CHAPTER 12

SUMMARY AND DISCUSSION

12.1. DISCUSSION

The aim of this chapter is to review and summarize the most important aspects of this study. At the onset of this thesis, it was stated that the main aim of this body of work was to study the mechanisms of enamel development in the Plio-Pleistocene South African hominids *A. africanus* and *P. robustus*. As was noted, technology has moved beyond the need to section original fossil material and thus a new, non-destructive imaging tool was used to access and image the enamel microstructure on post-mortem fractures of molars of fossil hominids. This enabled the candidate to undertake a series of studies using this novel method. These works, six in total, form the main body of this thesis.

As was noted in materials and methods, the prototype of a portable confocal scanning optical microscope (PCSOM) designed by T.G. Bromage, A. Perez-Ochoa and A. Boyde (Bromage *et al*.; 2003; 2005) was the primary microscopic tool applied to these studies of fossil enamel.

The works presented here are all based around a basic but well founded assumption, namely, that the microstructural markers preserved in mineralized enamel have a regular time dependency. This was based on the principle that cross striations represent daily secretion rates by ameloblasts. Robust experimental evidence, described in detail in Chapter 2, supports the circadian nature of cross striations (Boyde 1964, 1990; Dean 1987; Bromage 1991; Fitzgerald 1998; Antoine *et al*.; 1999; Smith 2006). Building from this point on, the candidate assumed that the periods of time between striae of Retzius and perikymata could be assessed.

In the introduction to this thesis, it was indicated that enamel thickness is determined by the number of active cells secreting enamel, the daily rate at which these cells secrete enamel matrix; and programmed cell death or apoptosis (e.g. Grine & Martin 1988; Beynon *et al*.; 1991; Macho 1995; Dean *et al*.; 2001). Each of these factors may independently contribute to the formation and thickness of the enamel crown. As a result, it has been established that enamel thickness can be achieved via very different developmental pathways (Beynon *et al*.; 1998; Dean 2000; Dean *et al*.; 2001) indicating that the phenotypic adult state of this character may not be synapomorphic across taxa (Beynon *et al*.; 1991; Schwartz 2000). As most of the fossil hominid samples used in previous studies of enamel microstructure derive from East African sites, the candidate identified that a gap of knowledge existed concerning these issues in South African fossil hominids. To illustrate this dearth of research and the opportunities it presented the candidate, only two studies had been published that were concerned with crown formation time in South Africa hominids representing four post-canine teeth, and a single canine (Dean *et al*.; 1993; Moggi-Cecchi *et al*.; 1998), while in contrast, more the 60 molars and premolars had been studied from East African deposits (Beynon & Wood 1987; Beynon & Dean 1987; Rozzi 1993, 1995; Dean *et al*.; 2001). Only a single specimen representing *P. robustus* - the sub adult SK 63 - comprised our entire knowledge on striae of Retzius periodicity in South African hominids (Dean *et al*.; 1993), and together with a single molar of Stw 151, represented the sum total of our knowledge of daily secretion rates in South African hominids (Dean *et al*.; 1993; Moggi-Cecchi *et al*.; 1998). The candidate therefore considered it important and necessary to increase our knowledge in this regard as this information holds the potential to clarify differences in tooth formation and development between fossil hominid taxa from across Africa, modern humans, and the great apes; and might elucidate further differences among the fossil taxa than has already been noted.

The theoretical emphasis in this study was therefore placed on the available knowledge of the biological controls operating during amelogenesis that were detailed in Chapter 2 (Thesleff & Hurmerinta 1985; Amar *et al*.; 1988; Lumsden 1988; Snead *et al*.; 1988; Ruch 1990; Sasaki 1990; Lesot 1991).

In our fossil sample, and based on the work of Boyde (1964,1990) and Shellis (1984), we have assumed that the inclination or the angles formed between the striae and the EDJ is indicative of the number of active secretory cells (Boyde 1964; Shellis 1984). Therefore the candidate focused on the following features: cross striation spacing, striae periodicity, striae/EDJ angles and crown formation time.

To better interpret the results obtained in this study, it is important to recognize that there is variability in developmental time between different cusps of different tooth families, and between maxillary and mandibular molars (e.g. Kraus & Jordan 1965; Dean *et al*.; 1993b; Reid *et al*.; 1998a,b). To address this issue, when comparisons were made between the variations observed in the fossil material analyzed in this thesis work and that observed by others in modern taxa, these comparisons were made on a matching bases. What is meant here is that for example, if the feature compared was crown formation time, or cusp formation time, the comparisons were made between the same cusp of the same tooth type in fossil and modern taxa. In the study of Stw 402 however, the cusp analyzed was the disto-buccal or metacone cusp, for which Reid & Dean (2006) had not published cusp formation time. In this instance, the comparisons were made between a corrected value of the whole crown of Stw 402 and the cusp formation time of the anterior cusps in modern humans.

The variation noted in Chapter 9 in appositional rates between *A. africanus* and *P. robustus* was considered within the context of the variation observed in the most comprehensive study conducted in primates (originally the thesis work of Smith 2004 more recently published as Smith *et al*.; 2007). The statistical analysis of Smith and co-

workers indicated that there was no significant variation in appositional rates between different cusps of different molar types in the chimpanzee. Furthermore, the author has recently reviewed a manuscript submitted to the Journal of Human Evolution in which appositional rates were compared between upper and lower molars and across the mesial and buccal cusps in human premolars. This study also showed that there was no variation in this feature. Given that appositional rates appear to be consistent between tooth families and cusps in chimpanzee molars and modern human premolars, the differences observed in Chapter 9 in appositional rates between *A. africanus* and *P. robustus* are more than likely the result of biological differences in enamel development between these two taxa rather than sampling differences between teeth or cusps.

In other areas of research, for example in the analysis of striae/edj angles, there is a marked paucity of data, and in some cases, it appears that different methodologies had been employed when recording this feature in several primate taxa. This point was discussed in Chapter 5. Despite the lack of comparative date with regards striae/edj angles across tooth types and cusps in modern taxa, it was assumed that, as other studies had done before (Beynon & Wood 1986, 1987; Beynon & Dean 1988; Ramirez Rozzi 1993; 2002), the differences observed between fossil taxa from relatively large samples of naturally fractures surfaces was probably indicative of biological differences. This point was addressed in detail in Chapter 10. Furthermore, counts of striae of Retzius in fossil samples were compared with works on modern humans and chimpanzees (Reid et al.; 1998a,b; Smith 2004). Here, as before, comparisons were made by matching tooth types and cusps in fossil and modern taxa. However, the paucity of data in modern taxa only allowed for some quantitative observations to be made, which were highlighted in Chapter 10.

It must be noted that Chapter 5 sampled striae/edj angles and counts of striae of Retzius in a modern human sample. This work represents a preliminary attempt to

interpret trends in the dentition of modern humans. For this reason, and given the relatively limited sample, no direct comparisons were made between the fossil and modern hominins and only descriptive statistics were included in this part of the study. Thus the work on striae/edj angles of Chapter 5, remains as a pilot study which needs further work using a more comprehensive data set but more importantly, given the differences in methodologies which were highlighted in this Chapter, it would be useful to standardize the protocols to measure striae/edj angles for example among researchers in dental enamel. In this thesis work, the protocol followed was the same as that employed by Schwartz *et al*.; (2003), which is similar to the method used by Ramirez Rozzi (1993, 1998, 2002) to measure this feature in fossil taxa, thus permitting direct comparisons with Ramirez Rozzi's data.

The ensuing discussions in the remaining part of this Chapter flesh out other significant and more specific aspects of the research undertaken in this thesis work. For instance, the duration of outward cuspal cell secretion (duration of growth from EDJ to OES by individual cells) could be estimated in a few specimens by measuring cuspal enamel thickness and dividing this value by the mean of cuspal secretion rates following methods detailed in Reid *et al*.; (1998b) and Reid and Dean (2006). These rates were assessed at specific sites on the enamel crown, which allowed for interpretations of the differences between the hominid species studied here. These results were presented in Chapter 9, in which the variation in cross striation spacing (which in 2-D is the equivalent to daily secretion rates) in fossil hominids was shown. This was one of the challenging hypotheses tested in this study and the testing one of the most time-consuming. The hypothesis tested was: given the differences in enamel thickness already noted between *A*. *africanus* and *P*. *robustus* (Robinson 1956; Grine & Martin 1988; Macho & Thackeray 1992), might these differences in part be accounted for by variation in daily rates of secretion? It was additionally hypothesized that given the greater enamel thickness in

these two fossil hominid taxa both in respect to thicknesses observed in modern humans as well as the great apes (Martin 1985; Beynon *et al*.; 1991; Schwartz 2000; Smith *et al*.; 2005), that differences in daily rates would be found between the fossil and extant groups. Our results showed that both *P*. *robustus* and *A*. *africanus* had much greater daily cell secretion rates than *H. sapiens*, *Gorilla*, *Pan troglodytes* and *Pongo*. For example, in the outer and middle cuspal enamel, the rate differences between modern humans and fossil taxa ranged between 24% and 30%. These results significantly contribute to the growing body of literature recognizing different and more rapid enamel growth rates in early hominid species. Even our reported mean values in bonobo (*P. paniscus*) presented in Chapter 6, which appeared to be higher than most other great ape values, were lower than values we reported for the fossil hominids despite that this value was established on only a single individual and a more in-depth study of bonobo enamel growth is required. Nevertheless, the results of this study are clear - all modern taxa showed means of daily rates which were markedly below those of the *P. robustus* and *A. africanus*.

Differences were also found between the two fossil hominid species studied. The taxon *A. africanus*, having slightly less thick enamel than *P*. *robustus* (Robinson 1956; Grine & Martin 1988; Macho & Thackeray 1992) was expected, based upon the candidate original hypothesis, to have daily cell rates slightly lower than those of *P*. *robustus*, and this was nominally supported by the results presented in Chapter 9. These differences, however, were not statistically significant except for daily secretion rates in the outer cuspal area. This study represents the largest study on cross striation spacing of South African fossil hominids. An interesting point of this research, only briefly discussed in the original Chapter 9, was that the values for cervical enamel *in P. robustus* differed from those of Beynon (1992). Our study noted that there was a decrease in the cervical rates with respect to the more cuspal values in this taxon, but

Beynon (1992) indicated that the rates appeared to be constant throughout the development of the crown. Figure 12.1, imaged on the cervical/lateral outer enamel of the *P. robustus* specimen SKW 4769, will be used here to illustrate a possible reason that may help explain the differences between the results presented in this research and those of Beynon (1992). Figure 12.1 illustrate that prisms do not run in the cuspal direction or even horizontally near the enamel surface, but rather, they appear to bend cervically. This change alone in prism orientation near the cervix could explain why the distance between striae appeared similar in the cervical third of the enamel crown to Beynon (1992), while at the same time recording lower rates than cuspal and lateral enamel. Because prisms cut through the striae, which are oriented upwards in the region, a change in prism orientation bending towards the cervix, changes the geometry of the relationship between both structures, and thus, even while ameloblasts secrete smaller amounts of enamel, the distance between striae remains unchanged.

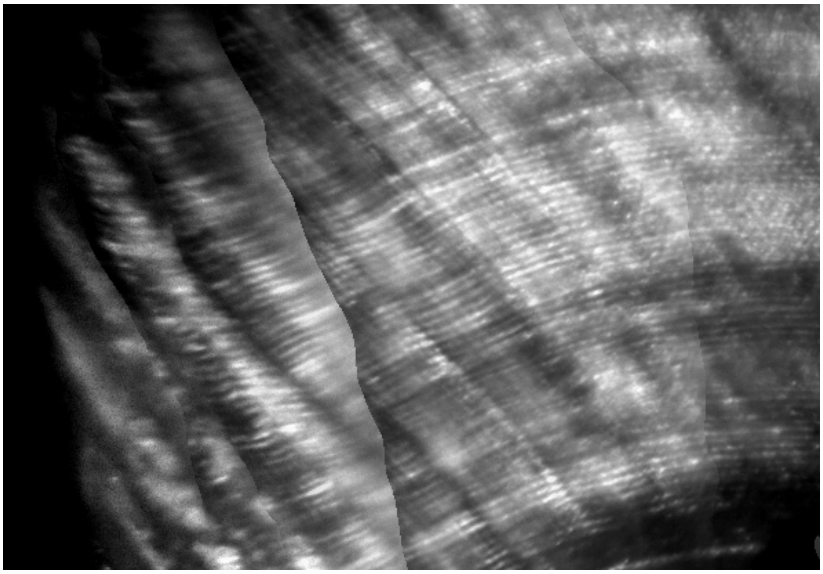


Figure 12.1. SKW 4769 where prisms can be shown to bend cervically in the outer half of the enamel. Cusp at top of image. FW app. 800 µm. Vertical line is an artifact of the montage.

Given the results from previous studies of East African taxa (Beynon & Dean 1987; Beynon & Wood 1987; Dean *et al*.; 1993; Dean *et al.*; 2001) and the values reported here for a relatively large sample of teeth, it seems clear that fossil hominids, which had greater enamel thickness than modern humans and great apes, formed their megadont molars partly by secreting enamel at faster daily rates than modern humans, and this result is in agreement with Dean *et al*.; (2001). In Chapter 11 the candidate presented additional data on a molar derived from the site of Kromdraai which was originally attributed to the taxon *P*. *robustus* (Grine 1982). Later research indicated that this specimen was best attributed to early *Homo* (Braga & Thackeray 2003). Our study of the daily secretion rates on the cusp and the lateral part of the enamel crown on the first permanent molar showed lower rates than those obtained for our sample of *P*. *robustus* and *A. africanus* and fitted best the limited values reported for early *Homo* in mid cuspal enamel (Beynon & Wood 1987). Besides this taxonomic issue, it was found that the values recorded for this specimen were higher than in modern humans as well as in great apes.

As stated earlier, it is clear that high daily secretion rates represent an important aspect by which early hominid megadontia was achieved, specifically in relation to enamel thickness. However, we can identify additional mechanisms in megadontia which include an increase of the dentinal area and patterns of expression of specific genes. The results of this body of work may also go some way towards explaining some of these mechanisms.

Based on our current knowledge of tooth developmental biology, it appears that the presence of (secondary) enamel knots greatly influence the location of cusps in molars (Jernvall 1995; Jernvall & Thesleff 2000; Kangas *et al*.; 2004). The assumption is that the further apart secondary enamel knots are, the further apart the cusps develop (Jernvall 2000), a process that appears to be mediated by the expression of sonic

hedgehog (*Shh*) (Kangas *et al*.; 2004). Shh has been identified as a signaling molecule and a marker for the activity and size of enamel knots (Jernvall *et al*.; 2000). Therefore, changes in the expression of *Shh* may result in changes of tooth size and shape (e.g. Kangas *et al.*; 2004).

McCollum and Sharpe (2001) indicated that larger teeth basically result from an upregulation of epithelial cell proliferation. However, in addition to epithelial upregulation, mesenchymally derived dentine may contribute to tooth crown increase or decrease. Crown size has several components, one of which is enamel thickness. It is well known that in modern humans for example, M3's tend to be the smallest in size in the dental arcade (e.g. Macho & Moggi-Cecchi 1992). However, this tooth type appears to have relatively greater enamel thickness than the other molar types (Macho & Berner 1993; Grine 2002). Grine (2002) noted in this regard that in modern human M3's, the relatively greater enamel thickness in relation to M1 and M2's is related to a reduction of the dentinal area on the M3. The megadont molar of *Paranthropus*, for example, have larger dentinal area and greater enamel thickness (Grine & Martin 1988) and shows as well greater measures between mesial dentine horns than modern humans (Lacruz & Jernvall in preparation). In this case, at least in this taxon, megadontia developed as a result of two processes: larger dentinal and great enamel thickness, which indicates differences in the genetic mechanisms controlling the location of secondary enamel knots (e.g. pattern of *Shh* expression). This example is used here simply to suggest that the problem of hominid megadontia is compound (e.g. McCollum & Sharpe 2001) and to fully understand this phenomenon from a biological perspective, one needs to consider not just the enamel thickness of early hominids, but the responses of the underlying tissues i.e. dentine, and patterns of *Shh* expression. It is also likely this process is affected by changes in expression patterns of enamel proteins (e.g. Paine *et al*.; 2001).

In addition to the daily secretion rates, another important contribution made by this study was the recording of data on striae of Retzius periodicity. In Chapters 8 and 10 we presented the largest account on variation of striae periodicity known for any fossil hominid species to date. Our results show that both *A*. *africanus* and *P*. *robustus*, which are considered small-bodied hominids (McHenry 1994, 2002; McHenry & Berger 1998a), have ranges of 6 to 8 cross striations between striae established from a sample of 15 teeth. The mean value in our sample was 7 days (Table 12.1), which is within the range of modern *H. sapiens* (6 – 11; Beynon 1992; Dean & Reid 2001) but lower than the mean and modal periodicity for this species, which is 9 days (Dean & Reid 2001; Reid & Dean 2006). The modal value of *Paranthropus* was 7 days while in *A. africanus*, depending on the periodicities of some specimens that could not be conclusively resolved, the modal value could be 6 or 7 days.

As pointed out by others (Dean & Scandret 1995; Dean 2000; Schwartz *et al*.; 2001; Smith *et al*.; 2003); there may be an association between periodicity and body size in hominoids. In Chapter 10 we indicated that *A. africanus* and *P. robustus* body size falls within the range known for *Pan troglodytes* (Smith & Jungers 1997) for which a range of 6- 8 cross striations have been reported (Reid *et al*.; 1998a; Smith 2004). In Smith (2004), the mean of a large sample of *P. troglodytes* molar sections was 6 days, which is less than the mean value obtained in our study for the fossil hominids. It must be pointed out here that the values reported for molars are below those reported for two anterior teeth in fossil hominids, where 9 cross striations were counted (Dean *et al*.; 1993; Bromage *et al*.; in press).

Taking into account all the periodicity values reported to date for anterior and posterior teeth of Plio-Pleistocene hominids by Beynon and Dean (1987); Dean (1987) and Dean *et al.*; (1993), as well as the studies included in this thesis, the mean and modal values for fossil taxa, which includes *P. boisei*, *P. robustus*, and *A. africanus* taxa,

is 7 days (Table12.1), lower than the values reported for modern humans of 9 days, but more similar to chimpanzee (Dean & Reid 2001; Smith 2004).

Table 12.1. Striae periodicity considering all samples reported in Chapters 8 and 10 for *A. africanus* and *P. robustus* (n=15), and those reported in Beynon and Dean (1987) and Dean (1987) for the specimen KNM-ER 733, and in Dean *et al*.; (1993) for SK 63.

N= 17	mean = 7.2	median = 7	mode= 7

Chapters 8 and 10 provided crown formation time (CFT) for two molars of the species *A. africanus* and one molar of *P. robustus.* In addition, numbers of striae of Retzius were observed in teeth of *A. africanus* (n= 4) and *P. robustus* (n= 5). Lateral striae are an important character in studies of enamel microstructure as they reflect differences in patterns of enamel crown development (Ramirez Rozzi 1998). Only one study on CFT or striae counts was available for molars of these species, which reported 50 perikymata on the M_1 of the *P. robustus* specimen SK 63 (Dean *et al*.; 1993). These authors also briefly reported 50 perikymata on another *P. robustus* specimen (SK 834). In our sample, we found differences between these taxa, with *P. robustus* having less lateral striae than *A. africanus*. Given that there are differences in striae number in different molar cusps of chimpanzees and modern humans (Reid *et al*.; 1998a,b; Smith 2004), and that our sample of Plio-Pleistocene molars sampled different cusps, it is difficult to confidently ascertain if this variation reflects clear developmental differences. However, as the anterior dentition of both fossil taxa, which has been more widely sampled, shows marked differences in perikymata number (Bromage & Dean 1985; Beynon & Dean 1988; Dean & Reid 2001a,b; Dean *et al*.; 2001), it is highly likely that the differences found by our study on molar striae reflects biological differences between these taxa.

In a recent summary of hominid crown development and life history, Kuykendall (2003) interpreted that the shorter crown formation time in *Paranthropus* was part of an adaptive strategy probably related to habitat and diet. The rationale hypothesized by Kuykendall (2003) is based on studies (Macho 2001; Macho & Williamson 2002) of mammals living in open habitats and consuming low quality diets - a life history pattern purportedly ascribed to *Paranthropus*. Animals with this life history pattern are also hypothesized to mature relatively earlier than closely related taxa of similar body size adapted to different diets and habitats (Kuykendall 2003). A similar distinction in primates was presented by Godfrey *et al*.; (2001) in which low quality diet (folivorous) primates matured faster than high-quality diet (frugivore) primates. This area of research holds great potential to better understand aspects of hominid life history.

Ramirez Rozzi *et al.*; (1999) indicated, based on their sample of molars and premolars derived from the Omo Formation in Ethiopia (n=66), that the number of striae and overall crown formation time increased from about 2.4 my. This change was broadly contemporaneous with dental changes developed across a wide range of African mammalian taxa in response to climatic change discerned by Turner and Wood (1993). However, based on the information presented in Chapter 10, it appears that striae number or CFT did not increase through time. *A. africanus* is generally recognized as a chronologically older species than *P. robustus*, and in our sample of molars from both taxa, it appears that *P*. *robustus* showed less number of striae than *A*. *africanus* suggesting a pattern that is probably better interpreted at genus level or among closely related taxa.

In our assessment of the CFT of the molars Stw 402 (M^1) 2.74 years; Stw 284 (M^2) 3.0 to 3.2 years, and SKX 21841 (M_3) 2.72 years; we found that the total duration of the development of the enamel crown was similar between these taxa but is less than in the corresponding tooth types of modern humans (Figure 12.2).

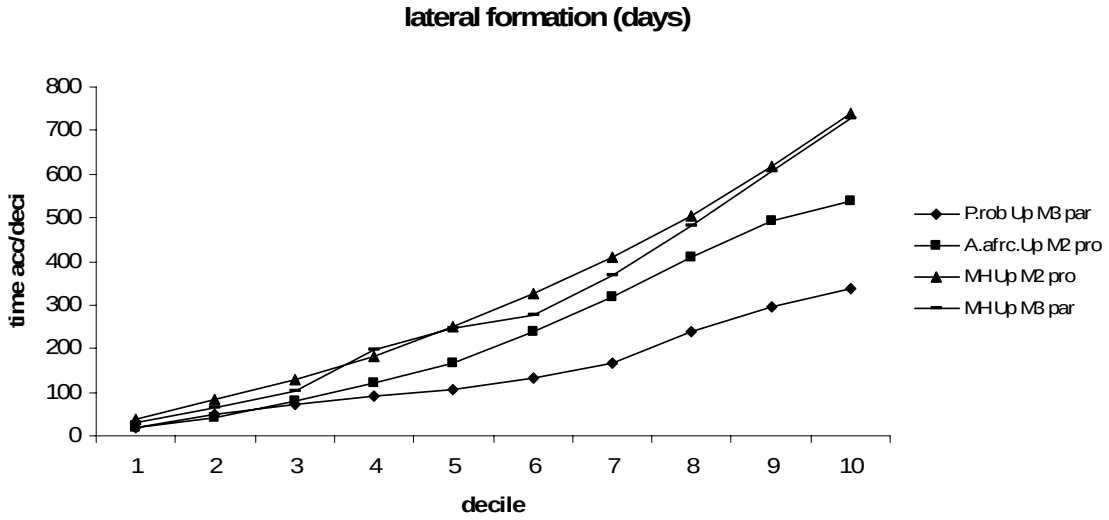


Figure 12. 2. Cumulative values of lateral CFT based on striae packing pattern per decile on two fossil specimens compared to modern humans. P. rob = *Paranthropus robustus*. A. afrc = *Australopithecus africanus*. MH = modern humans. Data on fossils is taken from Chapter 9 and are here compared with values obtained from Reid and Dean (2006) for cumulative values of *H. sapiens* (South African derived samples). Values of the *P. robustus* specimen SKX 21841 (M^3 paracone) can be compared with average values of *H. sapiens* (M^3 paracone). The *A. africanus* specimen STW 284 (M^2 protocone) is compared with the same tooth type and cusp in *H. sapiens*. Total values are: SKX 21841, 336 days, STW 284, 540 days, MH M^2 protocone 740 days and MH M^3 paracone 729 days.

We also noted differences between the fossil species by which the duration of the cuspal enamel in the *Paranthropus* specimen studied was more than 50% of the total crown development, while in two *A. africanus* molars the proportion of cuspal enamel was less than 50%. This is not surprising given the differences in enamel thickness between these species whereby *Paranthropus* has thicker cuspal enamel (Grine & Martin 1988). This is probably the main reason why others have noted the same pattern of enamel crown development in East African *Paranthropus* molars (e.g. Beynon & Wood 1987). It must be noted however, that the study of Dean *et al*.; (1993) reported a nearly equal distribution of cuspal and lateral crown formation on SK 63 M_1, a *P. robustus* specimen based on measurements of enamel thickness using CT scans.

A cautionary note must be added here, as daily appositional rates for molars in which we obtained CFT, could not be calculated in areas adjacent to the EDJ, but rather obtained some 150 microns away, yielding values of about 4 microns in inner enamel. A similar value in inner enamel was obtained by Beynon and Dean (1987) for a *P. boisei* premolar using SEM. However, Dean *et al*.; (1993) found a mean cuspal value of about 5 microns in the SK 63 M_1, although this study does not specify how this measurement was obtained. This value is lower than our estimated 5.5 microns and 5.8 recorded for cuspal mean values of *A. africanus* and *P. robustus* respectively obtained in this study. If we could have measured values near the EDJ, it may be possible that our values would be smaller than the ones reported in our inner cuspal enamel category shown in Table 2 of Chapter 9. However, these values would have to be significantly smaller than our values to have an effect in our calculation of cuspal and thus overall CFT. For example, even if we assumed values reported for modern humans in the inner cuspal enamel (2.8 microns, Table 2 Chapter 9) for calculating cuspal enamel development, our mean cuspal value for *P. robustus* would be approximately 5.6 microns. Assuming this value, cuspal enamel of SKX 21841 would be 1.70 years, giving a CFT of about 2.75 years, which is still lower than corresponding mean values reported for modern humans of 3.27 years (Reid & Dean 2006).

In Chapter 11, we calculated CFT for the M_1 of KB 5223, which ranged between 2.22 to 2.74 years depending on the periodicity used, 6 to 9 days (the periodicity could not be determined from direct examination of the specimen). The lower range value appears to be one of the shortest recorded for fossil hominid molars (Beynon & Wood 1987; Rozzi 1993, 1995; Rozzi *et al*.; 1997), and is considerably shorter than values for the same tooth type in modern humans, which reported a CFT for M_1 protoconids of 3.06 years (Reid & Dean 2006). For an M_2 of *H. rudolfensis*, Rozzi *et al.*; (1997) reported 2.38 years, 2.4 years were reported for the *P. robustus* SK 63 M_1 (Dean *et al*.; 1993),

and the low end value of 2.12 years is similar to values reported for *P. boisei* (Beynon & Wood 1987). The high end value of 2.74 years is similar to other values reported for *P. boisei* (Beynon & Wood 1987; Rozzi 1993, 1995) but closer to early *Homo* (Beynon & Wood 1987). The crown development of the M_1 metaconid of the *Homo* specimen from Sangiran reported by Dean *et al*.; (2001) was 2.5 years. As this value only refers to the development of a single anterior cusp, the growth of the entire crown is expected to be longer than this value. As noted above, the mean value of the protoconid on a large sample of M_1's from a large South African derived modern human sample was 3.06 years (Reid & Dean 2006), while Dean *et al*.; (1993b) reported 2.67 years for a single M_1. Thus the crown formation time of KB 5223 is lower than the mean obtained by Reid and Dean (2006) but could fall within the range obtained for modern humans.

Dean *et al*.; (2001) provided regression equations that purportedly best represent the growth trajectories of cuspal enamel growth in three early hominid groupings, based on data presented for two molars of *A. anamensis* and one of *P. boisei*, a canine of *P. robustus* and one premolar of *P. boisei* and one of *P. aethiopicus* (pp. 630-631). For this group of specimens, Dean *et al.*; (2001) suggested the following equation: $y = 6.64 + 0.21x - 0.00001x^2$; where "x" is enamel thickness. The *H. ergaster/H. erectus* group consisted of one molar and one premolar of *H. ergaster*, and one premolar of *H. erectus*, for which the proposed equation was: $y = 3.70 + 0.27x - 0.00003x^2$. Finally, for the early *Homo* group which most likely consists of a molar of *H. rudolfensis* and one premolar of *H. habilis*, these authors suggested $y = 3.76 + 0.26x – 0.00002x^2$.

In our study of cuspal enamel development we recorded the following values:

- The metaconid of SKX 21841 (*P. robustus*) developed in about 1.64 years
- The estimated cuspal development of the metacone of *A. africanus* molar Stw 402 was 1.02 years
- The protocone of the *A. africanus* molar Stw 284 was 1.50 years.

- In the molar KB 5223, which has been variously been assigned to *Paranthropus* and early *Homo*, the development of the cuspal enamel on the metaconid was estimated to be 1.04 years and 1.18 years for the protoconid.

All of these specimens would fall in the Dean *et al*.; (2001) australopith category, except perhaps KB 5223 which could fall in the early *Homo* group depending on its taxonomic allocation.

Calculating cuspal growth from these figures using the australopith regression equations proposed by Dean *et al*.; (2001), we obtained the following results:

- SKX 21841: 2.02 years
- Stw 284: 1.54 years
- Stw 402: 1.10 years

The specimen KB 5223 was compared using the australopith, early *Homo* and *H. erectus*/*ergaster* groups respectively yielding the following results:

- KB 5223: 0.97, 1.19 and 1.24 years (metaconid) and 1.10, 1.35 and 1.40 years (protoconid)

Interestingly, cuspal enamel development obtained using the method of Dean and co-workers and our results presented above, are very similar. The only exception is the *P. robustus* specimen SKX 21841, which is about 0.35 years longer using the proposed regression equations. Although the disparity is not large, there may be an explanation as to why these differences were recorded. *Paranthropus* taxa are regarded as having the thickest enamel amongst early hominids (Martin 1985; Grine & Martin 1988). In the Dean *et al.*; (2001) analysis, *A. anamensis*, which has less thick enamel than *Paranthropus*, was included. The difference in enamel thickness between these taxa may in part account for the disparity in the results. In addition, Dean and co-workers included in the same equation different tooth types (canines, premolars and molars) which may also had an influence on the results. For instance, Beynon and Dean (1987) reported daily

secretion values in the outer cuspal enamel for a premolar of *P. boisei* of about 6. 2 microns, while Beynon and Wood (1987) reported mid-cuspal values of over 7 microns on molars of *P. boisei*. Some of these factors may have contributed to the dissimilarity of results between both methods.

Chapters 8 and 10 recorded values for the striae/EDJ angles in relatively large samples of *A. africanus* (n = 15) and *P*. *robustus* (n = 11) molars. Low angles indicate high extension rates and vice versa. Given that previous studies (Beynon & Wood 1987; Grine & Martin 1988; Rozzi 1993, 2002; Rozzi *et al.*; 1997) noted differences between *Paranthropus* and other taxa whereby *Paranthropus* maintained high ameloblast extension rates, it was expected that South African *Paranthropus* would show a similar pattern. Our results indicated that the ameloblast extension rate in *P*. *robustus,* is also maintained high (low angles) during the whole development of the crown. In contrast, *A*. *africanus* developed the cervical part of the enamel crown at a slower rate than *Paranthropus* because of higher cervical striae/EDJ values found in the molars of this taxon.

In Chapter 11 we measured the striae/EDJ angles in the first permanent molar of KB 5223, originally attributed to *Paranthropus* by Grine (1982) and later to *Homo* by Braga and Thackeray (2003). Our results, summarized in Figure 12.3, indicate that the values obtained in this specimen were closer to values that Rozzi *et al.*; (1997) had reported for the specimen UR 501 attributed to *H. rudolfensis* (Bromage *et al.*; 1995), but different to values obtained for East or South African *Paranthropus* (Rozzi 2002; Chapter 10). The KB 5223 values also fall within the *A. africanus* range.

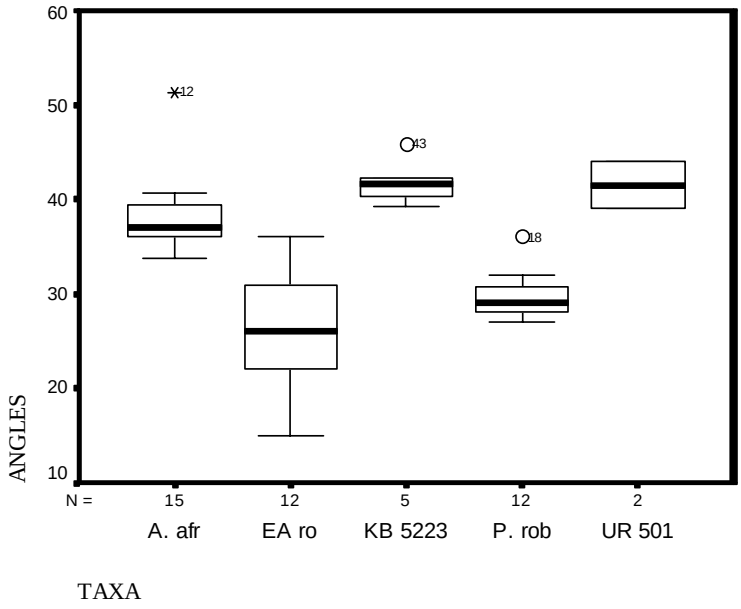


Figure 12.3. Box plots of the values of angles striae/EDJ in the cervical region of *A. africanus* (A. afr), *P. robustus* (P. rob), East African *Paranthropus* (EA ro), *H. rudolfensis* (UR 501) and the Kromdraai specimen KB 5223. It is noticeable that KB 5223 falls outside the ranges obtained for East and South African *Paranthropus*, but is similar to a single specimen of *H. rudolfensis* and our sample of *A. africanus*. Five angles were measured in the cervix of KB 5223 and values for UR 501 were taken from Rozzi *et al*.; (1997) by combining values from distal and lingual faces of the M_2. Data on East African robust taxa derives from Ramirez Rozzi (2002).

This study suggested that classifying KB 5223 as *Homo* based on evidence from the enamel microstructure could not be ascertained because the anterior dentition of this specimen showed features more allied with *Paranthropus* (number of perikymata and distribution on the first incisor) than with *Homo*, in agreement with the studies of Bromage and Dean (1985) and Dean and Reid (2001 a & b).

This complex pattern shown between the anterior and posterior dentition is intriguing, and to properly resolve this issue, it is important to increase our knowledge on the enamel microstructure of early *Homo* for which only limited information is presently available.

12.2. CONCISE SUMMARY

In the preceding pages we have reviewed the most important findings derived from the application of the PCSOM to the study of mechanisms of enamel growth in a sample of Plio-Pleistocene South African hominids. Our results for early hominid molars can be summarized as follows:

- *A. africanus* and *P*. *robustus* molars have the same mean value of striae periodicity, 7 days. The range for *A. africanus* was 6-9 and for *Parantropus* was 6 to 8 in our sample
- *A. africanus* and *P*. *robustus* have similar molar CFT
- Both taxa formed their molars in less time than modern humans in spite of having much larger crown areas and enamel thickness
- *A*. *africanus* shows greater number of lateral striae than *P. robustus*
- *P*. *robustus* dedicates longer time to the formation of cuspal enamel than *A. africanus*
- The angles formed between the striae/EDJ at the cervix in *A. africanus* are higher than in *P*. *robustus*, which are more similar to East African *Paranthropus*
- Daily appositional rates are higher in *P. robustus* outer enamel than in *A. africanus*, but both taxa have much greater rates than modern humans and the great apes
- The specimen KB 5223 cannot be confidently assigned to the genus *Homo* based on its enamel microstructure

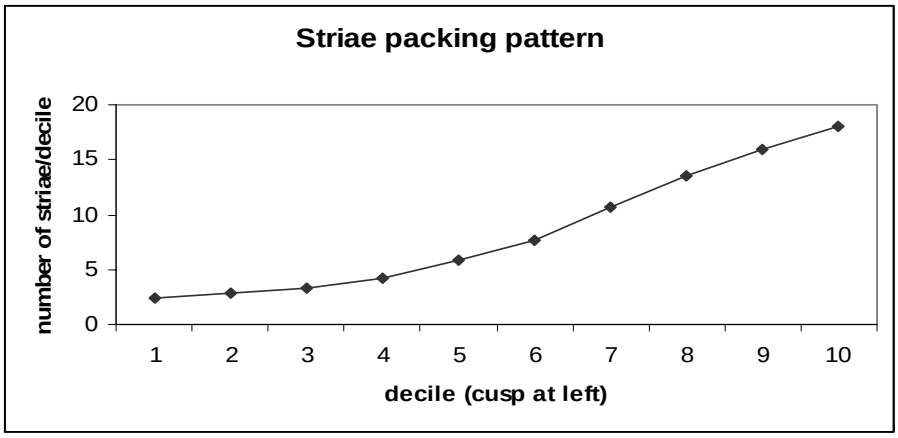
12.3. SUMMARY OF ADDITIONAL CHAPTERS

In addition to these studies of molar enamel microstructure using the PCSOM, detailed in Chapters 8, 9, 10 and 11, three additional studies were carried out on primate enamel microstructure using different microscopic techniques. The first of these is presented in Chapter 5, although some additional results were also presented in Chapter 9 for daily secretion rates in modern humans.

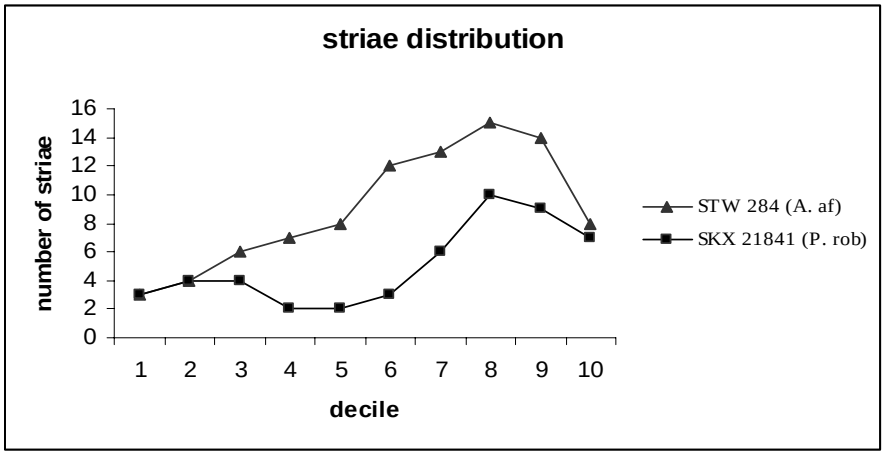
Chapter 5 shows striae packing patterns and striae/EDJ angles in a sample of modern humans. The analysis was conducted by separating the results obtained in each individual cusp first, and subsequently interpreting the results by pooling values from all faces. In general, the results presented here indicate that the striae/EDJ angles tend to increase from the cusp to the cervix, where the angles are highest. This pattern had already been noted by Beynon *et al.*; (1991) and Beynon and Dean (1995) in modern humans and great apes. Although our results from Chapters 8 and 10 on the fossil taxa are not directly comparable in most cases with those of modern humans reported in Chapter 5 (the number of divisions used in Chapter 5 along the EDJ were different between studies), it appears that *Paranthropus* shows significantly lower angles than modern humans, while the striae/EDJ values in *A*. *africanus* are closer to modern humans. However, these are only preliminary observations and need to be further investigated.

Striae packing patterns in molars of modern humans indicate that the number of striae tend to increase gradually from the cusp to the cervix (Figure 12.4a). This pattern appears to be different in fossil hominid molars where the number of striae decreased towards the cervix (Figure 12.4b). In the anterior teeth of modern humans, perikymata packing patterns described by Dean and Reid (2001) showed a slight decrease in the last decile, where in *Australopithecus* and *Paranthropus* the growth curves leveled off in the last three deciles. Our measurements cannot be compared with data on striae of Retzius reported by Reid and Dean (2006) because the methodology employed by these authors divided the length of the outer enamel a distance that was then divided in deciles. We measured the crown height and not the outer enamel length.

Figure 12.4. a and b. Striae packing pattern (SPP) for all of the sections of modern humans (Figure 12.2.a) combined showing a steady increase from cuspal (at left in this figure) to the cervix (right side). Figure 12.2.b shows the hominid pattern where striae decrease towards the cervix of the crown.



A



B

Chapter 6 comprises a preliminary study on the enamel development for a single female bonobo (*P. paniscus*) individual. No prior study has been carried out on this taxon but several works have been conducted on the common chimpanzee (*P. troglodytes*) (Reid *et al.*; 1998b; Smith 2004). The reason to conduct this study on bonobo was to record differences in patterns of enamel development between these two

closely related taxa. The upper central incisor and the first permanent molar of a juvenile female bonobo were sectioned. Incident and transmitted light microscopy were used to assess daily secretion rates, crown formation time and age at death of this individual. Age at death was estimated by assessing duration of M^1 crown development, to which the difference in I^1 crown development, which showed some delay in development with respect to the M^1, was added. As a minimal and negligible amount of root had developed on the I^1, crown development corresponded closely with the age at death, which was established at about 4.72 years. Two important features were noted on this specimen. The pattern of perikymata arrangement on the crowns of I^1 and M^1 differed from that of other great apes in that *P. paniscus* showed an increase in the number of perikymata towards the cervix. In addition, the daily rates of ameloblast secretion appeared to be one of the highest values recorded for the great apes and higher than mean values for *P. troglodytes* (Reid *et al*.; 1998b; Smith 2004). However, as noted in Chapter 6, these differences could partly reflect differences in the methodology employed in dividing the crown regions from where cross striations were measured. Relatively marked differences were noted between incisor growth in the two species of *Pan* whereby *P. paniscus* developed incisors in markedly less time.

Chapter 7 presented evidence for the presence of enamel defects found in the permanent dentition of one of the most well known fossil hominids, the Taung child. In addition, we provided a re-assessment of Taung's age at death based on root development and crown formation time. During the course of this study, it was noted that the first permanent molars of Taung showed a single linear enamel hypoplasia (LEH) near the cervix. These teeth were replicated and studied under light microscopy and SEM. All four permanent first molars of Taung showed LEH. The periodicity on this specimen was assumed to be 7 days based on our study in Chapter 10. Counts of normal perikymata from the LEH to the cervix and striae of Retzius periodicity indicated

that this abnormal growth phase had taken place about 11 to 13 perikymata before crown completion (roughly 3 months). Using the duration of crown formation from another M^1 of *A. africanus* (Stw 402), which was 2.74 years (Chapter 10) and mean rates of root development in hominids, about 13.5 microns/day, together with root development in the Taung child (5 to 6 mm), we proposed that the Taung child probably died at about 3.75 to 3.95 years of age. An interesting feature in this study was that the development of the LEH was coincidental with the possible presence of LEH in humans induced by weaning, which in humans is around 2.5 years of age. However, as no other *A*. *africanus* first molars showed LEH, we reasonably discounted weaning as the possible cause of the LEH.

12.2. CONCLUSIONS

As aptly noted by Dean (2000), there are a variety of approaches available to the study of human evolution. One such approach is to use the fossil record to try to understand the underlying mechanisms of morphological change during evolution. This was the central idea pursued in this body of work. Teeth are indeed remarkable structures that help piece together more closely the relationships between genes, development and phenotype (Jernvall & Jung 2000; Hlusko 2003). It is quite significant that changes in, for example, levels of *ectodysplasin* can alter mammalian tooth shape so distinctly (Kangas *et al.*; 2004), or that different levels of expression of enamel knots result in the addition of molar cusps in the same species of seals (*Phoca*) (Jernvall 2000).

The internal structures preserved in mature enamel are indelible records of its cellular development, reflecting the activity of single cells (i.e. cross striations) or fields of cells (striae of Retzius), as striae mark the position of cell cohorts at specific points during crown development. This perspective allows the understanding of tooth growth, following Hall (2002), at the level of developmental biology, a discipline through which

we study how development relates to evolution (Hall 2002). In this context, we can attempt to study the developmental biology of fossil hominid species through time.

Bromage and Dean's (1985) pioneering study of perikymata on hominid anterior teeth represented a new paradigm shift in the study of growth and development in fossil hominids. More recently, Dean *et al.*; (2001) showed more subtle mechanistic differences in the growth of enamel tissue on anterior and posterior teeth of hominids, great apes, and modern humans. Modern human molars appear to follow a unique growth trajectory characterized by forming thick enamel at initial slow rates which are maintained for a relatively long period of time. Fossil hominids on the contrary formed their thicker enameled-molars through presumed genetically controlled growth programs that invoked much higher rates of daily ameloblast secretions. Some additional differences among hominids were noted in the numbers of cells involved during amelogenesis (Beynon & Wood 1986, 1987; Beynon & Dean 1988; Rozzi 1993). In addition, hominids were thought to form their molars in less (Beynon & Wood 1987) or similar (Ramirez Rozzi 1994) time to modern humans.

Until now the above synthesis was largely based on studies conducted on samples of East African fossil hominids. Similar studies were needed on the large sample of South African taxa *P*. *robustus* and *A. africanus* to better comprehend Plio-Pleistocene hominid evolution. The overall differences between the fossil taxa studied here and modern humans are clear. Fossil hominids formed their thick enameled-molars at faster daily rates than modern humans, and they completed the development of molar crowns in less time.

Further differences were noted between *A*. *africanus* and *P*. *robustus* whereby the former showed lower daily secretion rates, had higher counts of lateral striae of Retzius, presumably forming the cervical crown at a slower rate than *P*. *robustus*, which made use of higher number of cells throughout crown development. Not surprisingly

given the differences in cuspal molar thickness between these species, *P*. *robustus* appears to have invested a longer time in forming the cuspal area than the lateral part of the crown, and the reverse appears to be the case in *A*. *africanus*. Importantly, we found that the mean value of striae periodicity in both fossil hominid taxa studied was 7 days. This contrasts with previous studies on fossil hominids in which a periodicity of 9 days had been assumed based on values obtained in large samples of modern human (Dean & Reid 2001; Dean *et al*.; 2001).

An ongoing debate with regards the south African fossil hominid record is whether the Sterkfontein Member 4 samples more than one species (e.g. Clarke 1988; Lockwood 1997; Moggi-Cecchi 2003). The samples used in this study did not suggest marked differences in enamel development among the specimens studied from Sterkfontein Member 4.

The patterns of enamel development described for East and South African *Paranthropus* appear to be very similar (Chapters 9 & 10). If we consider that similarities in patterns and rates of enamel development may represent similarities in underlying genetic programs, particularly if these similarities appear to be restricted to a few taxa for which some authors have suggested a close taxonomic link, then it probably means that both East and South African *Paranthropus* are more closely related than these are to *A. africanus*. Importantly, patterns of facial remodeling have been found to be more similar between East and South African *Paranthropus* than either is to *A*. *africanus* (Bromage 1989; McCollum 1999). While morphological differences do exists and have been recorded in facial and dental morphology between *P. boisei* and *P*. *robustus*, it should be expected that geographical variation, chronology, and perhaps environmental conditions may account for some of the morphological differences, while similarities in mechanisms of development may account for shared similar genetic controls and thus, for close phylogenetic relationships.

At present, little enamel microstructural information is known for the East African species *Australopithecus afarensis* and early *Homo*. Additional information on these species is critical to better understand relationships among early African hominids. This is likely to occupy the next phase of our research reported here.