INTRODUCTION

Recent attempts to reconstruct life histories of fossil hominids have focused on the dentition as a reliable source of information on developmental rates. This as it is assumed that dental maturation is highly synchronized with general body development (Dean et al., 1986; Smith, 1991). Most of the research carried out on dental development in fossil hominids has focused on studies of the later stages of crown and root development, estimated from enamel apposition rates (Beynon, 1992; Beynon and Dean, 1987; Beynon and Wood 1987; Ramirrez-Rozzi, 1993) or crown and root development estimated from radiographs or single C-t scans (Conroy and Vannier, 1987; Faerman et al., 1994; Mann et al., 1990; Skinner and Sperber, 1982). However it is also possible to study earlier stages of the ontogeny of these fossil teeth, since the dentine-enamel junction (DEJ) provides a permanent record of the balance achieved between cell growth and differentiation at an early stage of development. Moreover, this can be compared to the final shape of the crown seen at the outer enamel surface (OES), so providing information on two successive phases of growth within a single tooth.

The potential value of the DEJ for studying ontogeny and phylogeny has long been realized (Butler, 1968; Korenhof, 1960; Kraus, 1952, Kraus and Jordan, 1965), but its application to fossils has been limited until now by the difficulty of visualizing the DEJ without destroying either dentine or enamel. The development of three dimensional imaging systems has removed this obstacle, and Smith et al., (1997) used serial C-t scans to develop a three dimensional model for comparison of selected locations at the DEJ and OES of modern and fossil teeth.

In this paper we describe a new computerized three dimensional model derived from serial C-t scans, that can be viewed from any angle or superimposed on any other tooth for direct comparison. The model is extremely flexible and was designed to obtain information on global size and shape change in teeth. It has been applied here to analysis of growth in the first permanent (M1) and second deciduous (DM2) molars. We propose that the shape and size changes observed reflect growth trajectories within the teeth and can be used to reconstruct the ontogeny of tooth development in both living and fossil hominids. The rationale for using C-t scans and the accuracy of the method has been discussed in detail in Smith et al., (1997). The great advantage of the method is that it is non-invasive, can be applied to both recent and fossil teeth and provides excellent
delineation of both the DEJ and OES. The main limiting factor is the accuracy of identification of data points, but this can be estimated by repeated scanning and measurement of selected teeth.

MATERIALS AND METHODS

Mandibles of 14 infants recovered from archaeological excavations in Israel and dating to between 6000 B. P. and 200 A.D. were used in this study. Selection was based on the presence of an unworn DM2 and unerupted M1 with at least ¾ of the crown complete. Serial C-t scans were then taken along the mesio-distal axis of the lower DM2 and M1. The C-t scans were made using an Elscint 2400 model, with scans 1.2 mm thick, but taken at 0.5 mm intervals to give intercalated scans. Using a workstation and optimizing window settings for each reading (i.e., separate readings for the enamel-air and enamel-dentine boundaries), X and Y co-ordinates were registered for 8 sites on each scan as shown in Figure 1. The third coordinate, z, was calculated from the distance between successive slices. All scans were re-measured on separate occasions. Where differences exceeded 3%, teeth were re-scanned and if easily duplicable measurements could not be obtained they were excluded from analysis. When all scans of any one tooth had been examined in this way, the co-ordinates were entered into a data base and used to create a three dimensional image using the program described below.

Fig. 1. The C-t scan:
The CT scan results in a series of 2-dimensional images (slices) of a tooth profile. Such an image would typically look like that shown below.
Fig. 2. Computing the reference plane:
The contact points between the enamel and the dentine surfaces provide a set of points that
denote the base surface of the tooth. However, these points are not exactly on the same plane,
but rather distributed above and below any plane we would try to draw.
The situation is demonstrated here.

In order to find the best matching plane for all these points, a least-square analysis is used to
find the plane that minimizes the sum of distances to all points.

**Cusp detection:**
Refers to point ‘a’ in figure 1. For each CT scan section, this point is the “left maximum of the
enamel surface”. By taking all the a’s from all the sections, we get a set of such points, and we
can locate enamel cusps by finding maximum points within this set.

**Cusp detection:**
Refers to point ‘a’ in Figure 1. For each C-t scan section, this point is the “left
maximum of the enamel surface”. By taking all the a’s from all the sections, we get a set
of such points, and we can locate enamel cusps by finding maximum points within this
set. In this way, however, only points that were specifically measured can be located (as
mentioned above, the resolution of the C-t is normally 0.5 mm). Generally, the real cusp
might be in between two of the measured points. The situation is demonstrated in Figure
3: To gain a more accurate location of the cusp, an interpolation curve through the points
around the cusp was computed (like the curve in Figure 3), and the cusp was taken to be
the maximum of that curve. Assuming that the natural contour of the tooth is quite
smooth, the error for cusp location drops from 0.5 mm to about 0.2 mm. The same
technique is used for points ‘c’, ‘d’, and ‘h’ in Figure 1 to locate the other cusps.
**General description:**

The input to the program consists of two file-types:

1. A tooth topology-file, which contains information on the structure of the teeth and the method used to measure them. Hence different tooth types can be handled by the program.

2. A tooth data-file (or files), which contains a set of 3-dimensional tooth-coordinates obtained by performing measurements on the images created by the C-t scan. The result of such input is a 3-dimensional description of the enamel and the dentine surfaces of the tooth, and the program can work with one or more teeth.

The program analyzes the structure of the tooth, and performs the following computations:

1. Computes a reference plane which is an “average plane” for all the coordinates measured at the base of the tooth. The coordinate system is then transformed in such a way that further measurements will be related to that plane.

2. Detects the cusps: here the program applies an algorithm to find cusps on the enamel and dentine surfaces. Consequently a set of cusp-vectors are defined as the vectors connecting matching enamel and dentine cusps. When working with two teeth, a “center of gravity” is calculated for each one and the two teeth are aligned together so that their centers-of-gravity are united; their reference planes are parallel and the sum of distances between every two matching cusps (one of each tooth) is minimal (using mean-square analysis).

**Calculations and output:**

The program calculates:

1. All distances and angles in the 3-dimensional figure of the tooth, and in particular distances between cusps, angles between cusp-vectors and angles between cusp-vectors and the reference-plane (or the normal vector to that plane).

2. Average thickness of enamel, enamel and dentine areas, etc.

When working with two teeth, comparisons between them can also be performed.

For example, each of the following calculations can be made:

- Ratios between average magnitudes of each tooth (e.g. comparing average vector lengths).
- Angles between matching vectors in the two teeth.

Computation of deformation vectors, which are vectors between two matching cusps of the two teeth.

**Visualization:**

The image of the tooth is visualized by using interactive 3-dimensional graphics. This means that a 3-dimensional image appears on the screen, that the user can rotate, zoom or move, select and remove independently the enamel surface, dentine surface, reference-plane, cusp-vectors, and deformation vectors (in the case of two teeth). When working with two teeth, the user can manipulate each one of them separately or together.

**Implementation:**

The program can be used to measure a single tooth, to compare two teeth or to compare a tooth with a pre-defined tooth pattern. The C-t scan results in a series of 2-dimensional images (slices) of a tooth profile shown in Figure 1. In order to find the best matching plane for all these points, a least-square analysis is used to find the plane that minimizes the sum of distances to all points (Fig. 2).
Fig. 3. The maximum falls between sampled points. To gain a more accurate location of the cusp, an interpolation curve through the points around the cusp was computed (like the curve in fig. 3), and the cusp was taken to be the maximum of that curve.

Under the assumption that the natural contour of the tooth is quite smooth, the error for cusp location drops from 0.5 mm to about 0.2 mm. The same technique is used for points.

Computation of center of gravity:

There are a number of different options: the global center-of-gravity (the average point of all measured points) or cusps center-of-gravity (the average point of selected cusps).

Analyses:

From the large number of possible computations, the following variables were selected in order to estimate size and shape change between the DEJ and OES of the DM2 and M1: intercusp distances; surface area and shape of the pentagon joining cusp tips at the DEJ and OES and % area occupied by the DB-DI-DL triangle at each surface (Figure 4).

Fig. 4. Reconstruction of M1, with enamel removed, showing DEJ and vectors joining cusp tips at DEJ and OES.
Specifications:
Enamel / Dentine pentagon area:
The area of the enamel / dentine pentagon’s projection onto the reference plane.
Enamel / Dentine DB-DI-DL triangle area:
The area of the enamel / dentine projection onto the reference plane.
Enamel / Dentine pentagon angles:
Angles between neighbor edges of enamel / dentine pentagon.
Cusp tips:
MB-Mesio-buccal; DB- Disto-buccal; DI-distal; DL-Disto-lingual; ML-mesio-lingual: “e” is used as prefix to denote cusp tip at the OES and “d” is used to denote cusp tip at the DEJ.

RESULTS

One mandible was excluded from the study because of poor definition, and in a second the DM2 was damaged, so that data are presented for 12 DM2 and 13 M1. The computerized image of a typical M1 is shown in Figure 4. Here the enamel has been removed, leaving the dentine shell and vectors joining enamel and dentine cusp tips. The area of the pentagon defined as the projection between cusp distances on the reference plane, was significantly larger at the OES than that at the DEJ in both teeth (Table 1; p=0.001 in the DM2 and p=0.000 in the M1), and the OES and DEJ of the M1 were significantly larger than that of the DM2. In the DM2 the area occupied by the DB-DI-DL triangle at the OES deceased slightly relative to the area it occupied at the DEJ in 10/12 cases, but the differences were not statistically significant. In the M1, all 13 cases showed a decrease in the area occupied by the DB-DI-DL triangle at the OES and the differences were highly significant (P=0.001). This is reflected in shape differences in the two teeth shown in the pentagon joining cusp tips in Figure 5. In the DM2 there was a small but significant decrease in the DB angle at the OES (P=0.05). In the M1 the changes were more pronounced as the tooth assumed a more rectangular form. This is emphasized in the marked increase in the DB angle and reduction in DL angle, as the DI cusp approximates the mesio-buccal side of the tooth. Table 2 gives the mean distance of all cusp tips from the tip of the eMB cusp. Notably, the eMB-eDI and eMB-eDL distances are similar in the DM2, but very different in the M1. In this tooth the eMB-eDI distance is significantly less than the eMB-eDL distance.
Table 1. Pentagon area and shape change.

<table>
<thead>
<tr>
<th></th>
<th>M1 (n=13)</th>
<th>DM2 (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enamel</td>
<td>Dentine</td>
</tr>
<tr>
<td><strong>Total Area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>32.1</td>
<td>27.1</td>
</tr>
<tr>
<td>SD</td>
<td>3.98</td>
<td>2.40</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>%DB-DI-DC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>25.50</td>
<td>28.90</td>
</tr>
<tr>
<td>SD</td>
<td>6.43</td>
<td>7.20</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0.05</td>
</tr>
</tbody>
</table>

In M1, the percentage area of the triangle DB-DI-DL was always smaller at the OES and the differences were statistically significant. In DM2 the percentage area of the triangle DB-DI-DL was smaller in 10/12 cases, but the differences were not statistically significant.

Table 2. Distance of all cusps from tip of MB cusp on outer enamel surface

<table>
<thead>
<tr>
<th>DISTANCE</th>
<th>On dentine</th>
<th>On enamel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dm2</td>
<td>M1</td>
</tr>
<tr>
<td>MB-DB</td>
<td>Mean</td>
<td>3.57</td>
</tr>
<tr>
<td></td>
<td>Variance</td>
<td>0.14</td>
</tr>
<tr>
<td>MB-DI</td>
<td>Mean</td>
<td>6.37</td>
</tr>
<tr>
<td></td>
<td>Variance</td>
<td>0.41</td>
</tr>
<tr>
<td>MB-ML</td>
<td>Mean</td>
<td>3.86</td>
</tr>
<tr>
<td></td>
<td>Variance</td>
<td>0.16</td>
</tr>
<tr>
<td>MB-DL</td>
<td>Mean</td>
<td>6.65</td>
</tr>
<tr>
<td></td>
<td>Variance</td>
<td>0.24</td>
</tr>
<tr>
<td>MB-MB</td>
<td>Mean</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Variance</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Kraus and Jordan (1965) showed that the DM2 and M1 resembled one another in the early stages of morphogenesis, but differed in the relative ordering of morphogenesis and mineralization. The DM2 was characterized by early and rapid expansion of the talonid, and mineralization in the MB cusp of the DM2 began at an early stage of development, before all cusp tips were clearly defined (15 1/2 weeks *in utero*). Final definition of intercusp relationships at the DEJ defined as coalescence of centers of mineralization of individual cusps was completed by the 32nd week *in utero*. In the M1 mineralization commenced at a later stage of morphogenesis, after initial definition of all cusps. Kraus and Jordan (1965) reported that in one specimen studied by them, the first signs of
mineralization were seen at 28 weeks in utero, but that the average age for the initialization of mineralization in the M1 was 32 weeks in utero. There is relatively little information available on the later stages of morphogenesis and timing and sequence of mineralization at the interphase defining the DEJ in the M1, and most of that available is based on radiographic studies, summarized by Liversidge (1995). Moorrees et al., (1963) reported that cuspal coalescence in the lower M1 occurred at around 7 months, which would suggest that the process took twice as long as in the DM2. Thus the spatial and temporal patterning and rate of cell division and maturation appear to differ in the two teeth from an early phase of morphogenesis.

The results obtained here are in agreement with previous studies on decalcified teeth (Korenhof 1960) and cross sectional studies of tooth germs from archaeological samples (Smith et al., 1995) for the extent and pattern of differences observed between the DM2 and M1 at the DEJ. Butler (1968), reported that human fetal deciduous molars showed a marked increase in intercusp distances during the last stages of mineralization preceding coalescence. He theorized that this was due to the mineralized cusp tips tilting apart with continued growth at the cuspal base and Osborn (1993) presented a computerized simulation of cell division that showed how localized differences in cell division may affect the height and inclination of cusp tips. Experimental studies have now demonstrated that the spatial and temporal ordering of cell division and function within the tooth germ is regulated by a large number of genes (Slavkin and Diekwisch, 1996; Snead et al., 1988), so that small differences in their ordering and duration of activity can markedly modify tooth form.

![Image](image_url)

Fig. 5. Mean values for area and shape of pentagons joining cusps at the OES and DEJ, upper left-DM2 DEJ, Upper right DM2 OES. Lower left -M1 DEJ; Lower right-M1 OES.
In the three dimensional models of human teeth examined here, the angles between vectors joining individual cusp tips at the DEJ and OES, are assumed to define their direction of growth and produce size and shape differences between them. In this study they were defined by changes in intercusp distances and shape of the pentagon joining cusp tips at the DEJ and OES respectively. As shown in Tables 1, 2 and Figure 5, they demonstrate that there are significant differences in growth trajectories of the modern DM2 and M1. The relative reduction of the area defined by the DB-DI-DL triangle on the OES of the M1, reflects the reduced growth of the distal moiety of the tooth. The differences between the two teeth are not simply a function of size, but reflect specific differences in growth and maturation within the tooth germ.

We propose that the observed changes in cuspal divergence as measured by shape and size change at the DEJ and OES, provide a means of reconstructing the presumed pattern of growth. The continuous rapid growth of the talonid in the DM2 prior to coalescence with other cusps, is reflected in the marked divergence of cusps shown in our study and demonstrated by the extent and localization of change in size and shape at the OES. The change observed in the location of the distal cusps in the M1 is the result of differences in mitotic rates at the base of the developing DI and DL cusps, with the latter growing at the expense of the former.

CONCLUSIONS

The model developed for this study constitutes an innovative technique for studying growth trajectories in modern and fossil teeth. The specific results presented here support the hypothesis that evolutionary reduction in molar tooth size, characterized by talonid reduction is associated with reduction of mitosis in the later stages of morphogenesis, preferentially affecting the distal portion of the tooth.

LITERATURE CITED