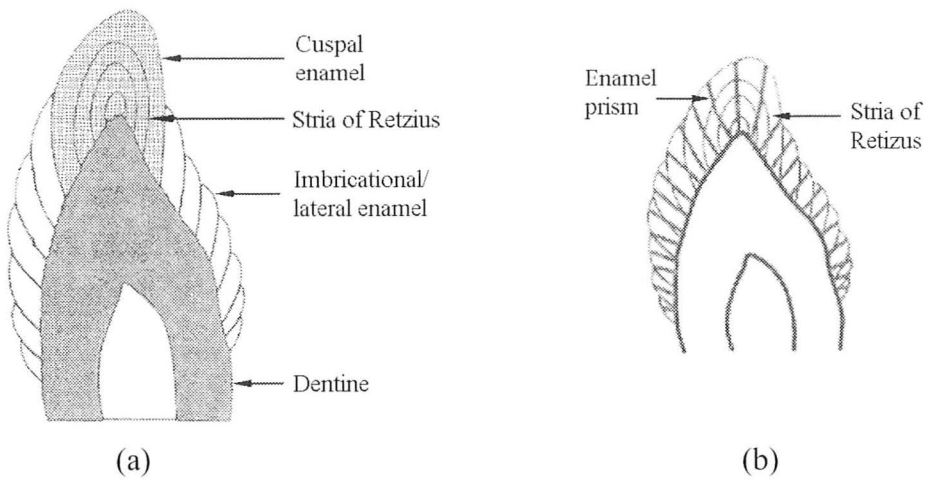

Figure 2-2. Schematic diagrams illustrating (a) cuspal and imbricational/lateral enamel; (b) prism pathways in association with long-period Striae of Retzius. Adapted from FitzGerald and Rose (2000:169,171).

Enamel formation proceeds via two modes of growth. Enamel apposition is the growth in enamel thickness outwards from the DEJ as a direct result of ameloblast secretions along the prisms, and enamel extension is the growth in crown height as ameloblast differentiation progresses cervically (Shellis, 1984).

Dentine formation occurs in a similar incremental fashion as does enamel formation, being deposited at a daily rate of about 4μm (Ten Cate, 1998). Odontoblasts, rather than ameloblasts, however, are the formative cells responsible for dentine matrix deposition. In contrast to enamel, the dentine matrix consists of type I collagen fibers and ground substance; mature dentine is less mineralized, consisting of 70% (by weight) of hydroxyapatite mineral (Ten Cate, 1998; Linde and Goldberg, 1993). During formation, the odontoblasts move downwards away from the DEJ towards the pulp, depositing dentine matrix in their wake. A cytoplasmic extension, called the odontoblast process, tails behind the odontoblast within the matrix; from this process, matrix vesicles containing hydroxyapatite crystallites bud off and are deposited between the collagen fibers. The crystals expand and rupture the vesicles, and fuse with adjacent crystal clusters to mineralize the organic matrix (Ten Cate, 1998). A lag exists between initial matrix deposition and mineral deposition (Ten Cate, 1998). This gap between mineralized dentine and matrix is called predentine; there is no corresponding tissue to predentine in amelogenesis.

2.1.4 Rates of crown development

Based on Bailit and Sung's (1968) research, Garn et al. (1979) suggest that rates of dental development at formation and calcification stages may affect crown size. External crown morphology is influenced by the growth potential, or the maximum growth ability of the ameloblasts, extending from the established DEJ (Massler and Schour, 1946). The growth potential, according to Massler and Schour (1946), is the product of the rate of apposition and the functional lifespan of the formative cells. Because this study is not concerned with the morphology of the pulp cavity as a result of odontoblast activity, the focus will be on the appositional rate and lifespan of the ameloblasts rather than on the dentine-forming odontoblasts.

Enamel appositional rates display particular patterns both within single teeth and across tooth types. First, the deciduous teeth undergo faster ameloblast differentiation than permanent teeth (Keene, 1982), as well as faster enamel extension rates as much as 5

times greater than the permanent dentition (Shellis, 1984). The resultant enamel prism pathways are significantly smaller than those observed in adult enamel (Shellis, 1984).

Secondly, a slight appositional rate gradient is present within each deciduous tooth, ranging from a maximum rate occurring at the growth center(s) and the minimum rate occurring at the cervix. FitzGerald and Saunders (2005) observe secretion rate variability in the post-natal enamel of deciduous crowns, although the data is not reported. In addition, Kraus (1959a) and Harila et al. (2003) claim that rates change during MD and vertical expansion similar to the permanent dentition, while Massler and Schour (1946) state that this change is less apparent than in the permanent dentition (Massler and Schour, 1946). Contrary to both, Shellis (1984) argues that deciduous rates are consistent over the entire crown in contrast to the permanent teeth which exhibit a decreasing gradient from cusp to cervix.

Lastly, upon comparison of the calcification rates between tooth types, Harila and colleagues (2003) observe that the rates of crown calcification are different. The greatest apposition rate occurs in the central incisors, and progressively decreases from anterior to posterior with the minimum rate occurring in the second molars (Massler and Schour, 1946).

2.1.5 Timing and sequence of deciduous tooth development

All of the deciduous teeth begin crown formation *in utero*. Kraus and Jordan (1965) suggest that maxillary teeth generally begin calcification before mandibular teeth, with m2 and sometimes i2 and c being exceptions. There exists no clear difference in developmental sequence pattern between populations, jaws or the sexes of deciduous teeth (Liversidge, 2003).

An extensive study of the timing of human deciduous development on 787 human fetal dentitions was conducted by Kraus and Jordan in 1965. Their findings indicate that a sequence of development is evident and predictable. Generally, the first macroscopic indication of the deciduous dentition occurs at the end of the first trimester. The i1, i2 and c appear at approximately 11 weeks *in utero*. The lower m1 appears next at 12 weeks,

while the lower m2 and upper m1 and m2 appear at 13 weeks *in utero*. Between this first macroscopic appearance and the initial calcification of the first cusp, there are marked increases in height and MD and BL diameters with slight swelling occurring at the presumptive cuspal areas; differential crown morphological characteristics are beginning to be observed. The first molar cusps to appear are the mesial cusps, followed by the distal cusps; the buccal cusps within these locations appear before the lingual cusps. The general pattern is as follows: mesiobuccal cusp (MB), mesiolingual cusp (ML), the distobuccal cusp (DB), the distolingual cusp (DL), and the distal cusp (D). The MB is the most pronounced cusp of the deciduous molars—appearing first and experiencing the largest amount of proliferative activity.

The initiation of crown calcification occurs throughout the second trimester. Initiation begins at a single occlusal center in the anterior deciduous teeth and on the MB cusp of the deciduous molars, followed by secondary initial calcification centers on the other cusps in the same pattern as the first macroscopic appearance (Kraus, 1959b). The initial calcification begins on i1 at 14 weeks, followed by m1 at 15.5 weeks, i2 at 16 weeks, the canine at 17 weeks, the lower m2 at 18 weeks and finally the upper m2 at 19 weeks. Calcification continues on the occlusal surface until all cusps have calcified and calcified coalescence has completed the occlusal surface. As calcification of the crown spreads, cessation of mitotic activity follows. Between the onset of calcification and final coalescence, the cusps which have not yet experienced initial calcification undergo marked growth to attain close to final size.

Complete occlusal coalescence of the molars is attained at the end of the third trimester and into full term. The lower m1 is the first to achieve occlusal completion at 32 weeks, followed by the lower m1 and m2 at 36 weeks and finally the upper m2 at 38 weeks. Calcification continues from the occlusal cusp tips down the vertical aspects of the teeth towards the cervical margin until crown completion.

Crown completion occurs after birth. At the time of birth, no deciduous tooth crown has fully calcified however the mineralization completion stages of the crown at birth ranges from the first incisors being 80% completely calcified, to the second molars

displaying only cuspal coalescence and not yet complete occlusal calcification (Lunt and Law, 1974a). The deciduous crowns are completed between 1.5 months and 11 months after birth, beginning with the upper i1 at 1.5 months; followed by the lower i1 and upper i2 at 2.5 months; the lower i2 at 3 months; the lower m1 at 5.5 months; the upper m1 at 6 months; the upper and lower c at 9 months; the lower m2 at 10 months; and the upper m2 at 11 months (Lunt and Law, 1974a).

Deciduous embryonic, fetal and post-natal development follows a predictable pattern of growth before calcification, onset of calcification, occlusal completion and crown completion. The prenatal and postnatal sequences have been established by Kraus and Jordan (1965) and Lunt and Law (1974a) respectively. It can be noted that the first incisors have the shortest coronal development time from the first macroscopic indication *in utero* to crown completion after birth at approximately 37-41 weeks, whereas the canines, lower m2 and upper m2 experience the longest coronal growth periods at approximately 64 weeks, 66.5 weeks and 69 weeks respectively.

For a more detailed chronology of deciduous dental development, see Schour and Massler (1940), Kraus and Jordan (1965), Nomata (1964), and Lunt and Law (1974a).

2.1.6 Sex differences in timing of crown development

In many respects, girls are more advanced than boys in the timing of somatic growth and development. These differences have been reported to range from 10-25% (Garn et al., 1958). For instance, females experience earlier appearance of ossification centers by 25%. They are ahead of males in the ossification of the carpals and the knee by 8% and 10% respectively, as well as in the appearance of secondary sex characteristics (Garn et al., 1958).

This advancement is not only evident in bony growth, but in the sequences of dental calcification and eruption. It is agreed that the permanent dentition exhibits sex differences in these respects although to a much lesser extent than are manifested in bony development (Demirjian and Levesque, 1980; Fanning and Brown, 1971; Burdi et al., 1970; Fanning, 1961; Garn et al., 1958). In general, sex differences in the developmental

chronology of the permanent dentition range from about 3-5%, with females being more advanced than males in crown completion and eruption of all permanent teeth except the variable third molar (Burdi et al., 1970; Garn et al., 1958). Sex differences in the timing of dental development increase with age, so that greater differences occur in the formation of roots compared with the crowns and in eruption rather than calcification; earlier forming teeth such as the first incisor exhibit smaller sex differences in calcification and eruption than later forming teeth such as the canine and second molar (Demirjian and Levesque, 1980; Fanning and Brown, 1971; Fanning, 1961; Garn et al., 1958). Hurme (1949) found sex differences favoring females in eruption which averaged 5%, ranging from 0.45 years to 0.93 years for the mandibular canine. Garn and colleagues (1958) found comparable differences at five defined stages of crown calcification and eruption which averaged about 3%; these differences however were insignificant. It can be noted that the average sex difference exhibited in crown calcification stages was 0.16 years, much smaller than the average difference of 0.52 years for alveolar eruption and attainment of occlusion.

Data suggests that sex differences in the timing of deciduous dental development are much more ambiguous. Furthermore, particular methods of charting dental formation, such as histological analysis and fetal jaw dissections are considerably more accurate than radiographic analysis; however, the majority of studies on dental development of living children are based on radiographic assessment (Hillson, 1996). The deciduous dentition has been found to exhibit no differences, or exhibit sex differences with male advancement in some cases and female advancement in other cases (Lysell and Myrberg, 1982; Demirjian and Levesque, 1980; Fanning and Brown, 1971; Burdi et al., 1970). Burdi and colleagues (1970) found that unlike timing patterns in the permanent dentition, males showed slight advancement averaging 11% over females during the first trimester *in utero*. Recall from the above section that during the first trimester the tooth germs are experiencing considerable growth from mitotic activity and do not begin calcification until the second trimester. In the case of deciduous crowns, it is possible that males undergo a slightly longer period of growth during the first trimester than do the females.

Lunt and Law's (1974b) review of deciduous eruption chronology shows that a consensus does not exist. Numerous factors may contribute to this lack in agreement; many studies define the eruption event differently and cannot be precisely compared to one another (Tanguay et al., 1984). For instance, Logan and Kronfield (1933) define eruption as 'attainment of occlusion', whereas later clinical studies generally define eruption as the appearance of teeth through the gingivae (Lunt and Law, 1974b). In any case, the definitions of timing landmarks must be considered when comparing sex differences in dental development. Some studies (Sandler, 1944; Robinow et al., 1942) suggest that males are advanced in eruption of all deciduous teeth except for the first molar, while others claim no significant difference in chronology exists (Lysell and Myrberg, 1982). The female deciduous dentition, on the other hand, appears to exhibit advancement up to 2 months over males with respect to root resorption (Fanning, 1961), suggesting that the general advancement of females over males occurs at some point shortly after birth approaching the time of eruption rather than prenatally. This advancement continues to increase with age becoming the most apparent in the longer and later-developing permanent dentition—maximizing up to 11 months difference for the permanent canine (Demirjian and Levesque, 1980).

In the analysis of Fanning (1961) and Fanning and Brown's (1971) results, it is evident that the male canine, first molar and second molar all reach crown completion shortly after the female. If these studies and that of Burdi and colleagues (1970) are indeed accurate, together they indicate that males experience a longer tooth germ and crown formation time than females. Moss and Moss-Salentijn (1976) believe that it is this difference in timing which allows for the male to achieve a greater crown size than females. They have attributed this ultimately to a difference in enamel apposition rather than to differences in dentine and pulp development. They however, did not have corresponding data to support this hypothesis, and various current studies contradict this supposition, reporting that females have relatively more enamel and less dentine than males (Saunders et al., 2007; Schwartz and Dean, 2005; Harris and Hicks, 1998; Alvesalo, 1997; Stroud et al., 1994).

2.1.7 Crown morphology and size

Tooth crown dimensions and shape are highly correlated, reflecting shared regulatory mechanisms governing their development (Harris, 2001). The human heterodont dentition exhibits non-random variation gradients of similarity between closely associated teeth (Scott and Turner, 1997). Tooth size communalities amongst the morphogenetic fields of the permanent dentition have been studied extensively however analogous data for deciduous teeth is limited (Harris, 2001). In his worldwide survey of the deciduous dentition of 58 groups, Harris (2001) reports that deciduous tooth size is variable among groups, yet exhibits identical ranking of relative average MD size differences amongst the 10 tooth types. He identifies that the mandibular m2 is the most variable tooth in MD size followed by the other maxillary and mandibular molars and the maxillary i1; the canine and other incisors on the other hand exhibit lower variability. Furthermore, DeVito and Saunders (1990) state that female teeth are more variable in size than male teeth except for the canines.

Two main hypotheses attempt to explain the heterdont organization of the human dentition; both are supported by molecular evidence. Butler (1939) explained mammalian dental gradients with the morphogenetic field concept. Dahlberg (1945) adapted this concept to the human dentition. According to this Field Theory, tooth germs in the early stage of their development have equal morphological potential. At a later stage in development, possibly a point preceding the histological recognition of the tooth germ, extrinsic morphogens (or field substances) are released and diffuse into three distinct regions for the deciduous dentition. The concentration of morphogens within each region directs morphogenesis of the three tooth types. At the center of each field, a 'polar' or 'key' tooth exists which exhibits a greater developmental stability and less overall morphological variation than the other teeth within the field (Dempsey and Townsend, 2001). The upper i1, lower i2, canines, and m1 have been identified as the polar teeth (Dempsey and Townsend, 2001; Dahlberg, 1945; Butler, 1939). 'Pattern genes' within each morphogenetic field control each germ response, dictating cusp patterns and tooth

shapes within classes. The differential expression of homeobox genes for each tooth type, directed by the ectomesenchyme, supports this theory (Thomas and Sharpe, 1998).

On the contrary, Osborn's Clone theory (1978) uses a slightly different approach to explain the morphological gradients in the dentition. Instead of equipotent tooth germ tissues at the earliest stages of development, the Clone theory describes the initiation of the different tooth primordia from a clone of cells. As the clone of cells divides and expands, it allows new primordial tissue to arise. All the primordia are different because they have experienced different numbers of cell divisions, thus cell ancestry (Osborn, 1978:171). Each primordial tissue corresponds to a different tooth type (incisor, canine or molar in the deciduous dentition). Unlike the Field Theory which uses extrinsic morphogens as directors of tooth germ growth, direction of tooth morphogenesis is intrinsic. Further, the Clone theory explains the gradient as the result of differential growth and division rather than an externally imposed gradient causing differential growth (Osborn, 1978).

In any case, a gradient of both tooth shape and size exists within the human dentition. Irrespective of the embryonic control mechanisms, it is known that mitotic cessation in the inner enamel epithelium determines the shape of the tooth (Ten Cate, 1998). Molecular lines of evidence show that genes within complex signal transduction pathways regulate cellular interactions, mediating the actions of ligands which target specific cell types. Ultimately, these genes are the "central regulators" (Thesleff and Mikkola, 2002:94) of tooth morphology. For an extensive review of the genetic regulation of the dentition, see Maas and Bei (1997).

During development, tooth shape is dependant on the interactions between the epithelium and mesenchyme tissues, and the resultant cascade of signals cause cellular changes including proliferation and differentiation—two growth events which lead to eventual DEJ completion (Ten Cate, 1998; Tonge, 1989; Slavkin, 1974). During the maturation phase, growth is constrained at the cervical loop and by the areas of maturation, continued cellular proliferation within the areas adjacent to the maturation zones cause the buckling of the inner enamel epithelium forming the cuspal outline, and

the subsequent upward thrusting of the future cusp towards the outer enamel epithelium (Ten Cate, 1998).

The difference in calcification timing between the MD and BL crown aspects also contributes to crown shape. According to Bell et al. (2001), the maximum MD dimension is fixed earlier than the maximum BL in incisors because it occurs at the occlusal incisal edge whereas the maximum BL distance occurs closer to the cervix at the cingulum bulge. Furthermore, the BL dimension is generally larger than the MD dimension, attributed to the greater developmental length of time that the BL crown sustains (Dempsey and Townsend, 2001). Early enamel calcification in the mesial and distal crown aspects rather than the buccal or lingual aspects hinders extensive germ growth in the directions of the former (Kraus and Jordan, 1965).

Two general factors contribute to final crown size: 1) cellular proliferation by means of mitotic activity during germ development, and 2) the appositional growth of enamel matrix and its subsequent mineralization. The majority of the crown volume is determined by the completion of the DEJ, and subsequent mineral deposition on top of the DEJ by ameloblasts (Zilberman et al., 2004).

Garn and colleagues (1967b) assessed the relationship between sexual dimorphism and size of adjacent teeth of the permanent dentition. They concluded that there exists a canine field in which the magnitude of size dimorphism in teeth adjacent to the canine, I2 and PM1, share a tendency for higher correlations with canine size dimorphism than more displaced teeth in the arcade (Garn et al., 1967b). Garn and colleagues in another study (1977) note that dimensional communalities exist at high correlations within the same morphogenetic classes of deciduous teeth, with males displaying slightly higher inter-correlations than females. According to Harris and Lease (2005), no analogous canine field is present in the deciduous teeth around which sexual dimorphic size differences occur.

The generation of crown morphology and size that may contribute to sexual dimorphism in the deciduous and permanent dentition is the result of enamel, dentine and pulp formation which are under: 1) *genetic control* (Miletich and Sharpe, 2003; Dempsey

and Townsend, 2001; Paine et al., 2001; Jernvall and Thesleff, 2000; Sharpe, 2000; Thesleff, 2000; Alvesalo, 1997; Maas and Bei, 1997; Simmer and Fincham, 1995; Salido et al., 1992; MacKenzie et al., 1992; Nakahori et al., 1991; Garn, 1977; Kollar, 1975; Lewis and Grainger, 1967; Garn et al., 1967b; Garn et al., 1965b; Garn et al., 1965c); 2) *hormonal influences* (Caton et al., 2005; Dale et al., 2002; Spelsberg et al., 1999; Hietala et al., 1998; Joseph et al., 1994; Garn et al., 1965a); and 3) *environmental influences* (Cardoso, 2007; Cardoso, 2005; Dempsey and Townsend, 2001; Newton et al., 1984; Guagliardo, 1982; Garn et al., 1980; Garn et al., 1979; Garn et al., 1965c; Stack, 1963). The next three sections of this chapter will serve to elucidate the potential genetic and epigenetic factors which may allow for tooth morphogenesis to manifest sex differences in the dental tissues. Hypothetically, any factors which differentially affect developmental duration, mitotic activity, and rates of post-mitotic mineralization of males and female crowns will alter the crown tissues and thus affect the level of sexual dimorphism.

2.2 Genetic Influences on Sexual Dimorphism

Genetic sex is determined by the presence or absence of the Y-chromosome. The Y-chromosome delegates the expression of several target genes required for the formation of the testes and sexual differentiation. Occurring at specific developmental periods, a series of complex genetic triggers signal the gonads to biosynthesize steroid hormones, which contribute to dimorphic phenotypes within targeted tissues (Parker and Schimmer, 2002). Thus, the complex set of genetically-determined pathways and interacting gene products within dental tissues provides the basis for all subsequent biochemical pathways to produce an endogenous base level of tissue-specific dimorphism.

The mechanisms of dental development are largely under genetic control (Garn, 1977), and according to Ten Cate (1998), the timing and rates of the establishment of the DEJ and enamel formation play a large role in the determination of final tooth size and dimension. Because tooth formation occurs very early and quickly in embryonic

development, the degree of sexual dimorphism may be more of a reflection of genetic activity rather than environmental or hormonal factors (Zilberman et al., 2004).

It was Garn and colleagues (1965b; 1965c; Garn et al., 1964), however, who first implicated the sex chromosomes in delegating tooth development, thus proposing a genetic basis for sexual dimorphism. Subsequent studies on sibling pairs (Alvesalo, 1971; Garn et al., 1965c), the teeth of individuals with aberrant sex chromosomes, and molecular analyses have demonstrated that expression of genes found on these chromosomes have *direct* effects on the differential shape and size on the permanent and deciduous human dentition of males and females.

2.2.1 Sibling Pair Studies

Family-line studies are conducted to estimate the heritability of developmental cues and patterns. Parent-child, sibling-sibling, and twin comparisons have enabled researchers to elucidate the role that genetics plays in dental developmental and dimensional events (Garn, 1977).

Garn and colleagues (1965b) suggested that the X-chromosome mediates tooth dimensional growth. Comparing like- and unlike-sex sibling pairs, they showed clear similarities in permanent tooth MD diameters between siblings—however they did find a range of correlations. The sister-sister correlations were the highest averaging 0.64, while brother-brother correlations averaged 0.38, and brother-sister correlations were the lowest, averaging 0.21. These correlation values are most easily explained by X-linked additive inheritance, where diploid sisters share a common paternal X-chromosome unlike haploid brothers (Garn et al., 1965b; Garn et al., 1965c). Parent-child tooth size correlations corroborate Garn et al's (1965b; 1965c) assumption of X-linked inheritance. Both mother-daughter and father-daughter pairs showed greater correlations in size than father-son pairs (Lewis and Grainger, 1967).

Garn and colleagues (1965c) propose that the Y-chromosome is involved in regulation of canine dimorphism. Within sibships, the magnitude of size dimorphism reaches 7-8% favoring males—suggestive of Y-linkage.

Similar results were demonstrated by Alvesalo (1971). His genetic and quantitative study of the sex-chromosome influence on tooth growth in normal individuals also suggests their involvement in producing dimorphism. He concluded that the X-chromosomal inheritance hypothesis was able to explain why a higher difference in correlations existed between the sister-sister pairs and brother-brother pairs, than between the sister-sister pairs and brother-sister pairs. Through cousin-cousin correlation analysis, Alvesalo (1971) also proposed a Y-chromosomal inheritance hypothesis. The low correlation between brother-brother pairs indicated that the Y-chromosome influence on quantity is different than the effect of the X-chromosome.

These above studies suggest that both sex chromosomes influence tooth size; however, they cannot say whether this influence is direct or indirect.

2.2.2 Aneuploid studies

Aneuploid studies of individuals with sex chromosome anomalies have demonstrated that the addition or deletion of sex chromosomes leads to measurable differences in overall crown size and structure, as well as tissue thicknesses. Furthermore, these studies suggest that both sex chromosomes differentially contribute to dimensional characteristics and developmental events resulting in dimorphism.

Androgen insensitive females (AIS) are phenotypically female, yet genetically male (46,XY). They experience testicular feminization due to a mutation of the androgen receptor gene (AR) on the X-chromosome (Brown, 1995). The BL and MD dimensions of the permanent dentition of these individuals show that tooth sizes are very similar to 46,XY control males, whereas they are generally larger than 46,XX control females—showing more significant differences in the BL dimension than the MD (Alvesalo and Varrela, 1980). By demonstrating that AIS phenotypic females have tooth crown sizes which are more comparable to genetically normal males than females, this study demonstrates that tooth growth is highly regulated by the sex chromosomes and is *directly* influenced by factors on these chromosomes. Tooth size, apparently, is not the direct result of androgen activity.

Alvesalo and colleagues (Alvesalo et al., 1985; Alvesalo and Kari, 1977; Alvesalo et al., 1975) showed that 47,XYY males experience excessive dental growth in the BL and MD dimensions of permanent and deciduous tooth crowns compared to those of genetically normal 46,XY male and 46,XX female control individuals (Alvesalo and Kari, 1977; Alvesalo et al., 1975). These two studies, however, fail to address the additive effect of the Y-chromosome, and therefore the nature of the role that the Y-chromosome plays in the establishment of sexual dimorphism. Although, it was concluded that Y-factors are already active in *utero* since their influence on permanent and deciduous teeth occurs at the same quantitative magnitude (Alvesalo and Kari, 1977; Alvesalo et al., 1975). Moreover, these authors note that additional Y-chromosomes may cause the delayed maturation time—as seen in 47,XYY males. This extended maturation period could allow for a longer mitotic growth period resulting in increased crown dimensions (Alvesalo et al., 1975), or perhaps postpone the differentiation time of ameloblasts and odontoblasts effectively extending the length of time for mitotic activity to occur.

In another aneuploid study, Alvesalo and Portin (1980) note that the crown dimension differences between 47,XXY Klinefelter's males and 46,XX females are greater than the differences between 47,XXY males and 46,XY, suggesting that the Y chromosome is more effective at increasing dental growth than the X-chromosome. No such comparison is made between 47,XYY and 47,XXY males to aid in the determination of Y-additive effects.

Chromosomal aberrations in 45,X Turner Syndrome females, 45,X/46,XX chromosome mosaic females and 47,XXX females demonstrate the additive effect of the X-chromosome on the development and growth of dental tissues. The dental development and maturation of 45,X females is accelerated compared to 46,XX normal females (Lahdesmaki and Alvesalo, 2006), while there is a tendency towards smaller teeth (Zilberman et al., 2000; Townsend et al., 1984; Alvesalo and Tammisalo, 1981; Kari et al., 1980). This implies that the X-chromosome either decreases the rate of cellular divisions, or increases the rate(s) and amounts of mineral deposition and/or cellular

differentiation. The faster mineralization or differentiation occurs, the lesser the amount of time does the enamel organ or dental papilla have for mitotic growth.

Lähdesmäki and Alvesalo (2006) suggest that reduced growth in Turner's syndrome individuals is due to a decreased number of dental-growth promoting sex-linked genes. Accordingly, 45,X females experience significant decreased dental growth in their deciduous and permanent crowns—being reflected more so in the MD than the BL dimensions of the deciduous mandibular first and second molars, and maxillary second molars (Kari et al., 1980). Interestingly, Kari and colleagues (1980) report that the dimensions of the deciduous canine are most similar in size between Turner's females and genetically normal females, and therefore is seemingly unaffected by the deletion of the X-chromosome. Similar results were demonstrated by Townsend and colleagues (1984), who reported that no significant reduction in size was seen in the deciduous incisors or canines of 45,X females, while all mandibular deciduous molars did show significant reductions in the MD and BL dimensions ($p<0.01$). The small sample sizes of deciduous tooth types, however, may affect the level of significance in both these studies. In contrast, the entire permanent dentition of Turner's syndrome females exhibits significant reductions in MD diameters (Townsend et al., 1984).

Studies on 45,X/46,XX chromosome mosaic females also demonstrate the developmental stability of the canine; the later-developing permanent canine of 45,X/46,XX individuals shows insignificant deviations from normal crown dimensions (Varrela et al., 1988). On the contrary, this study reports that earlier-forming teeth, such as the incisors and first molars, showed the greatest deviations—not the later-developing second molars as seen in Kari and colleagues' (1980) study. Varrela and colleagues (1988) note that the MD crown diameters of the permanent dentition are close to those of 45,X females, yet the BL dimension remains closer to 46,XX females. This suggests that the X-chromosome is implicated in the rate of mineralization or amount of enamel deposited—wherein the lack of an X-chromosome increases the rate of earlier phases of mineralization (mesial and distal directions) for all tooth types (Varrela et al., 1988).

Radiographic analysis of the deciduous and permanent dentition (Zilberman et al., 2000; Alvesalo et al., 1987; Alvesalo et al., 1985; Alvesalo and Tammisalo, 1981) has demonstrated the effects of the sex chromosomes on the hard tissues of the tooth crown. In general, the X-chromosome appears to affect enamel growth without changing dentine dimensions; the addition of X-chromosomes causes significant increases in enamel, while an X-chromosome deletion results in significantly thinner enamel (Harris et al., 2001; Alvesalo et al., 1987; Alvesalo and Tammisalo, 1981). Furthermore, the additions of X-chromosomes may influence enamel thickness differently between tooth types. Alvesalo et al. (1991) report that extra X-chromosomes influence enamel thickness differently between incisors and canines (36% and 21% increase respectively), despite isometric increases in crown size for both tooth types.

Various conclusions about the X-chromosome's affect on tooth size and sexual dimorphism can be drawn from the above studies. First, factors other than just the gene products responsible for amelogenesis are in part responsible for sexual dimorphism given that the canine is least affected by the X-chromosome aberrations while dimorphism is reported to center around this tooth. Secondly, the X-chromosome appears to be involved in the regulation of timing of growth events during dental development. Genes associated with the X-chromosome appear to have an effect on the early developmental phases—specifically the timing of onset and spread of mineralization (Varrela et al., 1988). Thirdly, crown tissue analyses demonstrate that the X-chromosome controls enamel apposition and dentine growth is independent of X-chromosome activity (Alvesalo, 1997).

The Y-chromosome, on the other hand, contributes to crown sexual dimorphism in a different manner. It appears to affect the growth of both dentine and enamel (Alvesalo et al., 1985), thereby having a similar quantitative effect on amelogenesis as the X-chromosome, and a different quantitative effect on dentinogenesis. The genes on the Y-chromosome are more effective at up-regulating dimensional growth through cellular proliferation of the tooth germ before the onset of mineralization (Harris et al., 2001; Alvesalo, 1997; Varrela, 1991; Alvesalo and Tammisalo, 1981; Alvesalo and Portin,

1980). It has been proposed that the presence of the Y-chromosome may lead to an increased potential in mitotic activity within the dental lamina possibly leading to an increase in dentine thickness; thus, explaining the increased crown size seen in males compared to that of females (Alvesalo, 1997).

2.2.3 The Amelogenin genes

Amelogenin is a matrix protein coded by the homologous genes AMELX and AMELY, found on the X and Y chromosomes respectively. This protein begins activity early and continuously throughout dental development (Alvesalo, 1997), regulating enamel thickness and tooth size through the mediation of the form and size of hydroxyapatite crystals during mineralization (Fincham et al., 1991). Amelogenin is first laid down as a hydrophobic, proline-rich protein by the ameloblasts in the inner enamel epithelium. This hydrophobic environment serves to initiate and promote the growth of mineral crystals (Salido et al., 1992). After secretion, the amelogenin protein undergoes proteolysis, and hydroxyapatite crystals are deposited within the organic matrix. After mineral deposition, the amelogenin-derived proteins are resorbed leaving a highly mineralized enamel tissue behind (Fincham et al., 1991).

Lau and colleagues (1989) presented results which demonstrated that two amelogenin genes existed: AMELX and AMELY which occur on the short arm of the X chromosome and in the pericentric region of the Y chromosome, respectively. These chromosomal locations are consistent with linkage analysis studies of the X-linked form of the disease Amelogenesis Imperfecta (AI), as well as with Alvesalo and de la Chapelle's (1981) hypothesis that the proximal long arm of the Y chromosome contained a region responsible for the regulation of tooth size.

Despite the 93% homology amongst the seven exons found within the sequences of the X and Y chromosomes (Nakahori et al., 1991), AMELX and AMELY mRNA transcripts show sex differences in expression due to splicing variation and greater percent sequence differences in the gene promoter regions (Salido et al., 1992). Both genes are functionally active, with AMELY being expressed at a level of 10% that of

AMELX within a male tooth bud (Salido et al., 1992). X-inactivation, however, may act on AMELX resulting in greater number of transcript products from AMELY—potentially allowing more gene products to be found in the male tooth bud than in the female tooth bud. The difference in expression is said to potentially translate into morphological differences—wherein which the increased tooth size generally seen in males is correlated to the higher level of AMELY expression in the male tooth bud (Roldan and Gomendio, 1999; Salido et al., 1992). The results of tissue proportions and aneuploidy studies counter this X-inactivation concept, but support the idea that tooth size is regulated by sex-linked genes.

2.3 Hormonal Influences on Sexual Dimorphism

Hormone involvement in tooth development and in sexual dimorphism is much more elusive than genetic involvement (Dempsey and Townsend, 2001). However, it is known that endocrine, paracrine and autocrine substances exert independent regulatory and signaling effects, as well as interact to achieve normal growth and developmental status (Clark and Rogol, 1996). Additionally, dimorphic phenotypes are generally attributed to the actions of gonadal hormones and sex-specific genes (Dempsey et al., 1999). Therefore, sex differences in fetal hormone and growth factor levels may influence the extent of the dimorphism exhibited between males and females. Levels of fetal androgen, which peak at the time of sexual differentiation, contribute to sexual dimorphism (Saunders, 1992; Stini, 1985; Weaver, 1980). Furthermore, sex hormones may affect dentine and enamel in a different manner (Schwartz and Dean, 2005). Therefore, differences may not be isomorphic in dental tissues, and a consideration of the effects of these substances is warranted.

2.3.1 Production of fetal gonadal steroids

The genital primordium first appears at around 29 days (Resko, 1977). At about 7 weeks, the testes are identifiable whereas only after 10 weeks do the fetal ovaries begin to

differentiate from the primordial tissues. The ovaries acquire their distinct morphological features by 20-25 weeks *in utero* (Grumbach and Conte, 1992). At about 8 weeks, steroidogenesis occurs in the fetal testes and the androgen testosterone is first detected in the male fetal gonads—with fetal plasma concentrations correlating to the testes biosynthetic activity (Grumbach and Conte, 1992). The level of testosterone formation increases to a peak plasma concentration at about 16 weeks. This circulating amount, ranging from 7-21 nmol/L, is comparable to levels within the adult male. After 16 weeks, the plasma concentrations decrease to about 3.5 nmol/L, and then decrease further after 24 weeks at the end of the second trimester (Grumbach and Conte, 1992). The peak plasma concentrations of testosterone coincide with the bell stage of deciduous dental development when all the tooth germs are experiencing extensive mitotic growth in both the inner enamel epithelium and dental papilla. The canines and second molars, in particular, have not initiated calcification at this point and therefore growth potential is at a maximum. The testosterone levels in the fetal ovaries, on the contrary, hovers around zero and maintains this level throughout gestation (Siiteri and Wilson, 1974). The sex difference in fetal testosterone is maintained during gestation by secretions from the fetal testes (Resko, 1977). The small amount of testosterone that is found in the fetal female is most likely due to the peripheral conversion of androstenedione, a precursor steroid, into testosterone (Resko, 1977).

In fact, the fetal female has little capacity in early and mid-gestation to biosynthesize steroid hormones; it is only during the late gestational period in which the ovaries begin biosynthesizing both estrogens and some androgens. Compared to the testis' capability to synthesize testosterone, the steroid precursors of the ovaries are 50-fold less capable of converting to estrogens (Grumbach and Conte, 1992). The level of estrogens within the female and male fetuses is comparable, likely originating from the maternal placenta (Dempsey et al., 1999; Norman and Litwack, 1987). According to Grumbach and Conte (1992), the fetal ovaries do not contribute to significant levels of circulating estrogens. The increased fetal plasma concentration of testosterone in males

between 11-24 weeks, therefore, is the only major sex differences in gonadal hormone levels (Grumbach and Styne, 1992).

Consequently, the high levels of testosterone which the fetal male experiences compared to the fetal female then should logically lead to "sufficient dimorphism for sex separation in fetal and early infant skeletons" (Saunders, 1992:16). Siiteri and Wilson (1974) state that the male phenotype results from androgen secretions from the male fetal testis, while Tanner and colleagues (1959) state that sex differences in the rate of development may be affected by fetal testosterone. It is reasonable to hypothesize then, that higher androgen levels in the fetal male will lead to their larger deciduous tooth crowns.

Gonadal steroid hormones do not act in isolation; their interactions with other endocrine and paracrine factors affect the somatic growth of the skeletal system, including the dental system. The extent of this influence of steroid hormones, growth factors and growth hormones on human deciduous tooth development is largely unknown. It is hypothesized that a finite number of cell cycles (cellular proliferation) are required before post-mitotic differentiation of the tooth germ tissues into mineral-depositing cells (Ruch, 1990); therefore, according to Young et al. (1995) the length of time it takes for teeth to develop depends on the availability of nutrients, growth hormones and growth factors. The vast majority of understanding of prenatal hormone influence comes from the *in vitro* immunohistochemical studies of the embryonic dental tissues of murines and non-human primates. Although mice and rats do not have two sets of dentition, early tooth development is very similar between mammals (Miletich and Sharpe, 2003). Receptor distribution patterns within specific cell and tissue types and across developmental stages of these organisms are used as proxies for an understanding of the *in vivo* chemical pathways in human tooth formation. Although the next discussion of hormone influence on human dimorphism is speculative, it is imperative to understand that is it not merely the influence of the single sex hormone testosterone that affects tooth size.

2.3.2 Gonadal steroid hormones and dental tissues

Initially, research studying the influence of steroid hormones on hard tissue development focused on osteological material. Various studies have shown a relationship between cellular proliferation and differentiation and the activity of gonadal androgens and estrogens. Specifically, osteoblasts are targets of these steroid hormones, which appear to promote osteoblastic activity and thus hard-tissue formation (Spelsberg et al., 1999; Kasperk et al., 1990). Turner and colleagues (1987) found a positive association between bone formation and estrogen treatment in ovarioectomized rats, while Kasperk and colleagues (1990) reported a positive effect of androgen treatment on bone cell proliferation. This presents strong evidence for implicating steroid hormone activity in the growth promotion of dental hard-tissues (Ferrer et al., 2005; Dale et al., 2002) and perhaps the establishment of dimorphic differences. Because odontoblasts and osteoblasts share similar mesenchymal origin, and their activities result in biomineralized tissue, their cellular events may also share similar endocrine and paracrine/autocrine regulatory mechanisms (Hietala and Larmas, 1992).

Various *in vitro* studies (Ferrer et al., 2005; Dale et al., 2002; Hietala et al., 1998; Svanberg et al., 1994; Hietala and Larmas, 1992) on both human and rat dental tissues have demonstrated the presence of sex hormone receptors in the enamel, dentine and pulp tissues suggesting that ameloblast and odontoblast activity, and pulp viability is facilitated by hormone activity. Further, differential presence of these steroid receptors in male and female tissues supports the idea that males and females respond differently to steroid hormones, and may experience different regulatory mechanisms of tissue development.

In general, both androgens and estrogens have a growth promoting effect on targeted tissues. Androgens play a role in the anabolic and growth-promoting pathways of sexual development both prenatally and postnatally (Martin et al., 1968). Estrogens promote cell proliferation and differentiation in various tissues, mediated by transcription of two types of estrogen receptors (ERs) (Ferrer et al., 2005).

The relationship between dentine apposition and estrogen was first established by Hietala and colleagues (1992) who demonstrated that ovariectomized adult rats displayed

enhanced secondary dentine formation in their molars. These researchers suggest that odontoblast metabolism is enhanced with ovary removal. Subsequent research reported a two-fold increase in dentine apposition in ovariectomized rats (Svanberg et al., 1994). An *in vitro* immunoblot of human odontoblast-predentine tissue detected estrogen-receptor-related antigen in the third molars of both sexes—suggesting the presence of estrogen receptors in odontoblast processes of later-developing teeth (Hietala et al., 1998). Although this *in vitro* study cannot conclusively state that estrogen is directly involved with dentine metabolism, these sex hormones may affect odontoblast activity later in life. Hietala and colleagues (1998) hypothesize that the activation of estrogen receptors may inhibit odontoblast activity and therefore decrease dentine formation.

In addition, ERs have been localized in the nuclear region of rat ameloblasts (Ferrer et al., 2005). Specifically, increased levels of ERs are associated with pre-secretory and secretory ameloblasts adjacent to the maturation zone, whereas levels decrease significantly in late-secretory mature ameloblasts. This study indicates a possible involvement of estrogen in both ameloblast differentiation and secretion.

Androgen receptors have also been found within the dental tissues. More specifically, Dale et al. (2002) localized these steroid receptors in human tooth pulp, within which androgen receptor mRNA transcript numbers were found to be greater in males than in females. Although there is a lack of human *in vivo* studies to provide evidence that androgens regulate pulp function, Molested et al. (1997) hypothesize that androgens are involved in dentinogenesis, signaling and/or facilitating the actions of odontoblasts. The masculinization of the canine complex of female rhesus Macaques by prenatal testosterone proprionate exposure suggests that endogenous fetal androgens activate genes encoding the male permanent canine complex—thus playing a role in the establishment of dental sexual dimorphism in the rhesus monkey (Zingeser and Phoenix, 1978). Yet, this prenatal activation period has not been identified, and the effects of testosterone on the deciduous canine complex are not commented on.

It is noteworthy, on the other hand, that Fitch and colleagues (1985) demonstrated that males who have deletions of the long arm of the Y-chromosome resulting in the loss

of the regulatory gene involving androgen receptors still show larger teeth than females. Furthermore, AIS phenotypic females have permanent teeth which are closer in size to males—supporting androgen-independent growth (Alvesalo and Varrela, 1980). Therefore, it remains unknown as to the full contribution of androgens to metric differences in sexual dimorphism.

The diffusion of fetal androgens from a male twin to a female twin *in utero* has been associated with increased permanent crown size of the latter. While conversely, no 'feminization' of crown size is seen in males sharing uterine environments. As such, this study provides evidence that fetal levels of androgens exhibit a much greater difference between males and females than do estrogens which show more similar levels early *in utero* (Dempsey et al., 1999). These findings suggest that differences in hormone levels are responsible for the differences in crown sizes, and that this same argument can be used to explain sex differences found in deciduous crown sizes.

Teeth that develop later ontogenetically, become more variable in size, and are thought to express greater dimorphism because of the increased difference in sex hormone production between males and females (Kondo et al., 2005). Additionally, it is possible that the greater length of developmental time of permanent teeth may result in a different responsiveness or receptiveness to sex hormones than seen in deciduous teeth. According to Zilberman and Smith (2001), sex hormones affect odontoblast activity later in life; specifically, dimorphism in this activity increases at puberty. The lower level of fetal and infant hormones in conjunction with very short peak times may account for both a different level of endocrine involvement as well as different level of physical manifestation in deciduous crowns compared to permanent crowns. Additionally, the very short developmental period of deciduous dentition may decrease the susceptibility to hormone influence such as that which the permanent dentition may experience.

Although the analysis of steroid hormone receptors is indicative of a direct effect of these endocrine substances on development of dental tissues, it is important to also consider their effect on and mediation by growth hormones and somatomedins. These

substances are known to affect various cellular events such as proliferation, DNA synthesis, differentiation and apoptosis.

2.3.3 Growth hormones and somatomedins

Growth hormones (GH) stimulate the growth of target tissues such as bone and teeth. GH is produced in the anterior pituitary gland and indirectly promotes cellular proliferation and somatic and skeletal growth through the mediation of gonadal steroid hormones, thyroid hormones and somatomedins (Zhang et al., 1997; Fisher, 1992; de Meyts et al., 1988).

Insulin-like growth factors I and II (IGF-I, IGF-II) are locally synthesized somatomedins which mediate somatic growth by means of ubiquitous receptors localized in various fetal tissues (Fisher, 1992; Norman and Litwack, 1987). They are heavily involved in the promotion of growth and differentiation of embryonic and fetal tissues (Fisher, 1992; D'Ercole, 1987). According to Fisher (1992), both GH levels and nutritional status are the main regulators of somatomedin activity. Food deprivation directly decreases the tissue levels of IGF mRNA due to the lack of dietary energy and protein. *In vitro* studies have shown that IGF-I mediates the activity of GH in odontogenic tissues, while GH up-regulates local IGF in GH-sensitive tissues (Young et al., 1995; Zhang et al., 1992; Fisher, 1992; Kostyo, 1988). Furthermore, GH production mediates androgen and estrogen effects on IGFs (Fisher, 1992; Grumbach and Styne, 1992).

Joseph and colleagues (1994) observed that immunohistostaining of GH and IGF-I receptors was greatest at times of histogenesis and cytodifferentiation in dental tissues. This finding indicates that both are involved with dental development by regulating the interactions between the epithelial and mesenchyme tissues and influencing tooth growth and differentiation events.

IGF-I was found to increase the fetal mouse molar germ volume by increasing dental papilla cell differentiation, odontoblast formation and dentine matrix production (Young et al., 1995). Strong IGF-I immunoreactivity was observed in the epithelium

during the late cap and early bell stages (Joseph et al., 1994), supporting the premise that IGFs also increase enamel matrix formation by inducing the transcription of matrix proteins amelogenin and ameloblastin (Caton et al., 2005; Takahashi et al., 1998).

Despite this strong evidence for somatomedin and GH-mediated odontogenesis, production of IGF-I and IGF-II does not peak until the 40th gestational week, thereby questioning the extent of involvement in deciduous crown matrix production (Fisher, 1992). Growth hormone plasma concentration, on the other hand does peak between 16-24 weeks (Fisher, 1992) during the initial calcification times of the canines and posterior dentition and early mineralization phase of all the deciduous crowns. Furthermore, the sexually dimorphic secretion pulses of pituitary GH established neonatally may be a causal factor in the appearance of postnatal somatic sex differences (Jansson et al., 1985). Although postnatal testosterone can increase GH secretions in children (Thorner and Rogol, 1988), suggesting a mechanism responsible for male somatic growth spurts (Martin et al., 1968), minimal research has addressed the growth-promoting effect of the testosterone-GH interaction on fetal odontogenic tissues.

The evidence demonstrating the growth promotion effect, the neonatally-established dimorphic secretion differences, and the interaction of GH with sex steroids is particularly notable with respect to the production of crown tissue dimorphism. On the other hand, no studies have been conducted on human fetal odontogenic tissues to provide evidence for their direct involvement in ameloblast or odontoblast metabolism in fetal tooth germs.

2.4 Environmental Influences

According to Cardoso (2005), environmental stress can affect the skeletal and dental developmental process in two ways. First, stress can decrease the rate of development thereby changing the timing of final size attainment. Secondly, stress may hinder growth at various stages, thus decreasing the final size attained. It is the second

somatic response to stress which is of interest here; the most critical period of deciduous crown growth occurs *in utero* (Stack, 1963).

It has been established that environmental insults can be reflected in the dentition as disruptions of normal matrix secretion through gross and histological abnormalities such as hypoplastic defects, accentuated brown striae of Retzius, and fluctuating asymmetry (Hillson, 1996; Guagliardo, 1982). These environmental factors may also affect the extent of sexual dimorphism by differentially affecting crown dimensions or tissue proportions (Harila, 2004). Cardoso (2005) argues that tissues which arise from different primordial origins, such as the enamel (from ectoderm) and dentine (from neural crest cells), may respond differently to environmental stress. According to the research of Newton and colleagues (1984), enamel appears to be more sensitive to environmental factors than dentine from the appearance of growth arrest lines. Townsend (1980) estimated that up to 42% of deciduous crown dimensions could be attributed to non-genetic factors—thus, indicating that environmental factors could play an extensive role in crown size variation.

Guagliardo (1982) reported that deciduous crown size of a prehistoric Amerindian sample was affected by population overcrowding—a source of stress through means of malnutrition, disease and warfare. Although he does not control for sexes in his study, Guagliardo (1982) raises the issue that insults on the deciduous dentition may not equally affect males and females. It is hypothesized that under stressful conditions, females are more buffered against the environment than males; skeletal populations which have suffered environmental stresses should logically have lower levels of sexual dimorphism (Stinson, 1985). More specifically, prenatal mortality rates are biased towards males during the third trimester, while mortality rates appear more or less equal between the sexes during the first two trimesters. Stinson (1985) however states that increased medical intervention has equalized survival rates at late gestation. In terms of prenatal sex differences in growth response to environmental (maternal) stress, the majority of investigations concerning birth weight and length indicate that males are more negatively impacted than females (Stinson, 1985).

Studies on dental responses to intrauterine stress have also been conducted which address this concern. Garn and colleagues (Garn et al., 1980; Garn et al., 1979) studied the effect of the intrauterine environment on odontogenesis, and found that maternal health status (maternal diabetes, hypothyroidism and hypertension) during gestation does affect MD and BL crown dimensions of canines and molars—possibly affecting boys more than girls. They concluded that the above maternal influences affected crown size more than infant size at birth or gestational length. Infants of diabetic mothers are more likely to sustain decreased levels of plasma calcium, increased prevalence of enamel hypoplasia and mineralization defects (Noren, 1984; Noren et al., 1978). These hypocalcemic newborns generally have wider neonatal lines demonstrating compromised ameloblastic activity, while postnatal enamel is significantly more affected than prenatal enamel. No sex differences in defective enamel development under this particular stress event are observed (Noren et al., 1978).

Some studies of the developing dentition of preterm birth children (occurring before 37 weeks of pregnancy) have shown a higher prevalence of enamel defects; results however, are varied and not significant (Harila et al., 2003; Harila, 2004). Enamel defects are more prevalent in postnatal enamel rather than prenatal enamel through the increased appearance of hypocalcemia and hypoplasia in the latter (Noren, 1983). Aine et al. (2000) found enamel defects at a prevalence of 78% in deciduous dentition, while Harila et al. (2003) and Harila (2004) concluded that short gestation is not significantly correlated with smaller tooth crowns. Furthermore, they noted that pre-maturity did not impede the presence of sexual dimorphism in the deciduous dentition. In fact the level of dimorphism ranged from 1-5% in favour of males in all tooth dimensions measured (Harila et al., 2003; Stack, 1963), concluding that sexual dimorphism is established early during deciduous tooth development.

Intrauterine undernutrition has also been hypothesized to affect the development process. However, using low birth weight as a proxy for undernutrition, Grahnen and colleagues (1972) determined that enamel hypoplasias in deciduous teeth were not significantly associated with intrauterine undernutrition.

An association between defective enamel development and enamel dimensions has not yet been made. It remains unclear from the literature whether or not defects in enamel translate to changes in enamel and overall crown dimensions. In addition, no study has confirmed a greater male sensitivity to environmental stress that is reflected in crown dimensions. The general consensus among researchers is that deciduous teeth are more buffered against extrinsic factors than either skeletal or permanent dental growth or development because of the protective uterine environment and short developmental period (Cardoso, 2005; Noren, 1984; Kreshover et al., 1958). Theoretically, deciduous teeth may provide a more stable assessment of the true levels of subadult sexual dimorphism. Therefore, the intrauterine environment cannot be definitively implicated in either increasing or decreasing sex differences in deciduous crown and tissue dimensions.

Bell and colleagues (2001) conducted a study on tooth sections of Down syndrome (DS) children to determine whether or not trisomy acts to change the crown tissues in an isometric manner. In DS individuals, reduced somatic growth is the result of decreased mitotic activity in trisomic cells (Bell et al., 2001). Microdontia of the permanent teeth has been reported as significant; however, deciduous teeth fail to show a significant size reduction compared to normal individuals. In fact, some DS patients displayed larger overall deciduous crown sizes than the normal controls (Bell et al., 2001; Townsend, 1983). Reported values of increased crown size in DS deciduous incisors reached 11% in the BL dimension and 2% in the MD dimension, but differences are inconsistent between central and lateral incisors. However, area measurements of both tissues did not exhibit significant differences between DS children and non-DS children. Decreased enamel thickness results from decreased secretory activity of ameloblasts, whereas decreased dentine thickness is the result of mitotic activity in the developing tooth germ. This finding supports the hypothesis that the majority of deciduous crown volume is established very early before the second trimester—thus the acceleration of growth via mitotic activity is not affected at this time *in utero*, and growth retardation that is notable in DS patients occurs after the second trimester (Zilberman et al., 2004).

Liversidge (2003) concludes that differences between modern populations, in both deciduous tooth formation and eruption, are less than one standard deviation. Therefore, tooth formation is not as affected by different environments as other non-dental growth systems.

2.5 Sexual dimorphism in tooth development

2.5.1 Crown Dimensions

Traditionally, crown size has been measured using buccolingual (BL) and mesiodistal (MD) diameters. Most studies have demonstrated that males possess larger and broader crown sizes in permanent teeth, with the most pronounced difference seen in the canines (Garn et al., 1967b; Moorrees et al., 1955). Analyzing absolute sizes, Garn and colleagues (1967b) found the largest dimorphism in the absolute measures of the mandibular first and second molars, followed by the maxillary and mandibular canines. When they calculated percent sexual dimorphism, the canines exhibited the greatest dimorphism (up to 7.3% for mandibular canines), followed by the molars, and the least for the incisors and premolars. These percent differences, however, change with the population being studied, as reflected in both Garn et al.'s (1967b) and Harris and Lease's (2005) research. The permanent dentition shows dimorphic percentages that range from 5-6% for the canines, to 2-3% for the other teeth (Garn et al., 1968; Garn et al., 1967a; Garn et al., 1967b) with a greater dimorphic difference in the BL dimension than for the MD dimension. It is further suggested that shape dimorphic levels exceed size dimorphic levels (Garn et al., 1967a).

On the contrary, deciduous teeth have shown variable results. A large overlap in crown size exists between the sexes, and no pattern of dimorphism is evident. Additionally, the lack of consistency in measurements poses difficulties for comparisons. In general, the mean crown size of males is larger than the female mean crown size (Moorrees et al., 1955), and according to Hillson (1996), the largest dimorphic differences in deciduous teeth are seen in the molars and canines.

The relationship between sexual dimorphism and MD tooth crown diameter is much weaker than that seen in the permanent dentition, averaging between 2-3% amongst geographically diverse modern and archaeological populations (Harris and Lease, 2005; Harris et al., 2001; Harris, 2001). Moorrees et al. (1955) found that male means in all deciduous tooth diameter measurements exceeded female means, and the mean MD diameters were significantly different between the sexes except for those of upper i1, m2 and lower m2. DeVito and Saunders (1990) found that the mean diameters (MD and BL) of male crowns in children are significantly greater than females. From the Burlington Growth Study (University of Toronto, Dental Research Institute), they reported percentage sexual dimorphism ranging from 1.91-6.44%. They found the highest differences between males and females in the MD diameter of the canine followed by the BL diameters of the upper i1, i2 and lower m2 respectively. Harris and Lease (2005) disagree with the above findings, reporting *median* percentages of sexual dimorphism that range from less than 1% for the upper i2 to greater than 2% for the lower i1; these measurements, however, were taken from the MD aspect rather than the BL aspect indicating that shape dimorphism may be present. Harris' (2001) early work agrees, finding that the MD diameter of the lower left i1 is the most sexually dimorphic tooth amongst all five geographically diverse populations he studied. On the other hand, another study conducted by Harris and colleagues (2001) found that the MD diameter of the m1 exhibits the greatest sex differences at 4%, complying neither with his own worldwide studies (Harris and Lease, 2005; Harris, 2001) or that of DeVito and Saunders (1990).

Black (1978) found that sex differences were greatest in the BL diameter of the upper m1 at 3.15%, also in disagreement with DeVito and Saunders (1990), however supporting Harris et al. (2001). He reports that male teeth are generally larger than females, but *females* show higher means for MD diameters of all four incisors, and of the BL diameter for the upper i1 (Black, 1978). These results are inconsistent with Harris and Lease (2005), Harris (2001), DeVito and Saunders (1990), and Moorrees et al. (1955).

Liversidge and Molleson's (1999) study of deciduous teeth from the Spitalfields collection showed a general trend for male tooth diameters to be larger than females, but like Black (1978), they found that the female mean MD dimension is higher than males for certain teeth. The upper m1, and lower m1 and i2 displayed larger MD diameters in females; however none of these teeth correspond to those reported by Black (1978). The BL diameter of the upper i2 was the only tooth measurement that displayed a significant dimorphic percentage at 6.8%, in which males were larger. This result agrees with DeVito and Saunders (1990), yet disagrees with Black (1978). Furthermore, Liversidge and Molleson (1999) found that the BL diameter of the anterior teeth displayed the greatest amount of variability, while DeVito and Saunders (1990) found the greatest coefficients of variation in the BL measurements and the least in the m2. This level of variability may provide an explanation for the incongruent results found in the degrees of dimorphism amongst the crown diameters of the incisors. Additionally, the greater variation of the teeth adjacent to the polar or key teeth within each morphogenetic field may explain the inconsistencies in the dimorphic levels of these teeth (Dempsey and Townsend, 2001; Dahlberg, 1945; Butler, 1939).

In a stereoscopic analysis of the fetal tooth buds of prenatal individuals aging 19 to 38 weeks *in utero*, Coughlin (1966) noted females possessed larger means in 25 of 28 dimensions measured in molar crowns—six of which were significantly dimorphic. He also suggested that post-natal sex differences in crown dimensions could change significantly in both MD and BL dimensions by enamel apposition until complete calcification. The majority of the tooth growth in these dimensions, however, occurs before the spread of calcification as the result of mitotic proliferation of the non-differentiated epithelial cells (Nomata, 1964).

The maxillary and mandibular teeth exhibit different distributions of sexual dimorphic teeth, with the canines and the first molars being the most dimorphic in the maxillary jaw, while the first incisors and canines are the most dimorphic in the mandibular jaw (Harris, 2001). These conclusions are supported by DeVito and Saunders (1990) who report that the maxillary dentition exhibited a higher significant dimorphic

difference than the mandibular dentition, particularly in both the MD and BL diameters of the upper i2, the BL aspect of the right upper i1, and the MD right lower canine. Harris and Lease (2005) also report that there is a significant difference in sexual dimorphism amongst the MD dimensions of maxillary dentition (due to the canine and first molar) however no significant sexual dimorphism amongst the deciduous mandibular dentition.

Currently, only two methods exist which use the crown dimensions of deciduous teeth for the sex determination of subadults, that of Black (1978) and DeVito and Saunders (1990). Black (1978) was unsuccessful in his stepwise discriminant function analysis; he achieved an accuracy range of 64-68% and up to 75.2% if ignoring the 5% probability level. His results therefore fail to meet the minimum criterion of at least 75% for the sex classification (Saunders, 2000). On the other hand, DeVito and Saunders (1990) used multivariate stepwise discriminant functions to achieve an accuracy range of 75-90% on a holdback sample. This method, although meeting the accuracy criterion, is limited by the population specificity of the sample, and the fact that their most accurate equations included three measurements of the permanent first molars. DeVito and Saunders (1990) compared the crown sizes of the Burlington children to six other geographically-diverse groups and found a range in crown sizes and percent sexual dimorphism amongst populations. Specifically, they found no positive correlation between crown sizes and the levels of dimorphism, and therefore no overall dimorphic pattern. They conclude that no one sample can be representative of a particular population due to inter- and intra-population variation in deciduous crown dimensions.

Therefore, there are several shortcomings when using the crown dimensions to examine sexual dimorphism. First, deciduous crown dimensions appear to be less dimorphic than their permanent counterparts. Secondly, linear measurements exhibit variability in the patterns of significant differences and percentage sexual dimorphism in the degree of significance both within and between tooth types. It can be noted that although discrepancies exist between studies, it is apparent that no similar patterning to the permanent dentition can be seen in the deciduous teeth; all of the above studies fail to report a peak of dimorphism centering on the canines as seen in the permanent dentition.

Thirdly, the extent of dimorphic differences in crown size varies within and amongst populations, and all conventional measurements of crown size are significantly and positively correlated (Harris and Bailit, 1988). Thus, tooth crown dimensions have relatively poor discriminatory power as a result of all being functions of total crown size (Harris and Bailit, 1988). Lastly, there has been no standardized method established to compare findings. The lack of consistency between MD and BL measurements on like tooth types suggests that dimorphism may be reflective in tooth shape as well as tooth size. All studies show, however, a *general* trend for a greater crown size in males and a range in percentage sexual dimorphism from less than 1% to 6.8%.

2.5.2 Enamel and Dentine Distributions

Crown size and dimensions are determined by the amount of enamel, dentine and pulp. The dimensions of these tissues do not necessarily co-vary or have similar correlation values in males and females; in fact the size difference in tooth crown linear measurements between males and females has been suggested to be the result of either a larger amount of enamel or dentine contributions (Saunders et al., 2007; Schwartz and Dean, 2005). More recent studies have analyzed relative dimensions of these individual tissues to assess which tissue(s) contributes to the sexual dimorphism seen in tooth crown size. The majority of these studies have analyzed permanent teeth and analogous research on deciduous teeth is minimal.

Moss and Moss-Salentijn (1976) were the first to suggest that different odontogenetic developmental mechanisms between the sexes were responsible for the different tissue amounts. They argue that dental sexual dimorphism in canines is a product of longer duration of amelogenesis in males causing greater enamel thickness. Moss (1978) further hypothesized that neither dentine thickness, length of the pulpal outline or length of the DEJ contributed to the sexual dimorphism in crown size or shape—therefore dismissing the dentine as a contributor to sexual differences. Some of the corresponding data used to support this supposition, however, was taken from Shillingburg and Grace (1973) who did not control for sex in their study of enamel and

dentine thickness. Many current studies contradict Moss and Moss-Salentijn's (1976) hypothesis, reporting that females have relatively more enamel and less dentine than males (Saunders et al., 2007; Schwartz and Dean, 2005; Zilberman and Smith, 2001; Alvesalo, 1997), or reporting that no differences in enamel can be found (Harris and Hicks, 1998; Stroud et al., 1994).

Many researchers have quantified tissue thicknesses for morphological comparisons and evolutionary purposes (Schwartz, 2000b; Macho and Berner, 1994; Macho and Berner, 1993; Shillingburg, Jr. and Grace, 1973) however, Stroud et al. (1994) were the first to control for sex in order to identify dimorphic differences in the linear measurements of dental tissues. Using bitewing radiographs, Stroud et al. (1994) concluded that males exhibited a greater MD dimension in the permanent mandibular premolars and molars due to significant differences in dentine thickness, not enamel thickness. Although females exhibited a slightly greater mean difference in enamel thickness in the molars, this difference was not significant. Sex differences in dentine thickness ranged from 0.3mm to 0.8mm, and percent dimorphism reached 9.20% for the M2. Additionally, males and females showed equal variability in dentine thickness of both these tooth types, while both sexes exhibited approximately twice as much absolute dentine thickness in the molars as in the premolars. Macho and Berner (1993) reported comparative enamel results when they examined sections of maxillary third molars from 11 Austrian individuals and found no dimorphic differences in enamel thickness.

Harris and Hicks (1998) found similar results as Stroud et al. (1994). Instead of examining the posterior permanent dentition, they analyzed the MD dimensions and enamel thicknesses of the four permanent maxillary incisors using periapical radiographs. Like Stroud et al. (1994), they found no significant dimorphic difference in enamel thickness at either the mesial or distal aspects. The maximum MD diameter for all teeth exhibited significant dimorphism which can be attributed to the significantly greater dentine width. The average sexual dimorphism was 6.5%, which is in agreement with the 5-7% relative dimorphic range reported by Stroud et al. (1994). Like Stroud et al.'s (1994) findings, Harris and Hicks (1998) found that dimorphism in dentine width

averaged slightly higher than MD width. Additionally, the lateral incisor exhibited higher dimorphism than the central incisor (Harris and Hicks, 1998). Interestingly, they found that females exhibited significant correlations between enamel thickness and either crown or dentine width, whereas males exhibited low correlations between enamel thickness and these other variables. Those variables, however, which involved either crown or dentine width exhibited moderate correlations at similar levels between the sexes.

Zilberman and Smith's (2001) study supports the findings of Stroud et al. (1994) and Harris and Hicks (1998). Dentine height was reported to be significantly larger in male mandibular first molars while the relative dentine height to crown width also showed significant dimorphism. Sex, according to Zilberman and Smith (2001), is the main contributing factor to dentine differences at 36.9%. In contrast to the aforementioned studies, this study demonstrated that relative enamel height and width were significantly larger in females—indicative of a greater enamel contribution.

Schwartz and Dean (2005) note that linear measurements may not be as optimal as area to represent the volume of tissue; an examination of area may present different patterns of dimorphism, and may perhaps take into account differences in tissue proportions and tissue quantities which thickness measurements may not. Therefore, they examined the tissue areas of the permanent third molar and permanent canines to determine the nature of the sexual dimorphism, and whether or not different tooth types exhibited similar patterns in tissue distributions across the sexes. They found that both males and females have the same amount of enamel in the canines and molars, but males have a greater amount of dentine in the molars than females (9.3% dimorphism). When they examined the relative amounts of tissues in both sexes, they found that the relative amounts of dentine in the canines were similar between the two, but females possessed a greater index of enamel thickness (Schwartz and Dean, 2005). Therefore, females show a greater proportion of enamel and a smaller proportion of dentine compared to the crown size than do males. A recent study by Saunders and colleagues (2007) also found that mean absolute dentine area exhibits the greatest levels of dimorphism (22% for lower canines) in the male direction. Unlike Schwartz and Dean (2005), however, they found

mandibular canines and 3rd premolars to have significantly dimorphic dentine area. Relative enamel areas of these two teeth also show significant levels of dimorphism but in the female direction.

From the above studies, it appears that dimorphic differences can be explained by a lower mitotic activity in females at the bell stage to initially produce a smaller tooth germ. Approximately equal thicknesses of post-mitotic enamel deposition between the sexes will logically result in a greater contribution of enamel relative to final crown size of the female, and a greater dentine contribution to final crown size in the male. Therefore, in order that dimorphism is observed in the dentine, sex differences in the permanent crowns need to be established before calcification at the time of DEJ establishment.

The tissue dimensions of the deciduous teeth display limited levels of sexual dimorphism. Gantt et al. (2001) looked at various enamel thicknesses, measured parallel to the cervix along the lateral enamel border and measured perpendicular to the DEJ at the occlusal surface, of sectioned human deciduous molars (m1, m2). He concluded that female deciduous molars had a significantly greater enamel thickness than males. However, they admitted their findings were premature due to small sample sizes and as well to the fact that significant results were obtained by combining inter-correlated variables from the all molar tooth types studied. Harris et al. (2001) examined bitewing radiographs of the deciduous molars of American "blacks" and "whites". They found that no sexual dimorphic difference in marginal enamel thickness existed. On the contrary, marginal dentine thickness did show significant differences in four of their eight measurements, although no intra-molar or inter-molar pattern of marginal thickness could be discerned. Overall, the percent sexual dimorphism was 4%, in which males exhibited a larger marginal dentine thickness (Harris et al., 2001). They concluded that males displayed enhanced, isometric growth of deciduous teeth as compared to females; thus the three tissue types occur in the same proportions in both sexes and teeth are simply larger in males.

It is recognized that the deciduous teeth exhibit proportionately less enamel than their corresponding permanent successors (Harris, 2001). This suggests that relative measures of the crown tissues will not parallel with the permanent dentition.

2.6 Method Limitations

2.6.1 Radiographic analysis

Radiographic analyses of molars (periapical and lateral bitewing) assess marginal enamel thickness because it is routinely imaged by dentists. Grine et al.'s (2001) assessment of the parallel film technique showed a general over-estimation of enamel thickness, as well as considerable measurement variation compared to measurements taken from sectioned teeth.

Therefore, the use of sectioned teeth in further research provides more reliable and reproducible results than do dental radiographs as it allows for the measurement of occlusal and cuspal enamel. Additionally, sectioned teeth—as opposed to radiographed teeth—can provide histological information about deciduous crown rates of growth which may differ between the sexes, as well as an individual's health status, which could deleteriously affect the accuracy of sex determination.

2.6.2 Coronal Parameters

Past research on the coronal measurements of permanent and deciduous teeth began with traditional maximum BL and MD lengths (Moorrees et al., 1955). Since then, various investigators have attempted to find better measures of the coronal tissues for such purposes as predicting sex (Saunders et al., 2007; Schwartz and Dean, 2005; Zilberman and Smith, 2001; Harris et al., 2001); identifying tissue patterning differences amongst extant and extinct hominid, hominoid and anthropoid taxa including modern humans (Smith et al., 2005; Smith et al., 2003; Schwartz, 2003; Grine and Martin, 1988); identifying systematic tissue patterning associated with functional use (Grine, 2005;

Macho and Berner, 1994; Macho and Berner, 1993); and testing the accuracy of different methods of tissue measurements (Olejniczak and Grine, 2006).

Linear measurements of maximum crown diameters eventually progressed into linear measurements of coronal tissues. Generally, measurements fall within two groups depending on what method is employed. The first are those measurements taken in a MD plane. Routine dental work includes bitewing radiographs of the posterior teeth, and therefore clinical studies generally use these images which display a MD tooth plane. The second grouping of measurements is taken in a BL plane. Physical sections of the tooth crown are cut in a plane along the first forming mesial cusps in the molars to encompass the earliest forming and latest forming enamel. Unfortunately, the results from these two groups cannot be directly compared not only because measurements are taken from different tooth planes, but discrepancies exist in radiographic distortion (described in section 2.6.1).

Additionally, the majority of studies which measure occlusal enamel are concerned with the functionality of molar enamel in the helicoidal plane and have standardized enamel thickness measurements within the occlusal basin between the crown cusps (Schwartz, 2000b; Macho and Berner, 1993). These measurements may not necessarily be good predictors of sex. In fact, because of the thin and porous nature of deciduous enamel, the enamel of deciduous crowns is more susceptible to wear than permanent crowns, thus compromising accurate measures of enamel cusp thicknesses, occlusal basin thicknesses and even dentine horn heights (as measured by Macho and Berner (1994)).

Nevertheless, "functional" enamel thicknesses taken from along the buccal and lingual crown margins rather than "radial" enamel thicknesses taken orthogonal to the DEJ within the occlusal basin are more impervious to wear and may prove to be a more accurate parameter for measuring the extent of dimorphism in deciduous teeth.

Schwartz and Dean (Schwartz and Dean, 2005) suggest that linear measurements, however, may not be the best predictors of crown volume and propose that coronal tissue areas may be better when examining crown sections. Such measurements seem ideal for

deciduous crowns since area measurements are most robust against occlusal wear than linear measurements. Additionally, average enamel thickness can be estimated from enamel and dentine areas if DEJ length is known (Grine, 2005; Grine, 2002; Martin, 1985).

Scaling measures need to also be considered. Although this is an important step in the comparison of crown morphology among individuals and/or species which may differ considerably in overall tooth size (Grine, 2002), this is not as of great importance for modern human deciduous teeth which are much more similar in size (Gantt et al., 2001). It can be noted, however, that appropriate scaling measures are in dispute, and according to Grine (2002) the BL cervical diameter and DEJ length are the two most common scaling measures for estimating molar enamel thickness—both of which are strongly correlated. Grine (2002) notes that the obliquity of the cervical line may affect the cervical diameter differently than the dentine core area as defined by the DEJ. In the case of deciduous teeth, use of the cervical diameter may be of greater use as a scaling factor since the DEJ may be disrupted by wear into the dentine horn.

Chapter 3
MATERIALS AND METHODS

3.1 The Sample

3.1.1 Participant Recruitment

A subset of the exfoliated teeth included in this study was collected from 28 children between the ages of 6 to 12 years old, residing in south-central and south-western Ontario, Canada. All participants in this subset were relatives, friends or acquaintances of the researcher (AC).

The recruitment process involved hand and e-mail distribution of a consent form to potential participants upon receipt of ethics approval from the McMaster University Research Ethics Board (MREB). The consent form included criteria specified by the MREB for graduate research; specifically, a brief outline of the background, purpose and research goals of this study were included (Appendix A). All participants were required to sign the consent form, as well as have their guardian provide consent. The only information required was the sex of the participant.

3.1.2 Collection of Specimens

In total, 93 exfoliated teeth were collected from these 28 individuals, of which 13 were females and 15 were males. In total, 48 of the teeth were from the female participants, and 45 of the teeth were collected from the male participants. The majority of teeth were in good condition, however 15 teeth needed to be glued together under a desktop magnifier using cyanoacrylate (GG Royal/SVM Crono Inc.) and two teeth were excluded due to half-missing crowns.

Each exfoliated tooth was placed into its own labeled re-sealable poly bag. Each label included the participant identification number (e.g. AC1-1), the sex of the

individual, tooth type in FDI notation, as well as an indication of bag number if there were more than one tooth from the same individual (e.g. Bag 1 of 2). Multiple teeth from the same individual were designated the same identification number but with a different dashed number (e.g. AC1-1, AC1-2). Anonymity was ensured by leaving the participant's name off of the sample bag. This sub-sample will be subsequently referred to as the *Chan* sample.

The remaining previously thin-sectioned exfoliated crown samples came from two other different research studies. This second sub-sample consists of teeth collected from healthy school children born between the years of 1985 to 1989 in Rome, Italy. This particular research project entitled '*Fatina*' was originally undertaken to study the dental histology and weaning period of modern children. The information collected along with these exfoliated tooth samples includes birth date, sex and length of pregnancy (Prowse et al., 2007). Of the 129 thin-sections provided to the researcher, 71 were selected for analysis representing 27 males and 32 females. This sub-sample will be subsequently referred to as the *Fatina* sample.

The third sub-sample consists of 45 previously thin-sectioned exfoliated crowns collected from 45 Canadian individuals residing in south-central Ontario or Montreal Quebec by Blyschak (2001), originally collected for the study of the neonatal line. The sex of the individual along with extensive birth history was collected. Of these 45 teeth, 34 teeth were analysed, representing 16 males and 16 females. Amongst those 32 individuals there are five brother-brother (B-B) pairs, four sister-sister (S-S) pairs and four brother-sister (B-S) pairs. This sample will be subsequently referred to as the *Blyschak* sample.

All three sub-samples were combined to provide a total sample of 161 crowns used for area measurements (75 males and 86 females), and 164 crowns used for linear measurements (77 males and 87 females). See commentary of sample composition in Chapter 5 (section 5.7.2) for a discussion of the combined sample.

3.2 Preparation of undecalcified ground tooth sections

The exfoliated deciduous teeth of the Chan sample required preparation as undecalcified thin sections, whereas the Blyschak and Fatina samples were previously prepared by other researchers into 100μm thin sections. Upon collection, each tooth was given a specimen number and the tooth type, jaw and side were identified using Kraus et al. (1969), Hillson (1996), and Simon Hillson's Insico-rite and Molar-o-matic sider and identifier (2002) as identification guides. The degree of attrition and macroscopic crown measurements were taken according to the following protocols.

3.2.1 Attrition Assessment

The degree of attrition on each exfoliated crown was measured using Smith's (1984) stages of occlusal wear. Although this chart is designed for permanent teeth, it served as a guide for the assessment of the degree of wear to which deciduous teeth reach while in occlusion. Table 3-1 illustrates the distribution of the wear stages within the Chan samples. Further analysis of occlusal wear is presented in Section 4-1.

Table 3-1. Breakdown of the distribution of Smith (1984) Stages of Occlusal Surface Wear within the Chan deciduous crown sample (n=93).

	Smith (1984) Stages of Occlusal Surface Wear								
FDI	1	2	3	4	5	6	7	8	Total
Ui1	0	2	2	2	3	1	2	0	12
Ui2	0	0	0	3	0	1	0	0	4
Uc	0	1	7	2	2	0	0	0	12
Um1	0	4	2	1	1	0	1	0	9
Um2	1	4	4	3	0	0	0	0	12
Li1	0	2	4	1	0	0	0	0	7
Li2	0	1	4	0	0	0	0	0	5
Lc	1	1	8	1	1	0	0	0	12
Lm1	1	6	2	2	0	0	0	0	11
Lm2	0	2	2	3	0	0	0	0	7
Total	3	22	35	18	7	2	3	0	91[1]

[1] Two crowns were eliminated from the 93 originally collected due to broken crowns.

3.2.2 Macroscopic Dental Measurements

Before sectioning, four measurements were taken from each exfoliated tooth crown. These measurements comprised of the maximum mesiodistal (MD) crown diameter, the buccolingual (BL) crown diameter, the MD cervical diameter and the BL cervical diameter. The latter two measurements were conducted if a clear cervical border could be identified. These measurements can be used if attrition interferes with conventional maximum crown diameter measurements (Hillson et al., 2005). According to the FitzGerald and Saunders (2006) tooth sectioning protocol, the former two measurements are not applicable on tooth crowns which have sustained gross amounts of wear. Using the Smith (1984) attrition measurements as a guide, it has been noted in this protocol that the maximum MD crown diameters cannot be measured accurately on the permanent incisors if they have sustained wear categorized above level 4 and on the permanent molars if they are categorized about level 5 wear. The maximum BL crown diameters are not accurate if their attrition levels are above level 6. These same precautions have been extended to the deciduous dentition. The teeth from the Chan sample which sustained these levels of attrition were removed from the study (see Exclusionary Criteria below). These wear stages were not available for either the Fatina or Blyschak samples.

The maximum MD crown diameter is defined as the distance between two parallel lines found at the most mesial and distal aspects of the crown sides respectively. The maximum BL crown diameter is defined as the distance between two parallel lines found at the most buccal and lingual aspects of the crown sides respectively (Hillson, 1996). For all teeth, these above diameters were measured with Mitutoyo® Absolute Digital Calipers which accurately measures to the 0.01mm.

The cervical diameters were taken at the cemento-enamel junction (CEJ) on the most apical aspect of the crown enamel. The MD cervical diameter is defined as the distance between the occlusal-most points along the cervix at the mesial and distal sides respectively. The BL cervical diameter is defined similarly, except it is measured at the

buccal and lingual sides along the CEJ (Hillson et al., 2005). These measurements were measured using Hillson-FitzGerald Calipers©.

3.2.3 Inscribing a cutting line

The designation of the cutting line was done in accordance to the FitzGerald and Saunders (2006) tooth sectioning protocol. A cutting line was inscribed along the buccal aspect of each tooth crown before embedding and sectioning to make sure that the proper orientation was maintained throughout the duration of the sectioning process, and most importantly, to outline the position of the dentinal horns within the thin section.

The sectioning plane for the incisor and canine crowns falls along the midline BL plane. Using an indelible, solvent-resistant Superfrost Securline® black marker, a single point was placed at the midpoint of the occlusal surface. A second point was placed on the buccal surface at the lowest point along the cervical margin. Both points were connected by a straight line on the buccal surface.

The molars were also sectioned in a BL plane which passed through the mesial cusps. Again using a Superfrost Securline® black marker, two points were plotted on the occlusal surface of the molars—one on the MB cusp, and a second on the ML cusp. These two points were connected by a straight line which was then extended down the buccal crown side to a third point which was located at the apical-most aspect of the buccal cervical margin.

3.2.4 Embedding

Due to the fragile nature of deciduous exfoliated crowns, all teeth used in this study were embedded in epoxy-resin prior to sectioning. Traditional Buehler plastic SamplKups® are too large for embedding small deciduous crowns; therefore, multi-mould 12-well plates designed at the McMaster Anthropology Hard Tissue and Light Microscopy Laboratory were used. Twenty-four hours before each embedding session began, all wells of the multi-mould were lined with a generous amount Buehler Releasing Agent to aid in the block removal process.

After 24 hours, a small amount of epoxy was made by mixing a 5:1.95 ratio (by weight) of Buehler's EpoThin Epoxy Resin to Buehler's EpoThin Epoxy Hardener. These two solutions were mixed together in a paper cup with a wooden stick for two minute until solution became transparent and contained no air bubbles. Afterwards, enough epoxy was pipetted into each well to cover the bottom, and provide an epoxy layer between the tooth crown and the well bottom. This epoxy was left to harden for at least 5 hours before continuing.

After the bottom epoxy layer had hardened, a single tooth crown was inserted into each well with the occlusal surface facing the well bottom, and the rest of the crown oriented vertically. A second batch of epoxy was mixed following the same procedure as outlined above. Enough epoxy was pipetted into each well around each tooth to just cover the crown. These epoxy "bullet" blocks were left overnight to cure.

The hardened "bullet" blocks were removed from the wells and placed in their respective labelled bags.

3.2.5 First Face: Sectioning

Before sectioning began, a Superfrost Securline® black marker was used to trace the cutting line (previously inscribed on the crown) onto the epoxy block in order to better visualize it during the alignment of the "bullet" block on an IsoMet® wafer chuck. The "bullet" block was then attached to the wafer chuck with DENTSPLY® Sticky Wax. A generous layer of dental stick wax was first melted onto the wafer chuck using a hot knife to provide a support base for the "bullet" block. The hot knife was used to melt a hole in the wax in which the embedded tooth crown was placed. Care was taken to ensure that that buccal cutting line and occlusal cutting line were parallel to the wafer chuck. This placement ensured a sectioning cut directly aligned with the dentinal horn of the tooth. The long axis of the tooth followed the long axis of the wafer chuck to ensure that the cuspal enamel would be the first enamel to be cut by the saw. Additional sticky wax was melted using the hot knife around the block for support.

Sectioning was undertaken using the IsoMet® 1000 Saw. A 4" diamond wafering blade with a 0.35mm kerf (supplied by Cedarlane Laboratories) supported by 2" flanges on either side provided sufficient blade clearance, the least amount of blade wobble and most suitable counterweight for sectioning embedded deciduous crowns. The wafering blade was dressed using a dressing stick before each sectioning session to remove the build-up of dental sticky wax and epoxy.

The wafer chuck-"bullet" block unit was fastened to the goniometer arm of the IsoMet® 1000 Saw at a 20-30° downwards angle so that the cuspal enamel was the first to be cut. While lifting the weight arm to lift the sample off the blade, the embedded tooth sample was moved using the positioning knob so that the blade was aligned slightly to the right of the inscribing line. The digital micrometer on the IsoMet® 1000 Saw was zeroed. The tooth was cut at a saw speed of 100 RPMs.

After this initial cut, the wafer chuck with the mounted newly sectioned tooth was removed from the goniometer arm. Using a hotplate, a small amount of dental sticky wax was melted onto a labeled "carrier" or non-permanent slide which was immediately placed onto the surface of the newly sectioned tooth. A flat weight was placed on top of the slide to ensure that the slide adhered parallel to the tooth section plane. The wafer chuck with the mounted tooth and "carrier" slide was remounted onto the goniometer of the IsoMet®. The micrometer was set to 1.0mm in order to allow room for blade thickness and subsequent lapping and polishing. The goal for the first cut was to obtain a tooth section on the microscope slide that was between 0.6-0.7mm. The first cut was also sawed at 100 RPMs.

3.2.6 First Face: Lapping

The thickness of the mounted tooth section on the "carrier" slide was measured at four locations (two on opposite occlusal edges and two on opposite cervical edges) on the tooth crown using the Mitutoyo digital calipers and recorded in a database. These measurements served as a guide for the time allotted for lapping the first face. The "carrier" slide was mounted in a jig specially designed in the McMaster Hard Tissue and

Light Microscopy Laboratory for the lapping and polishing of dental specimens mounted on 75mm x 25mm microscope slides. Coarse lapping was completed using CarbiMet 600 grit lapping disks and distilled water on Buehler's Minimet® 1000 at a speed of 50 RPMs. Lapping of the first face continued until all aspects of the tooth section reached a thickness of approximately 0.4mm.

All mounted specimens were sonicated in a water bath for 15 minutes to remove epoxy and sticky wax dust.

3.2.7 Second Face: Permanent Bonding

A second microscope slide (permanent slide) was etched with the identification number and cleaned with ethanol. Under a fumehood, a thin layer of Logitech OCON-186 UV Resin (Struers) was applied to the tooth mounted on the "carrier" slide. The new microscope slide was placed onto the tooth to effectively create a slide sandwich around the tooth section. While clamping the slides together with forceps, the slide sandwich was held over a black light UV lamp for 45 seconds to cure the UV glue. The slide sandwich was then placed on a hotplate with the "carrier" slide face down to melt the sticky wax. The "carrier" slide was discarded, and the excess sticky wax removed. The second face of the tooth section was then exposed and in need of lapping and polishing.

3.2.8 Second face: Lapping and polishing

The thickness of the tooth section on the permanent slide was measured in the same four locations using the Mitutoyo digital calipers. Similar to the method of lapping the first face, the second face underwent coarse lapping using CarbiMet 600 grit lapping disks and distilled water on Buehler's Minimet® 1000 at a speed of 50 RPMs. The section was taken down to 0.2mm. This particular lapping left fine scratch marks which needed to be polished away.

Subsequently, each specimen was polished using a MicroCloth Polishing cloth and 12.5μM aluminum oxide slurry on Buehler's Minimet® 1000 at a speed of 50 RPMs. The slurry was created at a 20g aluminum oxide to 100mL of distilled water ratio under

the fumehood. Each specimen was polished to a thickness between 0.15mm and 0.18mm, and then sonicated in a water bath for 15 minutes.

For the potential of additional histological analysis requiring thinner sections, no cover-slipping was carried out.

3.3 Photography

Each thin section was photographed with a Nikon Digital Sight DS-5M digital camera attached to a Nikon SMZ800 zoom stereomicroscope with a P-Plan Apo 0.53 objective and a 10x eyepiece. Reflected ectoscopic illumination was used, provided through a fibre–optic ring light by a Nikon MI-150 high intensity illuminator. The ring light was fitted with a polarizing system and all images were captured in polarized light with a fully crossed polarizer and analyzer at an effective magnification of 10x.

3.4 Reconstruction

The deciduous teeth used for this study were naturally exfoliated and therefore have all sustained varying degrees of occlusal wear. The majority of teeth, therefore, required occlusal cusp reconstruction. Adobe Photoshop CS2 was used to reconstruct the tooth cusps (Figure 3-1). The method used was similar to that specified by Saunders and colleagues (2007). See Section 3-6 for a description of the testing method and Section 4-1 for the results of the simulation testing on deciduous teeth.

First, the Zoom Tool was used to zoom in on the cusp tip. The Line Tool was then selected and two lines were drawn: the first line extended along the plane of the unworn lingual enamel surface just below the occlusal wear, while the second line extended along the plane of the unworn buccal enamel plane just below the occlusal wear. These lines were extended until they intersected at a point above the cusp tip (point 'a').

Secondly, the Pen Tool was selected and the Shape Layer and the Outline style options were chosen. Two anchor points were drawn: the first corresponded to the point

at which the first drawn lingual line meets the edge of lingual-occlusal wear (point 'b'), while the second anchor point corresponded to the point at which the buccal line meets the edge of the buccal-occlusal wear (point 'c'). The first anchor point drawn (on the lingual surface) was dragged downwards towards the cervical margin using the mouse. As the mouse was dragged in one direction (towards the cervix), a "direction line" formed and extended in the opposite direction (line'd'). When the direction line reached the point of intersection between the two lines described above, the mouse button was released and an arc was formed which approximated the position of an unworn cusp for that particular tooth ('e'). A similar procedure was used on the dentine horn if it required reconstruction as well. The same coronal reconstruction method was used on all deciduous tooth types.

Lastly, the cervical margin was delineated by a line between the lingual and buccal cervical loops created using the Line Tool. Figure 3-2 illustrates a crown with reconstructed enamel (a), a reconstructed dentine horn (b) and a delineated cervical margin (c).

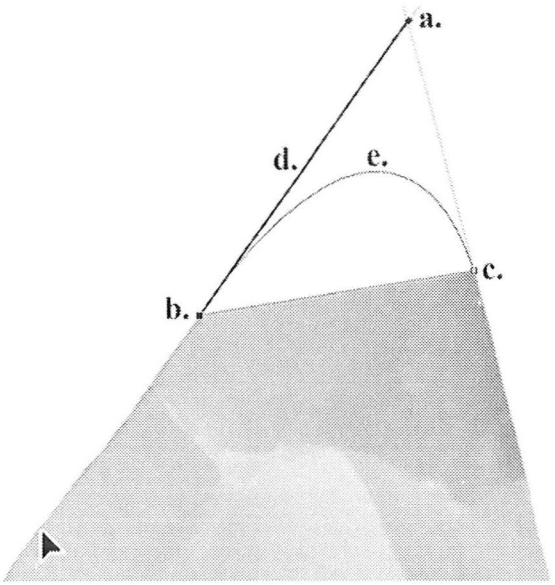

Figure 3-1. An illustration of the reconstruction method employed to reconstruct worn cusp tips and dentine horns using Photoshop CS2 (after Saunders et al. (2007)).

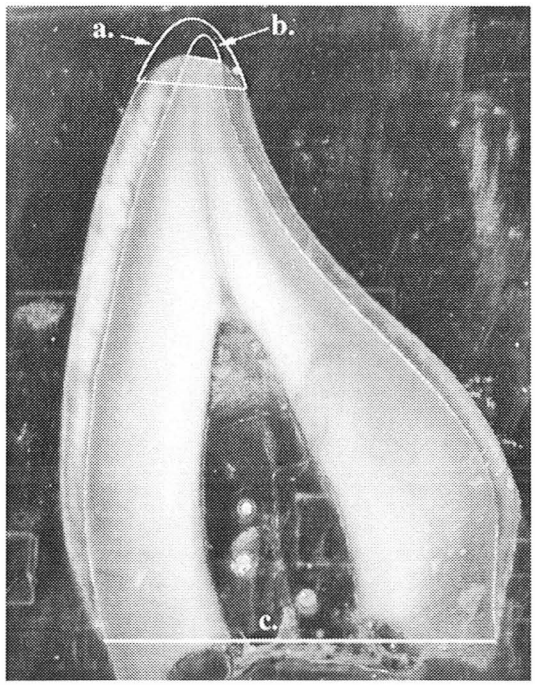

Figure 3-2. Illustration of a crown with enamel (a) and dentine (b) reconstruction, as well as a delineated cervical margin (c).

3.5 Crown Reconstruction Testing

Out of all 208 deciduous crowns initially collected and sectioned, 11 crowns were eliminated immediately due to extreme wear or damage. Of these 11 crowns, only two were eliminated because of cervical margin damage. Within the samples included, therefore, no significant portion of either the coronal enamel or dentine was lost due to resorption, and it can be assumed that the resorption process has little effect on the measurements of cervical dental tissues. Of the 197 remaining teeth, crown reconstruction was completed following the Photoshop CS2 protocol established by Saunders and colleagues (2007). A total of 145 out of 152 anterior teeth needed occlusal reconstruction, and 44 out of 46 molars needed reconstruction (Table 3-2). The anterior teeth sustained large amounts of wear, with 93% of the crowns needing both enamel and dentine reconstruction. The molar crowns also displayed occlusal wear; however, only 30% of these molars needed enamel and dentine reconstruction on both cusps. Therefore, the focus of this reconstruction test is on anterior teeth. For a list of molar elimination criteria, see section 3-6.

Table 3-2. The number of deciduous crowns requiring reconstruction. 'None' indicates no cusps reconstructed; 'E' indicates cuspal enamel reconstructed; 'E(1)' indicates enamel reconstructed on one cusp; 'E(2)' indicates enamel reconstructed on two cusps; E&D(1) indicates enamel and dentine reconstructed for one cusp; and E&D(2) indicates enamel and dentine reconstructed for two cusps.

	Incisors			***Canines***			***Molars***					***Total***
	n = 118			n = 34			n = 46					
Sample	None	E	E&D	None	E	E&D	None	E(1)	E(2)	E&D (1)	E&D (2)	
Blyschak	0	1	19	0	1	7	0	0	5	2	0	35
Chan	0	0	26	2	0	22	0	3	9	9	14	85
Fatina	3	4	65	0	0	2	2	1	0	1	0	78
Total	3	5	110	2	1	31	2	4	14	12	14	198

Saunders and colleagues' (Saunders et al., 2007) study performed cuspal reconstruction on permanent canines and third premolars, and through reconstruction simulations, reported that their reconstruction method had no effect on the accuracy of measured total crown area (TCA). Because the accuracy of this reconstruction method was only tested on the single parameter, it remains unknown if this method can be applied with accuracy, to more heavily worn deciduous crowns. Additionally, it is unknown how accurately it performs on incisors.

Of interest to this study, three questions were asked: 1) Is the reconstruction method developed by Saunders et al. (Saunders et al., 2007) applicable for use on worn deciduous crowns? 2) How do the absolute measurements of TCA, Enamel Cap Area (ECA), Dentine-Pulp Area (DPA) and Dentino-Enamel Junction length (DEJ) change depending on the degree (wear depth and angle) to which the occlusal surface is worn? 3) Is this reconstruction test applicable at all stages of occlusal wear? Ultimately, the answers to these questions will establish whether or not it is possible to use exfoliated deciduous teeth for analysis of crown tissue measurements.

3.5.1 Wear Analysis

Before proceeding with cuspal reconstruction simulations, it was necessary to assess the degree of occlusal wear sustained by anterior deciduous tooth types so that the simulated wear could best approximate actual wear patterns. Because Saunders and colleagues (Saunders et al., 2007) simulated wear on only the lower adult canine, it was not known whether deciduous occlusal wear followed the same pattern. Deciduous teeth are in occlusion for a short duration (Table 3-3), and compositional and structural crown tissue differences between deciduous and permanent teeth may lead to different levels or patterns of wear.

First, traced outlines of randomly chosen incisors and canines were created using the Pen tool in Adobe Photoshop CS2. Afterwards, all teeth within each tooth type were seriated in sequence from the least worn to the most worn to observe the pattern of wear on the occlusal surface. Figure 3-3 illustrates the variation in occlusal wear of randomly selected upper first incisors, upper canines and upper second molars.

Afterwards, the cervixes of all anterior crowns used in this study were oriented at a horizontal plane so that an angle of occlusal wear could be approximated from a standardized position using the Measure Tool. Wear sloping down towards the lingual crown aspect was recorded as a positive angle, whereas wear sloping down towards the labial crown aspect was recorded as a negative angle. Descriptive statistics were run in SPSS 13.0 for Windows to determine the extent of the variation in occlusal wear.

Table 3-3: The eruption times and exfoliation times of deciduous crowns of males and females. The third column represents the mean occlusion time of the deciduous crowns of males and females. Data extracted from Lysell et al. (1962) and Barnard and Hoffmann (1975).

	Eruption timing (mean year)*		**Exfoliation timing (mean±SE year)****		**Length in Occlusion (mean year)*****	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*
upper first incisor	0.83	0.87	7.0±0.05	6.8±0.04	6.17	5.93
lower first incisor	0.66	0.68	6.4±0.10	6.1±0.04	5.74	5.42
upper second incisor	0.93	0.96	7.7±0.06	7.4±0.07	6.77	6.44
lower second incisor	1.10	1.09	7.1±0.07	6.8±0.07	6.00	5.71
upper canine	1.61	1.60	10.5±0.17	10.1±0.19	8.89	8.50
lower canine	1.66	1.62	10.2±0.12	9.3±0.11	8.54	7.68
upper first molar	1.34	1.33	10.8±0.14	10.2±0.13	9.46	8.86
lower first molar	1.37	1.34	10.3±0.15	9.8±0.18	8.93	8.46
upper second molar	2.41	2.45	10.9±0.18	10.9±0.24	8.49	8.45
lower second molar	2.26	2.26	11.1±0.15	10.8±0.19	8.84	8.54

*Data extracted from Lysell et al. (1962)
** Data extracted from Barnard and Hoffman (1975)
*** Time in occlusion = Barnard and Hoffmann mean yr. – Lysell et al. mean yr.

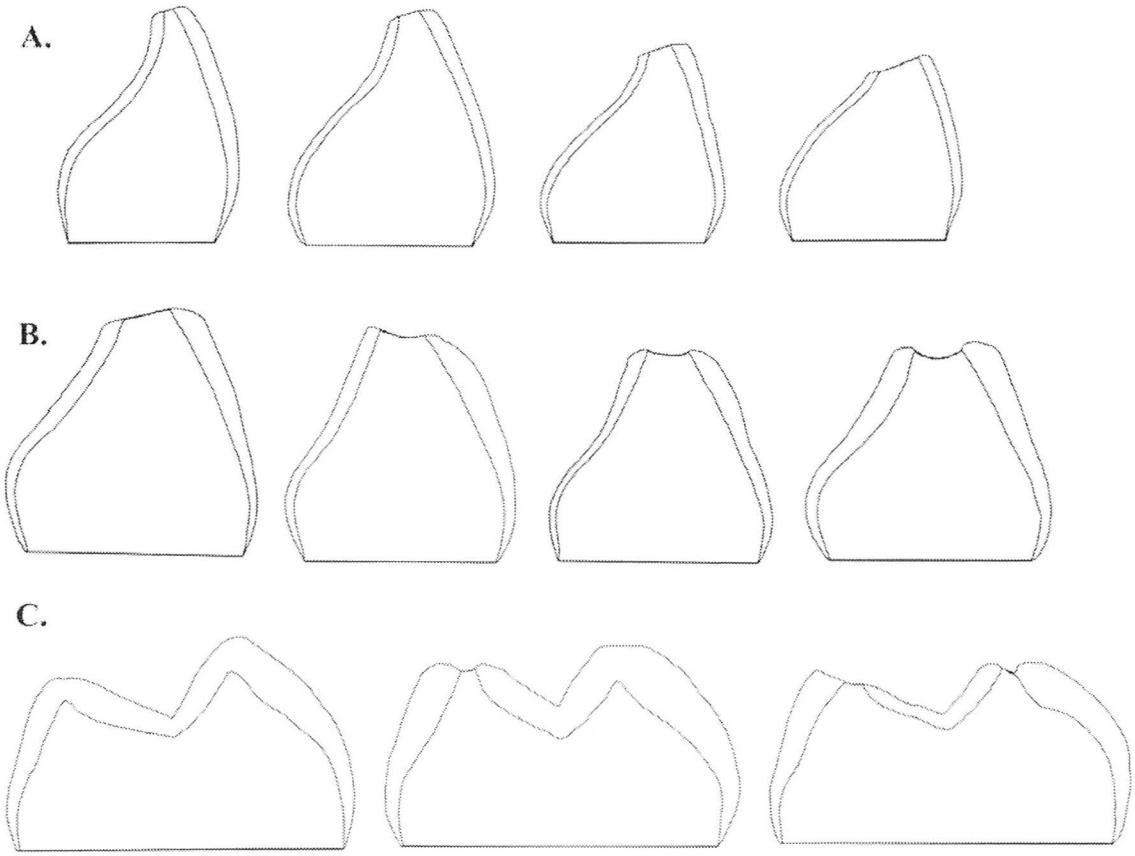

Figure 3-3. The upper first incisors (A), upper canines (B) and upper second molars (C) exemplify the variation in degree of wear observed in the deciduous crowns.

Using the image analysis software program SigmaScan Pro 5, a linear measurement of the exposed dentine at the occlusal surface was measured along with a measurement of the total crown thickness at the occlusal surface. Relative dentine thickness at the worn occlusal surface was determined for 123 randomly selected anterior crowns assessed in this study. The purpose of this was to devise elimination criteria of deciduous anterior crowns based on relative dentine exposure at the occlusal surface if the reconstruction simulations proved inaccurate based on degree of wear.

3.5.2 Reconstruction simulations

Reconstruction simulations were conducted on two unworn lower canines and three unworn lower incisors in Photoshop CS2. These crowns were the only five anterior teeth to exhibit no enamel or dentine wear at the occlusal surface. Initially, three arbitrary depths of wear were chosen at which six angles of wear were simulated. The three depths of simulated wear for single-rooted teeth (incisors and canines) were conducted at 0.5mm, 1.0mm and 1.5mm downwards vertically from the occlusal-most aspect of an unworn crown cusp. These depths were burned onto the image using the Nikon NIS software calibrated to the image. According to the angles of wear observed above, wear was simulated on the lingual occlusal surface at a horizontal (0°) plane, and at 10°, 20°, 30°, and 40° at each depth of simulated wear. Additionally, because a range of lower anterior teeth exhibit wear which sloped down towards the labial edge, angles of -10° and - 20° were also simulated at each depth. Figure 3-4 is a visualization of the wear simulations for the reconstruction test.

The Rectangle tool in Photoshop CS2 was used to cover the cusp tip at the specified simulated depth of wear (either 0.5mm, 1.0mm or 1.5mm), and was transformed to simulate the designated angle of wear at the specified depth. Using the method described in Section 3-4, a cusp was reconstructed. For each unworn canine and incisor tested, 21 cusps were reconstructed (seven angles, each at three depths). In total, therefore, 105 simulations were run.

After reconstruction, the 21 images were imported into SigmaScan Pro 5. The parameters TCA, DPA, ECA, and DEJ were measured. CD was not considered since the measurement did not change depending on the extent of occlusal wear. These four absolute measurements were also measured on the unworn tooth, and the differences between the simulated measurements and the true measurements were tabulated and statistically compared using SPSS 13.0 for Windows.

A 2x2 factorial ANOVA was conducted in order to see if the independent variables, simulated depth of wear and simulated angle of wear, had any main effects or interactions on the dependant measurements TCA, ECA, DPA and DEJ. Post-hoc

Bonferroni multiple comparison tests were also run to locate the significant differences for the variables with equal sample variance, or post-hoc Games-Howell multiple comparison tests were run for those variables with unequal sample variance.

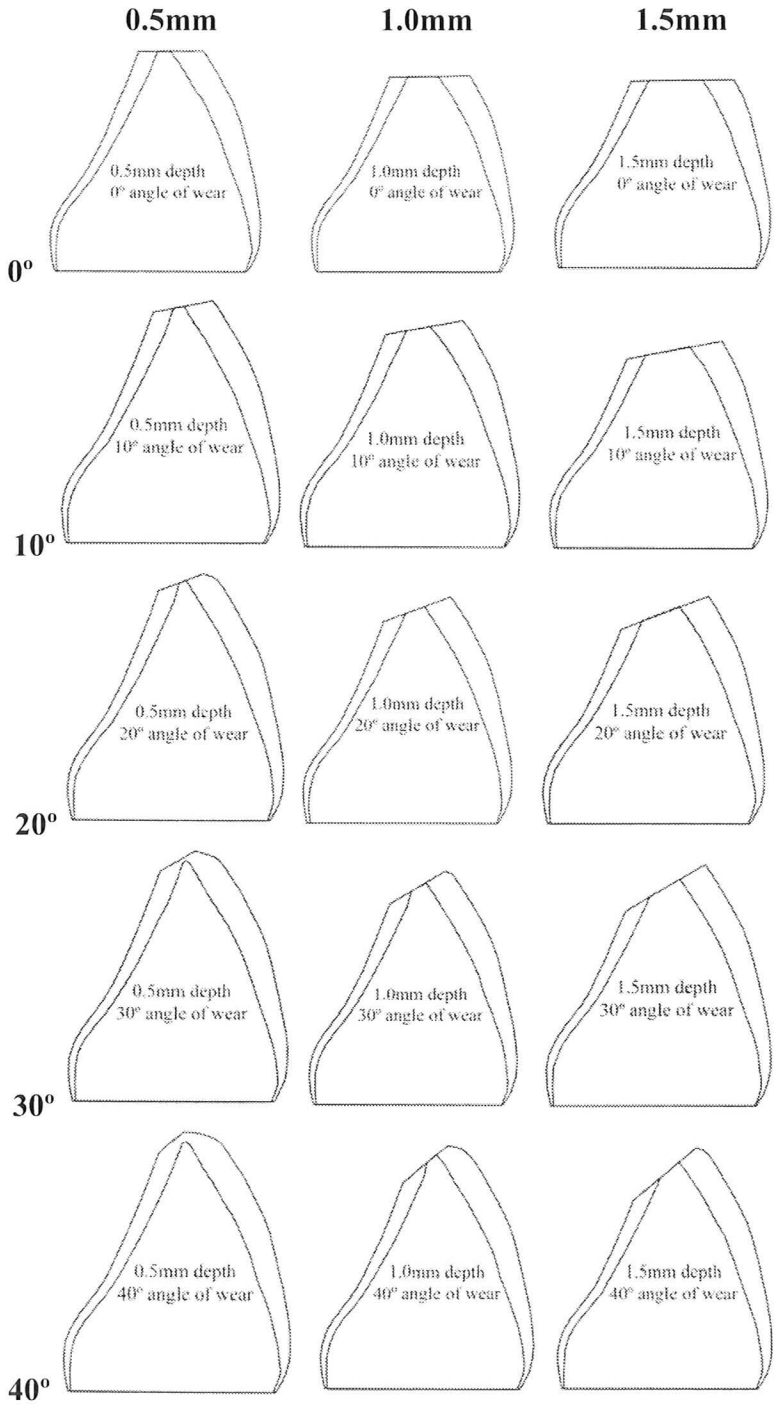

Figure 3-4. Visualization of the wear simulations for depths of wear approximating 0.5mm, 1.0mm and 1.5mm, and angles of wear approximating 0°, 10°, 20°, 30° and 40°.

3.6 Exclusionary Criteria

Not only do deciduous teeth exhibit greater levels of occlusal wear than permanent teeth, many teeth within this sample either came from the same individual or from related individuals. Therefore, criteria for excluding anterior and posterior crowns needed to be established. If one or more of these exclusionary criteria were applicable, the crown was eliminated from the study.

1. Cervical margins needed to be well defined in order that the cervix could be drawn to delineate crown tissues from root tissues. If more than the tip of one of the cervical loops was missing due to breakage or resorption, the tooth was eliminated from the sample.
2. Molars with fillings embedded in the occlusal basin were eliminated due to obstruction of the DEJ.
3. Crowns with abnormal wear patterns on the lingual or buccal/labial enamel were eliminated from the sample.
4. If both antimeres were present, the right thin-section was excluded unless the left thin-section was more poorly preserved.
5. If related individuals shared the same tooth type, the individual with the more poorly preserved crown was excluded from the study.
6. For linear measurement exclusionary criteria, any anterior teeth were eliminated from which not all 19 linear measurements (described below) could be calculated. Likewise, any molar teeth from which not all 13 linear measurements (described below) could be calculated were also eliminated from analysis.
7. For area measurements exclusionary criteria, anterior crowns were eliminated if the ratio of the relative thickness of dentine exposed at the occlusal surface to the cervical diameter was ≥ 0.39 for incisors, or ≥ 0.45 for canines. These criteria are explained in the above section and Results Section 4.1. Molar crowns were eliminated if fillings impeded reconstruction of the cusps.

Criteria 6 and 7 were sufficient in eliminating incisors which exhibited wear approximating a Smith (Smith, 1984) category 4, and molars which exhibited wear approximating a Smith (Smith, 1984) category 5.

Exclusionary criteria 4 and 5 apply to crowns within a single tooth type. Therefore, different tooth types may have teeth from the same individual or related individuals. This overlap could not be avoided simply due to already small sample sizes for each tooth type. Table 3-4 lists the sample sizes for each tooth type after the application of the elimination criteria.

Table 3-4. Sample sizes after applying the elimination criteria.

		Tissue Thickness Analysis		**Tissue Area Analysis**	
		Males	Females	Males	Females
Incisors	Upper First	17	20	16	19
	Upper Second	9	11	9	11
	Lower First	11	12	9	11
	Lower Second	5	13	6	13
Canines	Upper	6	7	6	8
	Lower	11	5	11	5
Molars	Upper First	6	2	6	2
	Upper Second	3	6	3	6
	Lower First	7	4	7	4
	Lower Second	2	7	2	7
		77	87	75	86

3.7 Measurements

All linear and area parameters were measured blind to the sex of the individual to avoid introduction of bias.

3.7.1 Area Measurements

Three absolute measurements: 1) total crown area (TCA); 2) dentine-pulp area (DPA); and 3) enamel cap area (ECA), along with the three relative area measurements of: 1) relative enamel cap area (RECA); 2) relative average enamel thickness (RAET); and 3) relative enamel cap area to cervical diameter (RECA to CD) were analyzed in this study. All six parameters were adapted from Martin (1985). The fourth relative area measurement included, relative dentine and pulp area (RDA), is described by Saunders et al. (2007). The linear crown measurements of cervical diameter (CD) and dentino-enamel junction (DEJ) linear lengths included in the relative area and/or thickness measurements are also described by Martin (1985). The descriptions that follow in Table 3-5 are illustrated in Figure 3-5.

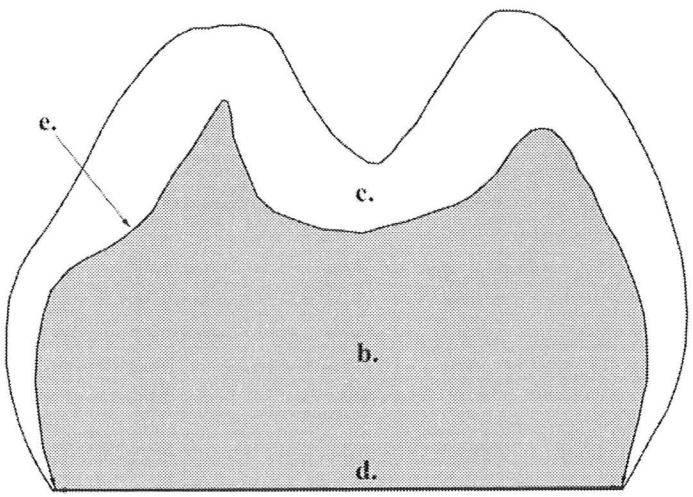

Figure 3-5. Illustration of the area measurements included in this study. Total crown area 'a' (TCA) is the combination of areas b + c. Measurement 'b' is the dentine-pulp area (DPA) and measurement 'c' is the enamel cap area (ECA). Linear measurement 'd' i s the cervical diameter (CD) and linear measurement 'e' is the dentino-enamel junction length (DEJ). Descriptions of these measurements are found in Table 3-5.

Table 3-5. Descriptions of the absolute and relative area measurements as well as the linear measurements of cervical diameter (CD) and dentino-enamel junction length (DEJ) embedded in the relative parameters. All parameters except measurement 'h' are adapted from Martin (1985).

a	Total area of the tooth crown section, defined as the area of the crown bounded by the outer enamel perimeter and a straight line between the buccal and lingual cervical margins	(b + c in Fig. 3-5)
b	Dentine and pulp area, defined as the area enclosed by the enamel-dentine junction (DEJ) and a straight line drawn between the buccal and lingual cervical margins.	
c	Enamel cap area, defined as the area of the crown bounded by the outer enamel perimeter and the DEJ.	
d	Cervical diameter, defined as the linear distance between the buccal and lingual cervical margins.	
e	Length of the DEJ, defined as the linear distance of the line separating the enamel cap area from the dentine and pulp area above the cervical margin.	
Based on the above variables, the following indices were then calculated for each tooth (the letter variables are defined in Fig. 3.5).		
f	RAET: Relative Average Enamel Thickness	$[(c / e)/ \sqrt{b}]$
g	RECA: Relative Enamel Cap Area	(c / a)
h	RDA: Relative Dentine Area	(b / a)
i	RECA to CD: Ratio of Relative Enamel Cap Area to Cervical Diameter	$(\sqrt{c} / d)$

3.7.2 Linear Measurements

Occlusal wear is problematic for the development of a systematic, consistent and repeatable method of taking linear measurements from deciduous crowns. As much as

93% of the anterior teeth and 57% of the posterior teeth examined in this study displayed occlusal wear into one or both of the dentine horns. All standardized linear measurements require two consistent points of reference to create a systematic measuring technique. With the destruction of the dentine horn in the majority of the anterior teeth, the apex of the DEJ could not be used as a standard reference point along with the cervical diameter (CD). However, in this particular sample of deciduous molars, occlusal wear had not affected the lowest point on the DEJ between the buccal and lingual cusps of molars; therefore, this point along with the CD (a) were used as the two reference points for posterior teeth.

For the molars, Photoshop CS2 was used to draw a line perpendicular to the CD to the lowest point on the DEJ between the cusps (a). This vertical distance was measured in SigmaScan Pro 5 and recorded. Additionally, the lingual enamel thickness, LET1; dentine-pulp thickness, DPT1; buccal enamel thickness, BET1; lingual enamel perpendicular to the DEJ, PLET1; buccal enamel perpendicular to the DEJ, PBET1; and total crown thickness, TCT1 (LET1 + DPT1 + BET1) were also measured at the ½ point on line b. Similar measurements were measured at the top of the line (the lowest point on the DEJ), designated by the LET2; DPT2; BET2; PLET2; PBET2; and
TCT2 (LET2 + DPT2 + BET2). Figure 3-6 illustrates these measurements.

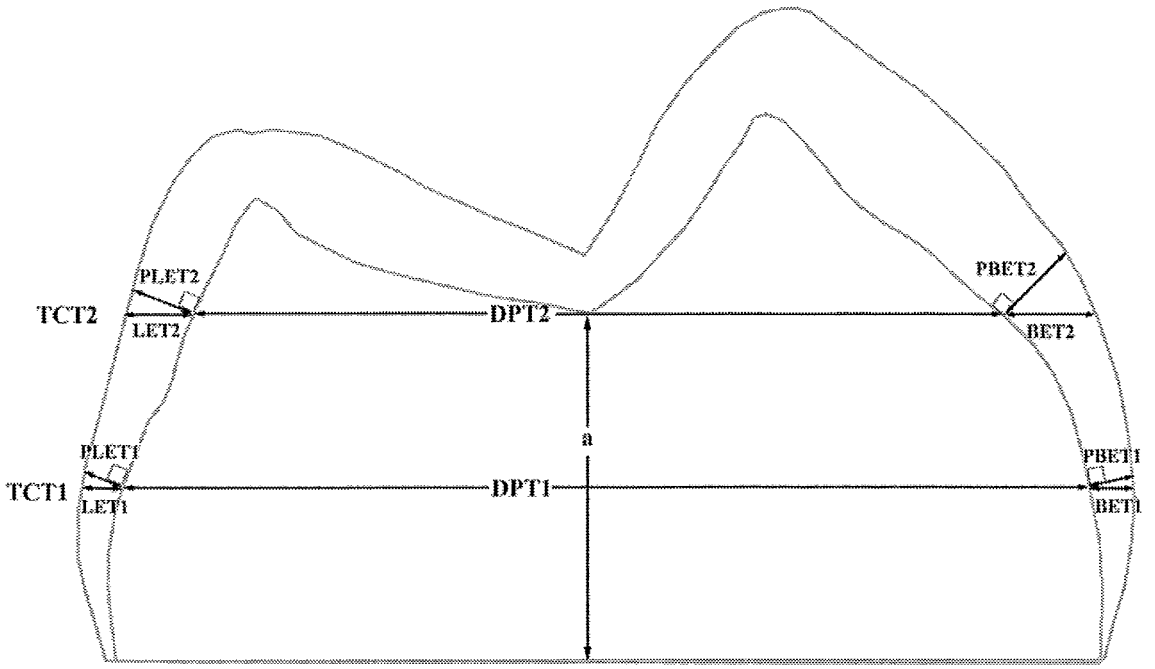

Figure 3-6. Illustration of the linear variables measured from deciduous molars.

For the anterior teeth, the CD was used as the first reference point and the second reference point was the lingual-most point on the edge of the lingual enamel. A line parallel to the cervix was extended from the lingual-most enamel edge to across the entire crown, the lingual enamel line (LEL); this line is not necessarily the maximum BL diameter at the mid-section since the line does not always extend to the labial-most enamel point. The vertical distance between the LEL and the CD was measured and recorded (a). On the LEL, the lingual enamel thickness LET1; dentine-pulp thickness DPT1; buccal enamel thickness BET1; lingual enamel perpendicular to the DEJ, PLET1; buccal enamel perpendicular to the DEJ, PBET1; and total crown width were measured (LEL or TCT1 = LET1 + DPT1 + BET1). The distance between the CD and the LEL (a) served as the standardized measurement at which two other horizontal lines of reference (TCT2 = LET2 + DPT2 + BET2 and TCT3 = LET3 + DPT3 + BET3) were drawn up along the height of the crown from which similar tissue thicknesses and perpendicular

tissue thicknesses were extracted. These anterior linear measurements are illustrated in Figures 3-7.

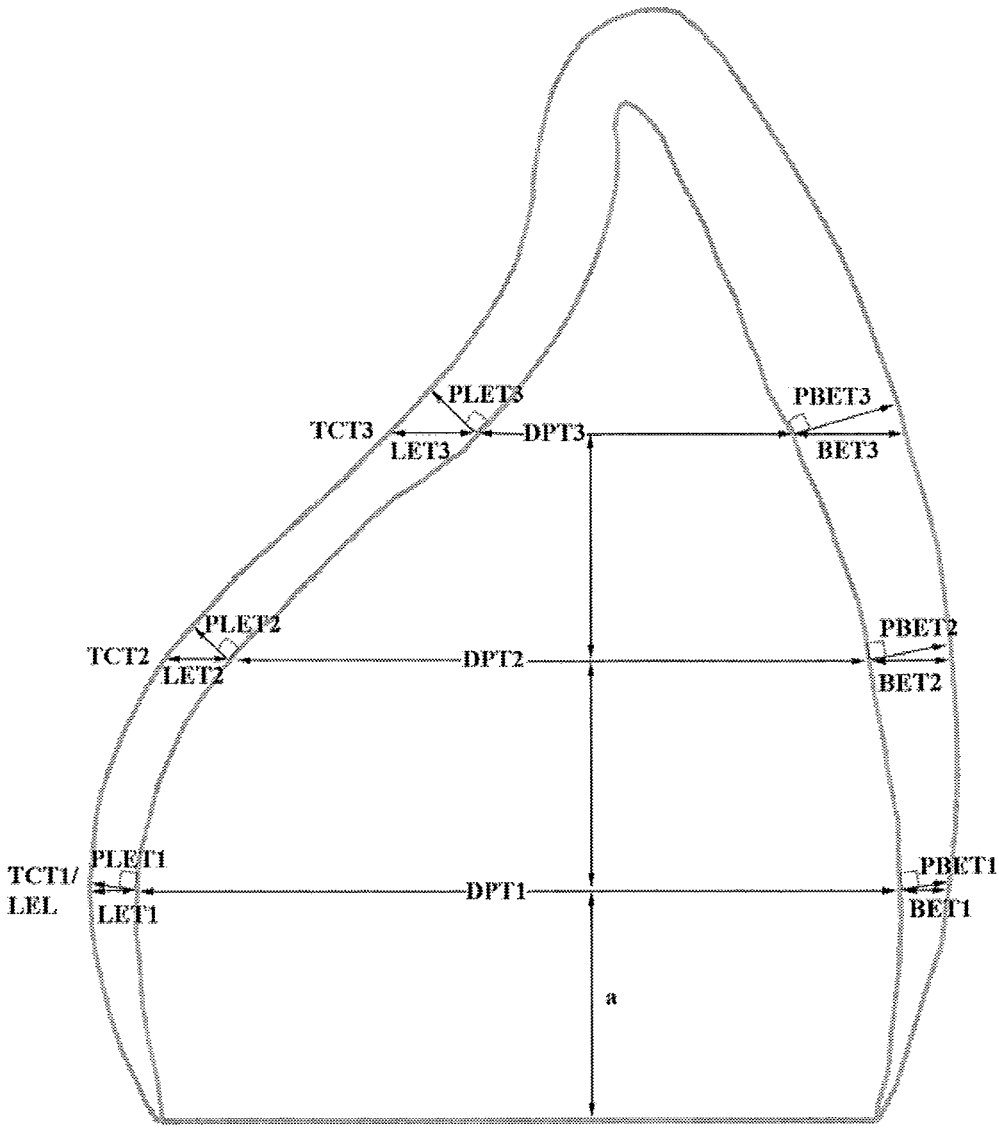

Figure 3-7. Illustration of the linear measurements taken from deciduous anterior teeth. The vertical line on the left side indicates the location of the lingual-most enamel point on the lingual crown aspect. Measurement 'a' represents a standard distance between the cervix and a parallel line extended from this lingual-most enamel point from which all subsequent tissue thicknesses are extracted.

3.7.3 Data Collection

All reconstructed images were imported into the image software analysis program SigmaScan Pro 5. Each photograph was calibrated to a standard measurement taken from

where O_i is the initial observation, O_f is the second observation and N is the total number of comparisons tested.

3.8.3 Univariate Analysis

All tests of significance in this study are two-tailed and considered significant if they were smaller than the specified Bonferroni corrected significance level. The Bonferroni corrected value was used to avoid making a type I statistical error (false positive) by carrying out several planned comparisons, and was calculated by dividing the true significance level (0.05) by the number of comparisons made (Aron et al., 2006). As such, separate values were calculated for crown area measurements for the incisors, canines and molars, and as well for the linear measurements for the anterior crowns and molars (Table 3-6). Discrepancies between the numbers of planned comparisons for each tooth type are explained by the elimination criteria in Section 4.1.

The null hypotheses were as follows: 1) there are no statistically significant differences between the mean crown tissue area measurements TCA, DPA and ECA of males and females, tooth types specified; 2) there are no statistically significant differences between the relative crown tissue area measurements RECA, RDA, RAET and RECA to CD of males and females, tooth types specified; 3) there are no statistically significant differences between the designated crown tissue linear measurements of males and females.

Descriptive statistics (mean, median, standard deviation (SD), minimum – maximum, variance and range) were calculated for all area and linear variables, with files split for sex and classified by tooth type.

For the variables whose distributions were normal, independent-samples t-tests were employed to compare the distribution of means between males and females for the specified area and linear variables. Although only one antimere was used within each tooth type and no related individuals had the same tooth within a single tooth type, no comparison in degree of sexual dimorphic difference could be made across tooth types because different tooth types may have contained teeth from the same individual or from

related individuals. These possibilities would confound statistical findings on the distribution of differences between male and female sample means.

For the variables whose distributions were not normal, a distribution free test, the Mann-Whitney U rank test, was used to compare the distribution of means between males and females for the specified area and linear variables. All data to be analyzed using this non-parametric test was first transformed into ranked order—from lowest to highest measurements. The null hypothesis for the non-parametric tests is slightly different from that of the parametric independent-samples t-test (Aron et al., 2006). The null hypothesis is that the median rank value of each variable is equal between males and females, since the middle rank of a rank-order test is equivalent to the mean in the corresponding parametric test (Aron et al., 2006). Again, across tooth type comparisons could not be done due to confounding factors of crowns included from the same individual or related individuals.

For all variables which showed significant differences between males and females, the percent sexual dimorphism was calculated using Garn et al.'s (1967b) formula: [(*male mean - female mean*) / *female mean*] x 100. These values, however, could not be directly compared across tooth types because different crowns belonging to the same individual or related individuals were included across different tooth types. On the contrary, similarities between percent dimorphism were compared amongst different significant variables for the same tooth type.

Table 3-6. A list of the Bonferroni corrected significance levels for multiple planned comparisons for each tooth type. The true significance level is 0.05.

Measurement	**Number of Planned Comparisons**	**Bonferroni Corrected Signficance Level**
Area: Incisors	2	0.025
Area: Canines	7	0.007
Area: Molars	9	0.005
Linear: Anterior crowns	19	0.003
Linear: Molars	13	0.004

Chapter 4
RESULTS

4.1 Crown Reconstruction Testing Results

4.1.1 Wear Analysis Results

Three main observations were made. First, the degree of wear exhibited within a single tooth type is highly variable; therefore, it is a safe presumption that at the time of exfoliation of a certain tooth type, there is a vast range in the degree of wear experienced by children of roughly the same age. Secondly, the degree of wear for all tooth types combined, as measured by the relative dentine thickness exposed at the occlusal surface, appears to not be highly correlated with the angle of wear (Pearson's r=0.156, Table 4-1). Lastly, the angle of wear exhibited by the maxillary anterior tooth crowns generally slopes downwards towards the lingual crown aspect, whereas the mandibular anterior tooth crowns tend to have a greater range of angles, sloping both to the lingual and labial crown aspects (Table 4-2). In general, the angle of wear ranges from 0 ° to 40° slopes along the lingual aspect, and from -0 ° to -20° sloping along the labial crown aspect. This observation is illustrated by the boxplots in Figure 4-1.

a stage micrometer with precision at the 0.01mm level. The above absolute measurements were carried out and imported into an Excel spreadsheet where the five remaining relative measurements were calculated. The same protocol was followed for the linear measurements.

3.8 Statistical Procedures

All statistical analyses of the deciduous crowns were carried out using SPSS 13.0 for Windows.

3.8.1 Normality and Homogeneity of Variance

The assumptions of normality and homogeneity of variance need to be met before proceeding with univariate parametric tests. Prior to conducting univariate analyses, the Shapiro-Wilk test was used to determine whether the data for each tooth type (split by sex) were normally distributed. This test was used because all sample sizes of all tooth types, for both area and linear measurements were less than 50. Likewise, Levene's test was used prior to univariate analysis to test for the homogeneity of variances. If the data was not normally distributed, non-parametric tests were employed. Where variances were unequal but the assumption of normality was met, the SPSS adjusted univariate parametric output for unequal variances was analyzed. If both assumptions were met, parametric tests were employed.

3.8.2 Intra-observer error

Eleven randomly selected teeth were selected for which the intra-observer error of the linear and area measurements was estimated. Each tooth was subjected to a re-measuring at least two months after the initial measurement. The average percentage of intra-observer error between two observations (%E) was calculated using the formula:

$$\text{Average } \%E = \Sigma \left[(O_i - O_f) / O_i \right] \times 100 \right] / N,$$

Table 4-1: Pearson's correlation between the angle of occlusal wear and the relative dentine thickness exposed at the occlusal surface. No significant correlation is observed between the two variables (r=0.156).

		Angle of Wear	Relative Dentine Thickness at Occlusal Surface
Angle of Wear	Pearson Correlation	1.000	-0.134
	Sig. (2-tailed)		0.156
	N	123	123
Relative Dentine Thickness at Occlusal Surface	Pearson Correlation	-0.134	1.000
	Sig. (2-tailed)	0.156	
	N	123	123

Table 4-2: Mean angle of wear of anterior deciduous crowns. The 'L' and 'U' denote lower and upper respectively, while 'c' and 'i' indicate canine and incisor respectively. '1' and '2' denote first and second respectively.

Tooth Type	Jaw	Mean	Std. Deviation	N
Lc	Mandibular	12.712	9.864	17
	Total	12.712	9.864	17
Li1	Mandibular	4.888	13.570	24
	Total	4.888	13.570	24
Li2	Mandibular	11.464	13.203	14
	Total	11.464	13.203	14
Uc	Maxillary	10.581	9.049	16
	Total	10.581	9.049	16
Ui1	Maxillary	19.585	9.391	34
	Total	19.585	9.391	34
Ui2	Maxillary	14.150	10.027	18
	Total	14.150	10.027	18
Total	Maxillary	16.028	10.086	68
	Mandibular	8.980	12.753	55
	Total	12.876	11.842	123

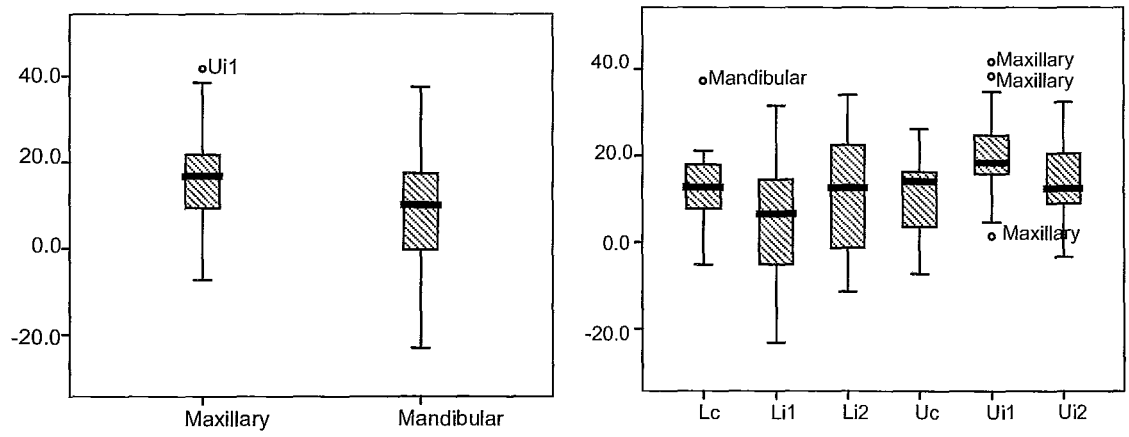

Figure 4-1: Boxplot 'a' demonstrates the variance of the angle of wear exhibited by maxillary and mandibular deciduous anterior crowns (incisors and canines). Boxplot 'b' demonstrates the range of wear exhibited by each deciduous crown type. The black horizontal lines represent the median angle of wear. The upper and lower hinges of the boxes represent the upper (75^{th}) and lower quartiles (25^{th}) respectively, while the whiskers represent 1.5(IQR). The outliers represent angles of wear which fall beyond the 1.5(IQR) range. Positive values are indicative of angles of wear sloping down towards the lingual crown aspect, whereas negative values are indicative of angles of wear sloping down towards the labial crown aspect. Maxillary crowns exhibit a range of wear in the positive range, whereas the range of mandibular wear is greater, spanning into the negative range.

4.1.2 Statistical Analysis of Reconstruction Simulations

For canines and incisors, normality of the difference values within the four variables (total crown area (TCA), dentine-pulp area (DPA), DEJ length (DEJ) and enamel cap area (ECA)) was assessed using the one-sample Komorogov-Smironov test. The distribution of the difference[1] values for all four parameters was normal (Table 4-3).

[1] In order to maximize the number of simulations to compare within the same tooth type, the differences between the true unworn measurements and the simulated measurements were calculated. The proceeding statistical analyses are based on the differences, not the absolute measurements.

Table 4-3: Komorogov-Smironov test for normality, calculated for incisor and canine samples separately. The sample distributions of all four parameters measured for the reconstruction simulations are normal.

Tooth Type	*Variable*	*N*	*Significance (2-tailed)*
Incisor	TCA	63	0.131
	DPA	63	0.062
	DEJ	63	0.129
	ECA	63	0.288
Canine	TCA	42	0.302
	DPA	42	0.634
	DEJ	42	0.354
	ECA	42	0.890

Levene's test for homogeneity of variance (Table 4-4) was conducted for the four variables for incisors and canines separately. The following table demonstrates that the DEJ sample variance for both the incisors and canines is unequal. Additionally the sample variances for TCA and DPA for incisors are unequal.

Table 4-4. Levene's test for homogeneity of variance, calculated for incisor and canine samples separately.

Tooth Type	*Variable*	*N*	*Significance*
Incisor	TCA	63	0.000*
	DPA	63	0.000*
	DEJ	63	0.000*
	ECA	63	0.154
Canine	TCA	42	0.072
	DPA	42	0.219
	DEJ	42	0.030*
	ECA	42	0.458

* denotes significant divergence from variance homogeneity

Based on the factorial ANOVA and post hoc statistics (Table 4-5, Table 4-6), the Saunders et al. (2007) reconstruction method is not completely sufficient for deciduous incisors or canines. In particular, it is unfavorable for depths of wear that approximate 1.5mm from the original occlusal-most point of an unworn cusp. Additionally, it fails to accurately approximate the DEJ length for both deciduous canines and incisors, and as well cannot be used to accurately measure the TCA and DPA for incisors. Therefore, the only absolute variable measurements which can be assessed for canines are the TCA,

DPA and ECA if a standardized criterion is developed to systematically exclude crowns which approximate a depth of wear of 1.5mm. The only absolute variable which is deemed statistically accurate for the incisors is the ECA if crowns which approximate a depth of wear of 1.5mm are excluded.

Table 4-5. Factorial 2X2 ANOVA results for canine and incisor wear simulations. Significant effects are denoted by '*'. SimDepth is the main effect for simulated depth of wear and SimAngle is the main effect of simulated angle of wear.

Source	df	F	Sig.	Partial Eta Squared
TCA Canines				
Corrected Model	20	3.936	0.010*	0.789
SimDepth	2	23.677	0.000*	0.693
SimAngle	6	2.526	0.053	0.419
SimDepth*SimAngle	12	1.350	0.264	0.436
TCA Incisors				
Corrected Model	20	9.463	0.000*	0.818
SimDepth	2	81.851	0.000*	0.796
SimAngle	6	1.559	0.183	0.182
SimDepth*SimAngle	12	1.351	0.228	0.278
DPA Canines				
Corrected Model	20	1.946	0.069	0.650
SimDepth	2	10.220	0.001*	0.493
SimAngle	6	1.304	0.298	0.271
SimDepth*SimAngle	12	0.888	0.571	0.337
DPA Incisors				
Corrected Model	20	11.683	0.000*	0.848
SimDepth	2	87.115	0.000*	0.806
SimAngle	6	5.689	0.000*	0.448
SimDepth*SimAngle	12	2.108	0.037*	0.376
DEJ Canines				
Corrected Model	20	7.544	0.000*	0.878
SimDepth	2	54.641	0.000*	0.839
SimAngle	6	5.568	0.001*	0.614
SimDepth*SimAngle	12	0.682	0.750	0.280
DEJ Incisors				
Corrected Model	20	21.852	0.000*	0.912
SimDepth	2	180.903	0.000*	0.896
SimAngle	6	8.633	0.000*	0.552
SimDepth*SimAngle	12	1.952	0.055	0.358
ECA Canines				
Corrected Model	20	2.454	0.024*	0.700
SimDepth	2	8.899	0.002*	0.459
SimAngle	6	3.835	0.010*	0.523
SimDepth*SimAngle	12	0.689	0.744	0.283
ECA Incisors				
Corrected Model	20	3.757	0.000*	0.641
SimDepth	2	32.621	0.000*	0.608
SimAngle	6	0.473	0.824	0.063
SimDepth*SimAngle	12	0.588	0.839	0.144

* denotes significance at $\alpha = 0.05$

Table 4-6: Post hoc tests (Bonferroni or Games-Howell) of the simulated depth of wear. Significant differences between depths are wear are denoted by '*'.

	Post Hoc test	SimDepth (I)	SimAngle (J)	Mean Difference (I-J)	Std. Error	Sig.
TCA Canines	Bonferroni	0.5	1.0	-0.036	0.055	1.000
			1.5*	-0.344	0.055	0.000
		1.0	0.5	0.036	0.055	1.000
			1.5*	-0.308	0.055	0.000
		1.5	0.5*	0.344	0.055	0.000
			1.0*	0.308	0.055	0.000
TCA Incisors	Games-Howell	0.5	1.0*	-0.106	0.030	0.003
			1.5*	-0.479	0.045	0.000
		1.0	0.5*	0.106	0.030	0.003
			1.5*	-0.373	0.048	0.000
		1.5	0.5*	0.479	0.045	0.000
			1.0*	0.373	0.048	0.000
DPA Canines	Bonferroni	0.5	1.0	-0.057	0.035	0.343
			1.5*	-0.154	0.035	0.001
		1.0	0.5	0.057	0.035	0.343
			1.5*	-0.097	0.035	0.031
		1.5	0.5*	0.154	0.035	0.001
			1.0*	0.097	0.035	0.031
DPA Incisors	Games-Howell	0.5	1.0*	-0.051	0.010	0.000
			1.5*	-0.176	0.020	0.000
		1.0	0.5*	0.051	0.010	0.000
			1.5*	-0.124	0.021	0.000
		1.5	0.5*	0.176	0.020	0.000
			1.0*	0.124	0.021	0.000
DEJ Canines	Games-Howell	0.5	1.0*	-0.322	0.077	0.001
			1.5*	-0.730	0.083	0.000
		1.0	0.5*	0.322	0.077	0.001
			1.5*	-0.407	0.103	0.002
		1.5	0.5*	0.730	0.083	0.000
			1.0*	0.407	0.103	0.002
DEJ Incisors	Games-Howell	0.5	1.0*	-0.405	0.055	0.000
			1.5*	-1.021	0.076	0.000
		1.0	0.5*	0.405	0.055	0.000
			1.5*	-0.616	0.091	0.000
		1.5	0.5*	1.021	0.076	0.000
			1.0*	0.616	0.091	0.000
ECA Canines	Bonferroni	0.5	1.0	0.020	0.055	1.000
			1.5*	-0.189	0.055	0.007
		1.0	0.5	-0.020	0.055	1.000
			1.5*	-0.210	0.055	0.003
		1.5	0.5*	0.189	0.055	0.007
			1.0*	0.210	0.055	0.003
ECA Incisors	Bonferroni	0.5	1.0	-0.054	0.040	0.541
			1.5*	-0.303	0.040	0.000
		1.0	0.5	0.054	0.040	0.541
			1.5*	-0.249	0.040	0.000
		1.5	0.5*	0.303	0.040	0.000
		0.5	1.0*	0.249	0.040	0.000

* denotes significance at $\alpha = 0.05$

All except one angle of wear have no influence on the accuracy of the above variables which are statistically considered to be sufficient for inclusion. The crowns which approximate an occlusal wear angle of -20° (sloping towards the labial crown aspect), however, cannot be used for estimations of ECA for canines (Table 4-7).

Table 4-7. Bonferroni post hoc test of the effect of simulated angle of wear on the accuracy of ECA for canines. Significant differences between depths are wear are denoted by '*'.

	Post Hoc test	SimDepth (I)	SimAngle (J)	Mean Difference (I-J)	Std. Error	Sig.
ECA Canines	Bonferroni	-20	-10	-0.232	0.084	0.243
			0*	-0.303	0.084	0.034
			10*	-0.330	0.084	0.016
			20*	-0.305	0.084	0.032
			30	-0.280	0.084	0.065
			40*	-0.320	0.084	0.021

* denotes significance at $\alpha = 0.05$

4.1.3 Exclusionary Criteria Based on Occlusal Wear

The DEJ length cannot be assessed using this reconstruction method for either canines or incisors, thereby eliminating use of the RAET which requires an accurate measure of the DEJ. The TCA and DPA cannot be accurately approximated for incisors, thereby eliminating the use of all relative measurements since all require an accurate measure of the TCA.

For canines, the variables TCA, DPA and ECA may be approximated accurately if crowns which approximate depths of occlusal wear of 1.5mm are eliminated, and likewise the ECA for the incisors. This criterion is difficult to assess because the initial occlusal outline for each crown is unknown. Therefore, using the simulations, two variables were measured in SigmaScan Pro: 1) the dentine thickness exposed at the occlusal surface due to wear; and 2) the total crown thickness exposed at the occlusal surface due to wear. Subsequently, the ratio of both these variables to the cervical diameter was determined to standardize the measure.

After this was completed, Pearson's correlation was used to assess the degree of relation between the aforementioned relative variables and the variables TCA, DPA, DEJ and ECA to determine whether occlusal dentine thickness or total crown thickness would be better criteria for systematically eliminating teeth with wear approximating 1.5mm.

Table 4-8. Pearson's product-moment correlation test (r) demonstrating a significant degree of relation between depth of occlusal wear and the ratio of dentine thickness at the occlusal surface to the cervical diameter. The proportion of total variance between that is explained by the relation between these variables is 0.653.

		Simulated Depth of Wear	R^2
Dentine Thickness at Occlusal Surface to Cervical Diameter	Pearson Correlation	0.808*	0.653
	Sig. (2-tailed)	0.000	
	N	105	
Total Crown Thickness at Occlusal Surface to Cervical Diameter	Pearson Correlation	0.605*	0.366
	Sig. (2-tailed)	0.000	
	N	105	

* denotes significant correlation at the 0.01 level (2-tailed)

Table 4-8 demonstrates that the ratio of dentine thickness at the occlusal surface to cervical diameter is significantly correlated to the degree of occlusal wear, and therefore would be a logical variable on which to base exclusionary criteria based on degree of wear.

The dentine thickness at the occlusal surface to cervical diameter ratio was examined for each of the 105 simulations and the smallest ratio that was found within the category of 1.5mm was used as the cut-off criterion for exclusion. A ratio of 0.45 or more was used as the exclusionary criterion for canines, and a ratio of 0.39 or more was used as the exclusionary criterion for incisors. For the canines, this exclusionary criterion is also sufficient to eliminate crowns approximating -20° wear, thus allowing use of the same teeth for ECA measurement.

For 34 canines and 113 incisors, dentine thickness at the occlusal surface and cervical diameter were measured. The ratio of these two variables was used to

systematically exclude crowns used for area measurement analysis. Seven incisors and one canine were eliminated, making a final total of 33 canines and 106 incisors to be considered for area measurements.

4.2 Sexual Dimorphism Results

4.2.1 Normality testing: Area variables

The Shapiro-Wilk test was not used to test the sample distributions of the incisor tooth types for TCA, DPA, DEJ and RAET since inaccuracies demonstrated by the above reconstruction tests did not justify their inclusion. Likewise, normal sample distributions for the DEJ and RAET of the canines were not tested for the same reason. The DEJ length and RAET sample distributions were analyzed only for the molars. No results for the lower second molars for males and the upper first molars for females are available because their respective sample sizes are only 2.

Results of the Shapiro-Wilk test for absolute and relative area measurements (Table 4-9) demonstrate that for males, the sample distributions of the TCA of the lower canine and the CD of the upper first incisors do not follow a normal sample distribution. Non-parametric Mann-Whitney U Rank tests were subsequently used for these variables.

For females, the Shapiro-Wilk test demonstrates that the sample distributions of CD for the lower canines and lower second incisors do not follow normal distributions. These variables were also subjected to non-parametric univariate analyses.

Table 4-9. Significant results ($\alpha = 0.05$) of the Shapiro-Wilk normality test for absolute and relative deciduous crown area measurement distributions as well as associated linear variables. Cases are split by sex and sorted into tooth type. The sample distributions of these following seven variables are nonnormal and will need to be tested using non-parametric means.

Sex	Variable	Variable Name	Tooth Type	Shapiro-Wilk		
				Stat.	*df*	*Sig.*
Male	a	TCA	Lc	0.802	11	0.010
	d	CD	Ui1	0.887	16	0.050
Female	d	CD	Lc	0.773	5	0.050
	d	CD	Li2	0.841	13	0.020

4.2.2 Normality testing: Linear variables

The results of the Shapiro-Wilk test for absolute linear measurements for anterior teeth demonstrates 17 instances in which non-parametric testing needed to be employed; males showed 13 nonnormal sample distributions while females showed only four. The rank distributions of the anterior crown linear variables for which the Mann-Whitney U Rank test was employed are listed in Table 4-10. The distributions of the absolute linear molar measurements show 5 variables which are nonnormal, three for males and two for females. These are also listed in Table 4-11.

Table 4-10. Significant results (α = 0.05) of the Shapiro-Wilk normality test for the sample distributions of the absolute linear deciduous anterior crown variables. Cases are split by sex and sorted into tooth type. The sample distributions of these following seven variables are nonnormal and will need to be tested using non-parametric means.

Sex	Variable Name	Tooth Type	Shapiro-Wilk		
			Stat.	*df*	*Sig.*
Male	TCT2	Uc	0.680	6	0.004
	BET2	Li2	0.733	5	0.021
	BET2	Uc	0.744	6	0.017
	LET2	Lc	0.738	11	0.001
	PBET2	Li2	0.725	5	0.017
	PBET2	Ui1	0.874	17	0.025
	PLET2	Lc	0.611	11	0.000
	TCT3	Uc	0.673	6	0.003
	LET3	Lc	0.805	11	0.011
	LET3	Ui2	0.831	9	0.046
	PLET3	Lc	0.807	11	0.012
	PLET3	Li1	0.830	11	0.024
	DPT3	Uc	0.670	6	0.003
Female	Height From Cervix to LEL[1] (a)	Ui2	0.843	11	0.035
	PLET2	Ui2	0.835	11	0.027
	DPT3	Li1	0.844	12	0.031

[1]LEL is the Lingual-Most Enamel Line, drawn horizontally across the crown tooth extending from the lingual-most point of the crown.

Table 4-11. Significant results (α = 0.05) of the Shapiro-Wilk normality test for the sample distributions of the absolute linear deciduous molar crown variables. Cases are split by sex and sorted into tooth type. The sample distributions of these following seven variables are nonnormal and will need to be tested using non-parametric means.

Sex	Variable Name	Tooth Type	Shapiro-Wilk		
			Stat.	*df*	*Sig.*
Male	TCT2	Um1	0.765	6	0.028
	PBET2	Lm1	0.791	7	0.033
	DPT2	Um1	0.753	6	0.021
Female	TCT2	Lm2	0.741	7	0.010
	DPT2	Lm2	0.717	7	0.006

4.2.3 Intra-observer Error

Intra-observer error for linear and area measurements was estimated in a randomly selected sub-sample of 11 randomly selected teeth. The mean average difference in linear measurements was 0.05mm and the average % intra-observer error was found to be 2.1%. The mean average difference in area measurements was 0.06mm^2, while the average % intra-observer error was calculated to be 0.7%.

4.2.4 Descriptive statistics: Area measurements

A summary of the descriptive statistics collected for the tissue area measurements of the deciduous incisors and canines for males and females can be found in Table 1 in Appendix B. For the incisors, only the absolute enamel cap area (ECA) and cervical diameter (CD) could be accurately measured; no relative measurements could be assessed because they incorporated variables deemed inaccurate by the reconstruction simulation tests.

For the upper first and second incisors, males have a greater mean absolute ECA. The lower first and second incisors, however, demonstrate that females have a greater mean absolute ECA.

For the upper first and second incisors, the mean CD for males is larger than females, following the same pattern as the ECA. The lower first incisors show females having a greater mean length, while males show a greater mean length for the lower second incisors.

A summary of the descriptive statistics collected for the tissue area measurements of the deciduous canines for males and females can be found in Table 1 in Appendix B. For the canines, three absolute area measurements (total crown area (TCA), dentine-pulp area (DPA), and ECA); the CD; and three relative area measurements (relative enamel cap area (RECA), relative dentine area (RDA), and relative enamel cap area to cervical diameter (RECA to CD)) were compared between males and females. Interestingly, the same pattern of differences between males and females was reflected in both upper and lower canines for all variables except the RECA to CD. In general, males exhibited

greater means in variables associated with the dentine and pulp, whereas females exhibited greater means in variables associated with the enamel.

For the variable TCA, males exhibit a higher mean area for both upper and lower canines. Males also show a higher mean area for upper and lower canines for the DPA, and RDA.

For the variable ECA, females show a higher mean area for the upper and lower canines than males. Likewise for the variable RECA, female upper and lower canines have a higher mean.

For the upper canines, the females show a larger mean CD than males, a pattern opposite than seen in the incisors. Conversely, the lower canines show larger male CD means.

The last relative area variable, RECA to CD exhibits conflicting results between the upper and lower canines. The mean RECA to CD is larger in the male upper canines, while larger in the female lower canines.

A summary of the descriptive statistics collected for the tissue area measurements of the deciduous molars for males and females can be found in Table 2 in Appendix B. All absolute area and relative area measurements were assessed for the molars. Unlike the incisors and canines which demonstrate similarly patterned tissue distributions between males and females, no similar patterns can be discerned in any of the molars.

With regards to absolute measurements, the upper first molars display opposite patterning than the anterior teeth; with the exception of the dentino-enamel junction (DEJ) length, female crowns appear to have larger dentine and pulp area dimensions, while male crowns appear to have larger enamel area dimensions. Females have a larger mean TCA, mean DPA and mean CD. The corresponding relative measurement, mean RDA is also larger in females. In contrast, males have a larger mean ECA, mean RECA, mean RAET, mean RECA to CD and mean DEJ length.

The upper second molar exhibits similar results as the upper first molar except that the mean DEJ length is larger in females. It is noteworthy to mention that the mean

ECA of the second molar is by far much larger than for the other molars in both males and females, exhibiting means of 12.093mm^2 and 12.048mm^2 respectively.

The lower first and second molars do not show a similar pattern of mean tissue area dimorphism. Furthermore, they do not show a similar pattern amongst one another. The mean measurements for the female lower first molars are larger than males for all variables except for the mean RDA, and the mean RECA to CD which are larger in the male lower first molars. In contrast, all the mean measurements for the male lower second molar are larger than the females except for the mean RDA which is larger in females.

The only variable mean which consistently leans in favour of males across all four molars is the mean RECA to CD.

4.2.5 Descriptive statistics: Linear measurements

A summary of the descriptive statistics collected for the linear crown tissue measurements of the deciduous incisors and canines for males and females can be found in Table 3 in Appendix B. The variable figure for these measurements can be found in Chapter 3, figure 3-7. Recall that these linear measurements are taken from three equidistant horizontal lines (total crown thickness 1 (TCT1), total crown thickness 2 (TCT2) and total crown thickness 3 (TCT3)) along the height of the crown. In general, for the anterior deciduous crowns, there is a decrease in dentine-pulp thickness (DPT) and in total crown thickness (TCT) from the cervix to the occlusal surface. Additionally, there is an opposite trend, for both the buccal enamel thickness (BET) and lingual enamel thickness (LET), to increase from the cervix to the occlusal surface. The perpendicular buccal enamel thickness (PBET) and perpendicular lingual enamel thickness (PLET) show a slighter increase towards the occlusal surface. The buccal enamel is thicker than the lingual enamel at all locations measured.

For the lower first incisor, females have a greater mean length for 16 of the 19 variables which are associated with both dentine and enamel thicknesses, where as males show a greater mean length for only three variables: height to the horizontal line extended across the crown at the lingual-most point ('a'); BET3; and PBET3.

The lower second incisor exhibits similar mean differences between the sexes as seen in the lower first incisor. The males exhibit greater mean lengths in the same variables 'a', BET3 and PBET3; males also show a greater mean length for PBET1. The females have larger mean lengths for 15 of the 19 variables, all of which are associated with both greater mean dentine and enamel thicknesses.

The upper first incisors exhibit a different distribution pattern of mean tissue thicknesses between males and females than the lower incisors, although males do also show a greater mean length in the same variables 'a' and PBET1. The males exhibit a greater number of larger mean tissue lengths than seen with the lower incisors. Males show 9 out of 19 larger mean lengths. Interestingly, males show a greater mean for the all three dentine variables (DPT1, DPT2 and DPT3), and also for all three crown thickness variables (TCT1, TCT2 and TCT3). Furthermore, females, who exhibit greater mean lengths for 10 out of the 19 variables, show greater mean lengths in all but two of the 12 measurements (BET1 and PBET1) associated with enamel thickness.

The upper second incisors show similarities to the upper first incisors. Although males do not show a larger mean length of measurement 'a', males again show larger mean lengths for all three of the DPT variables and all three TCT variables. Furthermore, females have larger mean enamel thickness lengths for all variables except PLET3.

A summary of the descriptive statistics collected for the linear tissue measurements of the deciduous canines for males and females can be found in Table 3 in Appendix B. The lower canine shows a similar pattern to the upper incisors; however the upper canine shows inconsistent results.

The males show larger mean tissue thicknesses of 6 of the 19 variables. These are the only 6 variables associated with dentine and total crown thickness—DPT1, DPT2 and DPT3, as well as TCT1, TCT2 and TCT3. Females, on the other hand, show a larger mean tissue thickness for 13 of the 19 variables—12 of 12 measurements which are associated with enamel thicknesses. Females also display a higher mean length of measurement 'a', the height from the cervix to the LEL.

The upper canines display the same results of dimorphic enamel thicknesses as the lower canines, but do not show a similar patterning of dimorphic dentine tissue thicknesses. While females have greater mean enamel thicknesses for all 12 enamel variables, males only show a greater mean tissue thickness for DPT3 and TCT3. Female means are greater for DPT1, DPT2, TCT1 and TCT2. In addition, males have a greater mean for variable 'a', the height from the cervix to the LEL.

A summary of the descriptive statistics collected for the linear tissue measurements of the deciduous molars for males and females can be found in Table 4 in Appendix B. The variable figures for these measurements can be found in Chapter 3, figure 3-6. Recall that the linear measurements are taken from two horizontal lines along the height of the crown (TCT1 and TCT2).

In general, the molars do not appear to follow the dimorphic tissue thickness pattern which is evident in the upper incisors and canines; this may in part be due to the extremely small samples sizes (n=2) of the lower second molars and upper first molars.

For the lower first molars, female mean tissue thicknesses for TCT1, TCT2, DPT1 and DPT2 were larger than males, while 6 of the 8 mean enamel thicknesses measured were also larger for females. Males displayed higher means for 'a', the height from the cervix to the lowest DEJ point, and for two enamel thicknesses, BET1 and PBET1.

The lower second molars displayed completely different results than the lower first molar. Males showed higher means for all the enamel and dentine thickness variables (12 of the 13 variables), while females had a higher mean length for only variable 'a'.

The upper first molars show males having greater mean lengths for 10 of the 13 variables measured, including the TCT1, DPT1, 7 of the 8 enamel thickness variables, and 'a'. Females have greater mean lengths for three variables: LET2, DPT2 and TCT2.

For the upper second molars, females exhibit greater mean lengths for 9 of the 13 variables measured. These include DPT1, DPT2, TCT1 and TCT2. Females also show greater mean enamel thicknesses for all lingual variables and for variable 'a'. Males, on the other hand, display greater means for the buccal thicknesses at TCT1 and TCT2: the BET1, PBET1, BET2 and PBET2.

In summary, for the upper first and second incisors, and the upper and lower canines, females have thicker enamel tissues than males. For the upper first and second incisors, and the lower canines, males have thicker dentine than females, while the upper canines do not consistently show males having thicker dentine. The lower first and second incisors generally show females having thicker enamel and dentine; however, males tend to exhibit thicker buccal enamel at TCT1 and TCT3.

In general, the molars show inconsistent results, and no tissue thickness is apparently greater in one sex. Females tend to exhibit both greater enamel and dentine thicknesses for the lower first molars, as well as greater dentine thickness for the upper second molars. Males tend to exhibit greater enamel thicknesses for the lower second molars and upper first molars.

4.2.6 Univariate analysis: Area measurements

A table of the independent t-tests for the anterior crown variables with normal sample distributions can be found in Table 5 Appendix B. The lower canine is the only deciduous crown which exhibits near-significant levels of relative tissue measurements between males and females at a Bonferroni corrected significance level of 0.007.

Several general patterns can be observed at a 0.05 significance level, although these are not considered statistically significant. They are noteworthy to mention, however, since the high stringency of the corrected significance level permits a greater chance of committing a Type II statistical error by decreasing the power of detecting a true difference. Females show greater relative enamel cap area than males at the 0.05 level ($p=0.015$), while males show greater relative dentine area than females ($p=0.015$). Additionally, females show a greater RECA to CD ($p=0.030$), thereby demonstrating that female lower canines have more enamel than males relative to the CD.

The results of the Mann-Whitney U Rank tests for the linear variables with nonnormal sample distributions can be found in Table 4-12. None of the four variables tested, TCA of the lower canine, CD of the upper first incisor, lower second incisor or lower canine, show significant differences between males and females.

Table 4-12. Mann-Whitney U Rank test results for the nonnormal sample distribution for the area variables and associated linear variables. The data are sorted by variable type and split by sex. A higher mean rank for a particular sex is indicative of a higher rank distribution of that particular variable (i.e. more high-end ranked measurements).

Tooth Type	**Variable**	**Mann-Whitney U Rank**						
		Sex	*N*	*Mean Rank*	*Sum of Ranks*	*Mann-Whitney U Rank*	*Z*	*Sig. (2-tailed)*
Li2	CD	M	6	11.67	70	16	-0.877	0.380
		F	13	9.23	120			
Ui1	CD	M	16	20.13	322	118	-1.126	0.260
		F	19	16.21	308			
Lc	TCA	M	11	8.91	98	23	-0.51	0.610
		F	5	7.60	38			
	CD	M	11	9.55	105	29	-1.303	0.193
		F	5	6.20	31			

Percentage sexual dimorphism for the three lower canine relative area variables approaching significance can be found in Table 4-13. The largest percentages of dimorphic differences are associated with the two relative enamel area variables: RECA (10.8%) and RECA to CD (11.6%).

Table 4-13. Percentage sexual dimorphism for the RECA, RDA and RECA to CD for the lower deciduous canines.

Lower Canine Variable	**Male Mean**	**Female Mean**	**Percentage Sexual Dimorphism[1]**
RECA	21.408	24.003	10.8
RDA	78.592	75.997	3.4
RECA to CD	47.682	53.973	11.6

[1]Calculated using [*male mean – female mean*) / *female mean*] x 100 from Garn et al. (1967a)

A table of the independent t-test for the molar crown variables with normal sample distributions can be found in Table 6 Appendix B. No absolute or relative variables exhibit significant differences between males and females. The lower second molar and upper first molar could not be tested for sexual dimorphism due to the low male sample sizes (n = 2) and low female sample sizes (n = 2), respectively.

4.2.7 Univariate analysis: Linear measurements

A summary of the significant independent t-test results for linear tissue measurements of incisors and canines can be found Table 4-14. A table of all the independent t-tests for the linear variables of the anterior crowns can be found in Table 7 Appendix B.

The upper canine is the only deciduous crown which shows significance at the Bonferroni corrected level of 0.003. Two variables, the BET3 and the PBET3 are significantly thicker in females than in males at $p = 0.002$ and $p = 0.001$, respectively.

Five other variables are found to approach significance levels. Four of these variables are associated with enamel thickness, in which females are larger than males. For the lower first incisors, females show a greater mean LET2 than males ($p = 0.047$), while the upper second incisors show a greater female means for the BET1 and PBET1 ($p = 0.012$ and $p = 0.009$, respectively), and the upper canines show a greater female mean for the PBET2 ($p = 0.017$). In general, females show thicker enamel than males, particularly on the buccal crown aspect closer to the incisal edge.

The only variable approaching significance for which males exhibit a greater mean is for variable 'a', the height from the cervix to the LEL ($p = 0.049$) for the lower second incisor. This finding suggests a slight tuberculum shape difference could exist between males and females for the second lower incisor.

Table 4-14. Significant results of the independent sample t-tests for the equality of means between male and female anterior crown linear variables.

Tooth Type	Variable	Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	*Sig.*	*t*[1]	*df*	*Sig. (2-tailed)*	*Mean Diff.*	*Std. Error Diff.*	*95% Confidence Interval of the Difference*	
									Lower	*Upper*
Li1	LET2	1.792	0.195	-2.114	21	0.047	-0.032	0.015	-0.064	-0.001
Li2	Height from cervix to LEL (a)	2.489	0.134	2.128	16	0.049	0.254	0.119	0.001	0.506
Ui2	BET1	0.001	0.970	-2.783	18	0.012	-0.04	0.014	-0.069	-0.01
	PBET1	0.794	0.385	-2.909	18	0.009	-0.036	0.012	-0.061	-0.01
Uc	PBET2	0.000	0.985	-2.801	11	0.017	-0.13	0.046	-0.232	-0.028
	BET3	3.075	0.107	-4.095	11	0.002*	-0.163	0.04	-0.25	-0.075
	PBET3	0.137	0.718	-4.429	11	0.001*	-0.124	0.028	-0.186	-0.063

[1]A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
*Significant at the Bonferroni corrected significance level of 0.003.

Results from the Mann-Whitney U Rank test for nonnormally distributed incisor and canine linear variables are reported in Table 4-15. No linear variables were significant at the Bonferroni corrected significance level. The only absolute linear variable to approach significant differences between males and females is the BET2 of the upper canine ($p = 0.015$), where females have a higher rank than males. This significant finding corresponds with the other significant buccal enamel variables identified from the independent t-tests for the upper canines, and confirms the trend associated with the patterning of increased sex differences in enamel thickness towards the buccal-incisal crown aspect.

Table 4-15. Mann-Whitney U Rank test results for the nonnormal sample distribution for the anterior teeth linear variables. The data are sorted by tooth type and split by sex. A higher mean rank for a particular sex is indicative of a higher rank distribution of that particular variable (i.e. more high-end ranked measurements).

Tooth Type	**Variable**	**Mann-Whitney U Rank**						
		Sex	*N*	*Mean Rank*	*Sum of Ranks*	*Mann-Whitney U Rank*	*Z*	*Sig. (2-tailed)*
Li1	PLET3	M	11	11.64	128	62	-0.246	0.806
		F	12	12.33	148			
	DPT3	M	11	11.18	123	57	-0.554	0.580
		F	12	12.75	153			
Li2	PBET2	M	5	9.00	45	30	-0.246	0.805
		F	13	9.69	125			
	BET2	M	5	9.20	46	31	-0.148	0.882
		F	13	9.20	125			
Ui1	PBET2	M	17	19.06	324	169	-0.030	0.976
		F	20	18.95	379			
Ui2	PLET2	M	9	9.11	82	37	-0.950	0.342
		F	11	11.64	128			
	LET3	M	9	11.00	99	45	-0.342	0.732
		F	11	10.09	111			
	Height CD to LEL (a)	M	9	8.22	74	29	-1.557	0.119
		F	11	12.36	136			
Lc	LET2	M	11	7.36	81	15	-1.416	0.157
		F	5	11.00	55			
	PLET2	M	11	7.09	78	12	-1.756	0.079
		F	5	11.60	58			
	LET3	M	11	7.55	83	17	-1.190	0.234
		F	5	10.60	53			
	PLET3	M	11	7.73	85	19	-0.963	0.336
		F	5	10.20	51			
Uc	BET2	M	6	4.17	25	4	-2.429	0.015*
		F	7	9.43	66			
	TCT2	M	6	5.67	34	13	-1.143	0.253
		F	7	8.14	57			
	DPT3	M	6	8.00	48	15	-0.857	0.391
		F	7	6.14	43			
	TCT3	M	6	7.67	46	17	-0.571	0.568
		F	7	6.43	45			

* denotes significant difference between the sexes at $\alpha = 0.05$, but not at Bonferroni corrected $\alpha = 0.003$.

Percentage sexual dimorphism for the significant and approaching significant linear variables of the deciduous incisors and canines can be found in Table 4-16. Of the two significantly dimorphic upper canine variables, the BET3 is observed to have the greatest percent sexual dimorphism, at 24.1%. In general, the 5 canine variables display higher percentages of dimorphism ranging form 19.4% to 26.9% while the incisors show slightly less percent dimorphism ranging from 13.3% to 26.8%.

Table 4-16. Percentage sexual dimorphism for the linear incisor and canine crown variables which exhibit significant and near significant mean differences between males and females.

Tooth Type	Variable	Male Mean (mm)	Female Mean (mm)	Percentage Sexual Dimorphism[1]
Li1	LET2	0.215	0.248	13.3
Li2	Height from cervix to LEL (a)	1.196	0.943	26.8
Ui2	BET1	0.186	0.226	17.7
	PBET1	0.185	0.224	17.4
Lc	PLET1	0.199	0.247	19.4
Uc	BET2	0.375	0.513	26.9
	PBET2	0.369	0.499	26.1
	BET3[2]	0.513	0.676	24.1
	PBET3[2]	0.495	0.620	20.2

[1]Calculated using [*male mean – female mean*) / *female mean*] x 100 from Garn et al. (1967)
[2]These variables are significantly different between the sexes at the Bonferroni corrected level.

A table of the independent t-test for the molar crown variables with normal sample distributions can be found in Table 8 Appendix B. Tests for the upper first molar and lower second molar were not run due to small sample sizes (n = 2 respectively). No variables displayed statistically significant levels of sexual dimorphism. The Mann-Whitney U Rank test results for the PBET2 of the lower first molar are presented in Table 4-17. No significant results are found.

Table 4-17. Mann-Whitney U Rank test results for the nonnormal sample distribution for the molar linear variables. Tests for the upper first molar and lower second molar were not run although nonnormal distributions were detected because sample sizes consisted of 2 respectively. The data are split by sex. A higher mean rank for a particular sex is indicative of a higher rank distribution of that particular variable (i.e. more high-end ranked measurements).

Variable Name	**Tooth Type**	**Mann-Whitney U Rank**						
		Sex	*N*	*Mean Rank*	*Sum of Ranks*	*Mann-Whitney U Rank*	*Z*	*Sig. (2-tailed)*
PBET2	Lm1	M	7	4.71	33.0	5.0	-1.701	0.089
		F	4	8.25	33.0			

4.2.8 Summary of Significant Dimorphism Results

In summary, significant differences in sexual dimorphism were observed only in two linear measurements of upper canine buccal enamel. BET3 and PBET3 showed significance at p=0.002 and p=0.001 respectively, and displayed high levels of percent dimorphism at 24.1% and 20.2% respectively. Measurements of the crown tissue areas failed to produce significant results.

Chapter 5
DISCUSSION

The main goal of this research is to determine if significant levels of sexual dimorphism can be detected by various standardized area and linear measurements of the human deciduous crown tissues. In addition to answering this question, this study examines the variation in tissue dimorphism within and between tooth types to predict which tissue and tooth type is the best predictor of subadult sex.

5.1 Univariate Statistical Results

Results show three general trends in the area measurements and linear measurements of deciduous crown tissues. First, the canines show the greatest level of sexual dimorphism of all the deciduous crowns. In particular, the lower canines exhibit greater dimorphic enamel and dentine area measurements, while the upper canines exhibit the greatest extent of dimorphic enamel thicknesses. Second, area and linear measurements associated with relative enamel area and enamel thickness are generally absolutely and relatively larger in females. This trend is most noticeable and consistent in the upper and lower canines, particularly for percent sexual dimorphism. Third, while not significantly different, measurements associated with overall crown area, dentine and pulp area, and dentine and pulp thickness are generally larger in males, both absolutely and relatively.

The area results do not show significant results when the significance level is adjusted for the number of measurements taken (Bonferroni corrected $\alpha = 0.007$). There is a weak trend, in the lower canines, however, which demonstrates that females have greater enamel area relative to the total crown area (RECA) as well as greater enamel area relative to the cervical diameter (RECA to CD), while males have greater dentine-pulp

area relative to the total crown area (RDA). The difference in percent sexual dimorphism seen in the RECA and RDA (10.8% and 3.4% respectively) shows that the RECA female and male means are farther apart irrespective of the extent of the variance overlap (both $p = 0.015$). The RECA to CD variable ($p = 0.030$) demonstrates that females not only have greater enamel area relative to an area representation of the crown size (RECA), but greater enamel area to a linear representation (CD) of crown size. This variable displays a higher percent sexual dimorphism than either of the relative crown tissue areas at 11.8%. This finding supports trend 2.

With respect to linear measurements, only the upper canine shows significant differences when the significance level was adjusted ($\alpha = 0.003$) to account for the 19 linear measurements taken from each crown. Females showed significantly greater mean enamel thicknesses of both the buccal enamel thickness at the 3rd line (BET3, $p = 0.002$) and perpendicular buccal enamel thickness at the 3rd line (PBET3, $p = 0.001$). The percent sexual dimorphism of these two variables was found to be 24.1% and 20.2% respectively. It is noteworthy to mention that significance therefore centers on buccal lateral enamel which is situated towards the incisal crown aspect, rather than cervical crown aspect.

No significant thickness differences were found in the other crowns, although the enamel thickness variables in the lower canine, the upper and lower second incisors, and the lower first incisor approach significance. The upper first incisors and all four molars types fail to show any dimorphic patterns with respect to linear tissue measurements. No thicknesses associated with dentine-pulp or total crown thickness are statistically significant, although an emergent pattern showed that males tend to have greater mean dentine-pulp thicknesses especially for the canines. Lower mean dentine-pulp thicknesses appear to be coupled with greater enamel thicknesses in females, particularly for the canines.

Therefore, this study is able to demonstrate: 1) the presence of significant sexual dimorphic deciduous coronal tissues through linear buccal enamel measurements, thereby supporting hypothesis one; 2) that the upper canine is the most dimorphic tooth when

linear measurements are considered; and finally 3) that for the deciduous crowns, the enamel is the most dimorphic tissue rather than the dentine or pulp tissues.

5.2 Comparison to Past Studies of Deciduous Crown Dimorphism

5.2.1 Overall Deciduous Crown Dimensions

Past studies of overall deciduous crown dimensions have been largely unsuccessful in demonstrating a metric difference between males and females. Low levels of dimorphism have been reported for overall crown dimensions (both MD and BL) ranging from 0.04% to 6.44% (Anderson, 2005; Harris and Lease, 2005; Harris, 2001; Liu et al., 2000; DeVito and Saunders, 1990; Axelsson and Kirveskari, 1984; Lysell and Myrberg, 1982; Black, 1978). Axelsson and Kirveskari (1984) report an anomalous percent dimorphism at 9.5% for the maximum MD diameter of the lower first incisor, but attribute this finding to the variability of a low sample size. Furthermore, contrary to this study, no peak dimorphic differences have been reported for the canines (Harris and Lease, 2005; Harris, 2001; Liu et al., 2000; Axelsson and Kirveskari, 1984).

In fact, no consensus has been reached as to which deciduous crown(s) are the most dimorphic (Table 5-1). For instance, Black (1978) reported the BL dimension of the upper first molar as having the greatest percent sexual dimorphism at 3.15% ($p<0.01$), while Lysell and Myrberg (1982) reported a 3.5% dimorphic difference for the MD dimension of the lower first molars (no p-value reported). Liu and colleagues (2000) reported the BL dimension of the lower second molar as the most dimorphic tooth at 2.86% ($p<0.05$). DeVito (1988) concluded that the BL dimension of the right upper second incisor displayed the greatest percent sexual dimorphism at 6.44% ($p<0.001$), while Harris and Lease (2005) found the lower first incisor to display the greatest percent dimorphic difference in the MD dimension at 2% (p-value not reported), and Axelsson and Kirveskari (1984) found the MD dimension of the lower second incisor to have the greatest percent dimorphism at 3.73%. Harris and colleagues (2001) found the MD dimension of the first molar to exhibit the greatest level of dimorphism at 4%, however

they analyzed only the MD dimensions of the molars from bitewing radiographs. The current study also failed to reveal significant levels of dimorphism for all the deciduous crowns through alternative estimations of overall crown size. No significant findings for any deciduous teeth were identified from the variables of overall crown area, total crown thickness (at standardized coronal locales), the cervical diameter or the DEJ length.

Table 5-1. A comparison of studies which report the most dimorphic deciduous crown based on overall crown dimensions.

Study	Most Dimorphic Tooth	Dimension	Percent Dimorphism	P-Value
Black (1978)	Um1	Max. BL	3.15	<0.01
Lysell and Myrberg (1982)	Lm1	Max. MD	3.50	n/a
Liu et al. (2000)	Lm2	Max. BL	2.86	<0.05
DeVito (1988)	Ui2	Max. BL	6.44	<0.001
Harris and Lease (2005)	Li1	Max. MD	2.00	n/a
Axelsson and Kirveskari (1984)[1]	Li2	Max. MD	3.73	n/a

[1]Axelsson and Kirveskari (1984) report a 9.5% dimorphism of the Li1, but dismiss finding due to low sample size.

5.2.2 Deciduous Coronal Tissue Dimensions

To date, only Gantt and colleagues (2001) and Harris and colleagues (2001) have published data pertaining to the level of dimorphism reflected in the enamel thickness and dentine thickness of the deciduous molars, respectively. No studies to date have analyzed crowns of the anterior deciduous tooth types in terms of either linear thickness or tissue area dimorphism. Gantt et al.'s (2001) study failed to disclose any significant levels of enamel thickness dimorphism in the BL section plane for individual deciduous molars; however these authors found that females had thicker enamel than males when the variables of all four molars were combined in order to increase sample size. The validity of this finding is under question because measures of different tooth types are positively

intercorrelated (Harris and Bailit, 1988); additionally, significant increasing trends noted for the permanent molars emphasize that enamel proportions are dependant on crown position, and thus would further confound such analyses (Smith et al., 2006; Grine, 2005; Smith et al., 2005; Grine, 2002; Schwartz, 2000b; Macho, 1994; Macho and Berner, 1993). Harris and colleagues (2001) conversely found that significant differences in tissue thicknesses could be isolated to the mesial and distal marginal dentine thicknesses taken at the maximum MD line of the deciduous molars. In particular, dentine thicknesses at the mesial aspect were significantly different between the sexes for all molars except the lower first molar. Unfortunately these measurements were taken from lateral bitewing radiographs and therefore cannot be directly compared to measurements taken from buccolingually sectioned molars. Moreover, the dentine thicknesses were measured relative to the sides of the pulp chamber, thereby introducing thickness inaccuracies based on potential secondary dentine deposition along the pulp chamber walls. In addition to these issues, bitewing radiographs have been demonstrated to provide an average overestimation in enamel thickness; significant differences between linear enamel measurements of mechanical sections and radiographs were exhibited in all cases by Grine et al. (2001). On the other hand, the results of Harris and colleagues (2001) may provide evidence that the longer and later developing second molars are more likely to exhibit dentine differences rather than anterior teeth, which begin to form earlier and take a shorter time for total crown development.

In comparison, the current study cannot make any conclusions about the dimorphic levels of the crown tissues of the four molars individually, due to small sample sizes.

The most striking finding of this study is that it is the enamel tissue which accounts for sex differences between males and females. Past studies of the tissue distributions of the permanent dentitions state that it is not the enamel, but the dentine tissue which is responsible for coronal dimorphic findings. A question arises at this juncture, therefore: Why is dimorphism of the deciduous canine centered in the enamel tissue, as opposed to the dentine and pulp tissues as seen with the permanent crowns?

5.3 Comparison to Past Studies of Permanent Coronal Tissue Dimorphism

In general, overall permanent crown dimorphism ranges from 1.5% to 9%, in which males are larger than females (Brown and Townsend, 1979; Alvesalo, 1971; Garn et al., 1969; Garn et al., 1967b; Garn et al., 1966; Moorrees et al., 1955). While much of the research on deciduous crown dimorphism has focused solely on metric data from overall crown measurements, studies of permanent coronal dimorphism have focused on using measurements of crown tissues as a means of identifying the main tissue contributor to overall crown dimorphism (Saunders et al., 2007; Schwartz and Dean, 2005; Zilberman and Smith, 2001; Harris et al., 2001; Harris and Hicks, 1998; Stroud et al., 1994).

Several past studies of permanent crown tissue dimorphism suggest that overall tooth dimension dimorphic differences are primarily attributed to the dentine component (Saunders et al., 2007; Smith et al., 2006; Schwartz and Dean, 2005; Zilberman and Smith, 2001; Harris and Hicks, 1998; Stroud et al., 1994). Zilberman and Smith (2001) found that the sex (as the dependant variable) was the main contributor to variation in the MD dentine thickness taken adjacent to the lowest point of the DEJ in the occlusal basin at 36.9%. Mean differences in dentine thickness at this location were observed to be significant ($p<0.01$) across all age groups examined, in which males exhibited greater thickness than females (Zilberman and Smith, 2001). The percent sexual dimorphism (calculated from Garn et al.'s (1967b) formula) across the age groups studies ranged from 10.5% to 17.6% for this variable. Females did exhibit statistically greater relative enamel heights and widths to maximum MD crown width, but did not show consistent results across age groups. The authors do not give an explanation for this age discrepancy despite the fact that both the DEJ and marginal enamel thickness of the lower permanent first molar are established well before 4 years of age—the earliest age at which crowns were studied.

Similar to Zilberman and Smith (2001), Stroud et al. (1994) used bitewing radiographs to measure the enamel and dentine tissue thicknesses at the maximum MD

crown width of mandibular premolars, and 1st and 2nd molars. They found similar results indicating that dentine thickness was the main contributor to significant differences in MD diameter, expressing differences towards males on the order of 5.9% to 9.20%. In contrast to Zilberman and Smith (2001), enamel thicknesses did not show any significant differences for lower premolars or molars (Stroud et al., 1994).

Linear measurements of the maxillary permanent incisors taken from periapical radiographs suggest that anterior teeth also display the same pattern of tissue distributions as the posterior crowns. Males had significantly greater dentine thickness than females at the maximum MD crown aspect, where percent dimorphism averaged 6.5%, while no differences in marginal enamel thickness were found (Harris and Hicks, 1998).

Both Schwartz and Dean (2005) and Saunders and colleagues (2007) agree that tissue area measurements of buccolingually sectioned permanent crowns demonstrate that the dentine tissue is primarily responsible for overall dimorphic size differences. While male mandibular third molars possess significantly greater dentine and pulp area than females at $p=0.0052$ (9.3% dimorphism), Schwartz and Dean (2005) found that the mandibular canines exhibit no dentine-pulp dimorphism ($p=0.2024$). Using a sample of historic teeth, Saunders and colleagues (2007) disagreed with this finding and proposed that male mandibular canines do have significantly greater dentine and pulp area ($p=0.001$, 22% dimorphism). Both studies provide results which indicate that the relative average enamel thickness also displays high levels of dimorphism in favour of females, reaching levels between 11.6% and 20.8% (Saunders et al., 2007; Schwartz and Dean, 2005).

Therefore, the main consensus of the above literature is that the larger absolute dentine crown component observed in males explains the significant differences in overall crown sizes between the sexes, and female crowns exhibit relatively thicker amounts of enamel due to a smaller dentine contribution. It appears, therefore, from a comparison between these studies and the results of the current research, that the mechanisms by which dimorphism are attained are different between deciduous and permanent crowns. While these studies provide evidence to support the hypothesis that

permanent crown dimorphism is established before the delineation of the DEJ, the current study speculates that dimorphism of the deciduous canine is established during the tooth crown mineralization phase, rather than beforehand.

5.4 Sexual Dimorphism: Mechanisms of Tissue Growth

Both aneuploid studies and molecular studies have provided insight on the possible genetic mechanisms for dentine dimorphism observed in permanent crowns, while they explain the lack of dimorphism in the enamel. Aneuploid males with extra Y-chromosomes (47,XYY) generally show larger deciduous and permanent teeth than normal males (46,XY) (Lahdesmaki, 2006; Lahdesmaki and Alvesalo, 2004; Varrela and Alvesalo, 1985; Alvesalo et al., 1985; Townsend and Alvesalo, 1985; Townsend, 1982; Alvesalo and Kari, 1977; Alvesalo et al., 1975), while aneuploid females with an extra X-chromosome (47,XXX) exhibit larger teeth than normal females (Alvesalo, 1997; Alvesalo et al., 1987). Furthermore, mean enamel thickness between 47,XXY and 47,XYY males is comparable while 47,XXY enamel is slightly thicker than 46,XX females (Alvesalo, 1997), and dentine thickness decreases in thickness from 47,XYY to 46,XY to 47,XXY to 47,XXX individuals (Alvesalo, 1997). In their review articles, Varrela (1991) and Alvesalo (1997) summarize the plausible mechanisms implied by the aneuploid studies. Because dentine thickness is the reflection of early mitotic growth of both the inner enamel epithelium and mesenchyme tissues, they postulate that the Y-chromosome increases mitotic potential at this early developmental stage. Both the X- and Y-chromosomes appear to increase metric enamel thickness at similar magnitudes, whereas the X-chromosome exerts little influence on dentine growth (Alvesalo, 1997; Varrela, 1991).

Molecular studies support aneuploid studies demonstrating that transcriptionally active genes which code for amelogenin reside on both the X- and Y-chromosome (Salido et al., 1992; Nakahori et al., 1991; Fincham et al., 1991; Lau et al., 1989). Despite this fact, it remains unclear how and/or if dimorphic alternatively spliced amelogenin products

deriving from the X- and Y-chromosomes translates into metric enamel thickness or histological differences (Brookes et al., 1995), especially since differences in the locations of sexual dimorphism are apparently different between the permanent and deciduous crowns. Therefore, speculations about the impact of amelogenin genes on metric enamel growth are beyond the scope of this study.

It has been demonstrated that Y-linked mitogens (active in *utero*) are responsible for sex differences in growth rate, and androgens both trigger the establishment of phenotypic sex differences and express mitogenic effects in prenatal development (Parker and Schimmer, 2002; Martin et al., 1968; Tanner et al., 1959). The lack of sex differences in deciduous dentine suggests that sexual dimorphism in the deciduous canine cannot be explained by mitotic activity or androgen fluctuations during the pre-mineralization phase of embryonic crown development. It appears therefore, that Y-linked factors and androgens which promote mitotic growth are not as active and/or given sufficient time during embryonic germ development to provide significant cellular proliferative differences in the germ tissues between males and females. Aneuploid studies of the *deciduous* crowns demonstrate that the presence of an extra Y-chromosome presents a trend for greater overall crown size in 47,XYY males (Alvesalo and Kari, 1977); however, the small sample size (n=6) and lack of research concerning the tissue distributions in the aneuploid deciduous crowns disallows speculations about the exact timing of excess growth. It may simply be the case that the additive effect of a second Y-chromosome is enough (within the short developmental time) to display enhanced growth in these aneuploid males, as opposed to being absent in normal males.

For the deciduous canine, sexual dimorphism appears to arise by a different mechanism, and later during crown development after the DEJ (thus dentine-pulp core area) has already been established—at the time of ameloblast differentiation and secretion.

Currently, research (particularly of the permanent canines) has progressed from tissue measurements to finer measurements at the enamel histological level, where researchers are interested in identifying ontogenetic mechanisms responsible for the

establishment of dimorphism by means of histological evidence (Smith et al., 2003; Schwartz and Dean, 2001; Schwartz et al., 2001; Dean, 2000). These studies however, are focused more on interspecific dimorphic data rather than directly on modern *Homo* dimorphism. Histological data collected from other hominoid or hominid species cannot be used to predict the ontogenetic mechanisms responsible for enamel thickness in humans simply because multiple developmental trajectories based on both rates and timing can result in similar enamel thicknesses between species or the sexes (Smith et al., 2003; Dean, 2000).

On the other hand, it is within this histological framework that potential reasons for deciduous canine buccal enamel dimorphism can be sought, and the mechanism(s) which determine metric crown dimorphism differences between permanent and deciduous crowns can be explained.

5.5 Deciduous Crowns: Speculations into Tissue Dimorphism

According to Grine and Martin (1988), three variables contribute to enamel thickness: 1) the duration of secretion; 2) ameloblast secretion rate; and 3) the total number of active secretory cells at any given time during development (rate of ameloblast metabolism or extension rate).

Logically then, a difference in a single factor or a differential combination of these factors between males and females may lead to varying enamel thicknesses between them. Little is known about the nature of the molecular growth mechanisms which underlie the regulation of the mineralization process with respect to final attainment size of enamel thickness and ameloblast metabolism because most molecular studies have been directed towards earlier stages of tooth development (Dean, 2000).

5.5.1 Crown Formation Times

Recent histological studies of four medieval French individuals (Reid et al., 1998a) suggest that enamel thickness is directly related to formation times, as exemplified

by the trend towards thicker enamel in similar cusps from faster-forming M1 to slower-forming M3. Additionally, from research based on *Afropithecus turkanensis*, Smith et al. (2003) suggest that cuspal crown formation times show a greater direct relationship to relative enamel thickness than the formation time of the total crown. For instance, the relative cuspal formation times of thick-enameled extinct and extant hominoids are greater than the relative cuspal formation times of thinner-enameled hominoids (Smith et al., 2003). Interestingly, histological research conducted by Schwartz and Dean (2001) failed to find a significant sex difference in the cuspal formation times and total crown formation times of lower modern human canines. On the other hand, they found that females possessed significantly greater mean relative cuspal formation times to total crown formation times than males (~28% and ~24%, respectively) (Schwartz and Dean, 2001). While not statistically significant, the male tendency towards higher mean formation times corresponded to slightly higher (but non-significant) mean crown heights, and females were found to possess a greater (but non-significant) average buccal enamel thickness (Schwartz et al., 2001). These particular findings together appear to argue against a positive direct relationship between enamel thickness and absolute cuspal/total crown formation times, but provide evidence that human enamel thickness dimorphism may be associated with relative cuspal formation times.

Unfortunately, comparative histological data is not available for the cuspal, lateral and total formation times of the deciduous dentition, and the duration of crown formation times based on tooth germ analysis and dissection are reported without sex discrimination. However, Garn et al. (1958) analyzed the calcification rates of permanent premolar and molar crowns using serial oblique radiographs and concluded that mean age differences within the stages of permanent crown calcification, averaging 3%, were not significant between the sexes. This finding is suggestive that ages at which males and females reach various stages of deciduous crown calcification may also not be significantly different. Therefore, differences in rates of enamel secretion may provide a better explanatory model for buccal enamel differences exhibited in the canine.

With respect to the discrepancy between the extent of dentine dimorphic levels exhibited in the deciduous and permanent crowns, crown formation time durations and observable sex differences provide justification for greater sex differences seen in the permanent crowns. The deciduous crowns take substantially less time than the permanent crowns to develop (Tables 5-2 and 5-3). Furthermore, developmental sex differences in the permanent dentition range between 3-5%, with females being more advanced in crown completion of all permanent teeth except the variable third molar (Burdi et al., 1970; Garn et al., 1958), while sex differences have not been reported in deciduous crown formation except for during the first trimester where males are slightly advanced (Burdi et al., 1970). Unfortunately, the recent histological assessment of permanent crown formation stages by Reid and Dean (2006) does not provide sex-differentiated data to support Burdi et al.'s (1970) and Garn et al.'s (1958) findings.

Calculated from reported data (Reid and Dean, 2006; Reid and Dean, 2000; Reid et al., 1998a; Lunt and Law, 1974a; Kraus and Jordan, 1965; Schour and Massler, 1940), deciduous crowns spend on average roughly 14.0-37.8% the amount of time as permanent crowns do on enamel formation, while they spend between 14.5-31.6% the adult duration on total crown formation (including the time of germ formation before the initial deposition of enamel).

Therefore, having an extended amount of time for cellular proliferation during the pre-mineralization growth period, along with a slightly longer period of male pre-mineralization growth, allows for a greater opportunity in male permanent crowns for Y-linked mitotic factors to compound differences between males and females. Although differences between the duration in the crown formation times may explain the significant dimorphic levels observed in the dentine and pulp tissue of permanent teeth but not the deciduous teeth, they do not explain why significant enamel thicknesses are observed in the deciduous crowns.

Table 5-2. Chronology of permanent crown formation times.

Tooth Type	Age of Crown Initiation (wks in utero*, wks after birth)[1]	Mean Age at Initial Mineralization (wks)[2]	Mean Age at Crown Completion (N.Eur.) (Protocone/Protoconid for molars) (wks)[3]	Mean Total Enamel Formation (wks)[4]	Mean Total Crown Formation (wks)[5]
UI1	20-21*	18.3	260.7	242.4	282.2
LI1	20-21*	12.9	198.1	185.2	219.6
UI2	20-22*	54.7	265.9	211.2	286.9
LI2	20-22*	20.9	219.0	198.1	240.0
UC	22-24*	39.1	276.4	237.3	295.4
LC	22-24*	28.6	323.3	294.7	342.3
UM1	14-16*	0.0	156.4	156.4	183.4
LM1	14-16*	0.0	172.1	172.1	199.1
UM2	34-36	151.3	328.5	177.2	293.5
LM2	34-36	151.3	323.3	172.0	288.3
UM3	182-208	417.1	594.4	177.3	399.4
LM3	182-208	417.1	584.0	166.9	389.0

[1]Data from Schour and Massler (1940)
[2]Data from Reid and Dean (2000) and Reid et al. (1998a)
[3]Data from Reid and Dean (2006)
[4]Difference between age at crown completion and mean age at initial mineralization time
[5]Difference between age at mean crown initiation and mean age at crown completion

Table 5-3. Chronology of the deciduous crown formation times.

Tooth Type	Age of Crown Initiation (wks in utero)[1]	Mean Age at Initial Mineralization (wks, in utero)[2]	Mean Age at Crown Completion (wks, after birth)[3]	Mean Total Enamel Formation Time (wks)[4]	Mean Total Crown Formation (wks)[5]
Ui1	7.0	14.0	6.0	34.0	41.0
Li1	7.0	14.0	10.0	38.0	45.0
Ui2	7.0	16.0	10.0	36.0	45.0
Li2	7.0	16.0	12.0	38.0	47.0
Uc	7.5	17.0	36.0	61.0	70.5
Lc	7.5	17.0	36.0	61.0	70.5
Um1	8.0	15.5	24.0	50.5	58.0
Lm1	8.0	15.5	22.0	48.5	56.0
Um2	10.0	19.0	44.0	67.0	76.0
Lm2	10.0	18.0	40.0	64.0	72.0

[1] Data from Schour and Massler (1940)
[2] Data from Kraus and Jordan (1965)
[3] Data from Lunt and Law (1974a)
[4] Determined by using 42 weeks as average gestational period
[5] Difference between mean age at crown completion and mean age at crown initiation

5.5.2 Secretion Rates

Secretion rates, the width of enamel laid down along a prism pathway per day, represent enamel thickness as a function of enamel apposition (Shellis, 1998). Schwartz et al. (2001) found that for humans, differences between male and female permanent canine daily secretion rates were absent, despite being significantly present in the canines of other extant hominids. This finding seems to explain the absence of significantly dimorphic average buccal enamel thicknesses in their study, as well as the general absence of absolute enamel thickness differences in permanent crowns. Although there is a large discrepancy between male (n=19) and female (n=9) sample sizes, this study brings into question the extent of the differences between permanent and deciduous ontogenetic pathways to achieve enamel thickness. If growth trajectories were similar between permanent and deciduous crowns, no difference in relative enamel thickness, relative enamel cap area or absolute enamel thicknesses should be observed in the deciduous crowns; however, these variables display differences in favour of females which either are approaching significance or are significant in the canines. Therefore, enamel growth trajectories appear not to be proportional.

In fact, the deciduous teeth display disproportionately thin enamel compared to permanent crowns (Harris and Lease, 2005; Gantt et al., 2001). Furthermore, their initial rates of enamel apposition within the inner enamel are much higher than permanent crowns averaging 4.5μM per day as opposed to 2.6 μM per day (Shellis, 1984). Potentially, therefore, an accumulation of greater female enamel thickness may be a product of a slightly greater secretion rate coupled with overall small crown size and relatively quick mineralization period. To date, unfortunately, no research has reported sex-differentiated data on secretion rates for any deciduous crowns.

5.5.3 Ameloblast Activity

According to Dean (2000), the differences in enamel thickness are best attributed to the rates of ameloblast differentiation rather than the secretion rate. Furthermore, implications of a lack of dimorphic findings in cuspal enamel formation (Schwartz and

Dean, 2001) and daily secretion rates (Schwartz et al., 2001) have prompted Schwartz and Dean (2001:278) to conclude that the "basis for sexual dimorphism in canine size lies wholly in the rate and/or duration of lateral enamel formation". The lateral enamel is precisely where the greatest levels of sexual dimorphism are found in this study, centering on the buccal enamel of the upper canines. On the other hand, work by Schwartz and Dean (2001) focused on interspecific analysis of the extent of dimorphism reflected in crown height rather than enamel thickness. While Shellis (1998) found a positive correlation between crown height and ameloblast differentiation rate in the permanent crowns, he failed to find a similar significant positive correlation in deciduous crowns. This finding emphasizes the likeliness that growth trajectories, in terms of establishing tissue dimorphism, are different between permanent and deciduous crowns. Therefore, extrapolation of data from permanent crowns is not practicable.

Lateral enamel thickness is a function of both the enamel extension rate (rate of ameloblast differentiation at the cervix) and appositional growth by means of ameloblast secretion outwards from the DEJ (Shellis, 1984). The extension rate is related to the angle in which the long-period incremental lines meet the DEJ (striae of Retzius), the angle at which the prisms meet the DEJ, and the daily secretion rate. In comparison to permanent crowns, deciduous crowns experience an enamel extension rate which is five times greater than their permanent analogs (Shellis, 1984), thereby reflecting their rapid mineralization phase.

The relationship between extension rates down the DEJ and enamel thickness (appositional rate) is explained by Shellis (1998). He proposes that not only do they simply co-vary in a strong negative correlative manner, but their timing is co-dependant so that the crown will have sufficient mineral maturation time before eruption. As such, sexual dimorphism in enamel thickness may be a by-product of the interplay between apposition and extension rates during the mineralization phase.

In summary, differences in crown formation times, secretion rates and extension rates may all contribute to the overall differential outcome of buccal enamel thickness which was observed between the male and female deciduous upper canine. For the

purpose of sex determination, these speculations shed light on avenues of further research into histological growth trajectories of deciduous crowns.

5.6 Measurements

Deciduous anterior crowns are subject to varying levels of wear which in most cases, affects both the enamel cusp and dentine horn. To date, no studies have been able to systematically and accurately measure tissue thicknesses of the deciduous anterior crowns, in part due to this issue. Despite this caveat, a standardized method of collecting linear data from anterior teeth was developed to test not only tissue thickness differences, but also to test basic crown shape differences. According to Harris et al. (2001), no static allometric regression coefficients for tissue thicknesses scaled to MD crown diameter showed significant differences between male and females for the deciduous molars. This finding concludes that no significant growth differences can be statistically associated with disproportionate tissue growth between males and females in the deciduous molars. Despite this finding, Harris et al. (2001) do state that it is unlikely that size differences exist without *shape* differences. Although accurate assessment of early developmentally-established shape differences at the occlusal and incisal locations of this crown may not be suitable due to excessive wear, this study provides a simple measurement to approximate shape differences at the cingulum bulge of anterior crowns.

In addition, the present study was designed to overcome the issue of incisal and occlusal wear sustained by the deciduous crowns so that area measurements could be assessed. Not only do the results here illustrate that particular cusp and dentine horn reconstruction can permit the use of tissue area measurements, they provide a stringent protocol for systematic elimination of crowns according to a metric assessment of the degree of wear.

With respect to the linear tissue thicknesses measured, only lateral enamel thicknesses were considered. Many past studies of both adult and deciduous molars (Kondo et al., 2005; Schwartz, 2000a; Schwartz, 2000b; Macho and Berner, 1994; Macho

and Berner, 1993) suggest a positive relationship between enamel thickness and masticatory forces. Therefore, a study of the occlusal and incisal surfaces of completely unworn deciduous crowns may yield different dimorphic results than measurements of lateral enamel.

While Schwartz and Dean (2005) state that area measurements better reflect the total crown volume, this study showed that they are ineffective at identifying particular locations of dimorphism. For instance, no significant dimorphic levels of relative average enamel thickness were identified for the upper canines, yet females showed significant greater enamel thickness at particular lateral buccal locales.

5.7 Limitations of the Current Study

5.7.1 Sample Size and Statistical Power

The primary limitation of this study is the small sample size of all tooth types. Although t-tests adjust for small sample sizes and are robust against small deviations from normality and equal variances, the predictive power is compromised by the small sample size for each tooth type considered as well as the significance level chosen (Aron et al., 2006). The small sample size of the upper canines, n (males) = 6 and n (females) = 7, compromises power due to a wider distribution of means than would be observed with a larger sample. Larger-scale studies will have more statistical power than the present study, and therefore the findings presented here are preliminary.

Multiple crown measurements were taken from each tooth, thereby increasing the likelihood of spurious positive results. A Bonferroni correction was applied in an attempt to avoid making a type I error. As a direct result of this stringency, the power of detection is decreased, and by default the designated rejection region was smaller. Therefore, this study is more likely to make a type II error (false negative) rather than a type I error (false positive)—particularly in terms of linear measurements since a more stringent significance level was used.

5.7.2 Sample Composition

A heterogeneous sample comprising Italian and Canadian children's exfoliated crowns was examined. Differences in measurements of overall crown differences were found to be not significant amongst the sub-samples, thereby justifying their combination. In addition, small male and female sample sizes, along with small tooth type sample sizes could not permit further division of the sample along country affiliation lines if statistical tests for the presence of sex differences were to be conducted. On the other hand, previous studies have demonstrated that overall crown dimensions provide little discriminatory power between populations (Harris and Lease, 2005; Harris, 2001), differences in tooth formation between populations fall below one standard deviation (Liversidge, 2003), and dental development is apparently buffered against environmental and socioeconomic impact (Cardoso, 2007; Cardoso, 2005; Noren, 1984; Grahnen et al., 1972; Kreshover et al., 1958).

In terms of histological analysis, differences are apparent between populations with respect to permanent crown formation times. Reid and Dean (2006) demonstrate that modern northern European populations have a shorter enamel formation time than modern southern African populations. This study, however, does not comment on the effect that such a difference in population crown formation time may have on the identification of sex differences in crown formation times. Thus, further research into dimorphism of enamel growth mechanisms may have to take population differences into consideration.

Lastly, across the 10 tooth types, related individuals were included to increase sample sizes. Although related individuals were not directly compared within the same tooth type, the univariate data values may be closer than expected amongst tooth types because of the inclusion of crowns of related individuals within different tooth types. Therefore, it is possible that the actual levels of dimorphic differences between tooth types are curtailed by dimensional similarities between related individuals and therefore maybe even higher than demonstrated in this study.

5.7.3 Measurements

In addition to sample sizes and composition, the last limitation of this study involves the linear enamel measurements. The measurements of perpendicular enamel thickness only approximate enamel prism pathways; therefore, any thickness measurement presented here is not completely reflective of the extent of dimorphism based on enamel apposition.

5.8 Future considerations

Ontogenetic mechanisms of deciduous crown dimorphism are unclear. Furthermore, because of the multiple possible histological pathways (interplay between formation times, secretion rates and extension rates) that are possible to acquire a certain enamel thickness, one is reluctant to extrapolate data collected from extant and extinct hominoids or from the permanent human dentition to the deciduous crowns. Furthermore, conclusions about the ontogenetic mechanisms of the development of sexual dimorphism in the permanent crown are generally based on small sample sizes.

Results of this research suggest that certain developmental aspects of enamel formation differ enough between males and females to provide statistically significant greater buccal enamel thickness in female upper canines. Therefore, these results identify at least three areas which need to be addressed in terms of the deciduous enamel growth before ontogenetic mechanisms of enamel thickness variation can be elucidated via histological explanations.

To date, no histological data exists which specifies total crown formation time, cuspal enamel formation time or lateral (imbricational) enamel formation time of the deciduous crowns, let alone addresses duration differences which may exist between the sexes. Although early data based on the tooth ring analysis and embryonic dissection of germ crypts by Schour and Massler (1940), Kraus and Jordan (1965), Lunt and Law (1974a), and Nomata (1964) have provided evidence for very similar mineralization timing between males and females, histological data provides a greater resolution in

minute microscopic changes and is better able to visualize differences at very early stages which may be missed by these methods (Reid and Dean, 2006).

Notably, deciduous crowns display both extreme occlusal/incisal wear and variation in the intra-crown levels of wear. This will ultimately prevent the accurate assessment of cuspal enamel formation times predicted from exfoliated deciduous crowns. However, because enamel thickness sex differences are conspicuously present in the lateral buccal enamel, the assessment of lateral enamel formation times (as opposed to cuspal enamel formation times) in a sex-differentiated sample may provide causal evidence for the dimorphic buccal enamel thickness demonstrated in the present study.

The second area of future research needs to focus on the secretion rate of the ameloblasts. Because significant differences were found in enamel thickness, one can presume that differences in secretion rates may exist. The literature demonstrates that rates may also change throughout the crown as the secreting ameloblast moves outwards from the inner enamel to the outer enamel, thereby affecting the total amount of enamel secreted. Overall secretion rates between males and females will reflect rate changes in these areas if proportional differences exist, whereas disproportional differences may effectively cancel one another out resulting in a lack of difference. Figure 5-1 provides a visualization of enamel thickness changes if the secretion rate is increased, resulting in a longer pathway.

In addition to the changes in rates which occur radially, literature has also suggested that rate changes occur longitudinally (Schwartz et al., 2001; Dean, 2000; Reid et al., 1998b). Daily secretion rates (DSR) have been found to decrease towards the cervix; it remains unclear whether or not this decrease is equal, proportionally different, or disproportionately different between males and females. Analysis of the rates between males and females in this direction will help resolve the question of why buccal enamel closer to the incisal crown aspect experiences a greater level of dimorphism than enamel closer to the cervical crown aspect as suggested by this study.

Therefore, analysis of the daily secretion rates, the rate changes along prism pathways and between pathways of males and females will aid in determining whether or not secretion rates are a direct causal factor of buccal enamel dimorphism.

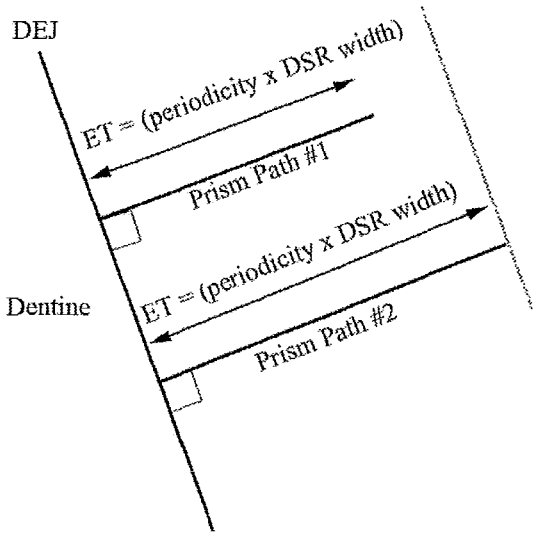

Figure 5-1. A diagrammatic explanation of enamel thickness (ET) changes when daily secretion rates (DSR) change and the angle at which the prism meets the dentino-enamel junction (DEJ) is held constant. Note that for simplicity-sake, the prisms have taken on an angle of 90° with the DEJ. The dotted line represents the outer edge of enamel.

In addition to determining average daily secretion rates of males and females for the deciduous crowns, the third area of future research should focus on the analysis of the prism pathway geometry—in particular, the angle at which it meets the DEJ. While an ontogenetic rationale for such a sex difference is not obvious, geometry states that a prism which is angled more steeply towards the incisal surface will result in a thinner enamel (measured perpendicular to the DEJ) if secretion rates are held constant. Therefore, enamel thicknesses could either by due to a difference in secretion rate, a difference in the angle at which the prism path meets the DEJ, or an interaction between both factors. See Figure 5-2 for visualization.

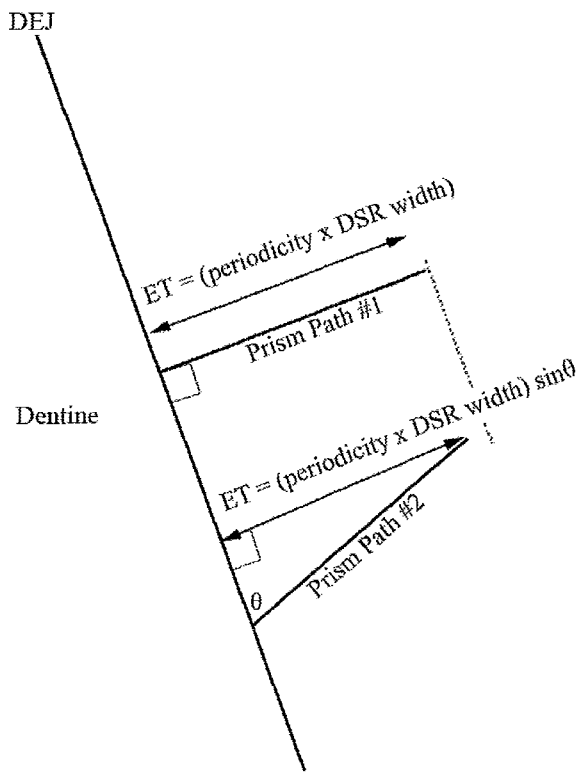

Figure 5-2. A visualization of the effect of the angle at which the prism pathway meets the DEJ has on enamel thickness (ET) if the daily secretion rate (DSR) is held constant. The dotted line on the right-hand side represents the outer edge of enamel. Note that the smaller angle (θ) results in thinner enamel.

Not only is pathway trajectory mapping a potential avenue for sex discrimination, it may prove to be a useful tool for cusp reconstruction. If cuspal and lateral ameloblast cytokinetics can be determined, in conjunction with the average pathway geometry for general crown locales, it may be possible to reconstruct deciduous crowns based on ameloblast secretion.

To date, no permanent data is based on a substantial sample size, nor has research been directed towards analysis of histological dimorphism of the deciduous enamel to aid in the elucidation of ontogenetic mechanisms involved in early crown development or the establishment of sexual dimorphism in humans. This study provides an important preliminary step towards understanding the mechanisms which dictate deciduous dimorphic characteristics derived from both metric and histological data. Beyond providing further avenues of research as well as a host of reconstruction and standardized measurement methods, this research suggests that the deciduous enamel thickness may provide a means for subadult sex determination of modern humans.

Chapter 8
CONCLUSIONS

The goals of this thesis were three-fold: 1) to establish the presence or absence of linear and area tissue sexual dimorphism in the human deciduous dentition, and determine which tissue(s) and tooth type(s) contribute the greatest degree of dimorphism; 2) to provide a set of standardized deciduous coronal measurements for the prediction of subadult sex; and 3) to provide a justification for the presence of sexual dimorphism in the deciduous dentition by outlining the genetic, developmental, hormonal and intra-uterine environmental agents which may potentially contribute to crown growth variation between the sexes; these agents are outlined in the literature review.

'Previous studies on the tissue dimorphism of human permanent crowns demonstrate that dentine is the main contributor to the overall crown differences between males and females, and the canine is the most dimorphic tooth. In the current study, univariate analysis of the deciduous crown data revealed that significant tissue sexual dimorphism also exists in the deciduous dentition and also centers on the upper canine. The present data, however, demonstrates that the enamel is responsible for sex differences in the deciduous crown, not the dentine. In particular, two lateral buccal enamel tissue variables (BET3 and PBET3) of the upper canine are significantly thicker in females ($p = 0.002$ and $p = 0.001$ respectively). The percent sexual dimorphism of these enamel variables are 24.1% and 20.2% respectively, displaying an order of magnitude which is comparable to the higher levels of percent dimorphism exhibited in the tissues of permanent crowns (Saunders et al., 2007; Schwartz and Dean, 2005; Zilberman and Smith, 2001). Although no other deciduous crowns exhibit significant sex differences in either linear or area measurements, there is a distinct trend for the females to have more enamel than males. Enamel tends to be thicker than male enamel in the lower first incisors and upper second incisors, and tends to show greater relative enamel area with

respect to total crown area and to the cervical diameter in the lower canines. No differences in dentine-pulp thickness were observed between the sexes; however the dentine-pulp area relative to the total crown area in the lower canines exhibited near-significant values in the male direction. The presence of significantly thicker female enamel in localized areas does not predicate the presence of significant dimorphism of overall crown size in favour of females, as demonstrated in the present study. The failure to identify overall crown size differences between males and females may be simply offset by the greater dentine area and thicknesses favoured in males.

In summary, all three goals were addressed. The current study concludes that dimorphism of the deciduous crowns arises during amelogenesis, rather than during the mitotic growth phase. Furthermore, it provides a standardized method for the systematic measurement of linear tissue dimensions, which takes into consideration the variation in the degree of wear sustained by deciduous crowns. These preliminary data provide a basis for further large-scale investigation into the degree of tissue dimorphism of the deciduous crowns for the purpose of subadult sex determination, as well providing a preliminary step towards understanding the mechanisms that dictate deciduous crown tissue dimorphic characteristics.

APPENDIX A

CONSENT TO PARTICIPATE IN RESEARCH

My name is Andrea Chan. I am a Masters student in the Department of Anthropology at McMaster University. I will be examining shed baby teeth to look for differences between boys and girls for the purpose of creating a method to aid physical anthropologists in the sex determination of children. Generally, anthropologists have a difficult time figuring out if skeletal remains of children are boys or girls because they are not fully developed into adults yet. However, since baby teeth are fully developed shortly after you are born, your baby teeth may be able to help answer this question.

Each tooth crown is comprised of three basic tissues: *enamel*, which covers the tooth; *dentine*, which is underneath the enamel and makes up the bulk of the crown; and *pulp*, which is at the center of the tooth and contains nerves and blood vessels. Through microscopic analysis, I will be comparing the amount of these tissues in your teeth to see if differences exist between boys and girls. Each tooth will be cut into a thin-section and mounted on a microscope slide. Each thin-sectioned tooth will undergo three measurements: firstly, I will measure the thickness of enamel, dentine and pulp; then, I will measure the area of each tissue; and lastly, I will look for differences in the microscopic structures of the enamel and dentine. After the study is completed, your sectioned teeth will be added to the deciduous dental collection of the Department of Anthropology.

I promise to keep your identity confidential and have provided you my email address if you wish to withdraw from the study. In return for your participation in this study and your personal contribution to scientific research, you will receive a digital and printed copy of your tooth as seen under the microscope.

If you require anymore information about the study, please feel free to contact me via email at chanahw@mcmaster.ca, or my supervisor Dr. Shelley Saunders at McMaster University at (905) 525-9140 ext. 24423. I sincerely appreciate your participation in my research.

This study has been approved by the McMaster University Research Board. If you have any questions or concerns, you may contact them at ethicsoffice@mcmaster.ca.

Thank you,

Andrea Chan

I understand the information provided for this study as described above. My questions have been answered to my satisfaction, and I agree to donate my shed baby teeth to this study. I have been given a copy of this form.

Participant's Name _____
Participant's Signature _____
If participant is a minor:
Parent/Guardian Signature: _____
Date _____
Investigator's Signature_____

APPENDIX B

Table 1. Descriptive statistics of deciduous incisor and canine absolute and relative areas and associated linear measurements.

Tooth Type (FDI)	Sex		N	Min	Max	Range	Mean		Std. Deviation	Variance
			Statistic	Statistic	Statistic		Statistic	Std. Error	Statistic	Statistic
Lower First Incisor	Male	ECA	9	1.991	3.058	1.067	2.405	0.108	0.325	0.105
		CD	9	2.749	3.148	0.399	2.997	0.049	0.148	0.022
		Valid N (listwise)	9							
	Female	ECA	11	1.879	3.687	1.808	2.599	0.165	0.546	0.298
		CD	11	2.923	3.444	0.521	3.138	0.049	0.163	0.027
		Valid N (listwise)	11							
Lower Second Incisor	Male	ECA	6	2.594	3.550	0.956	3.119	0.160	0.391	0.153
		CD	6	3.291	4.380	1.089	3.703	0.164	0.401	0.161
		Valid N (listwise)	6							
	Female	TCA	13	12.766	18.313	5.547	15.450	0.432	1.556	2.422
		DPA	13	10.345	14.488	4.143	12.053	0.364	1.314	1.725
		ECA	13	1.962	4.675	2.713	3.397	0.207	0.748	0.559
		CD	13	3.086	3.910	0.824	3.550	0.083	0.298	0.089
		DEJ	13	10.541	12.854	2.313	11.937	0.185	0.667	0.445
		RECA	13	15.369	30.121	14.752	21.922	1.154	4.160	17.309
		RDA	13	69.879	84.631	14.752	78.078	1.154	4.160	17.309
		RAET	13	5.486	12.333	6.847	8.214	0.507	1.829	3.346
		RECA to CD	13	42.388	65.451	23.063	51.797	1.734	6.251	39.072
		Valid N (listwise)	13							
Upper First Incisor	Male	ECA	16	2.261	4.467	2.206	3.511	0.132	0.527	0.278
		CD	16	3.859	5.010	1.151	4.203	0.074	0.297	0.088
		Valid N (listwise)	16							

Table 1. Continued.

Tooth Type (FDI)	Sex		N	Min	Max	Range	Mean		Std. Deviation	Variance
			Statistic	Statistic	Statistic		Statistic	Std. Error	Statistic	Statistic
Upper First Incisor	Female	ECA	19	2.391	4.144	1.753	3.363	0.122	0.531	0.282
		CD	19	3.384	4.460	1.076	4.040	0.064	0.279	0.078
		Valid N (listwise)	19							
Upper Second Incisor	Male	ECA	9	2.121	4.833	2.712	3.450	0.280	0.841	0.707
		CD	9	3.527	4.714	1.187	3.986	0.142	0.427	0.182
		Valid N (listwise)	9							
	Female	ECA	11	2.694	4.268	1.574	3.397	0.166	0.551	0.304
		CD	11	3.004	4.437	1.433	3.672	0.125	0.415	0.172
		Valid N (listwise)	11							
Lower Canine	Male	TCA	11	22.516	29.859	7.343	24.435	0.623	2.065	4.265
		DPA	11	17.439	22.240	4.801	19.177	0.394	1.306	1.707
		ECA	11	4.149	7.619	3.470	5.258	0.296	0.983	0.966
		CD	11	4.298	5.278	0.980	4.803	0.095	0.315	0.099
		RECA	11	18.154	25.517	7.363	21.408	0.751	2.490	6.198
		RDA	11	74.483	81.846	7.363	78.592	0.751	2.490	6.198
		RECA to CD	11	39.817	52.869	13.052	47.682	1.322	4.384	19.221
		Valid N (listwise)	11							
	Female	TCA	5	21.969	27.811	5.842	24.119	1.010	2.259	5.103
		DPA	5	16.486	21.530	5.044	18.345	0.870	1.945	3.785
		ECA	5	5.397	6.281	0.884	5.774	0.176	0.394	0.155
		CD	5	4.084	5.085	1.001	4.491	0.224	0.501	0.251
		RECA	5	22.586	25.072	2.486	24.003	0.555	1.242	1.542
		RDA	5	74.928	77.414	2.486	75.997	0.555	1.242	1.542
		RECA to CD	5	46.593	60.486	13.893	53.973	2.613	5.843	34.139
		Valid N (listwise)	5							

Table 1. Continued.

Tooth Type (FDI)	Sex		N	Min	Max	Range	Mean		Std. Deviation	Variance
			Statistic	Statistic	Statistic		Statistic	Std. Error	Statistic	Statistic
Upper Canine	Male	TCA	6	26.312	32.162	5.850	28.643	0.836	2.047	4.191
		DPA	6	20.755	26.168	5.413	22.850	0.756	1.853	3.433
		ECA	6	5.524	6.228	0.704	5.792	0.116	0.285	0.081
		CD	6	4.524	5.478	0.954	4.950	0.135	0.332	0.110
		RECA	6	18.637	21.193	2.556	20.263	0.386	0.946	0.894
		RDA	6	78.807	81.363	2.556	79.737	0.386	0.946	0.894
		RECA to CD	6	44.693	55.163	10.470	48.791	1.444	3.538	12.516
		Valid N (listwise)	6							
	Female	TCA	8	26.071	32.162	6.091	28.094	0.683	1.931	3.727
		DPA	8	19.872	25.510	5.638	22.020	0.585	1.656	2.742
		ECA	8	4.906	6.924	2.018	6.074	0.216	0.612	0.374
		CD	8	4.837	5.615	0.778	5.147	0.089	0.253	0.064
		RECA	8	18.620	24.443	5.823	21.632	0.653	1.848	3.415
		RDA	8	75.557	81.380	5.823	78.368	0.653	1.848	3.415
		RECA to CD	8	42.848	50.730	7.882	47.895	0.941	2.662	7.084
		Valid N (listwise)	8							

Table 2. Descriptive statistics of deciduous molar absolute and relative tissue areas and associated linear measurements for males and females.

Tooth Type	Sex		N	Min	Max	Mean		Std. Deviation	Variance
			Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
LowerM1	Male	TCA	7	24.559	33.729	30.106	1.300	3.438	11.821
		DPA	7	19.785	26.076	23.635	0.874	2.312	5.346
		ECA	7	4.774	8.036	6.472	0.448	1.185	1.403
		CD	7	5.178	6.010	5.614	0.114	0.303	0.092
		DEJ	7	13.436	16.269	14.830	0.374	0.989	0.978
		RECA	7	19.261	23.825	21.354	0.651	1.723	2.969
		RDA	7	76.175	80.739	78.646	0.651	1.723	2.969
		RAET	7	7.798	10.578	8.913	0.339	0.896	0.802
		RECA to CD	7	39.087	49.098	45.147	1.321	3.494	12.209
		Valid N (listwise)	7						
	Female	TCA	4	27.739	34.552	31.798	1.619	3.238	10.485
		DPA	4	21.572	26.729	24.215	1.063	2.126	4.521
		ECA	4	6.009	10.624	7.583	1.070	2.140	4.578
		CD	4	5.145	7.460	6.348	0.492	0.984	0.969
		DEJ	4	13.631	16.468	14.954	0.590	1.179	1.390
		RECA	4	19.611	30.749	23.643	2.441	4.882	23.835
		RDA	4	69.251	80.389	76.357	2.441	4.882	23.835
		RAET	4	8.021	14.852	10.365	1.536	3.073	9.443
		RECA to CD	4	36.784	48.480	43.491	2.916	5.832	34.011
		Valid N (listwise)	4						
LowerM2	Male	TCA	2	36.447	39.992	38.220	1.773	2.507	6.284
		DPA	2	25.437	27.982	26.710	1.273	1.800	3.239
		ECA	2	11.010	12.010	11.510	0.500	0.707	0.500
		CD	2	6.803	7.005	6.904	0.101	0.143	0.020
		DEJ	2	15.740	16.404	16.072	0.332	0.470	0.220
		RECA	2	30.031	30.208	30.120	0.088	0.125	0.016
		RDA	2	69.792	69.969	69.881	0.088	0.125	0.016

Table 2. Continued.

		RAET	2	13.307	14.424	13.866	0.558	0.790	0.624
		RECA to CD	2	48.775	49.474	49.125	0.349	0.494	0.244
		Valid N (listwise)	2						
LowerM2	Female	TCA	7	27.145	40.568	34.718	1.621	4.290	18.402
		DPA	7	19.758	30.911	24.932	1.376	3.640	13.253
		ECA	7	7.387	12.495	9.786	0.629	1.664	2.770
		CD	7	5.131	7.737	6.504	0.324	0.857	0.734
		DEJ	7	13.637	16.074	15.200	0.309	0.818	0.670
		RECA	7	23.805	33.146	28.256	1.457	3.856	14.868
		RDA	7	66.854	76.195	71.744	1.457	3.856	14.868
		RAET	7	10.709	15.783	12.951	0.791	2.093	4.380
		RECA to CD	7	40.167	55.343	48.508	2.302	6.091	37.097
		Valid N (listwise)	7						
UpperM1	Male	TCA	6	15.312	41.242	29.920	3.751	9.189	84.436
		DPA	6	12.181	32.904	23.004	3.006	7.363	54.208
		ECA	6	3.131	8.903	6.916	0.823	2.017	4.069
		CD	6	3.621	7.411	5.990	0.584	1.429	2.043
		DEJ	6	9.913	18.599	15.083	1.260	3.086	9.523
		RECA	6	20.218	27.988	23.188	1.167	2.859	8.176
		RDA	6	72.012	79.782	76.812	1.167	2.859	8.176
		RAET	6	7.816	11.966	9.586	0.572	1.402	1.965
		RECA to CD	6	36.042	50.905	44.370	2.456	6.015	36.178
		Valid N (listwise)	6						
	Female	TCA	2	28.090	34.933	31.512	3.422	4.839	23.413
		DPA	2	22.790	27.424	25.107	2.317	3.277	10.737
		ECA	2	5.301	7.509	6.405	1.104	1.561	2.438
		CD	2	6.735	6.896	6.816	0.080	0.114	0.013
		DEJ	2	14.775	14.952	14.864	0.088	0.125	0.016
		RECA	2	18.870	21.496	20.183	1.313	1.857	3.448
		RDA	2	78.504	81.130	79.817	1.313	1.857	3.448

Table 2. Continued.

		RAET	2	7.426	9.705	8.566	1.140	1.611	2.597
		RECA to CD	2	34.186	39.738	36.962	2.776	3.926	15.412
		Valid N (listwise)	2						
UpperM2	Male	TCA	3	39.799	46.658	43.674	2.030	3.515	12.357
		DPA	3	29.184	33.507	31.581	1.270	2.200	4.839
		ECA	3	10.615	13.151	12.093	0.762	1.319	1.740
		CD	3	8.446	8.580	8.504	0.040	0.069	0.005
		DEJ	3	16.231	17.153	16.822	0.296	0.513	0.263
		RECA	3	26.670	28.185	27.644	0.488	0.845	0.714
		RDA	3	71.815	73.330	72.356	0.488	0.845	0.714
		RAET	3	11.455	13.616	12.791	0.674	1.167	1.363
		RECA to CD	3	38.573	42.735	40.845	1.217	2.107	4.440
		Valid N (listwise)	3						
	Female	TCA	6	35.533	51.104	44.643	2.175	5.328	28.392
		DPA	6	26.437	38.974	32.594	1.820	4.457	19.868
		ECA	6	9.096	13.895	12.048	0.709	1.735	3.012
		CD	6	7.609	9.547	8.663	0.271	0.663	0.440
		DEJ	6	15.169	18.308	17.185	0.519	1.270	1.614
		RECA	6	23.736	31.920	27.048	1.261	3.090	9.546
		RDA	6	68.080	76.264	72.953	1.261	3.090	9.546
		RAET	6	10.621	15.559	12.350	0.753	1.845	3.406
		RECA to CD	6	36.480	48.543	40.194	1.913	4.685	21.947
		Valid N (listwise)	6						

Table 3. Descriptive statistics of deciduous incisor and canine linear tissue measurements for males and females.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Lower First Incisor	Male	a	11	0.736	1.224	0.962	0.039	0.128	0.016
		LET1	11	0.129	0.186	0.158	0.006	0.021	0.000
		DPT1	11	2.783	3.369	3.167	0.057	0.190	0.036
		BET1	11	0.115	0.231	0.170	0.010	0.034	0.001
		TCT1	11	3.119	3.780	3.495	0.060	0.198	0.039
		PLET1	11	0.125	0.190	0.160	0.007	0.022	0.000
		PBET1	11	0.122	0.241	0.171	0.010	0.033	0.001
		LET2	11	0.176	0.241	0.215	0.007	0.022	0.000
		DPT2	11	2.06	2.91	2.564	0.079	0.264	0.069
		BET2	11	0.200	0.319	0.258	0.010	0.032	0.001
		TCT2	11	2.508	3.383	3.037	0.078	0.258	0.067
		PLET2	11	0.151	0.200	0.178	0.005	0.017	0.000
		PBET2	11	0.207	0.319	0.257	0.009	0.030	0.001
		LET3	11	0.183	0.298	0.222	0.010	0.032	0.001
		DPT3	11	1.278	2.231	1.732	0.098	0.325	0.106
		BET3	11	0.254	0.427	0.320	0.014	0.046	0.002
		TCT3	11	1.864	2.708	2.275	0.087	0.290	0.084
		PLET3	11	0.161	0.276	0.193	0.010	0.033	0.001
		PBET3	11	0.252	0.414	0.315	0.013	0.043	0.002

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Lower First Incisor	Female	a	12	0.790	1.278	0.950	0.044	0.153	0.023
		LET1	12	0.139	0.203	0.172	0.006	0.022	0.000
		DPT1	12	3.034	3.498	3.278	0.046	0.159	0.025
		BET1	12	0.129	0.227	0.184	0.008	0.026	0.001
		TCT1	12	3.418	3.885	3.634	0.049	0.169	0.029
		PLET1	12	0.136	0.211	0.175	0.006	0.022	0.001
		PBET1	12	0.130	0.224	0.184	0.007	0.025	0.001
		LET2	12	0.142	0.317	0.248	0.013	0.046	0.002
		DPT2	12	2.250	3.080	2.672	0.070	0.241	0.058
		BET2	12	0.186	0.329	0.270	0.012	0.042	0.002
		TCT2	12	2.783	3.661	3.190	0.077	0.265	0.070
		PLET2	12	0.132	0.234	0.196	0.008	0.029	0.001
		PBET2	12	0.181	0.328	0.265	0.012	0.040	0.002
		LET3	12	0.129	0.339	0.227	0.015	0.053	0.003
		DPT3	12	0.922	2.081	1.767	0.095	0.329	0.108
		BET3	12	0.236	0.417	0.315	0.017	0.059	0.003
		TCT3	12	1.420	2.698	2.310	0.103	0.358	0.128
		PLET3	12	0.114	0.273	0.195	0.012	0.043	0.002
		PBET3	12	0.225	0.393	0.310	0.016	0.057	0.003

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Lower Second Incisor	Male	a	5	0.834	1.729	1.196	0.152	0.340	0.116
		LET1	5	0.112	0.210	0.173	0.018	0.041	0.002
		DPT1	5	3.375	3.912	3.640	0.089	0.200	0.040
		BET1	5	0.146	0.244	0.207	0.019	0.043	0.002
		TCT1	5	3.793	4.169	4.021	0.069	0.155	0.024
		PLET1	5	0.102	0.210	0.172	0.020	0.044	0.002
		PBET1	5	0.156	0.247	0.210	0.018	0.040	0.002
		LET2	5	0.190	0.279	0.225	0.017	0.037	0.001
		DPT2	5	2.380	3.470	2.834	0.188	0.421	0.177
		BET2	5	0.203	0.329	0.295	0.024	0.053	0.003
		TCT2	5	2.908	3.864	3.355	0.161	0.360	0.130
		PLET2	5	0.163	0.232	0.191	0.011	0.026	0.001
		PBET2	5	0.206	0.328	0.291	0.022	0.049	0.002
		LET3	5	0.193	0.298	0.230	0.019	0.043	0.002
		DPT3	5	1.105	2.695	1.825	0.261	0.583	0.340
		BET3	5	0.281	0.433	0.379	0.027	0.060	0.004
		TCT3	5	1.681	3.169	2.434	0.241	0.540	0.291
		PLET3	5	0.149	0.275	0.208	0.020	0.046	0.002
		PBET3	5	0.274	0.452	0.372	0.029	0.066	0.004

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Lower Second Incisor	Female	a	13	0.685	1.234	0.943	0.048	0.173	0.030
		LET1	13	0.129	0.224	0.177	0.009	0.032	0.001
		DPT1	13	3.322	4.146	3.679	0.072	0.260	0.067
		BET1	13	0.146	0.278	0.208	0.010	0.036	0.001
		TCT1	13	3.663	4.478	4.064	0.075	0.271	0.073
		PLET1	13	0.126	0.221	0.181	0.009	0.031	0.001
		PBET1	13	0.150	0.278	0.208	0.010	0.036	0.001
		LET2	13	0.186	0.312	0.246	0.011	0.040	0.002
		DPT2	13	2.470	3.810	3.118	0.108	0.388	0.151
		BET2	13	0.197	0.420	0.299	0.019	0.070	0.005
		TCT2	13	3.108	4.285	3.663	0.101	0.365	0.133
		PLET2	13	0.153	0.268	0.213	0.009	0.034	0.001
		PBET2	13	0.201	0.406	0.293	0.018	0.066	0.004
		LET3	13	0.183	0.427	0.267	0.019	0.067	0.005
		DPT3	13	1.386	3.105	2.184	0.138	0.498	0.248
		BET3	13	0.234	0.468	0.374	0.023	0.081	0.007
		TCT3	13	2.176	3.580	2.826	0.125	0.451	0.204
		PLET3	13	0.159	0.292	0.220	0.013	0.048	0.002
		PBET3	13	0.237	0.460	0.363	0.020	0.073	0.005

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Upper First Incisor	Male	a	17	0.715	1.441	1.066	0.052	0.214	0.046
		LET1	17	0.142	0.285	0.194	0.009	0.036	0.001
		DPT1	17	3.904	5.258	4.514	0.087	0.357	0.128
		BET1	17	0.129	0.298	0.222	0.011	0.046	0.002
		TCT1	17	4.293	5.610	4.930	0.094	0.387	0.150
		PLET1	17	0.139	0.270	0.195	0.008	0.033	0.001
		PBET1	17	0.133	0.299	0.222	0.011	0.045	0.002
		LET2	17	0.180	0.380	0.275	0.012	0.048	0.002
		DPT2	17	3.480	4.640	3.915	0.074	0.306	0.093
		BET2	17	0.186	0.369	0.308	0.012	0.049	0.002
		TCT2	17	4.043	5.217	4.499	0.071	0.293	0.086
		PLET2	17	0.171	0.293	0.222	0.008	0.031	0.001
		PBET2	17	0.187	0.354	0.304	0.011	0.045	0.002
		LET3	17	0.207	0.437	0.287	0.013	0.054	0.003
		DPT3	17	1.786	3.702	2.655	0.133	0.549	0.302
		BET3	17	0.241	0.461	0.365	0.016	0.064	0.004
		TCT3	17	2.576	4.149	3.306	0.118	0.486	0.236
		PLET3	17	0.153	0.260	0.202	0.008	0.031	0.001
		PBET3	17	0.228	0.430	0.351	0.014	0.058	0.003

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Upper First Incisor	Female	a	20	0.790	1.349	1.065	0.033	0.148	0.022
		LET1	20	0.153	0.251	0.197	0.006	0.029	0.001
		DPT1	20	3.919	5.038	4.460	0.059	0.265	0.070
		BET1	20	0.132	0.308	0.219	0.009	0.042	0.002
		TCT1	20	4.458	5.481	4.876	0.057	0.255	0.065
		PLET1	20	0.156	0.254	0.200	0.007	0.029	0.001
		PBET1	20	0.126	0.312	0.216	0.010	0.043	0.002
		LET2	20	0.183	0.399	0.282	0.013	0.058	0.003
		DPT2	20	3.100	4.590	3.858	0.084	0.376	0.141
		BET2	20	0.241	0.407	0.316	0.010	0.047	0.002
		TCT2	20	3.851	5.058	4.456	0.070	0.313	0.098
		PLET2	20	0.166	0.310	0.226	0.008	0.038	0.001
		PBET2	20	0.232	0.399	0.311	0.010	0.043	0.002
		LET3	20	0.207	0.447	0.299	0.015	0.065	0.004
		DPT3	20	1.315	3.668	2.578	0.133	0.596	0.355
		BET3	20	0.258	0.492	0.373	0.015	0.068	0.005
		TCT3	20	2.125	4.193	3.251	0.118	0.527	0.278
		PLET3	20	0.144	0.292	0.211	0.010	0.045	0.002
		PBET3	20	0.256	0.481	0.362	0.014	0.061	0.004

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Upper Second Incisor	Male	a	9	0.607	1.258	0.949	0.056	0.168	0.028
		LET1	9	0.125	0.207	0.166	0.010	0.029	0.001
		DPT1	9	3.586	4.854	4.096	0.161	0.483	0.234
		BET1	9	0.132	0.231	0.186	0.010	0.031	0.001
		TCT1	9	3.949	5.244	4.448	0.155	0.465	0.217
		PLET1	9	0.130	0.214	0.169	0.010	0.030	0.001
		PBET1	9	0.132	0.230	0.185	0.010	0.030	0.001
		LET2	9	0.200	0.305	0.252	0.011	0.034	0.001
		DPT2	9	2.610	4.540	3.587	0.216	0.649	0.421
		TCT2	9	3.166	5.108	4.126	0.209	0.626	0.392
		PLET2	9	0.175	0.269	0.219	0.012	0.036	0.001
		PBET2	9	0.219	0.339	0.284	0.013	0.038	0.001
		LET3	9	0.231	0.407	0.297	0.021	0.062	0.004
		DPT3	9	1.502	3.654	2.554	0.220	0.660	0.436
		BET3	9	0.275	0.454	0.369	0.022	0.065	0.004
		TCT3	9	2.251	4.431	3.219	0.221	0.664	0.441
		PLET3	9	0.133	0.307	0.221	0.017	0.052	0.003
		PBET3	9	0.269	0.436	0.359	0.020	0.059	0.004

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Upper Second Incisor	Female	a	11	0.841	1.525	1.058	0.055	0.184	0.034
		LET1	11	0.153	0.227	0.201	0.008	0.026	0.001
		DPT1	11	3.085	4.553	3.852	0.121	0.400	0.160
		BET1	11	0.190	0.295	0.226	0.010	0.032	0.001
		TCT1	11	3.583	4.908	4.278	0.111	0.366	0.134
		PLET1	11	0.160	0.231	0.205	0.007	0.024	0.001
		PBET1	11	0.187	0.288	0.224	0.009	0.031	0.001
		LET2	11	0.210	0.356	0.276	0.012	0.039	0.002
		DPT2	11	2.010	4.250	3.163	0.185	0.615	0.378
		BET2	11	0.261	0.431	0.329	0.016	0.051	0.003
		TCT2	11	2.702	4.756	3.768	0.173	0.573	0.329
		PLET2	11	0.193	0.263	0.228	0.009	0.029	0.001
		PBET2	11	0.252	0.387	0.318	0.013	0.044	0.002
		LET3	11	0.176	0.424	0.285	0.025	0.081	0.007
		DPT3	11	0.712	3.285	1.979	0.199	0.661	0.437
		BET3	11	0.319	0.573	0.420	0.026	0.086	0.007
		TCT3	11	1.461	3.966	2.684	0.192	0.637	0.406
		PLET3	11	0.122	0.304	0.216	0.015	0.051	0.003
		PBET3	11	0.304	0.512	0.392	0.020	0.067	0.004

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Lower Canine	Male	a	11	0.885	1.553	1.122	0.067	0.224	0.050
		LET1	11	0.149	0.251	0.199	0.011	0.038	0.001
		DPT1	11	4.500	5.569	4.964	0.108	0.357	0.128
		BET1	11	0.176	0.322	0.238	0.017	0.056	0.003
		TCT1	11	4.966	6.139	5.400	0.106	0.352	0.124
		PLET1	11	0.150	0.250	0.199	0.011	0.035	0.001
		PBET1	11	0.183	0.328	0.242	0.017	0.056	0.003
		LET2	11	0.183	0.635	0.291	0.038	0.127	0.016
		DPT2	11	3.150	4.800	4.134	0.146	0.483	0.233
		BET2	11	0.244	0.524	0.378	0.027	0.088	0.008
		TCT2	11	4.130	5.244	4.803	0.112	0.372	0.138
		PLET2	11	0.152	0.547	0.230	0.033	0.110	0.012
		PBET2	11	0.244	0.524	0.370	0.028	0.093	0.009
		LET3	11	0.210	0.644	0.328	0.038	0.127	0.016
		DPT3	11	1.409	3.698	2.905	0.194	0.643	0.413
		BET3	11	0.400	0.712	0.513	0.029	0.097	0.009
		TCT3	11	2.764	4.308	3.746	0.145	0.482	0.232
		PLET3	11	0.186	0.614	0.295	0.040	0.131	0.017
		PBET3	11	0.364	0.603	0.482	0.025	0.082	0.007

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Lower Canine	Female	a	5	0.658	1.715	1.327	0.189	0.423	0.179
		LET1	5	0.170	0.302	0.243	0.023	0.051	0.003
		DPT1	5	4.351	5.125	4.710	0.168	0.375	0.140
		BET1	5	0.119	0.353	0.273	0.042	0.095	0.009
		TCT1	5	4.851	5.591	5.226	0.135	0.301	0.091
		PLET1	5	0.170	0.305	0.247	0.024	0.054	0.003
		PBET1	5	0.122	0.360	0.280	0.043	0.096	0.009
		LET2	5	0.207	0.637	0.375	0.073	0.162	0.026
		DPT2	5	2.570	4.810	3.511	0.400	0.894	0.800
		BET2	5	0.258	0.658	0.494	0.067	0.149	0.022
		TCT2	5	3.634	5.275	4.380	0.284	0.636	0.404
		PLET2	5	0.190	0.510	0.314	0.057	0.128	0.016
		PBET2	5	0.258	0.614	0.479	0.061	0.135	0.018
		LET3	5	0.244	0.488	0.377	0.042	0.093	0.009
		DPT3	5	0.912	4.136	2.207	0.584	1.306	1.705
		BET3	5	0.353	0.858	0.630	0.085	0.191	0.036
		TCT3	5	1.880	4.732	3.138	0.504	1.127	1.269
		PLET3	5	0.193	0.480	0.352	0.049	0.109	0.012
		PBET3	5	0.338	0.687	0.571	0.063	0.142	0.020

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Upper Canine	Male	a	6	1.279	1.637	1.462	0.054	0.131	0.017
		LET1	6	0.227	0.293	0.263	0.011	0.026	0.001
		DPT1	6	4.986	5.902	5.376	0.133	0.325	0.106
		BET1	6	0.220	0.303	0.272	0.012	0.028	0.001
		TCT1	6	5.534	6.349	5.910	0.122	0.298	0.089
		PLET1	6	0.236	0.289	0.265	0.009	0.023	0.001
		PBET1	6	0.211	0.311	0.274	0.014	0.034	0.001
		LET2	6	0.153	0.721	0.441	0.078	0.191	0.037
		DPT2	6	2.850	4.340	3.853	0.213	0.521	0.272
		TCT2	6	3.186	5.130	4.669	0.303	0.743	0.552
		PLET2	6	0.135	0.403	0.313	0.041	0.100	0.010
		PBET2	6	0.191	0.457	0.369	0.038	0.093	0.009
		LET3	6	0.433	0.572	0.500	0.024	0.059	0.003
		DPT3	6	2.370	4.329	2.824	0.308	0.756	0.571
		BET3	6	0.417	0.567	0.513	0.022	0.054	0.003
		TCT3	6	3.385	5.258	3.838	0.289	0.709	0.502
		PLET3	6	0.355	0.489	0.415	0.023	0.057	0.003
		PBET3	6	0.402	0.542	0.495	0.021	0.050	0.003

Table 3. Continued.

Tooth Type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Upper Canine	Female	a	7	1.120	1.625	1.426	0.061	0.160	0.026
		LET1	7	0.220	0.303	0.265	0.013	0.035	0.001
		DPT1	7	5.221	5.918	5.509	0.087	0.231	0.053
		BET1	7	0.250	0.337	0.289	0.011	0.030	0.001
		TCT1	7	5.774	6.471	6.063	0.094	0.250	0.062
		PLET1	7	0.214	0.308	0.270	0.014	0.038	0.001
		PBET1	7	0.255	0.345	0.294	0.011	0.030	0.001
		LET2	7	0.231	0.639	0.445	0.063	0.168	0.028
		DPT2	7	3.760	4.530	4.147	0.116	0.307	0.094
		BET2	7	0.399	0.606	0.513	0.029	0.077	0.006
		TCT2	7	4.810	5.495	5.105	0.086	0.229	0.052
		PLET2	7	0.175	0.490	0.346	0.045	0.119	0.014
		PBET2	7	0.384	0.587	0.499	0.028	0.075	0.006
		LET3	7	0.407	0.668	0.534	0.037	0.098	0.010
		DPT3	7	1.841	2.938	2.377	0.133	0.353	0.125
		BET3	7	0.558	0.788	0.676	0.031	0.083	0.007
		TCT3	7	3.192	4.029	3.587	0.119	0.316	0.100
		PLET3	7	0.329	0.589	0.458	0.035	0.093	0.009
		PBET3	7	0.540	0.676	0.620	0.019	0.051	0.003

Table 4. Descriptive statistics of deciduous molar linear tissue measurements for males and females.

Tooth type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Lower First Molar	Male	a	7	3.064	4.019	3.677	0.133	0.353	0.125
		LET1	7	0.271	0.442	0.375	0.022	0.059	0.003
		TCT1	7	6.644	7.558	7.143	0.121	0.320	0.103
		DPT1	7	6.088	6.731	6.386	0.100	0.263	0.069
		BET1	7	0.285	0.462	0.383	0.024	0.064	0.004
		PLET1	7	0.273	0.434	0.373	0.021	0.055	0.003
		PBET1	7	0.275	0.437	0.373	0.025	0.065	0.004
		LET2	7	0.397	0.750	0.557	0.050	0.132	0.017
		TCT2	7	5.173	6.210	5.629	0.135	0.358	0.128
		DPT2	7	3.904	5.414	4.420	0.181	0.478	0.229
		BET2	7	0.400	0.774	0.652	0.051	0.135	0.018
		PLET2	7	0.390	0.638	0.505	0.037	0.098	0.010
		PBET2	7	0.379	0.627	0.555	0.032	0.084	0.007
	Female	a	4	2.824	3.870	3.396	0.266	0.532	0.283
		LET1	4	0.298	0.451	0.375	0.036	0.071	0.005
		TCT1	4	6.558	8.692	7.623	0.454	0.908	0.824
		DPT1	4	5.832	7.959	6.869	0.446	0.892	0.795
		BET1	4	0.315	0.413	0.379	0.022	0.043	0.002
		PLET1	4	0.310	0.452	0.388	0.031	0.063	0.004
		PBET1	4	0.316	0.392	0.357	0.016	0.033	0.001
		LET2	4	0.519	0.831	0.701	0.073	0.145	0.021
		TCT2	4	4.851	7.692	6.345	0.734	1.468	2.154
		DPT2	4	3.423	6.302	4.810	0.656	1.312	1.721
		BET2	4	0.586	1.217	0.834	0.135	0.269	0.072
		PLET2	4	0.474	0.829	0.656	0.074	0.148	0.022
		PBET2	4	0.553	0.927	0.689	0.082	0.164	0.027

Table 4. Continued.

Tooth type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Lower Second Molar	Male	a	2	3.000	3.437	3.218	0.219	0.309	0.095
		LET1	2	0.428	0.562	0.495	0.067	0.095	0.009
		TCT1	2	8.058	8.495	8.276	0.219	0.309	0.096
		DPT1	2	7.255	7.341	7.298	0.043	0.061	0.004
		BET1	2	0.375	0.591	0.483	0.108	0.153	0.023
		PLET1	2	0.435	0.582	0.508	0.074	0.104	0.011
		PBET1	2	0.380	0.617	0.498	0.119	0.168	0.028
		LET2	2	0.837	0.933	0.885	0.048	0.068	0.005
		TCT2	2	7.654	7.827	7.740	0.087	0.122	0.015
		DPT2	2	5.793	5.933	5.863	0.070	0.099	0.010
		BET2	2	0.885	1.101	0.993	0.108	0.153	0.023
		PLET2	2	0.797	0.898	0.847	0.051	0.072	0.005
		PBET2	2	0.806	0.919	0.862	0.057	0.080	0.006
	Female	a	7	3.010	3.475	3.235	0.074	0.195	0.038
		LET1	7	0.417	0.486	0.448	0.010	0.028	0.001
		TCT1	7	6.654	8.788	7.887	0.278	0.735	0.541
		DPT1	7	5.763	8.029	7.040	0.301	0.796	0.633
		BET1	7	0.274	0.519	0.399	0.034	0.090	0.008
		PLET1	7	0.408	0.491	0.443	0.011	0.029	0.001
		PBET1	7	0.260	0.529	0.394	0.034	0.091	0.008
		LET2	7	0.647	0.947	0.803	0.044	0.117	0.014
		TCT2	7	5.237	8.298	7.347	0.370	0.979	0.958
		DPT2	7	3.573	6.490	5.628	0.357	0.944	0.891
		BET2	7	0.779	1.017	0.916	0.033	0.088	0.008
		PLET2	7	0.613	0.931	0.779	0.046	0.122	0.015
		PBET2	7	0.650	1.058	0.828	0.047	0.125	0.016

Table 4. Continued.

Tooth type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Upper First Molar	Male	a	6	2.631	3.793	3.187	0.179	0.438	0.192
		LET1	6	0.258	0.462	0.358	0.031	0.077	0.006
		TCT1	6	6.400	8.846	7.935	0.352	0.863	0.745
		DPT1	6	5.776	7.971	7.230	0.315	0.770	0.594
		BET1	6	0.226	0.462	0.346	0.041	0.101	0.010
		PLET1	6	0.256	0.459	0.356	0.032	0.078	0.006
		PBET1	6	0.219	0.425	0.341	0.036	0.089	0.008
		LET2	6	0.542	0.769	0.674	0.043	0.106	0.011
		TCT2	6	5.861	7.274	6.879	0.213	0.523	0.273
		DPT2	6	4.661	5.912	5.569	0.189	0.464	0.215
		BET2	6	0.500	0.750	0.635	0.038	0.094	0.009
		PLET2	6	0.537	0.716	0.637	0.033	0.082	0.007
		PBET2	6	0.444	0.699	0.587	0.039	0.096	0.009
	Female	a	2	2.639	3.596	3.118	0.479	0.677	0.458
		LET1	2	0.269	0.394	0.332	0.063	0.088	0.008
		TCT1	2	7.587	8.106	7.847	0.260	0.367	0.135
		DPT1	2	7.034	7.356	7.195	0.161	0.228	0.052
		BET1	2	0.284	0.356	0.320	0.036	0.051	0.003
		PLET1	2	0.270	0.403	0.337	0.067	0.094	0.009
		PBET1	2	0.301	0.346	0.324	0.023	0.032	0.001
		LET2	2	0.514	0.861	0.688	0.174	0.245	0.060
		TCT2	2	6.889	6.923	6.906	0.017	0.024	0.001
		DPT2	2	5.341	5.880	5.611	0.270	0.381	0.145
		BET2	2	0.495	0.721	0.608	0.113	0.160	0.026
		PLET2	2	0.473	0.747	0.610	0.137	0.194	0.038
		PBET2	2	0.486	0.676	0.581	0.095	0.134	0.018

Table 4. Continued.

Tooth type	Sex	Variable	N	Min	Max	Mean	Std. Error	Std. Deviation	Variance
Upper Second Molar	Male	a	3	2.649	3.510	3.186	0.271	0.469	0.220
		LET1	3	0.353	0.534	0.417	0.058	0.101	0.010
		TCT1	3	9.255	9.698	9.505	0.131	0.227	0.051
		DPT1	3	8.490	8.817	8.647	0.095	0.164	0.027
		BET1	3	0.255	0.538	0.441	0.093	0.161	0.026
		PLET1	3	0.343	0.560	0.426	0.068	0.117	0.014
		PBET1	3	0.260	0.521	0.426	0.083	0.145	0.021
		LET2	3	0.841	1.303	1.040	0.137	0.238	0.056
		TCT2	3	8.756	8.971	8.837	0.067	0.117	0.014
		DPT2	3	6.488	7.322	6.800	0.263	0.455	0.207
		BET2	3	0.673	1.427	0.996	0.224	0.388	0.151
		PLET2	3	0.781	1.162	0.944	0.113	0.197	0.039
		PBET2	3	0.655	1.148	0.895	0.142	0.247	0.061
	Female	a	6	2.814	3.606	3.312	0.120	0.294	0.086
		LET1	6	0.329	0.607	0.469	0.046	0.112	0.012
		TCT1	6	8.766	10.500	9.589	0.247	0.606	0.367
		DPT1	6	8.031	9.505	8.727	0.232	0.568	0.323
		BET1	6	0.274	0.451	0.393	0.029	0.070	0.005
		PLET1	6	0.335	0.612	0.476	0.046	0.114	0.013
		PBET1	6	0.284	0.457	0.399	0.028	0.069	0.005
		LET2	6	0.556	1.332	1.077	0.122	0.300	0.090
		TCT2	6	8.132	9.457	8.901	0.195	0.477	0.228
		DPT2	6	6.369	7.303	6.927	0.126	0.308	0.095
		BET2	6	0.708	1.216	0.898	0.073	0.178	0.032
		PLET2	6	0.535	1.169	0.966	0.096	0.235	0.055
		PBET2	6	0.634	1.033	0.848	0.056	0.136	0.019

Table 5. Independent sample t-tests for area tissue measurements of incisors and canines with normal sample distributions. Those variables which reject Levene's hypothesis (<0.05) for equal variances are tested using the SPSS corrected t-test.

Tooth Type (FDI)		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Diff.	Std. Error Diff.	95% Confidence Interval of the Difference	
									Lower	Upper
Lower Canine	DPA	0.378	0.549	1.017	14	0.326	0.832	0.818	-0.923	2.587
	ECA	1.995	0.180	-1.116	14	0.283	-0.516	0.462	-1.507	0.476
	RECA	5.325	0.037[2]	-2.778	13.690	0.015*	-2.594	0.934	-4.601	-0.587
	RDA	5.325	0.037[2]	2.778	13.690	0.015*	2.594	0.934	0.587	4.601
	RECA to CD	1.034	0.327	-2.407	14	0.030*	-6.290	2.614	-11.896	-0.684
Lower First Incisor	ECA	2.468	0.134	-0.938	18	0.361	-0.194	0.207	-0.629	0.241
	CD	0.042	0.839	-1.997	18	0.061	-0.141	0.070	-0.289	0.007
Lower Second Incisor	ECA	2.830	0.111	-0.849	17	0.408	-0.278	0.327	-0.968	0.413
Upper Canine	TCA	0.033	0.859	0.513	12	0.617	0.549	1.069	-1.781	2.878
	DPA	0.022	0.885	0.884	12	0.394	0.831	0.940	-1.218	2.879
	ECA	1.571	0.234	-1.040	12	0.319	-0.282	0.271	-0.873	0.309
	CD	0.532	0.480	-1.263	12	0.230	-0.197	0.156	-0.536	0.143
	RECA	1.925	0.191	-1.648	12	0.125	-1.369	0.831	-3.179	0.440
	RDA	1.925	0.191	1.648	12	0.125	1.369	0.831	-0.440	3.179
	RECA to CD	0.071	0.794	0.543	12	0.597	0.896	1.651	-2.701	4.494
Upper First Incisor	ECA	0.162	0.690	0.825	33	0.415	0.148	0.180	-0.217	0.513
Upper Second Incisor	ECA	1.285	0.272	0.171	18	0.866	0.054	0.312	-0.603	0.710
	CD	0.225	0.641	1.666	18	0.113	0.315	0.189	-0.082	0.711

[1] A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.

Table 6. Independent sample t-tests for area tissue measurements of lower first molars and upper second molars with normal sample distributions. Those variables which reject Levene's hypothesis (<0.05) for equal variances are tested using the SPSS corrected t-test.

Tooth Type		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Diff.	Std. Error Diff.	95% Confidence Interval of the Difference	
									Lower	Upper
Lower First Molar	TCA	0.001	0.971	-0.800	9	0.444	-1.692	2.114	-6.474	3.090
	DPA	0.179	0.682	-0.411	9	0.690	-0.581	1.411	-3.773	2.612
	ECA	1.171	0.307	-1.130	9	0.288	-1.111	0.983	-3.335	1.114
	CD	6.919	0.027[2]	-1.453	3.329	0.234	-0.734	0.505	-2.256	0.788
	DEJ	0.091	0.770	-0.187	9	0.856	-0.124	0.662	-1.621	1.374
	RECA	4.227	0.070	-1.159	9	0.276	-2.289	1.975	-6.756	2.178
	RDA	4.227	0.070	1.159	9	0.276	2.289	1.975	-2.178	6.756
	RAET	5.809	0.039[2]	-0.923	3.295	0.419	-1.452	1.573	-6.215	3.311
	RECA to CD	3.838	0.082	0.599	9	0.564	1.657	2.766	-4.600	7.914
Upper Second Molar	TCA	0.393	0.551	-0.281	7	0.787	-0.969	3.450	-9.127	7.190
	DPA	0.934	0.366	-0.363	7	0.727	-1.013	2.791	-7.612	5.586
	ECA	0.081	0.784	0.039	7	0.970	0.045	1.151	-2.676	2.766
	CD	3.338	0.110	-0.400	7	0.701	-0.159	0.397	-1.098	0.780
	DEJ	2.689	0.145	-0.463	7	0.657	-0.363	0.784	-2.216	1.490
	RECA	4.714	0.067	0.318	7	0.760	0.596	1.874	-3.835	5.027
	RDA	4.714	0.067	-0.318	7	0.760	-0.596	1.874	-5.027	3.835
	RAET	0.871	0.382	0.371	7	0.722	0.441	1.188	-2.368	3.249
	RECA to CD	1.579	0.249	0.224	7	0.829	0.651	2.911	-6.232	7.534

[1]A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.

Table 7. Independent sample t-tests for linear tissue measurements of the incisors and canines. Those variables which reject Levene's hypothesis (<0.05) for equal variances are tested using the SPSS corrected t-test.

Tooth Type		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Lower First Incisor	a	1.135	0.299	0.211	21	0.835	0.012	0.059	-0.111	0.136
	TCT1	0.225	0.640	-1.820	21	0.083	-0.139	0.077	-0.299	0.020
	BET1	1.163	0.293	-1.132	21	0.270	-0.014	0.013	-0.040	0.012
	LET1	0.016	0.900	-1.606	21	0.123	-0.014	0.009	-0.033	0.004
	PBET1	0.443	0.513	-1.096	21	0.286	-0.013	0.012	-0.039	0.012
	PLET1	0.005	0.947	-1.636	21	0.117	-0.015	0.009	-0.035	0.004
	DPT1	0.163	0.691	-1.524	21	0.142	-0.111	0.073	-0.262	0.040
	TCT2	0.020	0.890	-1.397	21	0.177	-0.153	0.109	-0.380	0.075
	BET2	0.879	0.359	-0.792	21	0.437	-0.013	0.016	-0.045	0.020
	LET2	1.792	0.195	-2.114	21	0.047*	-0.032	0.015	-0.064	-0.001
	PBET2	0.586	0.453	-0.585	21	0.565	-0.009	0.015	-0.040	0.022
	PLET2	1.284	0.270	-1.793	21	0.087	-0.018	0.010	-0.038	0.003
	DPT2	0.125	0.727	-1.027	21	0.316	-0.108	0.105	-0.327	0.111
	TCT3	0.058	0.812	-0.256	21	0.801	-0.035	0.137	-0.319	0.249
	BET3	1.181	0.289	0.234	21	0.817	0.005	0.022	-0.041	0.051
	PBET3	1.348	0.259	0.263	21	0.795	0.006	0.021	-0.038	0.049

[1]A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.

Table 7. Continued

Tooth Type		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Lower Second Incisor	a	2.489	0.134	2.128	16	0.049*	0.254	0.119	0.001	0.506
	TCT1	2.377	0.143	-0.335	16	0.742	-0.044	0.130	-0.319	0.232
	BET1	0.427	0.523	-0.042	16	0.967	-0.001	0.020	-0.043	0.042
	LET1	0.319	0.580	-0.227	16	0.823	-0.004	0.018	-0.042	0.034
	PBET1	0.356	0.559	0.125	16	0.902	0.002	0.019	-0.039	0.044
	PLET1	0.329	0.574	-0.501	16	0.623	-0.009	0.018	-0.048	0.029
	DPT1	0.882	0.362	-0.298	16	0.770	-0.039	0.129	-0.313	0.236
	TCT2	0.053	0.820	-1.609	16	0.127	-0.308	0.192	-0.715	0.098
	LET2	0.267	0.613	-0.998	16	0.333	-0.021	0.021	-0.065	0.023
	PLET2	1.131	0.303	-1.271	16	0.222	-0.021	0.017	-0.057	0.014
	DPT2	0.003	0.956	-1.360	16	0.193	-0.284	0.209	-0.726	0.159
	TCT3	0.006	0.941	-1.570	16	0.136	-0.393	0.250	-0.922	0.137
	BET3	1.730	0.207	0.107	16	0.916	0.004	0.040	-0.081	0.090
	LET3	0.828	0.376	-1.131	16	0.275	-0.037	0.033	-0.107	0.032
	PBET3	0.538	0.474	0.235	16	0.817	0.009	0.037	-0.070	0.088
	PLET3	0.998	0.333	-0.499	16	0.625	-0.013	0.025	-0.066	0.041
	DPT3	0.000	0.983	-1.313	16	0.208	-0.360	0.274	-0.940	0.221

[1] A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.

Table 7. Continued.

Tooth Type		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Upper First Incisor	a	4.276	0.046[2]	0.019	27.774	0.985	0.001	0.062	-0.125	0.127
	TCT1	2.636	0.113	0.508	35	0.615	0.054	0.106	-0.162	0.270
	BET1	0.373	0.545	0.210	35	0.835	0.003	0.014	-0.026	0.032
	LET1	0.465	0.500	-0.292	35	0.772	-0.003	0.011	-0.025	0.018
	PBET1	0.336	0.566	0.459	35	0.649	0.007	0.015	-0.023	0.036
	PLET1	0.324	0.573	-0.477	35	0.636	-0.005	0.010	-0.026	0.016
	DPT1	1.706	0.200	0.531	35	0.599	0.054	0.102	-0.153	0.262
	TCT2	0.128	0.723	0.432	35	0.668	0.043	0.100	-0.160	0.247
	BET2	0.019	0.891	-0.486	35	0.630	-0.008	0.016	-0.040	0.024
	LET2	0.622	0.435	-0.347	35	0.731	-0.006	0.018	-0.042	0.030
	PLET2	0.345	0.561	-0.328	35	0.745	-0.004	0.012	-0.027	0.020
	DPT2	0.959	0.334	0.505	35	0.617	0.058	0.114	-0.174	0.289
	TCT3	0.251	0.620	0.332	35	0.742	0.056	0.168	-0.285	0.396
	BET3	0.094	0.761	-0.357	35	0.724	-0.008	0.022	-0.052	0.037
	LET3	1.402	0.244	-0.651	35	0.519	-0.013	0.020	-0.053	0.027
	PBET3	0.004	0.950	-0.587	35	0.561	-0.012	0.020	-0.051	0.028
	PLET3	1.118	0.298	-0.716	35	0.479	-0.009	0.013	-0.036	0.017
	DPT3	0.186	0.669	0.404	35	0.689	0.077	0.190	-0.309	0.462

[1] A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.

Table 7. Continued.

Tooth Type		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Upper Second Incisor	a	1.369	0.257	0.915	18	0.372	0.170	0.186	-0.220	0.560
	BET1	0.001	0.970	-2.783	18	0.012*	-0.040	0.014	-0.069	-0.010
	PLET1	0.794	0.385	-2.909	18	0.009*	-0.036	0.012	-0.061	-0.010
	DPT1	1.424	0.248	1.233	18	0.233	0.243	0.197	-0.171	0.658
	TCT2	0.177	0.679	1.335	18	0.199	0.358	0.268	-0.206	0.922
	BET2	0.420	0.525	-1.945	18	0.068	-0.042	0.022	-0.088	0.003
	LET2	0.185	0.672	-1.403	18	0.178	-0.023	0.017	-0.058	0.012
	PBET2	0.650	0.431	-1.778	18	0.092	-0.033	0.019	-0.072	0.006
	DPT2	0.128	0.725	1.496	18	0.152	0.424	0.283	-0.171	1.019
	TCT3	0.266	0.613	1.834	18	0.083	0.535	0.292	-0.078	1.148
	BET3	0.758	0.395	-1.479	18	0.157	-0.052	0.035	-0.125	0.022
	PBET3	0.144	0.709	-1.154	18	0.264	-0.033	0.029	-0.093	0.027
	PLET3	0.174	0.681	0.224	18	0.825	0.005	0.023	-0.043	0.054
	DPT3	0.217	0.647	1.935	18	0.069	0.575	0.297	-0.049	1.199

[1]A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.

Table 7. Continued.

Tooth Type		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Upper Canine	a	0.036	0.853	0.436	11	0.672	0.036	0.082	-0.145	0.217
	TCT1	0.143	0.712	-1.007	11	0.336	-0.153	0.152	-0.487	0.181
	BET1	0.033	0.859	-1.060	11	0.312	-0.017	0.016	-0.053	0.018
	LET1	1.835	0.203	-0.133	11	0.897	-0.002	0.017	-0.040	0.036
	PBET1	0.001	0.975	-1.153	11	0.273	-0.021	0.018	-0.060	0.019
	PLET1	5.239	0.043	-0.317	11	0.757	-0.006	0.018	-0.045	0.034
	DPT1	0.790	0.393	-0.864	11	0.406	-0.134	0.154	-0.474	0.207
	LET2	0.049	0.829	-0.037	11	0.971	-0.004	0.100	-0.223	0.216
	PBET2	0.000	0.985	-2.801	11	0.017*	-0.130	0.046	-0.232	-0.028
	PLET2	0.839	0.379	-0.528	11	0.608	-0.033	0.062	-0.168	0.103
	DPT2	0.391	0.545	-1.263	11	0.233	-0.294	0.233	-0.806	0.218
	BET3	3.075	0.107	-4.095	11	0.002**	-0.163	0.040	-0.250	-0.075
	LET3	2.888	0.117	-0.735	11	0.478	-0.034	0.046	-0.135	0.067
	PBET3	0.137	0.718	-4.429	11	0.001**	-0.124	0.028	-0.186	-0.063
	PLET3	2.608	0.135	-0.985	11	0.346	-0.043	0.044	-0.140	0.053

[1]A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.
**Significant at the Bonferroni corrected 0.003 level.

Table 7. Continued.

Tooth Type		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Lower Canine	a	1.482	0.244	-1.289	14	0.218	-0.205	0.159	-0.546	0.136
	TCT1	0.114	0.741	0.954	14	0.356	0.174	0.183	-0.217	0.566
	BET1	0.488	0.496	-0.927	14	0.370	-0.035	0.037	-0.115	0.045
	LET1	0.258	0.620	-1.993	14	0.066	-0.045	0.023	-0.093	0.003
	PBET1	0.670	0.427	-0.993	14	0.338	-0.037	0.038	-0.118	0.043
	PLET1	1.059	0.321	-2.148	14	0.050*	-0.048	0.022	-0.096	0.000
	DPT1	0.104	0.752	1.297	14	0.216	0.254	0.195	-0.166	0.673
	TCT2	2.356	0.147	1.694	14	0.112	0.423	0.250	-0.112	0.958
	BET2	0.692	0.420	-1.973	14	0.069	-0.116	0.059	-0.242	0.010
	PBET2	0.146	0.708	-1.893	14	0.079	-0.109	0.058	-0.233	0.014
	DPT2	2.752	0.119	1.838	14	0.087	0.623	0.339	-0.104	1.350
	TCT3	4.966	0.043[2]	1.160	4.680	0.302	0.608	0.524	-0.768	1.984
	BET3	2.443	0.140	-1.653	14	0.121	-0.117	0.070	-0.268	0.035
	PBET3	0.966	0.342	-1.599	14	0.132	-0.088	0.055	-0.207	0.030
	DPT3	3.088	0.101	1.463	14	0.166	0.698	0.477	-0.325	1.721

[1] A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.

Table 8. Independent sample t-tests for linear measurements of the lower first molars and second upper molars. Those variables which reject Levene's hypothesis (<0.05) for equal variances are tested using the SPSS corrected t-test.

Tooth type (FDI)		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Lower First Molar	A	3.038	0.115	1.063	9	0.316	0.280	0.264	-0.316	0.877
	TCT1	5.930	0.038[2]	-1.021	3.433	0.374	-0.480	0.470	-1.873	0.914
	BET1	0.891	0.370	0.113	9	0.913	0.004	0.036	-0.078	0.086
	LET1	0.679	0.431	0.002	9	0.999	0.000	0.040	-0.090	0.090
	PBET1	4.780	0.057	0.470	9	0.649	0.017	0.035	-0.064	0.097
	PLET1	0.118	0.739	-0.398	9	0.700	-0.014	0.036	-0.097	0.068
	DPT1	5.785	0.040[2]	-1.058	3.302	0.361	-0.483	0.457	-1.865	0.898
	TCT2	69.007	0.000[2]	-0.960	3.206	0.404	-0.716	0.746	-3.007	1.575
	BET2	1.653	0.231	-1.526	9	0.161	-0.182	0.119	-0.452	0.088
	LET2	0.060	0.812	-1.676	9	0.128	-0.143	0.085	-0.336	0.050
	PLET2	0.387	0.549	-2.061	9	0.069	-0.151	0.073	-0.317	0.015
	DPT2	10.792	0.009[2]	-0.574	3.463	0.601	-0.391	0.680	-2.401	1.620

[1] A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.

Table 8. Continued.

Tooth type		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t[1]	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Upper Second Molar	A	1.388	0.277	-0.504	7	0.630	-0.126	0.249	-0.715	0.464
	TCT1	1.542	0.254	-0.227	7	0.827	-0.084	0.372	-0.965	0.796
	BET1	5.414	0.053	0.641	7	0.542	0.047	0.074	-0.127	0.222
	LET1	0.142	0.718	-0.677	7	0.520	-0.052	0.077	-0.234	0.130
	PBET1	4.082	0.083	0.395	7	0.704	0.027	0.069	-0.135	0.189
	PLET1	0.017	0.901	-0.617	7	0.557	-0.050	0.081	-0.242	0.142
	DPT1	3.430	0.106	-0.232	7	0.823	-0.080	0.345	-0.896	0.736
	TCT2	3.903	0.089	-0.221	7	0.831	-0.064	0.289	-0.746	0.619
	BET2	3.099	0.122	0.545	7	0.603	0.099	0.181	-0.330	0.527
	LET2	0.451	0.523	-0.184	7	0.860	-0.037	0.200	-0.511	0.437
	PBET2	0.970	0.358	0.383	7	0.713	0.047	0.124	-0.245	0.340
	PLET2	0.119	0.741	-0.142	7	0.891	-0.023	0.159	-0.398	0.353
	DPT2	1.040	0.342	-0.501	7	0.632	-0.126	0.252	-0.722	0.470

[1]A positive critical t-value indicates a larger male mean whereas a negative value indicates a larger female mean.
[2] Equal variances not assumed; corrected t-value used.
* Significant at the 0.05 level.

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