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Primate enamel development with emphasis on South African Plio-Pleistocene fossil hominids

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CHAPTER 2

BACKGROUND FOR THIS STUDY

This chapter reviews aspects of the embryological processes involved in tooth development as well as the mechanisms of genetic control. Growth of enamel tissue is described from a structural and biological perspective. In addition, growth tracks as well as other microstructural features preserved in the enamel of the Hominoidea and their taxonomic and developmental applications are discussed.

SECTION 1- Embryological development and genetic control

2.1. Tooth Morphogenesis

The development of teeth involves complex processes of heterotypic and homotypic cell interactions followed by differentiation of tissue-specific secretory cells (Thesleff & Hurmerinta 1981). Tooth morphogenesis is similar to the development of other organs in that tissue interactions are mediated by reciprocal signals from ectodermally derived epithelium and neural crest mesenchyme (Tucker & Sharpe 1998). These signals induce or inhibit the expression of genes and molecules in tightly controlled stage-dependant sequences during odontogenesis (Jernvall & Thesleff 2000). Cell differentiation during odontogenesis is specific to the secretion of dental tissues. Epithelially derived ameloblasts secrete enamel matrix while odontoblasts forming on the opposite side of the folding basement membrane containing mesenchymal cells, secrete dentine matrix (Lumsden 1988).

The development of the dentition has a regional (tooth type or tooth family specification site) and temporal (e.g. deciduous vs. permanent teeth and sequential development of permanent teeth) component (Mackenzie *et al*.;

1992). Most studies on tooth development have been conducted on mice. However, the genetic bases controlling the different developmental stages are governed by homeobox genes and molecule complexes (Sharpe 1995) which are highly conserved in the animal kingdom (Carroll *et al*.; 2001) and thus the same principles of development can be applied to the formation of primate teeth (Wood 1995).

The origin and development of tissues and cell types involved in tooth morphogenesis as well as the process of amelogenesis are reviewed in the following sections.

2.1.1. Early developmental phases

During gastrulation, embryos become arranged into three concentric layers termed “germ layers” (ectoderm, mesoderm and endoderm). Cells outside the gastrula are epithelial cells and constitute the ectoderm. These cells are bounded by tight junctions and may consist of one or multiple layers of cells which rest on a sheet called basal lamina. The lamina is mainly composed of collagen and acts as the contact surface between tissues allowing for cellular communication (Moss-Salentjin & Hendricks-Klyvert 1990). The ectodermal cells are polarized with the apical surface facing away from the basal lamina. The inner germ layer, the endoderm, also consists of epithelial cells (Figure 2.1). Finally, the mesoderm contains mesenchymal cells, which are less tightly connected and more mobile.



Figure 2.1. Schematic drawing of the different embryonic tissues referred to in the text.

The neural plate folds over to form the neural tube which gives rise to the future brain and spinal chord. During closure of the neural tube, adjacent ectodermal cells which will form epidermal tissue, cover the neural tube, which now lies in the mesodermal area (Figure 2.2). When the tube finally detaches from the epidermal cells, a group of cells which are located at the crest area form a cell lineage called neural crest cells (NC) (Figure 2.2). These cells migrate to other developing organs giving rise to bone, cartilage, connective tissue and some dental tissues in the facial area. Neural crest cells express regulatory genes of the *Dlx*, *Msx* and *Pax* families (Carroll *et al*.; 2001) which are involved in the development and patterning of teeth (Jernvall & Thesleff 2000).

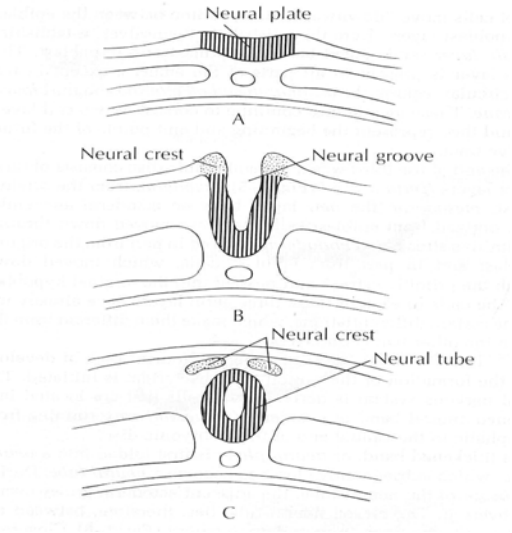


Figure 2.2. The neural plate folds during neurolation to form the neural tube. A cell linage located along the crest becomes de-attached (NC cells) which then move to other areas of the embryo. NC cells are involved in the development of dental tissues.

One important feature of the embryo at this stage is the formation of the pharyngeal arches, which are derived from the pharynx and adjacent mesodermal tissue. The oral area develops from the first pharyngeal arch and NC cells, first forming the mandibular and later the maxillary arches (Moss-Salentjin & Hendricks-Klyvert 1990). The brain organ forms prominences known as rhombomeres (r). It is from the midbrain and hindbrain rhombomeres that NC cells

migrate to different sites (proximal or distal) of the developing oral area (Imai *et al*.; 1996; Kontges & Lumsden 1996). Soon after the oral area has formed, tooth morphogenesis is initiated. This process involves the interaction of mesenchymal NC cells, epithelial cells from the oral area and basement membrane (Slavkin & Bringas 1976). Epithelial cells invaginate into the underlying mesenchyme and proceed to fold downwards incisally over the mesenchymal area. This process continues to form the tooth apex and finally the root (Osborn & Ten Cate 1983). Tooth development is a continuous process which can be divided in several stages referred to as dental lamina, bud, cup, and bell stage (Ruch 1990) (Figure 2.3). These stages are the same in the development of any tooth.

2.2. Tooth Formation Stages

2.2.1 Bud Stage

Following signals emanated from the mesenchyme, the first histological indication that the tooth is forming is a localized increase in mitotic activity of epithelial cells (Mackenzie *et al*.; 1992). These cells undergo changes in the orientation of the mitotic spindle to form the dental lamina (Ruch 1990) (Figure 2.3).The epithelium then invaginates into the underlying mesenchyme (Radlanski 1993). Invaginations occur at sites of tooth bud formation (Vainio *et al*.; 1993).

In the human embryo, 10 invaginations have been recorded (Radlanski 1993), the first at about 6 weeks (McCollum & Sharpe 2001). Budding in the mandibular region takes place earlier than in the maxillary area (Radlanski 1993). The underlying mesenchymal cells condense to form the supporting tissues during tooth development: the dental follicle and dental papilla (Ferguson *et al*.; 2000) (Figure 2.4).

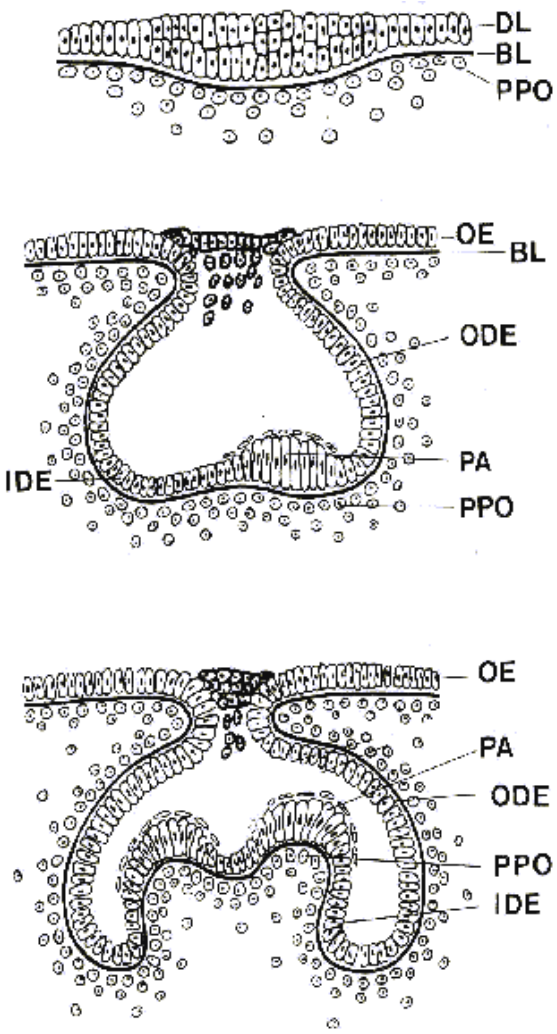


Figure 2.3. Schematic representation of the different stages of tooth development after Ruch (1990).Top figure represents the development of the dental lamina(DL) formed by oral epithelial cells with a modified orientation of the mitotic spindle. The basal lamina (BL) formed by collagen separates presumptive- pre-odontoblasts (PPO) from the DL allowing cell-tissue communication. In the first stages of tooth development, epithelial cells invaginate into the underlying mesenchymally derived PPO cells to form the tooth bud.

The middle figure represents the bud stage of tooth development. Cells of the IDE (inner dental epithelium) increase their mitotic rate at the PA (pre-ameloblasts) area. OE (outer epithelium).

The bottom figure represents a tooth germ in early bell stage. The pre-ameloblasts of the first developed epithelial fold are in a more advance stage of development and soon will become mature cells and will initiate enamel protein secretion. See next section of this chapter for details.

The transition from bud to cap stage marks the onset of crown development and the appearance of a signalling centre known as the enamel knot (Jernvall & Thesleff 2000). The enamel knot will be discussed in section 2.1.4.

2.2.2 Cap Stage

The tooth germ at this stage has a spherical shape contained within a follicle of connective tissue (Butler 1956) (Figure 2.4). It is during the cap stage that cell differentiation begins inside the germ (Osborn & Ten Cate 1983) and tooth cusp formation is initiated (Ruch 1990). The dental lamina, which is still folding, separates the dental papilla situated at the basal end and containing mesenchymal cells, from the enamel organ containing three different cell layers:

the inner and outer epithelium, and the *stellate reticulum* (Butler 1956) (Figure 2.4). Growth of the enamel organ and the dental papilla is fast exerting reciprocal pressures on their individual growth trajectories. The cells of the *stellate reticulum* secrete a hydrophilic polysaccharide which results in an increase of the intracellular space. As the cells are connected via desmosomal junctions, an increase of the intracellular fluid results in these cells taking a star-like appearance, and hence the name (Zhao *et al*.; 2000). This increase of intracellular fluid results in an increase in the pressure to which the enamel organ is subjected, but also probably protects the inner epithelium from distortion due to the growth of the dental papilla (Butler 1956). The inner and outer epithelial layers play very distinct roles. The outer enamel epithelium regulates water intake into the follicle and do not undergo mitosis (Butler 1956). Differential mitotic rate are observed in cells of the inner epithelium (pre-ameloblasts) increasing their mitotic rate from a 10 hour cell cycle to 1.2 hours (Ruch 1990). Experimental evidence has confirmed that this mitotic activity is controlled by the dental papillae (basal lamina) (Olive & Ruch 1982). Outer epithelial cells when in contact with this matrix experience an increase in mitotic rate. Wild type does not increase mitotic rates (Olive & Ruch 1982). It remains unknown what mechanisms initiate cell competence of mature ameloblasts but it has been proposed that it is controlled by the number of cell cycles (Amar *et al*.; 1989) which in turn is determined by ameloblasts and odontoblasts genetic programmes (Lesot 1991).

The cells of the inner epithelium join the outer enamel epithelium at thecervical loop. This region of the developing tooth generates the production of the different cell types within the follicle. The expression of fibroblast growth factor (FGF) 10 in the cervical loop appears to be necessary for cell differentiation (Kawano *et al*.; 2003).

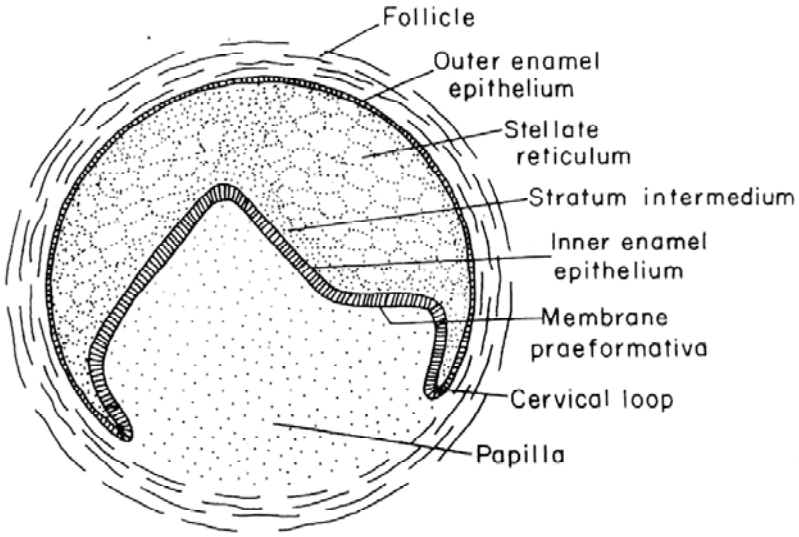


Figure 2.4. Schematic representation of a tooth germ during the late bell stage of development indicating the different tissues within the enamel organ (after Butler 1956).

2.2.3. Bell Stage

It is during the bell stage that the final pattern of crown morphology is established via the number of folds in the inner enamel epithelium. This layer continues mitosis and grows over the dental papilla which has decreased its mitotic activity at this point (Butler 1956). A new cell layer in the enamel organ appears during the bell stage, the *stratum intermedium*, whose probable function is to manufacture materials which will form part of the enamel matrix (Osborn & Ten Cate 1983, Sasaki 1990). The junction between the enamel organ and the dental papilla forms the enamel/dentine junction as the lamina breaks down (Osborn & Ten Cate 1983). This layer will become the region from where ameloblasts begin enamel matrix secretion. As crown development proceeds towards the cervical end, the enamel organ forms a cuff which extends out as a tube called the Hertwig's sheath, which marks root formation (Hillson 1996).

2.3. Genetic controls and tooth identity

Tooth morphogenesis is a good model system that illustrates general principles of epithelial and mesenchymal cell interactions during development (Jernvall

2000). The inductive role in signalling sequences shifts between the epithelium and mesenchyme during tooth development (Kollard & Baird 1970). These signals involve the expression of a variety of homeobox-containing genes and transcription factors (McCollum & Sharpe 2001). The most important are Bone Morphogenetic Proteins (BMP), Fibroblast Growth Factors (FGF) and the Wnt and Hh families (Jernvall & Thesleff 2000). These molecules induce and/or inhibit the expression patterns of homeobox transcription factor genes at different sites along the mandible and maxillary areas, allowing regional development and differentiation of tooth types (Ferguson *et al*.; 2000). How the patterning of tooth type differentiation occurs, has been critical issue in tooth morphogenesis.

A morphogen-based model proposed that the development of the dentition took place and was controlled by a “morphogenetic gradient”. Thus, a “field of induction” in specific regions (in this case the oral region) produces a substance (morphogen) which diffuses in the growing region. Depending on the concentration of this morphogen along the dental primordia, different tooth types develop (Butler 1967). In this model, primordia tissue has an equivalent power to develop various tooth types. It is the concentration levels of the morphogen which determines the tooth type (Ruch 1987).

Osborn (1978) proposed a different model referred to as the “clone model”. Homogeneous self proliferating cell groups differentiate from a single unit or clone. This clone grows posteriorly generating new primordia after a number of cell divisions as the clone expands. Three dental clones are formed which correspond to the growth of incisors, canines and molars. The formation of new primordia is regulated by the inhibitory action of the previous primordia. As cells self proliferate and divide, each new generation has undergone more divisions than the previous one and thus a “cell linage” is established. A gradient exists in the cell lineage. The resulting structures are therefore a combination of the

developed primordia and the gradients of this cell linage (Osborn 1978). This model differs from the morphogenetic gradient model in that the proliferation of cells is self induced and that each primordium is different (Ruch 1987). This model has been experimentally supported by the work of Lumsden (1979) in which fragments of mouse mandibles were grafted into the anterior eye chamber of a fly giving rise to full molar dentition.

Recently, a new model has been proposed based on advances made in identifying the homeobox-containing genes and their field of expression. Interactions between BMP, FGF and other signalling molecules govern the induction/inhibition of transcription factor genes whose domains are associated with the development of incisors or molars (no canines or premolars in adult murine dentition). The Odontogenic Homeobox Code (OHC) has been proposed to identify sites of tooth type development (Sharpe 1995; Thomas *et al*.; 1998). This model was influenced by the discovery that in mice lacking some *Dlx* homeobox genes molar development was arrested (Qui *et al*.; 1997). *Dlx* genes are associated with the proximal mandibular arch or presumptive molar (multicuspid) field and their mutants only affect molar but not incisor development (Thomas *et al*.; 1998). In other instances, the spatial information of tooth sites is provided by the overlapping domain of certain genes. *Barx 1* for example is associated with molar cells and *Msx 1* with incisor cells (Tucker *et al*.; 1998). When *Barx 1* is negatively expressed and *Msx 1* is positively expressed, incisors will develop. When *Msx 1* is negatively expressed and *Barx 1* is positively expressed, molars will form. Thus, the boundary between cells that express *Msx 1* and *Barx 1* determines the development of molars or incisors (Tucker *et al*.; 1998). A number of genes inducing the expression of incisor development (*Msx 1*, *Msx 2* and *Alx 3*) and molar development (*Barx 1* and *Dlx 2*) have been identified (e.g. Zhao *et al.*; 2000; McCollum & Sharpe 2001). It has been proposed that the development of canines and premolars

(tooth types absent in mature mice) occurs by the overlapping expression of perhaps *Dlx* and *Msx* genes (Tucker & Sharpe 1999; McCollum & Sharpe 2001). However, given that the upper and lower jaws derive from different embryonic tissues (Zhao *et al*.; 2000) and that upper and lower dentition are under different genetic controls (Qui *et al*.; 1997; Thomas & Sharpe 1998); the OHC model still remains to be conclusively demonstrated (Zhao *et al*.; 2000).

2.4. The Enamel Knot as Key Determinant of Tooth Shape

The shapes of mammalian tooth crowns develop by the inward folding of inner enamel epithelium (Butler 1956). The number of cusps present in a tooth depends on the number of folds of the epithelial layer (Jernvall 1995a). During the cap stage of tooth morphogenesis, a cluster of non-dividing cells called the enamel knot appear in the centres of epithelial folds (Jernvall & Jung 2000). This structure was initially recognized by Ahrens (1913) and Butler (1956) (Figure 2.5).

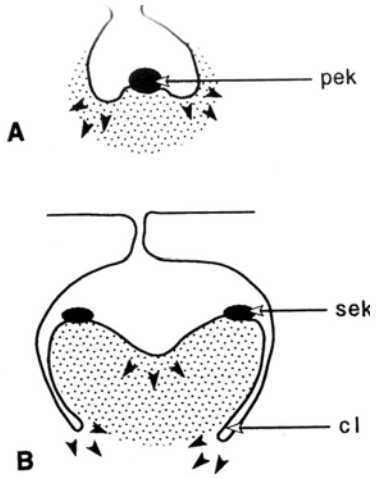


Figure 2.5. Simplified representation of the positions of primary (pek) and secondary (sek) enamel knots. Arrows indicate the direction of growth. Diagram taken from Zhao *et al*.; 2000.

The enamel knot acts as a signalling centre (Vaahtokari *et al*.; 1996) whose function is similar to that of other signalling molecules during mammalian organogenesis such as apical ectodermal ridge (AER) or zone of polarizing activity (ZPA) (Jernvall 1995b). The primary (also called first) enamel knot is

located in the central part of the dental epithelium facing the dental mesenchyme (Vaahtokari *et al*.; 1996). The cells of the enamel knot have a different gene expression pattern to the rest of the tooth germ and contain more than 10 signalling molecules of the BMP, FGF, Hh and Wnt families (Jernvall & Thesleff 2000). The primary enamel knot however (the only enamel knot in single cuspid-teeth), has a different gene expression to the secondary knots (present in multicuspid teeth) (Jernvall & Jung 2000). The lifespan of the enamel knot is short. The disappearance of this centre due to cell apoptosis appears to be associated with the presence of *Bmp4* (Jernvall *et al*.; 1998).

Although growth stimulating *Fgfs* are present in the enamel knot, it is non-proliferative and includes the inhibitor *p21* (Parker *et al*.; 1995; Jernvall & Jung 2000). Secondary enamel knots quickly appear in multi-cuspid teeth at the places of future cusp tips after apoptosis of the primary enamel knot (Jernvall & Thesleff 2000b). The shape of the cuspal area in multi-cuspid teeth is controlled by the inter-relationship of the spatial and temporal pattern of the enamel knots (Jernvall & Jung 2000). Tooth development begins at the tallest cusp (dentine horn). The folding of the inner enamel epithelium by the secondary enamel knots proceeds until the smallest cusp is formed (Butler 1956). Thus, the shape and relief of the cuspal surface of a multi-cuspid tooth essentially depends on the number of enamel knots, their spatial closeness and the time difference between the onsets of their formation (Jernvall & Jung 2000).

Jernvall (2000) has proposed the *Cascade Mode of Cusp Spacing* to explain a possible path for the evolution of new cusps, as well as the cusp arrangement pattern of the occlusal tooth surface. Jernvall (2000) interpreted that a broad inhibition field (non proliferation of cells) around the primary enamel knot will displace the location and delay the onset of formation of the secondary enamel knots. This will have an effect of the distance between the cusps and their relative heights, determining the morphology of the tooth (Figures 2.6 a,b).

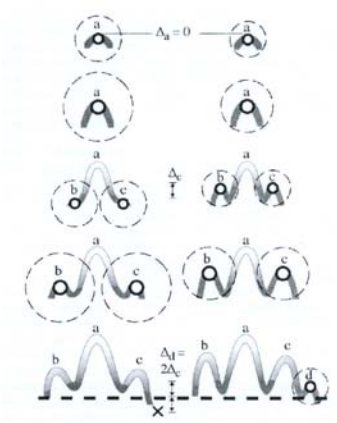


Figure 2.6b

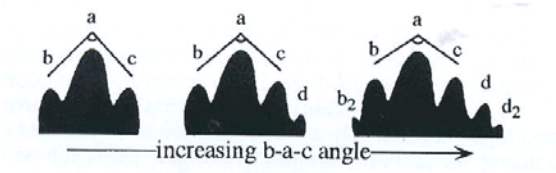


Figure 2.6a

Figure 2.6a & 2.6b. Figure 2.6b indicates the spatial separation of the different cusps in *Phoca* according to the inhibition field generated by the first forming enamel knot. Smaller inhibitory fields correspond with less widely spaced cusps, which allows the formation of more enamel knots and therefore more cusps (Figure 2.6a).

The Cascade Mode model was tested on a population of seals of the same species which displayed large degree of variation on the number of accessory cusps (Jernvall 2000). It was proposed that the angle formed by the tallest of the cusps in relation to the second and third main cusps (i.e. the distance between the enamel knots of each cusp) influenced the presence and number of accessory cusps (Jernvall 2000). More acute angles were associated with a reduced number of cusps (Figures 2.6a & 2.6b).

An important point discussed by Zhao *et al*.; (2000) is that at present, it appears that it is the pattern of the entire crown and not individual cusps that is under genetic control. These authors have interpreted this information to suggest that while the process of crown patterning maybe homologous among species, there maybe not be strict homology for individual cusps (Zhao *et al*.; 2000).

SECTION 2- Amelogenesis and enamel structure

2.6. Amelogenesis

The cytodifferentiation of ameloblasts begins after completion of their mitotic phase following signals from the already functional odontoblasts (Karcher-Djuricic *et al*.; 1985). Enamel formation undergoes two phases: secretion and maturation. The former relates to the changes which transform epithelial cells of the enamel organ to pre-ameloblasts via ultrastructural, size, and polarity changes in these cells. It also concerns the transformation of these polarized cells into enamel matrix secretory cells (Moss-Salentjin *et al*.; 1997). Maturation involves ion uptake, loss of protein matrix, and mineralization.

Early ameloblast cytodifferentiation involves a series of changes marked by an increase in the number and size of organelles, especially those implicated in the synthesis and or secretion of proteins (Sasaki 1990). Epithelial cells of the enamel organ located near the basement membrane are randomly arranged before pre-ameloblast development. Later, these cells begin to elongate to become pre-ameloblasts attaining 25 μm high on average and position themselves parallel to each other at sites distally located from the cervical loop along the basal membrane (Skobe *et al*.; 1981). The membrane disappears as the pre-ameloblasts become differentiated cells.

Differentiated ameloblasts consist of tall columnar cells (50 μm high) with polarized intra-cellular structures (Reith 1960) (Figure 2.7). Very small intercellular spaces exist between ameloblasts, which are closely apposed to each other (Boyde 1989). Externally, at the terminal end of the differentiating ameloblasts, junctional complexes between adjacent ameloblasts develop as well as a network of microtubules, filaments and terminal webs (Sasaki 1990) (Figure 2.6). Gap junctions are also present and have been suggested to serve as cell-cell communication mechanism which coordinates the movement and activity of the ameloblast layer (Warshawsky 1978).

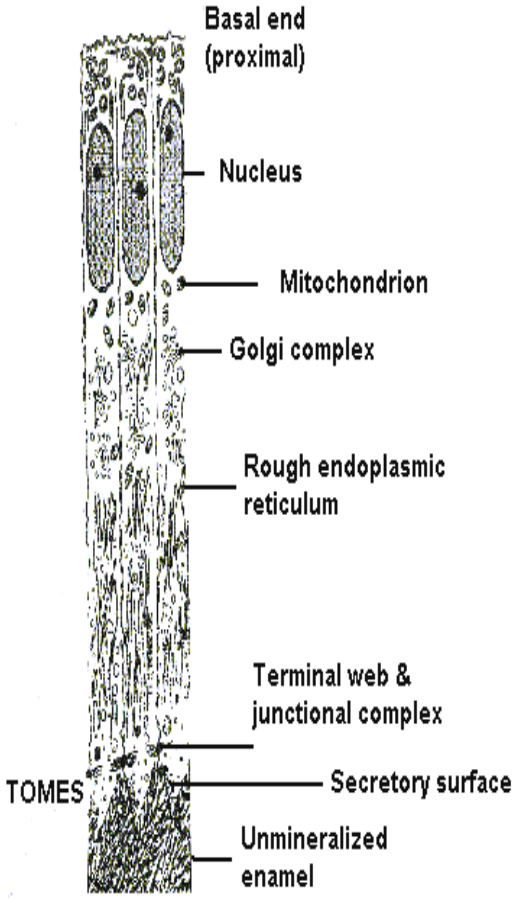


Figure 2.7. Schematic representation of mature ameloblasts during the secretory phase. These tall columnar cells have undergone some ultrastructural changes. The locations of the main cell organs during this phase are shown. The cell's secretory structure, the Tomes process, is located distally or apically. At this stage, enamel is unmineralized and consists mostly of proteins (amelogenins and enamelins).

The cell organs contained in the proximal end are the nucleus and the mitochondria, while the distal end contains the endoplasmatic reticulum. The Golgi apparatus moves towards the distal endoplasm prior to ameloblast differentiation. Enamel matrix is transported via granules to a cell process, the Tomes process, located at the distal end of the cell to which the granules fuse. Matrix is exocytosed through the Tomes process but how exactly exocytosis takes place is a matter of debate (Simmelink 1982). The Tomes process is unique to the secretory ameloblasts and is therefore the cell organ responsible for enamel matrix secretion. Its morphology plays an important role in the process of enamel prism arrangement (Warshawsky *et al*. 1981) (see next

section) although the mechanisms that control its development and morphology are not clearly understood (Sasaki 1990). The Tomes process can be striated or ruffle-ended, and smooth-ended (Reith & Boyde 1981; Sasaki 1990). The former appears at the beginning of the maturational stage and is functionally related to the calcification of maturing enamel (Sasaki 1990). In addition, secretory and non-secretory regions can be recognized (Moss-Salentjin *et al*.; 1997) (Figure 2.7).

Initiation of extra-cellular enamel matrix secretion appears to be controlled by the presence of odontoblasts (Karcher-Djuricic *et al*.; 1985). It has been proposed that the cytodifferentiation of ameloblasts is correlated with the presence of pre-dentine, while enamel secretion is programmed by dentine tissue (Slavkin & Bringas 1978). The early stages of enamel matrix secretion near the enamel-dentine junction may occur before the formation of the Tomes process, which results in the formation of aprismatic enamel (Moss-Salentjin *et al*.; 1997). Enamel matrix is secreted from the distal end of the cell (Sasaki 1990; Risnes 1998). Matrix secreted at the end of the Tomes process become arranged in a perpendicular fashion to the cell's long axis, whereas protein secreted form the sides of the Tomes Process become arranged at an angle (Paine *et al*.; 2001). At the proximal end, the ameloblasts are in contact with the cells of the *stratum intermedium* as they travel away from the dental papilla. Ameloblasts move in coordinated groups maintaining a cohesive front or ameloblastema (Boyde 1989; Risnes 1998). The exact forces or the possible mechanisms that control ameloblast movement are not yet clear. Two main models have been proposed. The first model (Boyde 1978) indicates that genetic mechanisms determine the ameloblastic path. However, Osborn (1970) proposed that biophysical processes, related to intra and extracellular pressures exerted during exocytosis and independent of genetic influence control the movement of ameloblasts.

Newly secreted enamel matrix contains a high component of organic matter, mostly proteins, and only becomes a mineralized network of crystals after maturation (Robinson *et al*.; 1983). Prior to the maturation phase of the enamel matrix, ameloblasts undergo cellular changes that involve a decrease in height, loss of protein synthesizing organelles and the disappearance of the Tomes process (Moss-Salentjin *et al*.; 1997). After the loss of the Tomes process, the last segment of enamel secreted by the ameloblasts near the outer enamel surface (OES) forms rodless enamel. At this stage, the *stratum intermedium*, *stellate reticulum* and outer enamel epithelium are no longer present (Sasaki 1990).

2.7. Enamel matrix and Mature Enamel

Enamel matrix is composed of three main kinds of proteins, which amount to 80-90% of the total volume of the tissue. The remaining components are water and a 10-20% of mineral content (Moss-Salentjin *et al*.; 1997). The largest protein content at this stage is composed of the hydrophobic protein *amelogenin*, which forms 90% of the protein volume (Hu *et al*.; 2001). This protein self-assembles extracellularly forming nanospheres which interact with the growing hydroxyapatite crystals influencing the orientation of the crystals – see next section- (Paine & Snead 1997; Paine *et al*.; 2001). Mutations of *amelogenin* gene on the X chromosome cause a widely recognized enamel defect known as amelogenesis imperfecta (Snead *et al*.; 2002).

The second most prominent enamel structural protein is *ameloblastin*. A third type of protein, the hydrophilic *enamelins* are found in smaller quantities (Fincham *et al*.; 2000). These proteins have different immunological properties, ph tolerance and molecular weights (Boyde 1989) Although the specific functions of these proteins are not very well understood, *amelogenin* appears to be involved in controlling the growth and the bounding of the enamel crystals (Hart *et al*.; 2002). Hart *et al.*; (2002), have reported hypoplasia and

hypomineralization of mature teeth induced by defects produced in the formation of *amelogenin*. Similarly, Paine *et al.*; (2001) observed that overexpression of *amelogenin* influences crystallite habit and enamel rod morphology, thus potentially influencing tooth function (Paine *et al*.; 2001). Interestingly, the aminoacid sequence of amelogenin is highly conserved across species (Paine *et al*.; 2001).

The sites of protein deposition vary according to the protein type. *Amelogenin* is dispersedly deposited while *enamelins* are deposited with their long axes perpendicular to the membrane of the Tomes process (Moss-Salentjin & Hendricks-Klyvert 1990; Paine *et al*.; 2001) and *ameloblastin* in the interod enamel (Dhamija *et al*.; 1999). Enamel protein secretions ceases at the cemento-enamel junction. This event is associated with the disappearance of the cells of the *stellate reticulum* and *stratum intermedium* and the joining of the inner and outer epithelium (Hu *et al*.; 2001).

Maturation of the enamel matrix consists of the rapid degradation of the protein content, and rapid absorption of mineral salts (Osborn & Ten Cate 1983). This process is initiated shortly after the matrix has been secreted (secretory phase) and continues into the maturational phase (Boyde 1989). Mineral absorption is associated with a reduction in height by 50% of the ameloblasts and the loss of the Tomes process (Robinson *et al*.; 1982). Protein degradation affects mostly the disappearance of *amelogenins*, while *enamelins* are conserved in an altered state in mature enamel (Termine & Young 1991).

These two proteins form supporting structures for the ensuing phase of mineral uptake. The three main mineral salts involved in the maturation of enamel are calcium, phosphate and fluoride (Bawden *et al*.; 1982) but others, magnesium and potassium, are also present. Rates of mineral uptake vary in different species (Boyde 1989). The precipitation of apatite crystals requires an increase of inorganic ions. The mitochondria play an important role at this stage

as it stores calcium ions (Eisenmann *et al*.; 1982). It appears that the first crystalline structures (crystallites), which have needle-like shape, are formed in vesicles bound to the protein content of the enamel matrix (Osborn & Ten Cate 1983). These crystallites grow rapidly forming clusters of mineralized matrix. Extracellular calcium ions appear to be incorporated from cell organelles via the ameloblast cell membrane, a process controlled by the enamel organ (Eisenmann *et al*.; 1982; Reith 1983). Calcium uptake takes place during the maturation phase. In contrast, the largest fluoride uptake occurs during the secretory phase with very little uptake during the maturation phase (Bawden *et al*.; 1982). Mature enamel is the hardest biological tissue produced by the human body, formed by hydroaxyapatite crystals **[**$Ca_{10}(PO_4)_6(OH)_2$**]** (Cuy *et al*.; 2002).

2.8. Enamel prisms

The structure of mineralized enamel matrix consists of enamel rods and inter-rod enamel separated by prism-sheaths (Warshawsky *et al*.; 1981; Daculsi *et al*.; 1984). Composition varies between them, being the enamel rods formed by hydroxyapatite crystals and very little matrix, while the prism sheaths contain relatively more enamel matrix (Moss-Salentjin & Hendricks-Klyvert 1990). Each enamel rod is formed by individual ameloblasts, while the inter-prismatic region is the product of several neighbouring cells (Warshawsky *et al.;* 1981; Nancy & Warshawsky 1984; Risnes 1998).

The building blocks of mammalian enamel are the crystallites (von Koenigswald & Clemens 1992). Prisms or rods consist of bundles of crystallites arranged in a specific structure or pattern (Boyde 1989) and have been considered the basic structural unit of enamel (Nishikawa 1992). Their orientation has functional properties further discussed in section 2.11 (von Koenigswald & Clemens 1992). Prisms originate only a few microns from the enamel-dentine junction (EDJ) and course towards the outer enamel surface

(OES). Near the EDJ, prisms do not follow straight paths but undulate (Boyde 1969; Osborn 1990), continuing then towards the outer enamel surface (OES) in a more linear path. A few microns away from the OES prisms tend to change their trajectory reaching the OES at an angle (Radlanski *et al*.; 1985). The first enamel secreted at the cusps shows a spiral-like arrangement and has been referred to as gnarled enamel. Prisms show variation in size, shape and the way in which they appear to be arranged in relation to each other (Boyde 1964; Risnes 1998). The area covered by the OES is greater than the surface area covered by the EDJ (Kimura *et al.*; 1977) and yet the number of ameloblasts that initiate enamel matrix near the EDJ is the same of cells that reach the OES (Risnes 1998). To explain how these differences in surface area are matched by ameloblasts, it has been proposed for example that prisms increase in diameter towards the OES (Fosse 1968; Skobe & Stern 1980). However, Radlanski *et al*.; (1995) indicated that the 30% increase in surface area at the OES with respect to the EDJ is related to changes in prism orientation near the OES. Interestingly, about 50% of the ameloblasts populations that differentiate and become secretory cells die prior to the onset of the maturational phase (Smith & Warshawsky 1977). This massive cell death, according to Smith and Warshawsky (1977), appears to require the re-organization of the remaining ameloblasts to cover the larger surface area of the OES.

The presence and morphology of the Tomes process influences enamel matrix deposition and thus prism morphology (Nanci & Warshawsky 1984). The Tomes process extends beyond the junctional complexes of the ameloblasts into the enamel matrix and has variable shapes which appear to be different across species (Sasaki 1990). The projecting Tomes process into the enamel matrix forms a pit, the Tomes process pit (Boyde 1989). Two secretory areas are recognized in the distal or secretory end of the cell. The distal-most section of the Tomes process produces the enamel rod, while the most proximal area to

the ameloblast secretes the inter-rod enamel (Warshawsky *et al*.; 1981). Inter-rod enamel appears to be secreted prior to the formation of rod enamel (Warshawsky 1978). The orientation of crystals varies between these two areas partly due to the morphology of the Tomes process and partly to the binding of a specific protein, probably *enamelin* (Simmelink 1982; Nanci & Warshawsky 1984). In the distal end, crystals are deposited parallel to the direction of movement of the ameloblast, while in the proximal end the crystals are perpendicular (Warshawsky *et al*.; 1981). In between them, an abrupt change in prism orientation is evident, marking the presence of prism-sheaths (Daculsi *et al*.; 1984).

Boyde (1964, 1989) indicated that when prisms are studied in cross section they appear to form three predominant patterns based on their shape. These three predominant types of prism arrangements were used as distinguishing features at various taxonomic levels (Boyde 1964, 1989; Boyde & Martin 1984) but not at the species level. Although it appears that there is some overlap across taxa, a predominant type seems to characterize the bulk of the enamel in each taxonomic group considered (Boyde 1989, von Koenigswald & Clemens 1992). In primates for example, the prisms have a keyhole like appearance in cross section and are surrounded by inter-prismatic enamel. This keyhole shape gives the impression that the prisms have a “head” and a “tail” area. Rows of prisms in cross section are stacked with the tails of one prism layer interlocked with the heads of the second layer. These patterns can be briefly described as follows (Boyde 1964, 1989):

1) Pattern 1: Characterized by the presence of circular prism
2) Pattern 2: Horseshoe –shaped prisms stacked in longitudinal rows
3) Pattern 3: Horseshoe-shaped prisms stacked along horizontal rows

Boyde and Martin (1984) indicated that the region of the tooth which best reflects the predominant prism pattern is in the deep layers of the enamel. The predominant pattern in Hominoidea appears to be pattern 3 (Martin 1985), although patterns 1 and 2 can also be identified in human enamel (Boyde 1989). Martin (1985) associated pattern 1 with slow forming enamel and pattern 2 with fast forming enamel. In Martin's description of the predominant patterns in the great apes, it was noted that slow forming enamel characterized the outer enamel areas of *Pan* and *Gorilla* and Pongo, while fast forming enamel is found in the inner regions. This was interpreted as the cause for their thin enamel (Martin 1985). However, Beynon *et al*.; (1991) re-assessed this interpretation based on the morphology of microstructural features. Enamel thickness in *Pan* and *Gorilla* does not develop by decreasing the enamel secretion in the outer region of the enamel but by a reduction of the secretory period (Beynon *et al.;* 1991).

2.9. Development and calcification of human molar cusps

The primary or deciduous dentition develops *in uteruo*, and precedes the formation of the permanent teeth. Positional information determines patterns of cuspal arrangement and timing of tooth type development (Jernvall & Jung 2000). A sequential pattern of development is also present in the formation or calcification of the individual cusps, the development of the mesial or distal regions of the tooth and the bridging between cusps (Kraus 1965).

Knowledge about the sequential pattern of cusp formation was stimulated by controversy surrounding the issue of which cusp is phylogenetically the oldest, (now known to be the paracone; Butler 1965).The mesial, or trigonid part of the molars forms prior to the formation of the distal, or talonid region (Butler 1967). At approximately $12^{1/2}$ weeks of embryonic development in humans, a tubercle appears on the mesiobuccal region of the occlusal area of the first mandibular deciduous molar, which marks the appearance of the protoconid (Kraus & Jordan

1965). About two weeks later, a tubercle appears in the mesio-lingual area which develops into the metaconid. The appearance of the soft tissue tubercles in the posterior or talonid region may or may not be preceded by the onset of calcification of the forming mesial cusps (Kraus & Jordan 1965). Individual tooth types of the upper dentition are formed after their corresponding mandibular type has initiated their development (Osborn & Ten Cate 1983). In the maxillary molars of the primary dentition, there is a similar developmental pattern of cusp formation to the mandibular teeth. The same pattern can be observed in the permanent dentition, summarized for the mandibular molars in Table 2.1 based on Kraus (1965).

Table 2.1 Calcification sequence of maxillary and mandibular cusps (Kraus 1965).

Mandibular cusps	Calcification sequence	Maxillary cusps
Protoconid	Mesiobuccal	Paracone
Metaconid	Mesiolingual	Protocone
Hypoconid	Distobuccal	Metacone
Entoconid	Distolingual	Hypocone
Hypoconulid	Distal cusp	

The temporal delay between the formation of the first (mesiobuccal) and the last cusp (distolingual) is only a few weeks. Bridging of the individual cusps takes place after all cusps are present (Kraus 1965). The bridging of cusps is summarized in Table 2.2 following Kraus (1965).

Table 2.2. Coalescence sequence of mandibular permanent molars (Kraus 1965).

First	protoconid-hypoconid
Second	hypoconid-hypoconulid
Third	protoconid-metaconid
Fourth	hypoconulid-entoconid
Fifth	metaconid-entoconid

Calcification of the first permanent molars and central incisors begins around or soon after birth. Initial calcification of the first permanent molar takes place after 28 weeks of foetus development and probably around 32 weeks as the mean, but no coalescence of cusps has taken place at this stage (Kraus & Jordan 1965). As birth commonly takes place between 38 and 42 weeks, the neonatal line will be present in only a small percentage of the population.

SECTION 3- Deriving biological information from mature enamel

2.10. Microstructural markers preserved in enamel

There are two main periodic cycles reflected in the microstructures of mature enamel (Dean 1987; Bromage 1991; Fitzgerald 1998; Shellis 1998; Schwartz & Dean 2000). These are the short term cross striations, and long term striae of Retzius. Other microstructural structures will be additionally described below: perikymata and Hunter Schreger Bands (Figure 2.8).

It is important to remember that the enamel caps of teeth are 3-dimensional structures (Risnes 1987). Ameloblasts move in longitudinal and transverse planes, forming complex structures difficult to be fully appreciated in 2-dimensions (Macho *et al*.; 2003). Notwithstanding this fact, there is a large body of work in tooth histology (or 2-D analysis) spanning over 100 years which has elucidated many aspects of the development and growth of enamel tissue.

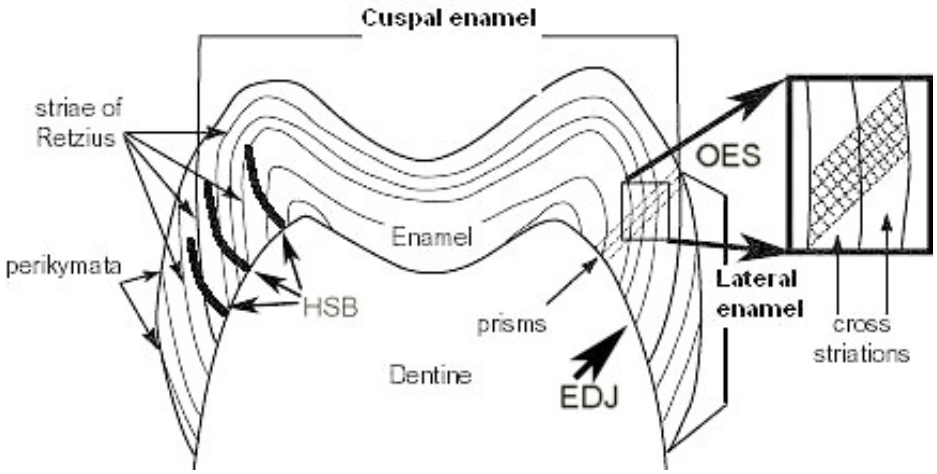


Figure 2. 8. This schematic drawing represents a cross section of a modern human molar. The main incremental markings are shown in the figure. The crown has been divided into the cuspal (or appositional) and the lateral (or imbricational) regions following Beynon and Wood (1988) (Figure modified from Ramirez Rozzi 1998b).

Thus, the analyses of enamel structures from sectioned teeth or from broken vertical surfaces, *consistent within itself*, provide strong bases for the interpretation of biological information with regards cellular behaviour during tooth growth.

2.11. Cross striations

As ameloblasts move from the EDJ towards the OES, changes in cell behaviour are reflected in the development of structures known as cross striations (Boyde 1964, 1989). These are represented as either transverse lines running perpendicular to the prisms main path, or constrictions and varicosities (Boyde 1989) depending on the microscopic technique used (i.e. SEM or transmitted light). Both features represent circadian or daily increments of enamel secreted by ameloblasts (Boyde 1964, 1989, 1990) (Figures 2.8 and 2.9). Slow secretion phases are represented by constrictions or cross striations, and fast secretory phases are regarded as varicosities (Boyde 1979, 1989). The presence of varicosities and cross striations alternate along the prism. Boyde (1990) emphasizes the point that the presence of cross striations does not represent a temporal cessation of enamel secretion, as ameloblasts do not stop their secretory activity, but rather, that there is an alternation of secretory phases between fast to slow secretion.

The distance between these transverse lines allows for 2-D measurements which can be described as the daily secretion rates or appositional rates of the ameloblasts (e.g. Schwartz & Dean 2000). The reflective properties of light at the sites where cross striations are present are different to other sites along the prism rod (Boyde 1964). Variations in mineral content have also been detected in cross striations, the fast enamel secretion phases having increased carbonate (CO_3) concentrations (Boyde 1979). Variations in sodium (*Na*) composition corresponded with the frequency of cross striations measured at ca. 6 μm (Driessens *et al*.; 1984). In addition, Simmelink and Nygaard (1982) reported differences in porosity at sites where cross striations appear.

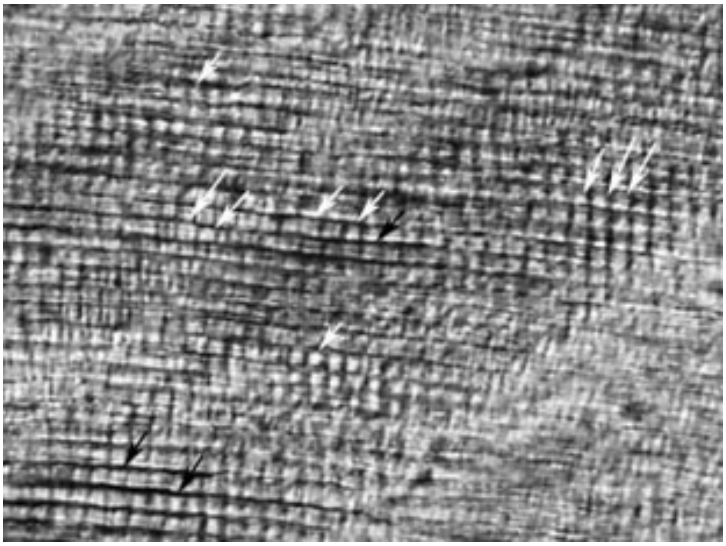


Figure 2.9. Cross striations, marked with white and black arrows, at high magnification in a modern human premolar imaged at the mid cuspal enamel. Transmitted light, FW 160 microns.

Other features, manifested also as transverse lines, have been detected along prisms which do not correspond to the cross striations. These lines, intradian lines, appear at shorter intervals than cross striations (Gustafson & Gustafson 1967; Dean & Scandret 1995; Smith *et al.*; 2003). The periodicity of these lines has been reported to be about 12 hours (Smith 2006).

2.12. Striae of Retzius

Functional ameloblasts move from the EDJ towards the OES as tight cohorts but with a slight time delay between the movement of the most occlusal and the most cervical of the secreting cells. This forms the ameloblastema or enamel forming front (Shellis 1984). As the ameloblastema moves towards the outer area of the enamel, systemic disruptions affect the secreting cells creating enamel bands known as striae of Retzius (Figures 2.10 and 2.11) (Dean 1987; Risnes 1998). Shellis and Poole (1977) referred to the striae of Retzius as “a fossilized record of the developing enamel front at a point in its history” and Wood (2000) referred to them as representing “an isochronous surface”.

Microscopically, striae appear as brown markings in transmitted light and blue in reflected light (Dean 1987).

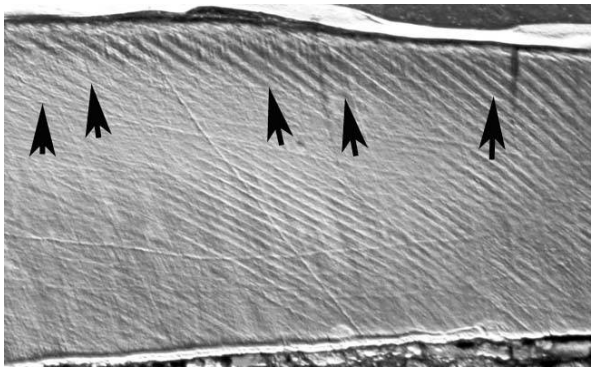


Figure 2.10. Striae of Retzius are shown in this picture running from the EDJ (left side of the picture) towards the OES (top of image) on the specimen SK 63 a canine of *Paranthropus robustus* at low magnification after acid etching. Stereoscopic microscope, FW 1.5 mm.

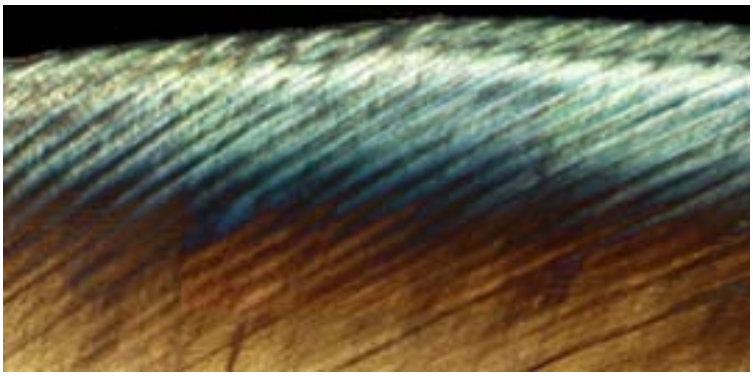


Figure 2.11. Striae of Retzius on a modern human premolar on the outer surface (top of image) of the lateral enamel at higher magnification. Transmitted light, FW 800 microns.

The aetiology of brown striae of Retzius lines is still a matter of debate (Weber *et al*.; 1974; Risnes 1990). Newman and Poole (1974: p. 1139) proposed that the striae appear as a result of an out of phase circadian cycle with other internal systems which may follow a 27 hour cycle. The same authors also considered that ameloblast apoptosis would have an effect on the secretory activity of the remaining active cells. Risnes (1990) indicated that the striae could result as the switching between a predominantly secretory to a more mineral absorptive phase

The presence of Retzius lines has been associated with changes in chemical or mineral composition (Gustafson & Gustafson 1967; Boyde 1964;

Driessens *et al.;* 1984), and changes in prism direction or bending planes (Gustafson 1959; Osborn 1973; Weber & Ashrafi 1979). Structurally, the striae of Retzius have been defined as having a staircase configuration in the outer half of the enamel (Weber *et al*.; 1971). In addition, enamel crystals appear to have a different orientation in the region of the prism forming the striae (Weber *et al*.; 1971). Risnes (1990: 144) proposed a possible mechanism which induces the formation of the striae. Striae are formed when a large amount of interprismatic material accumulates between the Tomes process and the pit, constricting the area of the pit available to the Tomes process. The movement of the Tomes process becomes limited, necessitating moving at a different angle than normal, to overcome the apparent constriction. Ameloblasts may pause at this point, however it is not known why they may do so.

Boyde (1964) interpreted that the ameloblast extension rate - the rate of differentiation of ameloblasts along the EDJ - can be assessed from the inclination of the striae. A subsequent study (Shellis 1984) elaborated on Boyde's original observations and mathematically represented this concept, which is discussed in more detail in section 2.10. Dean and Shellis (1998) and Beynon *et al*., (1998) noted that the striae is S-shaped in *Pongo*, *Proconsul* and *Hylobates* (Figure 2.12). Dean and Shellis (1998) interpreted this morphology as possible evidence for a shared developmental mechanism by these taxa. This has been recently contested by Macho *et al*.; (2003) who interpret that the S-shape only reflects biophysical processes related to the movement of ameloblasts following Osborn's (1970) model.

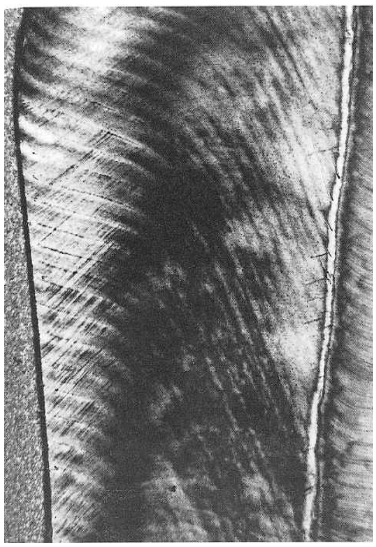


Figure 2. 12. Lateral enamel of a Pongo P_3 showing the S-Shaped striae shown in Figure 1 of Dean and Shellis (1998). It has been considered that this morphology may be of developmental and/or taxonomic value. However, Macho *et al*.; (2003) indicated that S-Shaped striae are probably the result of biophysical processes related to the movement of the ameloblasts. FW was not provided in the original.

Striae of Retzius do not appear as a generalized feature in mammalian enamel. While many extant and extinct artiodactyls and perissodactyls form striae (Macho & Williamson 2002; Macho *et al*.; 2003), some carnivores (e.g. cats and dogs) do not show this feature (Skobe *et al*.; 1985).

Abnormal striae or striae other than the regular brown striae of Retziushave been described. These are associated with disturbances occurring during amelogenesis due to illness, malnutrition and may even appear as a result of emotional stresses (Rose *et al;* 1978; Rose 1979; Bowman 1991). These lines have been referred to as Wilson lines (Rose 1979). Wilson lines can be markedly recurrent and periodic throughout the life of the organism, which in some cases can be indicative of the effects of seasonality (Macho *et al*.; 1996; Macho *et al*.; 2003). Prominent striae in the human primary dentition, the neonatal line, have been associated with the stress of birth (Weber & Eisenmann 1971). These are sometimes formed a few microns away from the dentine horn on first permanent molars or central incisors.

2.13. Perikymata

In some mammalian enamel, including that of humans, concentric rings or perikymata are visible on the outer surface of the enamel (Risnes 1984). These represent the surface manifestations of the striae of Retzius (Newman & Poole 1974) (Figure 2.13). As the striae approach the OES and ameloblasts end their secretory functions, the striae form troughs (perikymata) on the outer surface of the enamel (Boyde 1989). As perikymata represent an external manifestation of the striae, they too represent incremental growth lines (Dean 1987, 1989). All perikymata are formed by striae, however, not all striae reach the OES.

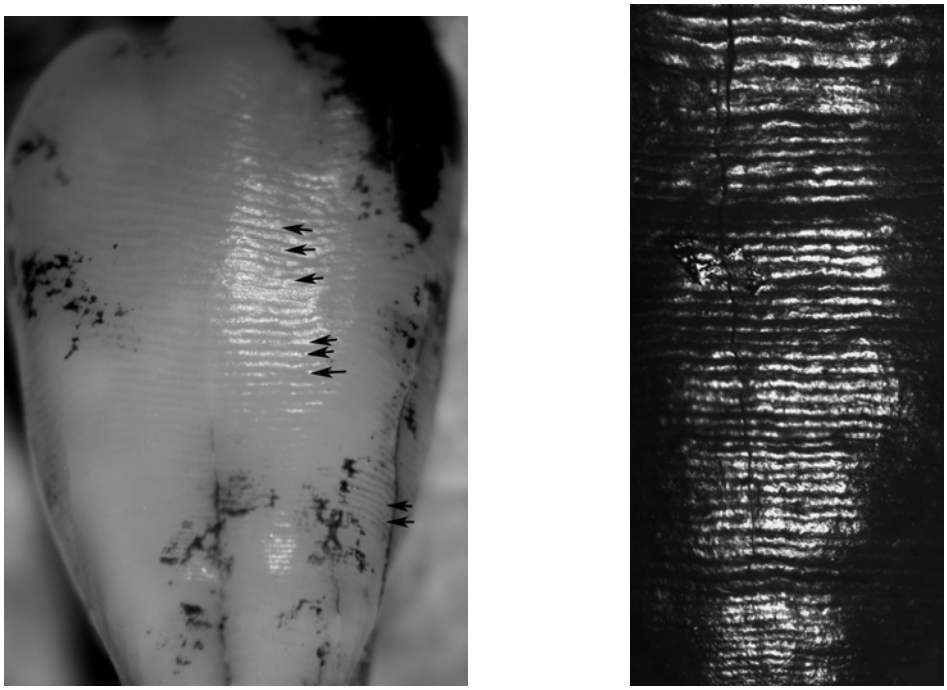


Figure 2.13a, b. Left, perikymata, marked in black arrows, shown in the labial surface of a hominid incisor using light microscopy. Stereoscopic microscope, FW 3.0 mm. Right, perikymata on a modern human incisor using confocal microscopy, FW 2.5 mm.

There are a number of incremental lines not visible from an external examination of a tooth. This aspect of enamel structure has been used to differentiate two main regions of a tooth (Beynon & Wood 1988) (Figure 2.8). Appositional, or cuspal, enamel is regarded as the area of the tooth cusp where the striae do not reach the surface. Here, the striae constitute dome-shaped layers

around the tip of the dentin (Risnes 1998). Imbricational, or cervical, enamel represents the region of the tooth where striae project to the OES and where perikymata may be present. Perikymata appear to be more numerous in anterior teeth (Dean 1987, 1989) due to the higher percentage of the crown formed by imbricational enamel.

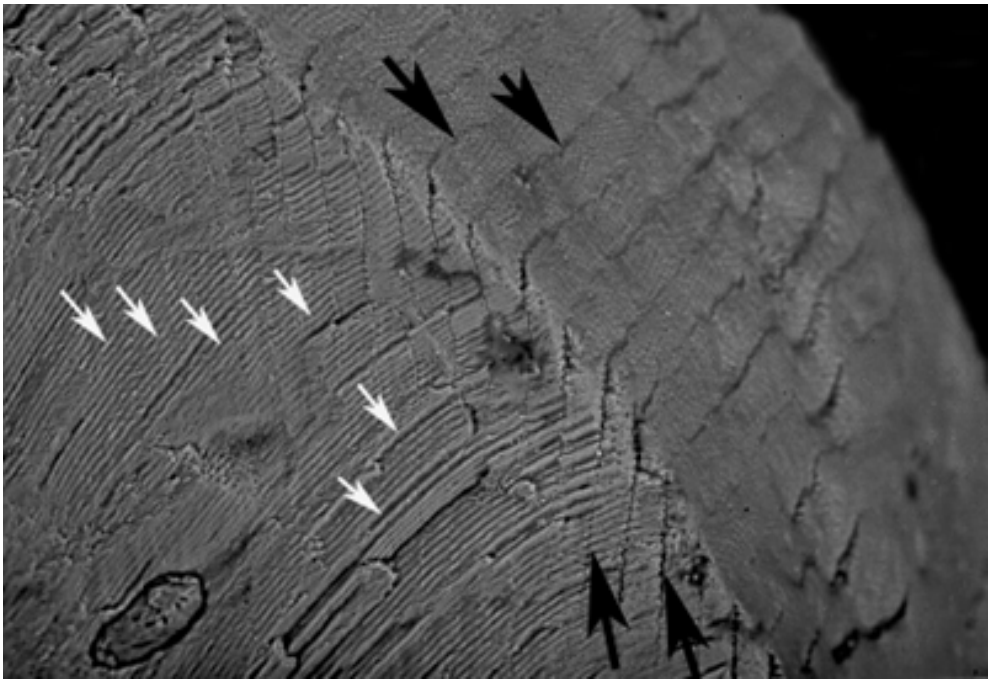


Figure. 2. 14. This SEM image, courtesy of J. Kelley and T. Smith, shows the relationship between the different enamel microstructural markers discussed in the text. Upward black arrows = Striae of Retzius, downward black arrows = perikymata; white arrows = enamel prisms. SEM image, FW 750 microns.

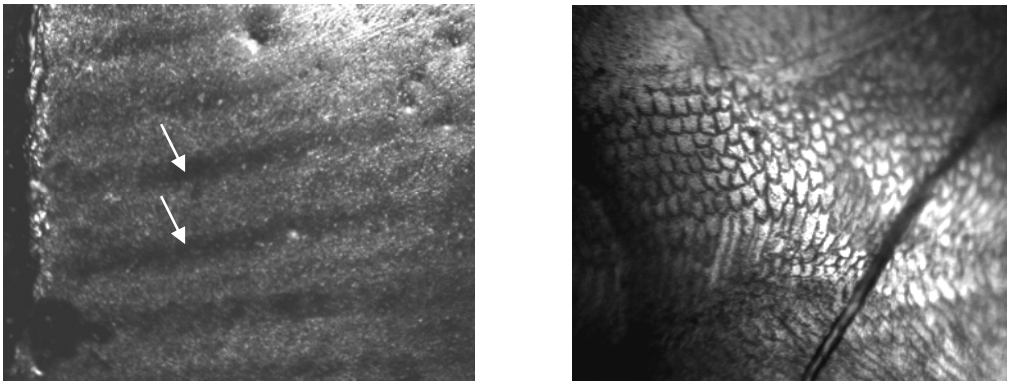
Imaged with the scanning electron microscope (SEM), perikymata appear as smooth bands pock-marked with depressions, where the enamel thickness is slightly deficient (Shellis & Poole 1977; Boyde 1989). The smooth surfaces are prismless, while the depressions represent the remnants of the Tomes process pits (Shellis & Poole 1977). Perikymata are variably expressed. In human teeth where enamel secretion slows as the ameloblasts are near the OES perikymata do not appear to be very prominent (Beynon 1992).

2.14. Hunter-Schreger Bands

In longitudinal sections, the undulating trajectory of the prisms near the EDJ gives rise to a structural phenomenon known as Hunter-Schreger Bands (HSB) (Boyde 1989). They appear as light and dark bands in reflected light (Osborn 1990). These bands have been defined as “zones in which the prisms have a common orientation property which contrasts with that in adjacent zones” (Boyde & Fortelius 1986). The HSB have been associated with functional properties of enamel as a fracture stopping mechanism (von Koenigswald *et al.*; 1987). Human teeth show that the HSB are more prominent in the inner 2/3 of the enamel (Osborn 1990). The directions of the prisms within the HSB are not parallel to the direction of HSB (Skobe & Stern 1980). X-Ray diffraction indicates that prisms twist into two sections: parazones and diazones (Hirota 1982). Parazones are formed when prism bundles are cut longitudinally in relation to their outward direction. Diazones occur when prisms are cut end-on. However, as pointed out by Osborn (1965, 1990) observations made on HSB are affected by several factors. Thinness of the sections, manner of polishing, the degree of prism orientation in successive bands and the shape of the EDJ are some of the variables that influence the shape of the HSB.



Figure 2. 15. HSB in a modern human molar using incident light. EDJ at right on the image. Stereoscopic microscope, FW 1.5mm.



Figures 2. 16 a & b. Left, HSB on a *P. robustus* molar. EDJ is at left on this image. HSB appear to be long, straight and thin in this taxon. Image on the right is a close up of the prisms cut end on (diazones). Both images were taken using PCSOM. Left image FW 950 microns, right image FW 160 microns.

2.15. Evidence for the periodicity of cross striations

In the previous sections it was noted that cross striations represent the daily (circadian) secretory activity of functional ameloblasts. It was also noted that an average of 7-9 cross striations separate adjacent striae of Retzius in apes and humans (Dean 1987; Beynon 1992). This section provides a summary of some of the experimental evidence which has documented the periodicity of these growth lines in enamel.

Schour and Poncher (1937) injected sodium fluoride to a human infant with a terminal medical condition. An average of 4 μm a day (cross striation length) between the markings was reported. Schour and Hoffman (1939) administered sodium fluoride and alizarine red to macaques and rats. A rate of 4 μm a day was given for the dentin growth of macaques. Emphasis was made on the understanding that calcification and appositional rates, although different processes, reflected the periodicity of the markings in enamel and dentin. Massler and Schour (1946) described rates of enamel growth in different human tooth types. It was noted that the rates decreased towards the EDJ. Differences were reported between the rates of permanent and deciduous teeth with a faster rate in the latter. For the first permanent molar, an average value of 4.5 μm a day was reported for maxillary and mandibular molars. Similar values were later obtained

by Shellis (1984) for permanent teeth (2.6 and 5.5 μm a day in the inner and outer enamel respectively).

Several Japanese researchers in the 1930s and 1940s applied similar methods of administering dogs, rabbits, pigs and monkeys with labelling substances. These works have been reviewed by Dean (1987) and Fitzgerald (1998). Briefly, the substances injected in animals produced marked lines in the enamel. The number of cross striations counted between these marked lines corresponded to the number of days between the injections.

Similarly, and more recently, using controlled labelling of mineralizing tissues in pig-tailed macaques (*Macaca nemestrina*), Bromage (1991) correlated the spacing between the labels preserved in the enamel with the time intervals of the injections. This correlation demonstrated the daily incremental periodicity in enamel represented by cross striations. Antoine *et al*.; (1998) used a different method in which counts were made of cross striations from the neonatal line to the last formed enamel increment in an individual for whom the age at death was known. The number of cross striations was 1190, which was very similar to the age at death of this individual (1216 days).The most recent study by Smith (2006) replicated Bromage (1991) study on a larger sample of teeth from the same sample, showing additional images of labelling and counts between these markers more clearly. Smith's (2006) findings indeed support Bromage's (1991) study and again indicate the daily nature of cross striations.

It is important to recognize that daily appositional cycles have been recorded for dentine (see section 2.18), which indicates the similar rhythm of ameloblasts and odontoblasts in the formation of their respective tissues (Schour & Hoffman 1939; Bromage 1991; Dean 1995; Dean *et al.*; 1993b; Dean & Scandret 1995; Fitzgerald 1998).

The physiological bases for the daily or circadian rhythmic cellular functions are well known in various organisms (most recently reviewed in Smith

2004, 2006). Daily or circadian rhythms can be traced at cellular levels in other organisms from *Cyanobacteria* to *Drosophila* (Dunlap 1999). Alterations in circadian rhythms have now been detected through mutations in the genes *tau* and *double-time* of hamsters and flies respectively (Young 2000). Mutations of the gene *tau* in hamsters produced 20 hour rhythm of sleep-wake cycles as well as 20 hour cycle in the production of melatonin by their eyes instead of the normal 24 hour cycle (Young 2000). Circadian rhythms are influenced by extra-cellular events, sometimes due to variation in temperatures (Liu *et al*.; 1998). As Dunlap (1999) pointed out, for a circadian clock to operate, the biochemical basis for this process has to be established. In the case of the cross striations, changes in CO_2 have been described by Boyde (1979) at sites along the enamel rods that would coincide with the average length (in microns) of cross striations. Dean (1995) pointed to changes in physiological levels of CO_2 during sleep, which may alter the levels of CO_2 in the enamel. Although this has not been tested, it could explain the chemical changes observed by Boyde (1979).

2.16. Cross striations spacing patterns

Cross striations show different growth patterns in different taxa but generally two characteristics are noted. First, the rate of secretion of ameloblasts decreases from cuspal to the cervical enamel and second, the rate of ameloblasts secretion increases from the EDJ towards the OES (measured as an increase in the length between cross striations) (Beynon *et al*.; 1991; Reid *et al.*; 1998b). Beynon *et al*.; (1991) demonstrated that in occlusal, lateral and cervical enamel of *Pongo, Pan, Gorilla* and *Homo* molars and premolars; the widths between cross-striations measured in microns (μ) increased from the EDJ to the outer enamel. Cross striation repeat intervals vary across species and in different areas of the enamel. Shellis (1984) for example reported values of 2-3 μm per day in the inner enamel which increased to 5-6 μm per day in the outer enamel of modern humans Averages for a larger number of primate species was given by Shellis (1998)

ranging from 3.4 µm per day in *Varecia variegates* to 4.4 µm per day in *Saimiri sciureus.* Table 2.3 summarizes the cross striation length in hominoids.

Table 2. 3. Values of cross striation spacing reported by Beynon *et al*.; (1991)

		Cuspal			**Lateral**			**Cervical**	
		Outer	Middle	Inner	Outer	Middle	Inner	Outer	Inner
Homo	premolars	4.9	4.2	2.7	4.9	4.1	2.8	3.0	2.3
	Molars	5.1	4.3	2.7	5.0	4.0	2.6	2.8	2.3
Pan	premolars	4.4	4.2	3.1	4.4	4.7	2.5	4.2	2.7
	Molars	5.0	4.4	3.1	4.1	4.1	3.1	3.8	2.8
Gorilla	premolars	6.1	5.3	2.9	5.9	5.6	2.8	4.3	2.7
	Molars	6.1	5.2	3.2	6.1	4.9	3.3	4.4	3.2
Pongo	premolars	4.7	4.4	3.4	4.9	4.0	3.0	4.0	3.1
	Molars	5.3	4.7	3.3	5.1	4.0	3.4	3.7	2.9

Interestingly, in a large sample (n= 69) of ground sections of molars of *P. troglodytes*, daily secretion rates appear to be consistent between different cusps, within each region of each cusp (i.e. inner, middle and outer) and between molar types (Smith 2004).

Subsequent studies (Beynon *et al.*; 1998; Dean 1998; Dean 2000; Dean *et al*.; 2001; Dean 2004) indicated that there are more subtle differences in the monthly growth of enamel than Beynon *et al.*; (1991) had suggested. Dean (1998) for example noted that when average secretion rates per month are plotted, human enamel forms more slowly for a longer period of time than in chimpanzees (Figure 2.10). This study also reported an average of 4 µm per day for *Pan, Pongo* and *Homo* molars.

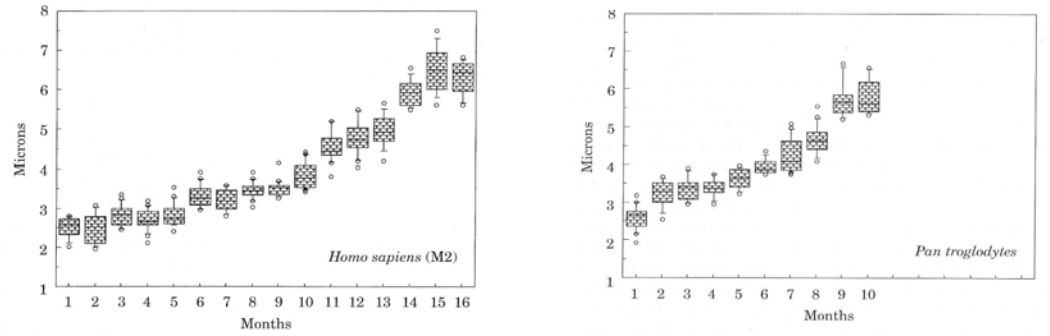


Figure 2.17. Dean (1998) showed that the growth of human enamel (left) developed at a slower rate for a longer period of time than *Pan* (right).

Dean *et al*.; (2001) showed differences in enamel formation between great apes, Miocene hominoids and hominids (Figure 2.18). These kinds of studies are critically important as they show that an adult phenotype, such as enamel thickness, can be achieved via a variety of multiple cellular mechanisms.

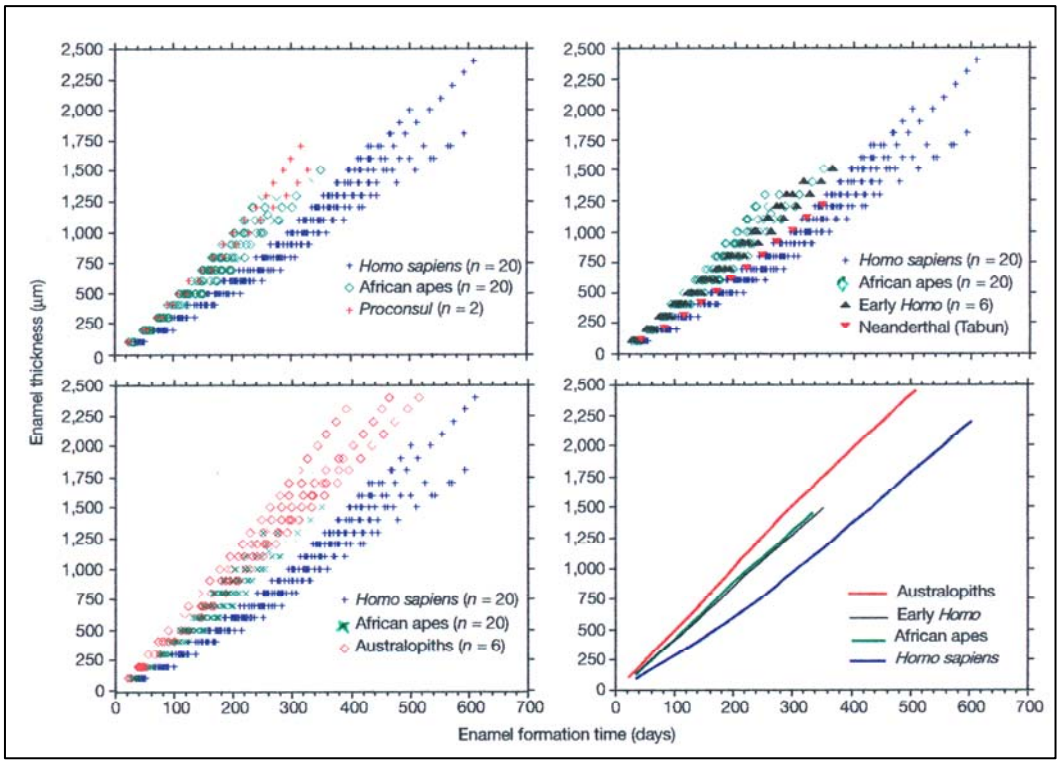


Figure 2.18. Lowess regression analyses plotting increments of enamel thickness (Y axis) versus duration of growth (X axis). It is noteworthy that in all cases studied, modern humans show slower growth rates than fossil hominids and extant African apes. It is also interesting to note that early *Homo* and australopiths appear to follow different growth trajectories.

The periodicity of the striae of Retzius is variable and appears to be influenced by body size in primates (Dean & Scandret 1995). Newman and Poole (1974) reported average values of approximately 8 cross striations separating adjacent striae. For humans and great apes, ranges of 6-12 have been recorded, but the top and bottom values are considered rare (Beynon 1992). The most common periodic appearance of the striae is 7-8 or 9 days for this group (Boyde 1989; Dean 1998) although other intervals ≥10 have been noted by Shellis (1998). Dean and Reid (2001) indicated that there is a mean and median periodicity of 9 cross striations for human and apes. The same number of cross striations was counted in a canine attributed to *Paranthropus robustus* (Dean *et al*.; 1993). In a molar and premolar of *P.*

boisei, Dean (1987) and Beynon and Dean (1987) counted 7 cross striations, while Dean *et al.*; (2001) noted 9 cross striations in a Neandertal molar from Tabun.

In smaller cercopithecoid monkeys, a periodicity of 4-5 days for the Retzius lines has been reported (Bowman 1991) and four cross striations were reported in *Hylobates lar* (Dirks 1998). The difference in the number of cross striations between striae between large-bodied and small-bodied primates has lead to considerations of a possible association between body mass and the periodicity of these short term markers (Dean & Scandret 1995; Dean 2000, Smith *et al.*; 2003). In artiodactyls, Macho *et al*.; (2003) have reported a periodicity of 4-6 striae.

Although there is a known variation across species and within the same species (e.g. humans), the cross striation periodicity does not vary in different sections of the same tooth (Reid *et al.*; 1998) and there are no differences between the teeth of the same individual (Fitzgerald 1998). This is important in estimations of crown formation times. It must be noted however, that a single study (Huda & Bowman 1994) found differences of cross striations in the same tooth in modern human teeth derived from archaeological populations.

2.17. Criticism on the validity of using enamel growth markings in estimations of the crown formation in hominids

An important problem faced in studies of enamel incremental markings is based on the fact that the exact aetiology of these features is not known. Indeed, at present, although the correspondence of cross striations with changes in daily secretory rhythms appears to be generally accepted (e.g. Anemome 2002), the physiological inductors of the formation of both striae of Retzius and cross striations remains obscure. However, some criticism on the interpretations of these features has been put forward by dental histologists and anthropologists. Weber and Glick (1975) and

Weber and Ashrafi (1979), have claimed that cross striations are not related to biological rhythms but that they are artefacts created on the tooth surface during preparation, some of which may interpret sectioned prisms as cross striations. Others have indicated that striae correspond with boundaries of interprism enamel of decussating cell cohorts (Warshasky *et al.*; 1984) without any rhythmic appearance. However, these studies have been aptly contested by other investigators (Boyde 1989; Ramirez Rozzi 1995; Risnes 1998), exposing the weaknesses of these claims.

In addition to the discrepancies in interpretations of enamel incremental markings, there are other studies in which the use of perikymata has been brought into question as a valid discriminator of fossil species, specifically the interpretations made by Bromage and Dean (1985) and Dean *et al*.; (1986) on Neanderthals and australopithecines. Most notably, Mann *et al*.; (1990, 1991) highlighted the possibility that cross striations were not time dependant growth markers, citing the works of Weber and Warshasky. In addition, they suggested, based on their own study of perikymata on archaeological samples of modern humans and additional Neanderthal samples, that the purported differences observed in perikymata number between fossil hominid and modern human anterior teeth grossly overlapped, and hence the presumed growth differences between groups. More recent studies appear to confirm the original separation between fossil taxa and modern humans (Dean & Reid 2001a,b; Ramirez Rozzi & Bermudez de Castro 2004; but see Guatelli-Steinberg *et al*.; 2005).

A more recent study by Macho *et al*.; (2003) cast serious shadows on some previous interpretations on the processes of enamel development based on incremental markings. The claims made by these authors centre on some conceptual and some practical issues that need to be discussed here. Much of the criticism of Macho *et al*.; (2003) is directed at the works of Dean and co-workers (Dean 1998;

Dean & Shellis 1998; Schwartz & Dean 2001; Dean *et al*.; 2001). A detailed reply to the issues raised by Macho *et al*.; (2003) can be found in Dean (2004) and in addition, some of the shortcomings of Macho's (2001) work were highlighted by Smith (2004) and Smith *et al.*; (2004).

Macho *et al*.'s, (2003) mathematical model for interpreting prism decussation was originally discussed in Jiang *et al*.; (2003). Their model is based on Osborn (1970) theory for ameloblast movement discussed earlier in this Chapter. This theory, however, has not gone without criticism (Boyde 1978). The conclusions reached by Macho and co-workers highlight the shortcomings of investigating the 3-D arrangement of prisms in a two-dimensional plane. Specifically, they claimed, "the linear one-dimensional 'secretion rates' are inappropriate for the description of the volume of material secreted, cell activity and/or growth rates" (Macho *et al.;* 2003. p 87). With regard interpretations of secretion rates by Macho *et al.*; (2003), it is quite true that in order to be able to express the total volume of enamel secretion, it is critical to know the diameter of the prism, not just the linear measurement of cross striations.

There are two main schools, one of which supports an increase in prism diameter from the EDJ to the enamel surface in order to cover the greater outer enamel area with respect to the area covered by the EDJ (Skobe & Stern 1980), and the other which advocates for a change in prism direction at the enamel surface whereby diameter remains unchanged (Radlanski 1995). It is yet unclear if or how prism diameter may vary in different teeth or cusps of different taxa. As already discussed, there is an apparent pattern of cross striation spacing in which the distance increases from the EDJ to the enamel surface and decreases from the cuspal area to the cervix. The "daily rates" used by these studies (e.g. Macho & Wood 1995) are obviously linear. The implicit assumption is that a change in cross striation

spacing probably indicates a change in secretion rate, thus an increase in the distance between cross striations indicates an increase in secretion rates. The only way in which this would not be the case is if an increase in spacing is met by a decrease in volume, that is, a decrease in the diameter of the cross striations. Such model has not yet been discussed in the literature. In fact, based on the limited available information presented by Dean (2004), prism diameter averages 4.5 to 5.5 microns, perhaps increasing to higher values in the outermost enamel. In the studies presented in later chapters of this thesis work, it is acknowledged that resolution of this issue remains uncertain, but that given the results reported by Dean (2004) and our discussion above; variation on secretory rates in a one dimensional linear fashion obtained by measuring the distance between adjacent cross striations reflects changes in the cell's rhythmic activity, as proposed by Boyde (1978, 1989, 1990), and thus has great potential for interpreting differences in patterns of enamel growth in fossil hominids. Finally, as noted by Dean (2004), and regardless of how volume relates to rates, knowledge of striae periodicity, or the number of days between striae, yields important biological and life history information about the organisms studied (e.g. Schwartz *et al*.; 2002).

2.18. Methods Used to Estimate Crown Formation Time

Based on the periodicity of the cross striations and perikymata and the number of striae of Retzius, it has been possible to assess the total crown formation time of different extant and extinct primate taxa. Several approaches have been used. These differ on whether the samples consist of thin sections, broken enamel surfaces or if the study is conducted on the enamel surface. Many of these studies have commonly applied Beynon and Wood's (1987) model of tooth regions which identifies the cuspal or appositional enamel, and the lateral or imbricational enamel (see Figure 2.6).

Following a method championed by Boyde (1964); Bromage and Dean (1985) first studied crown formation time in hominid incisors from perikymata counts. These authors used a seven day periodicity interval for the perikymata spacing based on a study from Newman and Poole (1974) on modern humans. In addition, a period of hidden increments of about six month's of cuspal enamel in incisors and a three month period which reflects the time between birth and the onset of calcification were added to the total count of perikymata to more accurately represent the age at death of the immature individuals considered. In a similar study, Beynon and Dean (1988) using perikymata counts calculated crown formation time for incisors, premolars and canines on samples of *A. afarensis*, *A. africanus, P. boisei, P. robustus* and early *Homo*. Their study assumed a 1.3 year period for the formation of the cuspal enamel. This was based on modern *H. sapiens* standards and observations made on one fossil premolar previously described by Beynon and Dean (1987).

Using histological thin sections, Shellis (1984) developed mathematically the concept of "ameloblast extension rate" originally discussed by Boyde (1964) to estimate the rate at which enamel formation extends over the crown. Shellis' model is based on trigonometric calculations using sine and cosine values of the angles formed by the striae of Retzius and the prism rods near the EDJ. The formula c = d [(sin *I* /tan *D*)-cos *I*] was reported by Shellis (1984), where c is enamel extension rate, d is the length of prism formed in one day and D is the angle formed between the enamel surface and the EDJ. This formula indicates that low values of *D* are associated with high extension rates (▼***D*** = ▲***c***). Extension rates decrease as tooth growth proceeds cervically. Significantly greater values were obtained for deciduous teeth. Shellis noted errors in *D* values of ≤ 8 degrees. Using the extension rate values, Shellis (1984) found a significant correlation (Spearman rank r_s = 0.81) between crown formation times developed by this method and those from published sources.

Risnes (1986) used histological sections to assess daily rates of enamel apposition. Risnes (1986) assumed that one ameloblast is responsible for the formation of individual enamel prisms. The path of the enamel rod at the tip of the dentin was followed from the EDJ to the OES. The Retzius line present at that point of the OES was then traced to its origin at the EDJ. The enamel prism at this point was then followed to the OES and the identified Retzius line was traced back to the EDJ. This process was repeated to the most cervical aspect of the tooth. According to Risnes (1986) the time that it takes for the ameloblasts to move from the EDJ to the OES at the locations studied, would correspond to the crown formation time.

A simplified model was used by Beynon *et al*.; (1991). Using histological sections, cuspal enamel and lateral striae of Retzius could be added to obtain total crown formation time. In lateral enamel, this method multiplies the number of striae by the striae periodicity. Cuspal enamel was assessed by measuring the length of a prism and multiplying this value by a correction factor (1.15). This correction factor originally developed by Risnes (1986) was used to adjust for prism decussation.

Read *et al*.; (1998) used simple formulas to calculate cuspal and imbricational enamel making use of Risnes' correction factor as follows:

$$\text{Cuspal Enamel} = \frac{\text{cuspal enamel thickness} \times 1.15}{\text{mean daily secretion rate}} \times \frac{1}{365}$$

$$\text{Imbricational Enamel} = \frac{\text{number of striae}}{365} \times \text{periodicity}$$

Dean (1998) used cross striations were counted from thin sections in a single enamel prism just lateral to the gnarled enamel from the EDJ to the OES. Near-monthly intervals (30 cross striations) were counted in photomontages. In prisms lateral to the main prism used, the length of six cross striations were measured and divided by five to obtain daily rates. In this manner, nearly monthly counts were assessed in relation to the average secretion rate per month (see Figure 2.9).

Teeth tend to fracture along occluso-cervical planes (Beynon 1992). Naturally occurring broken surfaces of enamel have been studied using replicas of the broken areas or by direct counts of striae. Complications in the use of broken enamel surfaces may arise as the fractured areas may not pass through the dentine horn. Thus, most of the methods employed make use of alternative ways of exploring the information not available for certain enamel regions. In Beynon and Wood's (1987) study of a large sample of naturally fractured teeth attributed to *Homo* and "East African robust australopithecines" specimens were immersed in ethanol, which enhances the visualization of striae. The use of ethanol in fossil teeth would increase the refractivity properties of porous enamel, as proposed by Poole *et al*.; (1981). Fractured enamel surfaces were then illuminated with oblique polarised light and observed with a stereo microscope. In their study, a mean daily incremental rate of 5.8 μm for *Homo* and 7.3 μm for the robust australopithecines was used in addition to measurements of enamel thickness [which included a correction factor different to Risnes (1986)] to determine cuspal enamel formation time. A similar study of a *P. boisei* premolar was conducted by Beynon and Dean (1987). These authors were able to obtain cross striation spacing in subdivisions of 0.5 mm of cuspal enamel. In addition, lateral striae counts were employed to calculate crown formation time for this specimen.

A large sample (n > 65) of isolated teeth from the Omo/Shungura Basin were analysed by means of counting striae on naturally occurring broken surfaces of molars and premolars (Ramirez Rozzi 1993; 1994, 1995; 1998; 2002) using a similar method employed by Beynon and Wood (1986). No records of cross striation spacing were noted as only an incident light stereo microscope was used. In this comprehensive study, cuspal enamel development was inferred from counts of cuspal striae. The work of Ramirez Rozzi differs from previous studies (e.g. Beynon & Wood

1987) in that formation time differences between mesial and distal cusps were taken into consideration. Thus, the last lateral stria visible in the anterior cusps was traced to the corresponding perikyma. Subsequently, this perikyma was followed to the distal cusps and counts were made of the perikymata cervical to it. Using this approach, Ramirez Rozzi (1993; 1995) indicated that crown formation times of hominid teeth were greater than those suggested by Beynon and Wood (1987). His results also suggested, by comparison with molar development from radiographic studies by Moorreess *et al*.; (1963), that fossil molar development was similar to modern humans. In addition, Ramirez Rozzi (1993, 2002) studies included an analysis of variations in the angles of the striae and the EDJ in the cuspal, lateral and cervical enamel to assess differences in the extension rates during the formation of these regions of the tooth. His results indicated similar trends to those of Beynon and Wood (1987) in which *Paranthropus* taxa showed low angle values.

2.19. Using Enamel Microstructure in Hominid Taxonomy

Bromage and Dean (1985), using counts of perikymata, pointed out that there were differences in growth between the fossil hominid taxa *Australopithecus*, *Paranthropus* and early *Homo*. Beynon and Wood (1986) studied fractured teeth of east African *Paranthropus* and *Homo* and concluded that differences were observed in the shape and form of the Hunter Schreger Bands (HSB) of these two genera. *Paranthropus* appear to show thin and straight HSB, while these features are curved and wider in *Homo*. In addition, the angle formed between the striae of Retzius and the EDJ in the mid or central are of the crown varied with a mean of 23^{o} in *Paranthropus* and 31^{o} in *Homo*, a difference that was also appreciable in deciduous teeth of these genera. A subsequent study (Beynon & Wood 1987) indicated that the pattern of enamel deposition varied between *Paranthropus* and *Homo*. The occlusal area of *Paranthropus* teeth accounted for about 90% of the enamel while in *Homo* this was

probably about 25% less. Beynon and Dean (1987) reported crown formation time for a *P. boisei* premolar and indicated that the period of growth for this specimen was markedly shorter than a similar tooth in *H. sapiens*, a result later supported using a greater sample by Ramirez Rozzi (1995). Beynon and Dean (1988) showed that perikymata spacing in the incisors of *Australopithecus* and *Paranthropus* presented different patterns, which became more obvious in the cervical aspect of the teeth. In *Paranthropus* perikymata remained more spaced out, while in *Australopithecus* there was a tendency to develop crowding of perikymata near the cervix. They also summarized differences in crown development between these two genera as well as in early *Homo* whereby *Paranthropus* showed less number of total perikymata, as Bromage and Dean (1985) had already pointed out. Further differences in enamel microstructure between South African *Paranthropus* (*P. crassidens* and *P. robustus*) and *A. africanus* were indicated by Grine and Martin (1988). These authors analysed sections of molars attributed to *P. boisei, P. robustus, P. crassidens* and *A. africanus*. Differences were observed between the proportions of the EDJ covered by the first striae reaching the surface (occluso-cervically; in effect, differentiating cuspal and lateral enamel). In *A. africanus* this ratio is less than 100 (84-97) while, in all *Paranthropus* species, it surpasses 100 (114-132). In addition, differences in the striae-EDJ angles were noted between *P. robustus* on one hand and *P. boisei* and *P. crassidens* on the other, with *P. robustus* indicating a slower cervical enamel development than the other two species (Grine & Martin 1988).

Beynon (1992), while revising the periodicity of the long term incremental markings in hominid posterior teeth, indicated that appreciable differences could be observed between East and South African *Paranthropus* species. These relate to enamel deposition in the cervical aspect of the teeth. In *P. boisei* there was a decrease in the rate of enamel growth while in *P. robustus* a rapid rate of enamel

growth was maintained in the cervical region of posterior teeth (Beynon 1992). In *A. africanus*, there was a marked reduction of striae spacing towards the cervix.

More detailed information was provided by Ramirez Rozzi (1993, 1994, 1995). Teeth of *P. aethiopicus* and *P. boisei* from the Omo-Shungura basin were compared using up to thirteen variables. These variables related mostly to differences between the shape and numbers of appositional and imbricational striae. It was found that clear differences existed between the proportions of cuspal and appositional striae (larger in *P. aethiopicus*), the angles of the striae to the EDJ (more acute in *P. aethiopicus*) and the total crown formation time between these two species (shorter in *P. aethiopicus*) (Ramirez Rozzi, 1993, 1994). The length of the striae was longer in *P. aethiopicus* indicating that more ameloblasts were present in the formation of enamel. In a more recent study, Ramirez Rozzi (1998) compared a large sample of teeth from the Omo Shungura Formation on the basis of their enamel microstructure. This was done to assess if enamel microstructural features could be used to separate taxa as they have been identified on the basis of gross morphological features. Results from this study indicated that micro- and macroanatomy do not relate to one another in the samples studied (Ramirez Rozzi 1998).

Several recent studies have added to the utility of enamel microstructure to interpret differences among extant and fossil primate taxa. Dean *et al*.; (2001) showed that patterns of enamel growth per unit of time were different in australopiths (a group which included *A. anamensis* and *Paranthropus*), early *Homo, H. ergaster,* and *Homo sapiens*. In addition, differences could be observed between these taxa, modern African apes and the Miocene Hominoid *Proconsul* (Dean *et al*.; 2001). Dean and Reid (2001) using perikymata spacing on anterior teeth indicated that, at the generic level, differences could be observed between African great apes, *Australopithecus*, *Paranthropus* and *H. sapiens*. Interestingly, they also noted perikymata spacing

differences between the three species of early *Homo*: *H. rudolfensis*, *H. habilis* and *H. ergaster* (Dean & Reid 2001).

One of the most recent studies on enamel incremental markings on fossil hominids by Ramirez Rozzi and Bermudez de Castro (2004) has shown marked growth differences among European hominid species and Upper Palaeolithic modern humans using perikymata packing patterns. In a relatively short period of evolutionary time, statistically significant differences can be observed between *H. antecesor*, *H. heildebergensis*, *H. neanderthalensis* and *H. sapiens* (Ramirez Rozzi & Bermudez de Castro 2004). These differences are irrespective of crown height. However, in a more recent interpretation of differences in perikymata distribution, specifically comparisons of Neandertals with other and more widely distributed populations of modern humans, it was found that South African peoples have faster rates of development than the observed growth in Neandertals (Guatelli-Steinberg *et al*.; 2005). It must be noted that Mann *et al.*; (1990, 1991) did not find differences in the number of perikymata of anterior teeth between modern humans and Neanderthals.

2.20. Summary of current knowledge of hominid dental microstructure

Two main trends in enamel growth can be observed in the great apes, *H. sapiens* and most Plio-Pleistocene hominids. The first is that enamel grows faster in the cuspal regions than at the cervix. In addition, enamel secretion rates increase from the EDJ towards the OES and decreases towards the cervix of the tooth (Beynon *et al*.; 1991; Macho & Wood 1995). However, exceptions have been recorded. At least one hominid species from the Omo/Shungura sequence displays an increase of enamel secretion rates towards the cervix (Ramirez Rozzi 2002). Among the Plio-Pleistocene hominids, the dentition of the robust australopithecines (*P. boisei* and *P. robustus*) appears developmentally unique. Anterior and posterior teeth of these two species are probably the best known of all hominids at microstructural level. The available

information indicates that enamel development of the anterior dentition of both species differs quite substantially from early *Homo* and *Australopithecus,* being much shorter (Bromage & Dean 1985; Dean 2000; Dean & Reid 2001a,b). Both *A. afarensis* and *A. africanus* have a shorter period of incisor development than the great apes and *H. sapiens* (Dean 2000; Dean & Reid 2001a,b). While the postcanine dentition of the gracile australopithecines is not well documented at this level, it appears that *Paranthropus* has achieved relatively thick, or hyperthick, enamel capping via two unique mechanisms: a rapid extension rate and increased ameloblasts secretion rate (Beynon & Wood 1988; Beynon & Dean 1988; Ramirez Rozzi 1998). Similar fast enamel formation rates are found in the deciduous dentition of *H. sapiens*. However, subtle differences can be recognized between molars of *P. boisei* and *P. robustus* (Beynon 1992; Grine & Martin 1988).

Interestingly, while the developmental mechanisms of enamel growth in molars of hominid species appears to be different; the duration of growth is not dissimilar from that of *H. sapiens* and the great apes, roughly about 2.5 years (Moorreess *et al.*; 1963; Macho & Wood 1995; Dean 2000).

In contrast, premolar crown formation of Plio-Pleistocene hominids was abbreviated in relation to that of *H. sapiens* (Beynon & Wood 1987; Rozzi 1995). While premolar crown formation in *H. sapiens* ranges between 3.2 to 4.5 years (using histological sections; Moores *et al*.; 1963; Schour & Massler 1940; Shellis 1984), Plio-Pleistocene hominids (*Paranthropus* and early *Homo*) had a formation period of about 2.8 years (Ramirez Rozzi 1995; 1998) or less (2.5 years, Beynon & Wood 1987). However, premolar and incisor crown formation differs among these taxa.

2.22. Dental Development of Humans and Great Apes

Modern humans are characterized by a slow postnatal developmental period in comparison to the great apes (Bogin 1999). This extension has been associated with the

need to increase the time necessary to develop the learning capabilities of the young in a more socially complex context (Lovejoy 1981). The growth schedules of apes and humans are reflected in their dental development (Anemone *et al*.; 1991, 1996). Significant differences are observed in the eruption time of each tooth family between these taxa (Dean & Wood 1981; Kuykendall 1996; Schwartz & Dean 2000; Kuykendall 2002) as well as in the overall period of growth. While humans reach dental maturity variably at the age of 18-20 years of age, that is all teeth have erupted and are in full occlusions, the great apes are dentally mature at about 11 years of age (Figure 2.12).

However, comparisons of apes and humans reflect complex *patterns* of dental emergence and *rates* of development. Molar crowns of apes and humans, for example, develop in broadly similar time periods (Macho & Wood 1995). However, root growth is faster in the great apes than in humans (Dean & Wood 1981, Dean 2000). This characteristic, in combination with the earlier onset of calcification of successive molar families in apes results in the temporal overlap of molar emergence of these taxa (Dean & Wood 1981; Kuykendall 1996).

It must be noted that the few studies that have been concerned with interpreting rates of root growth in hominid teeth have indicated that root growth was fast in early hominids, similar to values obtained for the great apes (Dean 1995, 2000; Dean *et al*.; 2001).

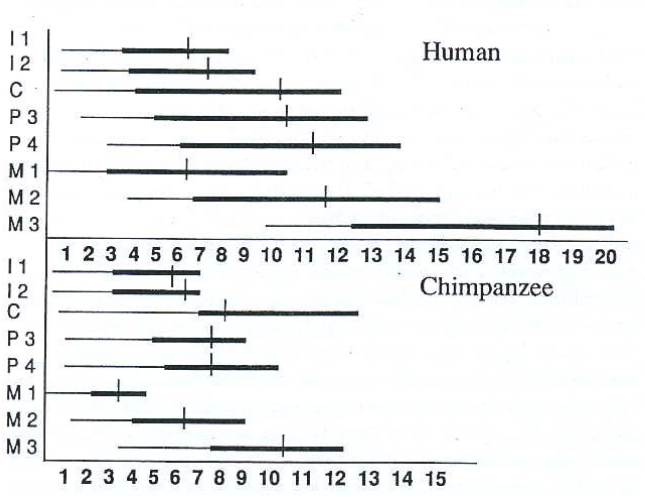


Figure 2.19. Dental development chart showing cuspal growth (−), root growth (represented by thicker line), and eruption times (vertical line) of chimpanzee and modern humans (after Anemone 2002).

An important marker in primate life history appears to be the eruption of the first permanent molar (Smith 1991). The timing of eruption of this tooth in *Pan* usually takes place between 3 and 4 years of age (Nissen & Riesen 1964; Smith *et al.*; 1994) and between 5 and 6 years of age in humans, (Demirjian 1986) which reflects differences in the overall developmental schedules of these taxa (Smith 1988, 1989, 1991).

Importantly, the eruption of the central incisors in relation to the first permanent molars characterises one important difference between the patterns of dental development of apes and humans. Chimpanzee central incisors erupt close to the appearance of the M_2, while in humans the incisors appear close to the eruption time of the M_1 (Conroy & Vannier 1991a; Anemone *et al.;* 1996). The characterization of these patterns in humans and apes has been useful to infer the growth schedules of fossil hominids (see section 2.16).

2.22. Jaw growth in relation to tooth growth

The development of the deciduous dentition is initiated *in uteruo*. The tooth germs develop some time prior to the development of the maxillary and mandibular dental arches (Tonge 1976). For the permanent teeth, dental development studies tend to show

a correlation between the development of teeth and the growth of the maxilla and mandible (e.g. Richardson 1977). For example, it was found that mandibular length in M3-impacted modern human individuals was shorter than individuals for which the M3 was not impacted (Richardson 1977). Later studies (Forsberg *et al.*; 1989; Hattab & Alhaija 1999) demonstrated that there is a threshold of about 25mm of retromolar space in modern humans, below which the M3 was not likely to emerge. However, more recent studies (Boughner & Dean 2003) have shown that in the case of *Pan* and *Papio,* there are no correlations between the growth of the mandible and that of teeth, suggesting that these two systems function independently.

2.23. Enamel Thickness and Prism Direction: Taxonomic and Functional Implications

The primary function of teeth is the breakdown of food particles which, for more efficient processing, involves a number of associated characters like crown and cuspal shape, enamel thickness and specific arrangements of the underlying enamel microstructures (e.g. Spears *et al*.; 1993; Macho 1995; Rensberger 2000; Jiang *et al*.; 2003). Thus, it is the function performed by teeth which appears to relate these characters with one another. However, earlier studies used enamel thickness as a taxonomic marker. The Miocene taxon *Sivapithecus* (*Ramapithecus*) possessed thick enamel, a character known for humans and which lead to the placing of this taxon at the base of the hominid line (Simmons & Pilbeam 1972). Kay (1981) and Martin (1985) subsequently developed a thickness “scheme” to categorize hominoid enamel.

Measurements of enamel thickness are generally complex and have involved a number of measurement schemes. For instance Martin (1985) developed the concept of relative enamel thickness (RET). This method consists of measuring the volumes of enamel and dividing this value by the surface area of dentine. In practical terms, sections were cut through the tips of mesial cusps of molars and dividing the

area of the enamel cap by the length of the EDJ. Martin (1985) noted that in anthropoid primates, enamel thickness was associated with body size. Martin's (1985) formula is: c/e x 100: √b

where "c" is the area of the enamel cap; "e" is the length of the EDJ and "b" is the combined area of the dentine and pulp.

A similar scheme has been more recently used by Smith *et al*.; (2005) in a larger sample of primate teeth. Alternatively, linear measurements have been designed and used in fossil and extant primates. Beynon and Wood (1986) and Macho and Berner (1993) used a set of eight linear measurements along the cuspal area of the crowns. It is noteworthy that in measuring enamel thickness, one has to be cautious with oblique cuts as they influence linear measurements (e.g. Smith *et al*.; 2005). A less destructive approach for measuring enamel thickness is the use of CT scans and micro CT. In these situations, one can either measure enamel thickness linearly (e.g. Macho and Thackeray 1992) or the total volume of enamel can be measured for the whole tooth (Kono *et al*.; 2002).

The original Martin's (1985) index recognized four categories: 1) thin, 2) intermediate-thin; 3) intermediate-thick and 4) thick enamel. It was established that the ancestor of the great ape and humans (at that time though to be *Sivapithecus*) had "thick" enamel. This scheme identified a reduction of enamel thickness in the African apes from thick to thin, a reduction in *Pongo* from thick to intermediate-thin while *Homo* retained the ancestral "thick enamel" condition (Martin & Boyde 1984). Later studies however have shown that this scheme was incorrect and that the ancestral condition of the great apes and humans was not thick enamel, but rather, thin (Beynon *et al.;* 1991), an interpretation that is maintained today. Beynon *et al*.; (1991) showed that enamel thickness can be achieved through different developmental mechanisms. Therefore there is no need for considering enamel

thickness as having any particular interspecific taxonomic valence, but, as in *Sivapithecus* and humans, a case of evolutionary parallelism. However, the studies of hominid enamel by Beynon and Wood (1986) and Grine and Martin (1988) have shown differences in the enamel thickness of samples attributed to *Paranthropus*, *Australopithecus* and *Homo* from East and South Africa. Grine and Martin (1988) additionally suggested that the "hyper-thick" enamel of the *Paranthropus* species indicated a shared taxonomic character of these taxa. At a more general level, Macho (1995) pointed out that enamel thickness is better interpreted not as a taxonomic character but as an indication of the dietary niche of the animal.

Robinson (1954, 1956) first speculated on an association between enamel thickness and diet in the South African australopithecines. Thick cuspal enamel (Jolly 1970; Dumont 1995) and more specifically, low-cusped and thick enamelled teeth are associated with the processing of hard foods (Molnar & Gantt 1977). Wear development of thick enamelled teeth usually results in a flat occlusal surface providing efficient grinding areas and thus increasing the functional life span of a tooth (Schwartz 2000). In contrast, thin-enamelled primates usually prefer softer diets and have more pointed cusps. In the thin-enamelled *Gorilla*, this character maybe a mechanism by which sharp cusps are retained later in life to allow the processing of these soft food types (Ungar & Williamson 2000). Shearing and crushing is undertaken primarily by different cusps of the same tooth (Spears & Crompton 1996). For this reason, enamel is not uniformly built on the occlusal area but different cusps show differences in enamel thickness (Macho & Berner 1993; Schwartz 2000; Kono *et al*.; 2002). Further, posterior molars in humans decrease in size in relation to anterior molar families (Macho & Moggi Cecchi 1992) and show thicker enamel, probably as a result of an increase of functional demands (Macho & Berner 1993). A recent study

has shown that this decrease in size of third molars in humans, once corrected for size, is related to a decrease of the dentinal area (Grine 2002).

Functional demands on molar families thus appear to show variations in enamel thickness of individual cusps and indicate something of the predominant food types consumed. The efficacy of enamel depends on its mechanical capacity to dissipate the forces applied to this tissue (Macho & Spears 1999). Thus, the various food types, especially when these are hard objects, require the presence of protecting mechanisms to prevent cracking of the enamel (Popowics *et al*.; 2001). Enamel is anisotropic as it can better resist stresses when these are applied in a certain direction than others (Rasmussen *et al*.; 1976; Spears *et al*.; 1993; Spears & Crompton 1994). A mechanism developed in response to enamel stress is enamel prism decussation (e.g. Hunter Schreger bands (HBS)) which limit the propagation of vertical cracks (von Koenigswald *et al*.; 1987; Jiang *et al*.; 2003). The shape of HBS, which is related to enamel prism orientation, has been shown to have taxonomic and functional potential (e.g. Rensberger & von Koenigswald 1980; Boyde & Fortelius 1986). More recently, chemical composition in addition to the orientation of enamel prisms (Rasmussen *et al*.; 1976; von Koenigswald *et al*.; 1987; Jiang *et al*.; 2003) has been shown to be related to the mechanical behaviour of enamel (Cuy *et al*.; 2002).

There may be a relationship between tooth size and sex chromosomes (Garn *et al*.; 1958, 1965). Alvesalo *et al*.; (1985, 1987 and 1991) further indicated that the X chromosome promotes enamel deposition while the Y chromosome may be implicated in enamel thickness.

2.24. Estimating Life History Variables from Dental Eruption Patterns and Crown Formation Time

A recurrent question in human evolution has been whether early hominid ontogeny followed a human or an ape-like pattern. In general, the development of the great apes

is slower in comparison to the growth of monkeys, and human development is in fact slower in relation to any other primate species (Bogin 1999). A shift from fast-growing monkey-like development to a slower more-ape like development appears to have occurred in the Miocene (Kelly 2002). By estimating the eruption of the first permanent molar, Kelly (2002) and Kelly and Smith (2003) indicated that the early Miocene taxon *Afropithecus* showed indications of a grade shift in development, which was more definite in the later hominoid *Sivapithecus* (Kelly 2002; Kelly & Smith 2003).

Humans are characterized by a prolonged post-natal period of growth unique to our species (Lovejoy 1981; Bogin 1999), which has been important in assessments of growth and development in early fossil hominids. While earlier descriptions of juvenile hominid specimens (e.g. Mann 1975) assumed similar growth trajectories to that of modern humans, later research has challenged such notions. Growth periods are related to life history traits, which can be defined as the "timing or scheduling of life stages" (Kay 2002: 223). Knowledge of these traits is important for an understanding of organismal biology. In primates, a positive correlation has been indicated between life history and body size (Harvey & Clutton-Brock 1985) while the chronology of dental eruption has been shown to be an important developmental marker (Conroy & Vannier 1991a; Smith 1991). In general, the stage of development of the tooth crowns and roots of any individual, fossil or living, can be compared with charts of dental development of apes and humans. This comparison allows the classification of the specimen into either of these patterns.

Based on these patterns which were assessed from skiagrams and wear stages, Mann's (1975) influential study of juvenile hominids from South African caves found no indication of an accelerated developmental pathway similar to that of the chimpanzee. The only one exception was the specimen SK 841a for which Mann indicated that its eruption pattern implied an age of one year younger than that of a young human (Mann

1975). His study suggested that the South African hominids had, as in modern humans, a prolonged post-natal growth period of parental care.

Many authors have since questioned Mann's original work (Bromage 1985; 1987; Bromage & Dean 1985; Beynon & Dean 1988; Smith 1986, 1991; Smith *et a*l.; 1995; Ramirez Rozzi 1995). Bromage and Dean (1985) using a novel approach to infer the age at death of individual fossil by counts of perikymata (and thus crown formation) of anterior teeth, which was then compared to dental emergence patterns, concluded that australopithecines did not have a prolonged growth period. Other studies based on dental evidence, either using histological methods or tooth eruption sequences (e.g. Beynon & Wood 1987; Beynon & Dean 1988; Conroy 1988; Anemone *et al*.; 1991; Conroy & Vannier 1991a,b; Anemone 1995) have confirmed Bromage and Dean's work. The pattern of eruption of the anterior dentition indicated that *Australopithecus* showed a delayed emergence of the incisors in relation to the first permanent molar and in the overlapping of successive molar families, a pattern similar to that of chimpanzees. In the case of *Paranthropus*, superficial similarities were initially observed between this taxon and modern humans on the similar timing of eruption of the incisors and the M_1 (Dean 1985). This pattern however has no taxonomic relevance as the eruption of these tooth families was achieved via different mechanisms (Conroy 1988; Conroy & Vannier 1991a&b).

Especially important in this discussion has been the correlation between M_1 eruption and other life history traits, in particular cranial capacity. The work of Smith (1986, 1988, 1991, 1994) and Smith *et al*.; (1995) has shown a high correlation between M_1 eruption and cranial capacity in a wide range of primate species. Interpretations of hominid development based on these correlations were also consistent with a more ape-like pattern of development of the australopithecines. If cranial capacity is considered such a prominent "pace maker" in the life history of a species (Sacher 1975), Bromage

(1987) questioned why then would australopithecines have had such small brains (and body size) in comparison with modern humans and yet follow a similar developmental strategy.

Some of the discussions involving ontogenetic trajectories of the australopithecines relate to the use of pattern to infer timing of developmental events (Macho & Wood 1995). As pointed out by Simpson *et al*.; (1991), pattern and rate may not indicate similarities of growth strategies although both are bound within the available period of growth (Bromage 1987). Simpson *et al*.; (1991) also indicated that some of the similarities observed between australopithecines and apes based on interpretations of eruption of the anterior dentition, should be better interpreted within the general development of the facial structures, which are especially relevant to the development of the anterior dentition. However, when the rate of growth is inferred from crown formation time of primate first permanent molars, it indicates, as the eruption time does, a high correlation with life history traits, most notably age at weaning, cranial capacity and body mass (Macho 2001).

When all evidence is taken together, it strongly suggests that the australopithecines (e.g. *Australopithecus* and *Paranthropus*) did not have a slow growth trajectory similar to that of modern humans (Anemone 1995, 2002; Anemone *et al.*; 1996; Kay 2002). In fact, recent studies have suggested that a modern human growth schedule has only developed in recent evolutionary time. Early *Homo* from Africa (ca. 1.7 Ma) and the Middle and Late Pleistocene European hominids did not have growth rates that characterize *H. sapiens* (Bromage 1987; Dean *et al*.; 2001; Ramirez Rozzi & Bermudez de Castro 2004).

2.25. Tooth Microstructure and the Environmental Context

In section 2.6.2 it was indicated that some striae of Retzius and abnormal or accentuated striae could be associated with some external (epigenetic) factors. In an

early study by Massler *et al*.; (1941), it was indicated that some markings (e.g. Wilson bands) could be associated with disturbances due to illness or malnutrition. Later, Rose *et al*.; (1978) noted an increase in the proliferation of these bands in human archaeological samples marking the transition from hunter-gatherers to agriculture. Rose (1979) attempted to classify the various types of morphologies visible in Wilson bands. Interestingly, Bowman (1991) noted that emotional stresses in juvenile macaques could be traced as pronounced striae. In human enamel, it has been shown that teeth developing in one individual during the same stress period will show similar abnormal striae. This can be used to determine whether isolated teeth belong to the same individual (Gustafson 1959).

Macho *et al*.; (1996) studied histological sections of fossil *Theropithecus* from Kenya and observed a recurrent pattern in the presence of accentuated striae. This pattern appeared to reflect seasonality/food availability which varied across the sampled fossil populations (Macho *et al*.; 1996). In a similar study, Macho *et al*.; (2003) found that seasonality was an important factor in the spacing of accentuated striae in several ungulate species from the *A. anamensis* site. Differences could be elucidated for grazers (two striae per year) and browsers (three striae per year) (Macho *et al*.; 2003).

At a more general level, Ramirez Rozzi *et al*.; (1999) found that an increase in the number of appositional striae (associated with thicker enamel) was present in the Omo Shungura hominid teeth at ca.2.4 Mya. These authors noted that thicker enamel in hominids was in line with a general trend towards hypsodonty across other mammalian faunas during this period in response to overall climatic change (Ramirez Rozzi *et al*.; 1999).

An important external marker of stress is the presence of linear enamel hypoplasia (LEH). Several types have been described (Rose 1979) (e.g. pits and

grooves) but the most common is the presence of “furrows” in the same direction as the perikymata (Hillson 1986). The aetiology of LEH has been related to periods of physiological disruptions during enamel formation due to illness, malnutrition and in some cases, emotional stress (Sarnat & Schour 1941; Rose *et al*.; 1978; Hillson 1986; Goodman & Rose 1990; Bowman 1991). For primates, the social environment is considered to be an important factor that influences their life histories (Johnson 2003). Weaning has been suggested as a cause for the appearance of LEH in humans, as a result of the stress the infant experiences when shifting from breastfeeding to new food types (Moggi-Cecchi *et al.*; 1994; Simpson 1999; but see Katzenberg *et al.*; 1996). In modern human societies, the timing of weaning is a variable process heavily influenced by availability of prepared foods. Even in less developed societies, where breastfeeding is common practice, there is high variability in weaning (Kennedy 2005). However, weaning can be said to take place around 2.5 years of age in humans or about three to four years before M_1 eruption (Kennedy 2005). However, in chimpanzees weaning takes place at about 5 years, which is about two years after the eruption of the first permanent molars (Smith *et al.*; 1994; Bogin 2003; Kennedy 2005). Dirks (1998) and Dirks *et al*.; (2002) have discussed other possible causes related to the development of LEH. In infant baboons and gibbons, LEH can be developed due to the mother’s rejection for nursing, which has been associated with the mother’s first postpartum mating period. In modern sympatric populations of *Gorilla* and *Pan,* hypoplastic grooves have been associated with seasonality patterns (Skinner 1986).

Recent studies on the presence of LEH in fossil hominids have suggested that the apparent high levels of stress in Neandertals, was in fact similar to that of modern Inuit’s (Gautelli-Steinberg *et al*.; 2004). When the presence of LEH in the Plio-Pleistocene hominid fossil genera *Australopithecus* and *Paranthropus* from South

Africa were compared, it was shown that the latter showed, in general, higher levels of LEH (Gautelli-Steinberg 2004). It was noticeable in the Guatelli-Steinberg (2004) study that of the low percentage of postcanine teeth with LEH, especially relevant was the fact that no first permanent molars of *A. africanus* showed LEH.

2.26. Dentine and Cementum: Concise Summary of Growth Processes

2.18.1- Dentine

Dentine tissue formation begins at the late bell stage, after ultra structural changes of the pre-odontoblasts (Osborn & Ten Cate 1983). Differentiated odontoblasts, the dentine forming cells, are displaced toward the centre of the dental papilla forming tracks similar to enamel prisms known as dentine tubules (Jones & Boyde 1984). Pre-dentine is originally secreted as a matrix that later mineralises into mature dentine forming three types of tissues. These are differentiated according to whether collagen fibres or a non-fibrous substance (ground substance) form the mayor constituent (Hillson 1996):

1- Intertubular dentine: This is the largest component of the dentine in the root and the crown, formed by fine collagen fibrils.

2- Mantle dentine: Also found in the crown and root, this type is characterized by a dense accumulation of collagen fibres.

3- Peritubular dentine: Mostly composed of ground substance.

Dentine tubules are perpendicularly orientated to the basal lamina, but become S-shaped as they approach the pulpal area (Hillson 1996). Odontoblasts develop incremental structures within this tissue that have a similar time dependency as those described in enamel. Daily secretions are known as von Ebners lines, and have a similar secretion rate to that observed in enamel cross striations (Boyde 1990). Long period markers are known as Andresen lines which are the equivalent of Striae of Retzius in enamel, and have a similar periodicity which is variable according to

varying body sizes in primates (Dean 1987; Dean *et al.*; 1993b). In fact, there is correspondence between accentuated lines in dentine and those found in enamel (Dean & Scandret 1995).

An important property of dentine is that, in contrast to enamel, there is tissue repair after initial mineralization which may continue at its pulpal surface during the life of the tooth (Osborn & Ten Cate 1983). Layers of pre-dentine form the floor of the pulp chamber and the roots, where these layers run parallel to the basal lamina. The roots are covered by cementum.

2.18.2- Cementum

Dental cement is formed by specific cells known as cementoblasts, and is composed of ground substance and collage fibres. The cement layer covering the roots has a variable thickness which increases with age. The main function of the cement is to attach the periodontal ligament to the root (Hillson 1996). Cement is laid down through the life of the tooth.