APPENDIX 1

A.1. Preliminary observations on prism decussation in A. africanus.

Introduction

During this thesis work, it was noticed that some teeth of *A. africanus* showed prisms decussating near the EDJ (Figures A1.1 and A1.2). It was also observed that HSB in this taxon appeared to be curved in some molars, but less so or even straight in others. In most cases, HSB in *P. robustus* did not generally curve but remained straight and perpendicular to the EDJ, similar to observations made by Beynon and Wood (1986) and Ramirez Rozzi (1993) in East African *Paranthropus*. This difference in prism decussation in some *A. africanus* specimens prompted the use of Risnes (1986) correction factor to calculate CFT. To clarify the use of correction factors, prism decussation was quantified in a molar of *A. africanus* (Stw 284).

Materials and Methods

To image prisms on Stw 284, a portable confocal scanning optical microscope (PCSOM) (Bromage *et al.*; 2003, 2005, in press) was used by direct observation of prisms on the protocone of this specimen. The tooth was imaged dry using 5x, 20x and 50x lenses placing it perpendicular to the lens. This specimen was selected because it had been previously sectioned along the apices of the mesial cusps using a diamond saw (Grine & Martin 1988) and therefore it showed relatively flat surfaces. In addition, during a different study in which this specimen had been used (Lacruz *et al.*; in press) it was noticed that in several areas of the cusp, prisms could be followed from the EDJ for a few hundred microns. An area was then selected along the EDJ located in the cuspal third of the crown, about 600 microns cervically from the apparent dentine horn of the Stw 284 protocone (Figure A1.3). This same area was imaged by SEM. Coltene President putty and light body moulds were used to obtain expoxy resin replicas. These were then coated and studied under SEM.

Results

Figure A1.4 shows an enlarged image of the same area using a 20x lens and 0.5 adapter. The main prism direction can be observed (long white arrow) as well as general patterns of prism decussation in this area. The short white arrow marks the location of point A, which is used in Figure A1.5 as an arbitrary landmark to measure prism decussation. Point A is located about 70 microns from the EDJ.

To measure prism decussation, we used a 50x lens and 1:1 adapter without applying a clearing medium. Images were then montaged and analyzed measuring prism path using ImageJ software which allows measurements of curved lines. The field width of Figure 4 is 230 microns. Figure A1.6 shows the same area as Figure A1.5 imaged in SEM.

It was difficult to follow a single prism across the entire field. Instead, the montaged field was divided into zone 1 and zone 2, separated by a thick black line (Figure A1.5). In zone 1 we followed prism 1 from point A to point B located in the black vertical line where it became difficult to confidently identify the course of this prism. The distance traveled was about 96 microns. A second prism, prism 2, located about 15 microns cervically from prism 1 was identified in zone 2. This prism was followed from point C in the black line for about 159 microns to a point D. The distance in a straight line measured between A and B was 92 microns and between C and D was 152 microns. This indicates that prisms decussate about 4.3 % in zone 1 and 4.6 % in zone 2. If these are considered together, decussation in this region of Stw 284 amounts to approximately 8.9%. Based on Figure A1.4, where additional prism decussation could be observed between the EDJ and point A, situated about 70 microns from the EDJ, a conservative 1% of decussation was estimated, indicating the likelihood that decussation maybe about 10% in this area.

Summary and Discussion

In several of the previous Chapters, Risnes (1986) correction factor was used to calculate CFT. It was noted, specifically in Lacruz *et al.*; (in press) that prisms decussated in some specimens of *A. africanus*. Here some information about prism decussation in this taxon was presented. Decussation in some areas was about 10 %, less than the observed decussation in some modern human premolars (Risnes 1986). Although the use of correction factors may not significantly increase cuspal formation time (Macho *et al.*; 2003), it is important to recognize enamel prism decussation as this may hold the potential for interpreting functional adaptations of fossil and extant taxa (Macho *et al.*; 2003). It appears that *A. africanus* shows a degree of prism decussation, as judged by this study and by the shape of HSB, that maybe different from *Paranthropus*.



Figure A1.1. Metacone of Stw 11 indicating the cuspal region where prisms were imaged in Figure A1.2.



Figure A1.2. Cuspal area of the metacone of the *A. africanus* specimen Stw 11 imaged at 20x using PCSOM. Prisms noticeably bend on the longitudinal plane following the main prism direction indicated by a white arrow. FW 480 microns.



Figure A1.3. Protocone cusp of Stw 284 indicating the area of the enamel (white square) about 600 microns from the dentine horn, in which we identified prisms that could be followed for a few hundred microns away from the EDJ. The area in the box has been enlarged in Figure A1.4. Image taken using 5x lens and 2.5 adapter. FW 3.5 mm.



Figure A1.4. This image shows the location of point A which is used in Figure A1.5 as an arbitrary point where we measured the beginning of prism decussation in this specimen. The distance between the EDJ and point A is about 70 microns. The long white arrow indicates the predominant prism direction. Image taken with 20x lens and 0.5 adapter using no clearing medium. FW 360 microns.



Figure A1.5. Montage image of prisms about 70 microns from the EDJ. Prism 1 was measured from point A to point B which is located at the vertical black line for a distance of about 96 microns. From this point on, prism 1 could not be confidently followed. The vertical black line separates zones 1 and 2. In zone 2, prism 2 was followed from point C in the black line, to point D for about 159 microns. The distance in a direct line between points A and B is 92 microns and the distance between points C and D is 152 microns, giving an estimated decussation of 4.3 % and 4.6 % in zone 1 and zone 2 respectively. Cross striations were visible about 270 microns from the EDJ (insert on this image). The average cross striation spacing in this area was 4.0 microns. FW 230 microns.



Figure A1.6. SEM montage of the same area shown in Figure 4. Prisms are noticeably bending in the longitudinal plane. Cross striations are shown near the area where these were highlighted in Figure A1.5, and the same mean (about 4.0 microns) was obtained. The white line indicates the division between zones 1 (left) and zone 2 (right) used in Figure A1.5. Specimen un-etched.