Chapter 14

Computerized Reconstruction of Prenatal Growth Trajectories in the Dentition: Implications for the Taxonomic Status of Neandertals

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Abstract The hierarchical pattern of tooth formation means that successive phases of development can be identified in fully formed teeth offering a unique insight into ontogenetic processes. The spatial geometry of the cusps expressed in the topography of the dentin-enamel junction (DEJ) records the partitioning of cell proliferation and differentiation as well as the timing of these events. The final stage of development is expressed in the topography of the fully formed crown (OES). Here, the overlying shell of enamel increases crown volume, while modifying cusp relations seen at the DEJ reflecting local variations in enamel thickness.

Using serial scans taken with a micro-CT at 16 µm we have developed a three-dimensional model that enables us to identify, and more importantly quantify, all these developmental features. We have applied this model to reconstruct growth trajectories and their impact on tooth size and cusp relationships in teeth of varying size and tooth classes. The results are used to interpret the extent of developmental variation expressed in Neandertal molars. They indicate that the characteristic features of Neandertal teeth, expressed in inter-cusp distances and proportions, thin enamel and taurodont roots represent a different partitioning of cell division and differentiation from that observed in *Homo sapiens sapiens*.

Our findings indicate the existence of differences between Neandertals and other hominins in genes controlling the switch-on/switch-off mechanism that regulates the timing, rate and spatial organization of cell proliferation and differentiation of epithelial derived tissues. The thin Neandertal enamel results in teeth that wear down fast, resulting in loss of dental function. We propose that the changes observed in Neandertal teeth, may be secondary to those occurring in other organs developing from epithelial-mesenchymal interaction, with greater adaptive significance.

Keywords Tooth Formation • Ontogenesis • Neandertal - *Homo sapiens sapiens* • micro-CT

Introduction

Over 150 years after their first discovery, there is still no consensus concerning the taxonomic status of the Neandertals. Hrdlička (1930: pp. 319), complained that Neandertal skeletal remains were too few and too poorly preserved to provide adequate samples for the morphometric analyses necessary to resolve this issue. Today, the number of fossils available for analysis has greatly increased, demonstrating that the temporal and geographic distribution of Neandertals was greater than originally perceived and was accompanied by a correspondingly larger range of morphometric variation (Bailey and Hublin 2006; Harvati 2003; Hublin 1998, 2011; Rosas et al. 2006; Smith et al. 2005; Stringer 2002; Stringer et al. 2004; Wolpoff et al. 2001). Moreover, some features originally considered as having arisen *de novo* in Neandertals have been reported in other fossil hominins including Upper Paleolithic *Homo sapiens sapiens* (Soficaru et al. 2006; Trinkaus et al. 2003), renewing speculation as to the possibility of gene flow between them. Even aDNA techniques (Gutiérrez et al. 2002; Hebsgaard et al. 2007; Pääbo et al. 2004; Weiss and Smith 2007) have not yet provided a conclusive answer to the question of Neandertal admixture. The application of theories that relate the timing and pattern of developmental variation to evolutionary diversity provides an alternative starting point for investigating the phylogenetic status of Neandertals.

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While an ‘Evo-Devo’ approach cannot be directly applied to the study of fossil remains, the concepts can be utilized to reconstruct growth trajectories from examination of the fully formed teeth. In contrast to the bony skeleton, tooth crown form and size once finalized does not undergo remodeling or repair so that the dental phenotype is not affected by environmental factors related to function (Dahlberg 1961, 1985). Most importantly, the hierarchical pattern of tooth formation means that the partitioning of cell division and differentiation, expressed during morphogenesis, is recorded in the topography of the dentin-enamel junction (DEJ) and outer enamel surface (OES) (Korenhof 1979; Kraus 1952; Sasaki and Kanazawa 2000; Smith et al. 1997, 2000).

Both oral ectoderm and neural crest ecto-mesenchyme participate in tooth formation. This process is governed by numerous growth factors some of which act specifically on epithelial derivatives and others primarily on mesenchymal tissues (Jernvall and Thesleff 2000; Butler 1956, 1967, 2000; McCollum and Sharpe 2001; Zaho et al. 2000). Each tooth germ is initiated by down-growth of a fold in the oral epithelium. This forms a cap-shaped structure, the dental organ, that partially encloses a local condensation of mesodermal cells. The cusps are initiated one after another in response to growth factors secreted by the primary and secondary enamel knots (Jernvall et al. 2000). Biomineralization begins when cells of the inner enamel epithelium and underlying layer of cells of the dental papilla differentiate into ameloblasts and odontoblasts that form enamel and dentin respectively. The process starts at the cusp tips and proceeds apically (Fig. 14.1).

Cusp initiation in all hominids follows a constant sequence of protoconid, metaconid, hypoconid, entoconid and hypoconulid (Butler 1956, 1967, 2000; Kraus 1952; Kraus and Jordan 1965; Swindler and McCoy 1965; Swindler et al. 1968; Swindler and Meekins 1991; Winkler et al. 1996), but the amount of growth between the initiation of successive cusps varies as does the duration and rate of further growth (Butler 1967, 2000; Jernvall et al. 2000). Differences in growth rates along the inner and outer slopes of the cusps, as well as those between adjacent cusps, are reflected in their height, shape (that is thin or rounded), as well as the order of coalescence with adjacent cusps. These features are preserved in the topography of the DEJ, on which is superimposed a layer of enamel of varying thickness, that increases the volume of the tooth while modifying the outline defined at the DEJ. Thus, crown size and cusp pattern seen on the outer enamel surface of the tooth reflect not only the order of cusp initiation but also the spatial organization of cell division and differentiation as reflected in cusp size, proportions and location defined at the DEJ, modified by the overlying thickness of enamel.

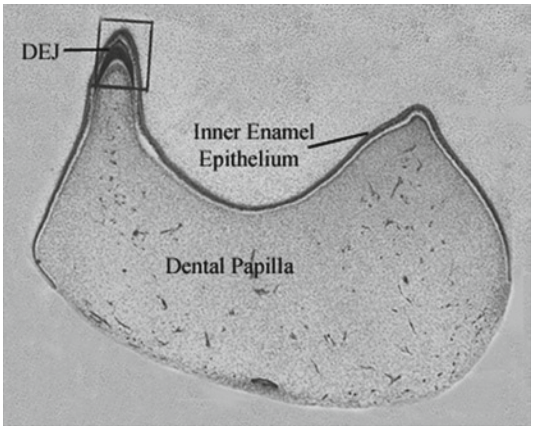

Fig. 14.1 Developing lower 2nd deciduous molar showing initial phase of biomineralization. Note that enamel and dentin formation have begun on the protoconid, enclosed in the *rectangle*, but not yet on the metaconid (Modified from Kraus and Jordan 1965)

Recent advances in imaging techniques have now made it possible to obtain extremely accurate high resolution serial scans that differentiate between dentin and enamel and can be compiled to create accurate 3-D reconstructions of both tissues. Using this approach we have developed a model that facilitates quantification of individual cusps and their relationship to one another at the DEJ and OES. The accuracy of the model has been tested using developing tooth germs (Avishai et al. 2004) and found to provide a reliable estimate of the partitioning of cell proliferation and biomineralization. These processes are reflected in cusp volume and distances between cusp tips and coalescence points. The model is used here to examine the effect of variation in growth trajectories on crown size and cusp pattern of contemporary molars and applied to interpret the significance of developmental variation that results in the characteristic morphology of Neandertal molars.

The Model

Our model is derived from serial micro-CT scans taken at 16 μm intervals. We used a standard protocol for scanning and subsequent reconstructions and analyses that was developed by R.M. The landmarks were located by G.A. directly from the micro-CT workstation. They included XYZ coordinates of cusp tips and points of coalescence between cusps, the cemento-enamel junction defining the base of the crown and enamel and dentin contours of individual cusps and crown circumference. The total measurement error calculated from reconstructions derived from repeated scans was 1.2% (Avishai et al. 2004).

We have now scanned 30 lower second deciduous and first permanent molars of recent humans using the method detailed in Avishai et al. (2004). We oriented all specimens along a horizontal plane defined by the cusp tips of the protoconid, metaconid and hypoconid, which are the first cusps

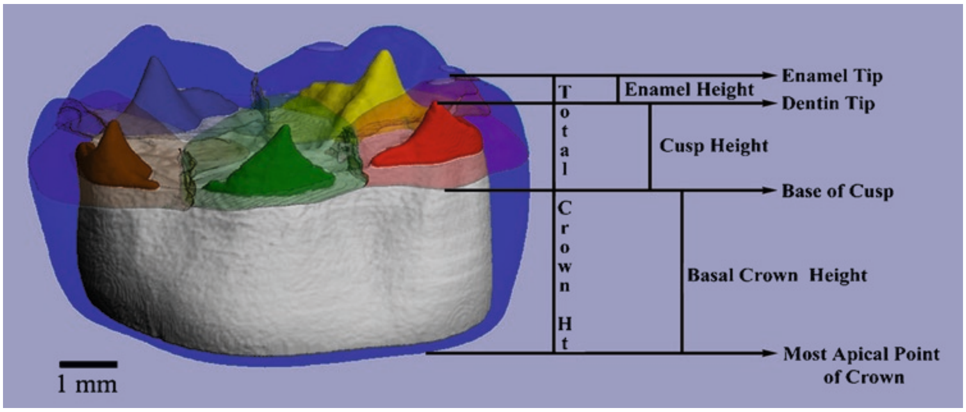

Fig. 14.2 Buccal view of micro-CT reconstruction of lower second deciduous molar showing major reference points measured

initiated (Kraus and Jordan 1965), and used this as our reference plane for evaluating subsequent tooth development. Computerized reconstructions of each tooth were compiled using an algorithm developed by R.M. The vertical distance between cusp tips was used to estimate the order of cusp initiation and the distance between the most occlusal and most apical points of coalescence of the cusps, defined as Maximal Fusion Height (MFH). This was used to assess the amount of growth between them. A line joining the first two coalescence points of each cusp was used to construct a virtual plane defining the base of each cusp and used in subsequent calculations. The area of cusp base was defined by morphing to compensate for height differences between coalescence points. Analyses included calculation of: cusp height and volume at the DEJ and OES, intercusp distances, enamel thickness and volume, cross-sectional area and thickness of enamel at standard points along the cusps, crown volume and maximum perimeter and area of the crown.

For ease of interpretation we partitioned Euclidean distances between points of measurements into their vertical and horizontal components, with apical growth reflected in dentin cusp height (DCH) and maximum coalescence height difference (MFH), while horizontal growth is reflected in distances' between cusp tips. The ratio of horizontal to vertical cusp distance provides an estimate of the amount and direction of growth between successive cusps (Fig. 14.2).

In modern deciduous and permanent molars, cusps first coalesce on their outer surfaces, while still continuing to grow independently on their inner slopes (Fig. 14.3). The protoconid first coalesces with the metaconid and hypoconid, the next coalescence takes place between the hypoconulid and entoconid. This is followed by the hypoconid-hypoconulid. The metaconid-entoconid coalescence is the last to occur. Once this occurs, the cusps are united together by a continuous ring of dentin fixing their relations to one another, even though they continue to grow on their inner slopes. The amount of further growth on their inner aspects before coalescence is reflected in the depth of grooves and fovea. Since cusps expand in basal area as they grow, their height before coalescence is reflected in intercusp distances and overall dimensions of the crown. Occasionally additional folds appear in the inner enamel epithelium, in which independent

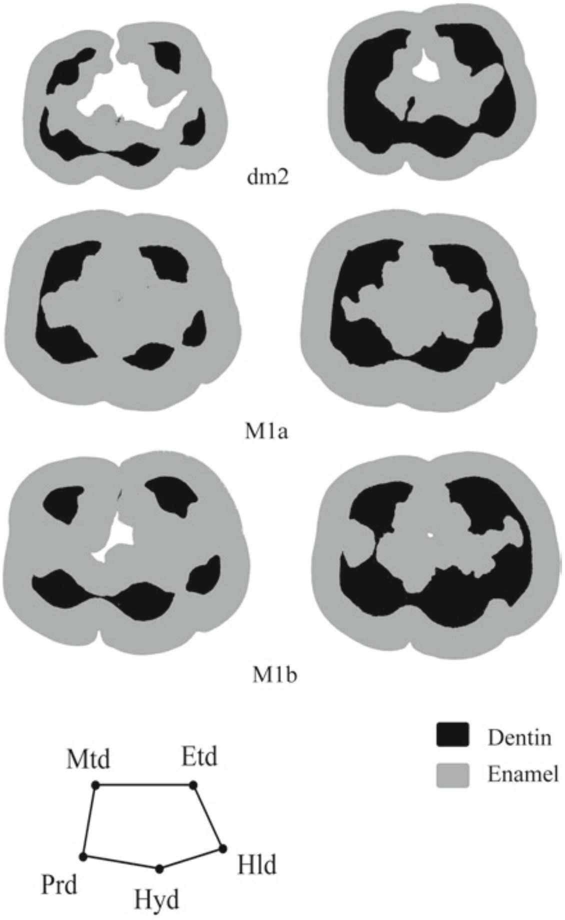

Fig. 14.3 Reconstructed axial slices of three lower molars at first point of coalescence (*at left*) and last coalescence prior to metaconid-entoconid coalescence (*at right*). All slices oriented as outlined in diagram at *bottom*. *Top* – second deciduous molar, note: thin enamel. *Middle* – small first permanent molar. *Bottom* – large first permanent molar

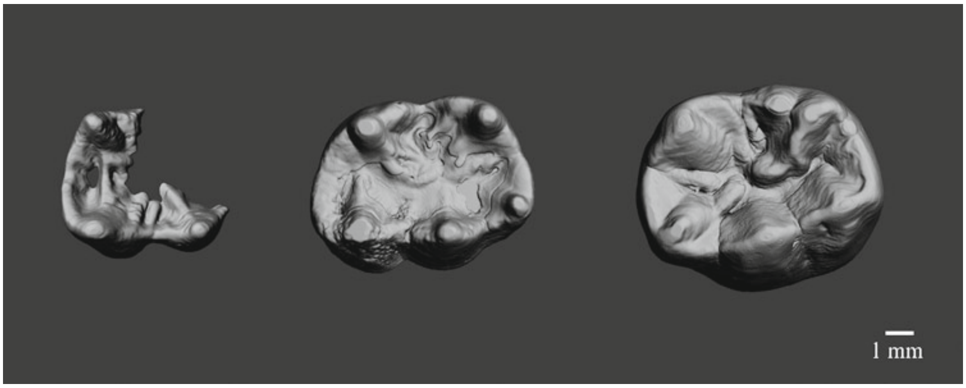

Fig. 14.4 Occlusal representation of three lower 2nd deciduous molars, showing changes in surface topography in different stages of development

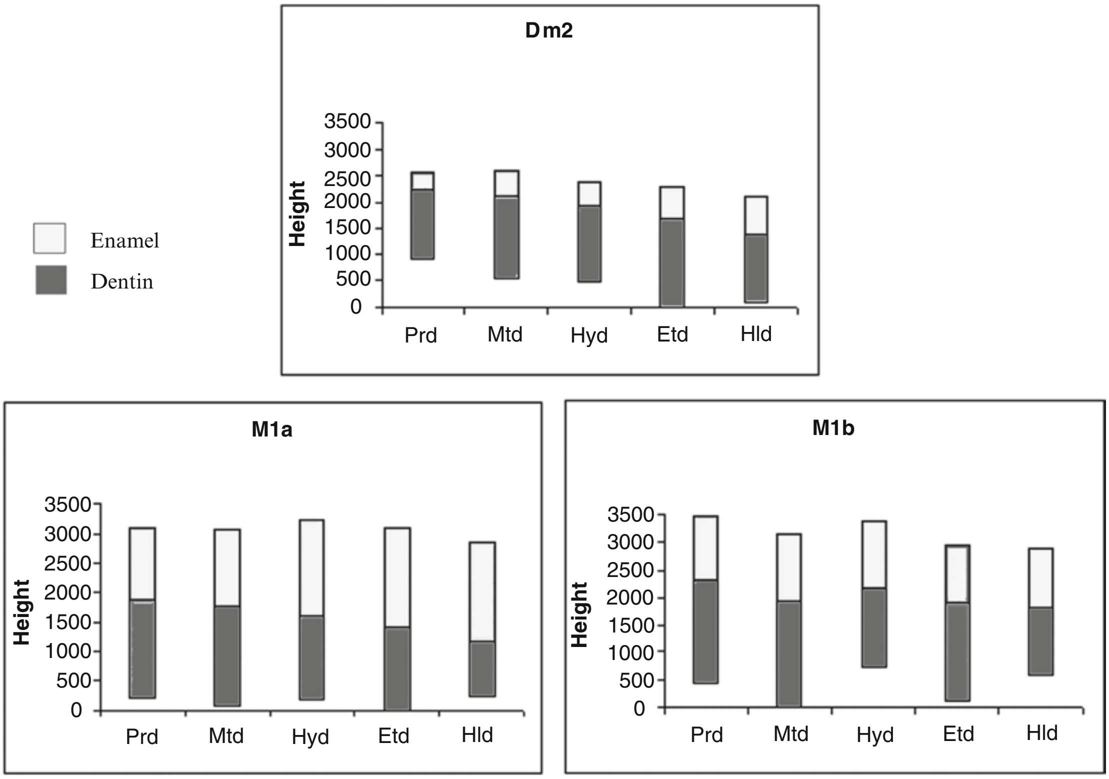

Fig. 14.5 Vertical location of enamel tip (*top of bar*) and cusp base (*bottom of bar*). Height measured in µm. Note differences in enamel and dentin height between cusps and between teeth. The metaconid is tallest in the permanent molars and the entoconid tallest in the deciduous molar. Enamel is thicker in the permanent teeth than in the deciduous tooth and is thickest on the hypoconid and hypoconulid

centers of bio-mineralization develop, producing extra ridges and cuspules (Fig. 14.4). Measurements taken at the DEJ show that the amount of vertical growth expressed by MFH is correlated with horizontal growth expressed by intercusp distances (Smith et al. 2007).

The spatial components of growth expressed by distances between cusp tips and coalescence points at the DEJ indicate major differences between tooth classes as well as between large and small teeth of the same class (Fig. 14.5 and Table 14.1). In the deciduous molars, the entoconid, which is the fourth cusp initiated, is consistently the tallest followed by the metaconid, protoconid and hypoconulid (Fig. 14.5). The hypoconid, the third cusp initiated, is consistently the shortest. At the DEJ of the first permanent molar, ranking of cusp height is more variable. In small teeth, the metaconid is taller than the entoconid and the hypoconid is taller than the hypoconulid.

The contribution of cusp height to total crown height also differs markedly between the deciduous and permanent molar, reflecting the different partitioning of cusp formation relative to crown formation. In the fast growing second deciduous molar, biomineralization begins some 6 weeks after initiation of the tooth germ and horizontal and vertical distances between cusp tips are similar, reflecting the rapid growth in length and breadth as well as apically in this tooth while the cusps are forming. In the first permanent molars, biomineralization only begins some 4 months after the initiation of the tooth germ and continues for nearly three times as long as that of the deciduous molars. In the permanent molar height differences between cusp tips are small, relative to horizontal distances, indicating that the tooth germ is larger when biomineralization begins and that less growth takes place between the initiation of successive cusps. This may reflect the slower rate of growth of this tooth but is also expressed in a different spatial partitioning of cell proliferation within the tooth (Butler 1967, Kraus and Jordan 1965). In both large and small first molars, the hypoconid is finalized at an early stage of development, when it coalesces with the protoconid, while the size of the hypoconulid is dependent on that of the entoconid (Fig. 14.5).

Comparison of the DEJ and OES of deciduous and permanent molars demonstrates the contribution of the thickness of the enamel shell to tooth size and proportions. The volume as well as the shape of the tooth crown seen at the OES reflects the combined volume of the tooth defined at the DEJ that results from cell proliferation, plus the amount of enamel matrix laid down by a fixed number of differentiated cells-the ameloblasts. Obviously, the thicker the enamel, or the more it varies in different locations, the greater the extent of modification of the crown from the underlying template defined by the DEJ. Like the covering of snow that smoothes out jagged mountain peaks and fills in crevices, thick enamel obscures details present at the DEJ.

Table 14.1 Basal cusp area at DEJ and OES (mm^2)

Area at DEJ

Tooth	Prd	Mtd	Hyd	Etd	Hld
dm$_2$	3.6	5.43	3.27	5.55	2.62
M_1 a	4.89	6.52	2.7	6.02	1.72
M_1 b	5.85	8.72	4.12	9.72	3.15

Area at OES

Cusp	Prd	Mtd	Hyd	Etd	Hld
dm$_2$	13.59	13.7	12.42	12.06	14.73
M_1 a	21.63	21.23	15.01	18.9	11.77
M_1 b	20.66	22.04	17.06	20.96	17.33

Note area of buccal cusps changes more than area of lingual cusps because of thicker enamel. This changes cuspal proportions at the OES. Note that in both permanent molars the entoconid is larger than the hypoconid at the OES, while the deciduous tooth with a larger talonid maintains the conservative sequence with the hypoconid larger

Prd Protoconid, *Mtd* Metaconid, *Hyd* Hypoconid, *Etd* Entoconid, *Hld* Hypoconulid, M_1a is a small 1st permanent molar, M_1b is a large 1st permanent molar

Examination of enamel thickness in different locations over the cusps shows that the thickness of the enamel shell varies over the surface of each cusp. Consequently cusps are more rounded and the topography seen at the OES is much smoother than that at the DEJ. At the same time, local variation in enamel thickness creates additional features such as marginal cuspules not represented at the DEJ. Enamel on the outer surface of all cusps is thicker than that on the inner slopes (Grine 2005; Keinan et al. 2006; Suwa and Kono 2005). It is thicker on the buccal cusps than on the lingual cusps and is usually thickest on the hypoconulid followed by the hypoconid, so that these cusps occupy a relatively larger area on the crown surface than at the DEJ. This applies even to the thin-enameled deciduous molars so that the rank order of cusp area at the OES may change from that measured at the DEJ (Table 14.2). However, the angle formed by the cusps to one another also affects intercusp distances. Where the cusps diverge from one another, distances at the OES are greater than those at the DEJ. Where there is little intercusp growth, cusps may be parallel or incline towards one another and distances between them at the OES are similar or smaller to those seen at the DEJ (Smith et al. 1997, 2000). This is demonstrated here by the differences shown between intercusp distances at the DEJ and OES of the dm2 and permanent molars in Table 14.1.

In the small M1a represented in Figs. 14.3 and 14.5 and Tables 14.1 and 14.2, the hypoconid and hypoconulid are very much smaller than other cusps at the DEJ, but the thicker enamel obscures this at the OES. In the permanent molars, the increase in hypoconid cusp area is more than double that of the metaconid. Moreover, when the hypoconulid is extremely reduced, the thicker enamel may obscure the boundary between it and the entoconid (Korenhof 1979; Kraus and Jordan 1965).

Table 14.2 Distance between cusp tips (mm) at DEJ and OES, and maximum area (mm^2) at OES

Tooth	Area		Prd-Mtd	Prd-Hyd	Prd-Etd	Prd-Hld	Mtd-Hyd	Mtd-Etd	Mtd-Hld	Hyd-Etd	Hyd-Hld	Etd-Hld
dm$_2$	–	DEJ	3.51	3.58	6.46	6.41	5.00	4.71	6.80	4.64	2.92	3.94
dm$_2$	72.1	OES	3.65	3.93	7.12	7.18	5.65	5.20	7.67	5.60	3.45	4.75
M_1 a	–	DEJ	4.15	4.18	7.26	7.24	6.27	5.55	8.07	5.44	3.26	4.50
M_1 a	83.4	OES	4.85	3.64	7.45	6.89	6.35	5.67	8.42	5.58	3.29	4.97
M_1 b	–	DEJ	4.31	4.56	6.97	6.85	6.48	5.32	7.67	4.88	2.50	4.04
M_1 b	99.4	OES	5.28	4.78	7.45	7.16	7.40	5.99	8.68	5.02	2.51	4.31

For each tooth first row = DEJ, second row = OES. Tooth area in mm^2. Calculated from maximum convexity of crown

Note that the Prd-Hld intercusp distance is smaller than Mtd- Hld distance in all teeth, despite the variation in location of the Hld

Implications for Neandertal Tooth Formation

The results obtained using our three dimensional model show how differences in the partitioning of cell division and differentiation between cusp initiation and coalescence of individual cusps, are reflected in spatial geometry of the cusps at the DEJ and the extent to which the DEJ is modified on the crown surface by the enamel shell. They show differences in the partitioning of growth between deciduous and permanent molars as well as between large and small teeth of the same tooth class. They also show that the hypoconid is incorporated into the developmental sequence of the modified trigonid, while the early entoconid and hypoconulid coalescence means that these two cusps grow independently until the coalescence of the hypoconulid with the hypoconid. The location of the hypoconulid relative to the entoconid is therefore defined before its position relative to the hypoconid is finalized. Finally as shown here in Tables 14.1 and 14.2 and Fig. 14.5, differences in enamel thickness modify the crown surface defined at the DEJ, so that while the topography of the DEJ reflects epithelial-mesenchymal interactions, the epithelial derived ameloblasts "have the last word" in terms of fine-tuning enamel thickness and so crown form. These findings provide a developmental framework with which to interpret the significance of morphological features that distinguish Neandertal teeth from those of other fossil or contemporary hominins.

Neandertal deciduous and first permanent molars are characterized by thin enamel and taurodont roots (Macchiarelli et al. 2006; Molnar et al. 1993; Ramirez Rozzi 1996; Smith 1990; Zilberman and Smith 1992; Zilberman et al. 1992). The occlusal surface shows deep anterior fovea, a high frequency of mid-trigonid crests and a tendency for cusp tips to be more closely aligned on the occlusal surface than those of other hominins (Bailey 2002a, b; Bailey 2005). Radiographic studies of Neandertal deciduous teeth also indicate accelerated development and eruption relative to that of modern humans (Faerman et al. 1994; Legoux 1966; Skinner and Sperber 1982). However, the extent to which this accelerated growth pattern is maintained in the permanent dentition is unclear, since conflicting results have been published from both radiographic studies and those based on counts of perichymata or incremental lines (Dean et al. 1986; Thompson and Nelson 2000; Guatelli-Steinberg et al. 2005, 2007; Macchiarelli et al. 2006; Mann et al. 1990; Ramirez Rozzi and Bermudez De Castro 2004).

Enamel Thickness

The thin enamel that distinguishes Neandertal teeth means that the OES in permanent as well as deciduous Neandertal molars retains a more faithful resemblance to the underlying DEJ than the similar sized but thicker enameled molars of other hominins. This may account for the greater frequency of features such as midtrigonid crests and 6th and 7th cusps that are more commonly present at the DEJ of contemporary humans than at the OES where they tend to be obscured by the overlying enamel (Korenhof 1979; Kraus and Jordan 1965; Sasaki and Kanazawa 2000).

Cusp Pattern

Molar cusp pattern in *Homo sapiens sapiens* is directly linked to tooth size, with smaller teeth changing from the Dryopithecine 5Y pattern to 5+ and eventually 4+ as the hypoconulid rotates lingually and is eventually integrated with the entoconid. Bailey (2002b) reported that in anatomically modern *Homo* and Upper Paleolithic *Homo sapiens sapiens* cusp size rank was predominately protoconid>metaconid>hypoconid>entoconid>hypoconulid, but changed in contemporary teeth to protoconid>metaconid>entoconid>hypoconid>hypoconulid. These differences within fossil and modern *Homo sapiens sapiens* illustrate the changing cusp relations associated with reduction in tooth size. In the Neandertals, cusp pattern differs from that predicted from their large size, and clusters with that found for contemporary teeth, emphasizing yet again their unique pattern of development. Moreover these differences appear to be even more pronounced in the early developing deciduous teeth (Fig. 14.6).

The ratio of distances between cusp tips in deciduous teeth of Neandertals, anatomically modern *Homo* and a modern sample is shown in Table 14.3. In both anatomically modern *Homo* and contemporary deciduous teeth, the cusps are more evenly spaced than is the case for the Neandertal molars and the distance between the protoconid and hypoconulid is greater than that between the metaconid and hypoconulid. In the Neandertal molars, intercusp distances vary markedly and the shape of the tooth differs, with the hypoconulid internally located and much closer to the entoconid than in any of the other teeth. Placed within the developmental pattern shown by our model, this suggests a major shift in the partitioning of cell division and differentiation within the tooth germ, expressed in a striking reduction of the talonid.

The thin enamel of Neandertal molars also means that the contribution of enamel thickness to tooth volume is smaller than in similar sized molars of *Homo sapiens* or other hominins. This implies a larger dental papilla and so a larger surface area at the DEJ with more ameloblasts even though they produce less enamel. This makes the reduced functional activity of the ameloblasts shown by the thin enamel even more striking. It indicates a major difference in the expression of growth factors regulating cell division and differentiation of the inner enamel epithelium in Neandertals.

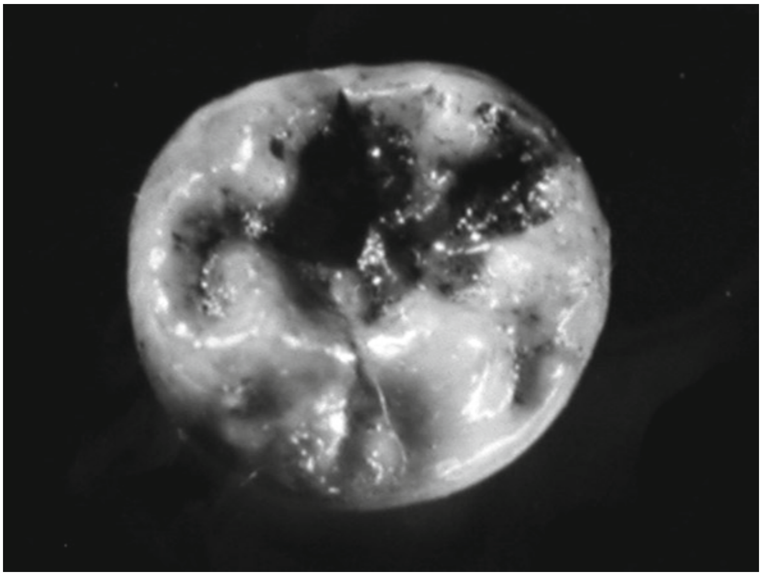

Fig. 14.6 Occlusal view of the second deciduous molar from Kebara 1. Note the internally located cusps and rounded outline of this tooth

Table 14.3 Intercusp distances measured at OES and standardized as % of Prd-Hld distance

	dm$_2^a$	dm$_2^b$	KEB I	KEB IV	SKHUL X
Prd-Mtd	51	58	63	56	59
Prd-Hyd	55	54	61	55	50
Prd-Etd	99	101	104	106	99
Prd-Hld	100	100	100	100	100
Mtd-Etd	72	70	64	68	58
Mtd-Hld	107	107	94	94	101
Hyd-Etd	78	81	73	79	73
Hyd-Hld	48	50	42	50	51
Etd-Hld	66	65	54	55	69

Neandertals: KEB I – Kebara I, KEB IV – Kebara IV. Anatomically modern human: SKHUL X. Modern specimens taken from archaeological collections where ^{a}dm$_2$ – measured from micro-CT reconstructions, ^{b}dm$_2$ mean value for 20 teeth. This and fossil specimens measured directly from occlusal surface using "Galai" image analyzer as described in Smith et al. 1995. Note: Similar ranking of intercusp distances measured directly from the occlusal surface of the dm2 and those computed using the 3D model. In the two Neandertal specimens, Prd-Hld, Prd-Etd ratios are reversed, with the Hld closer to the Etd and further from the Prd than in all other specimens

Root Formation

Root formation in Neandertals is also unique. In Neandertals the cervical region lacks the cervical constriction typical of *Homo sapiens sapiens* molars, while the common root stem is elongated and bifurcation of the roots is apically located (Kallay 1963). This is due to a delay in the change of direction of the cells of Hertwig's epithelial root sheath that define root form. While this condition does occur in modern humans as well as in other fossil hominids, it is not combined with thin enamel, but rather associated with short roots and so differs from the pattern seen in Neandertal molars that are larger with longer roots than most modern teeth (Bailey 2005).

Macchiarelli et al. (2006) reported that the Neandertal first permanent molar shows a slower initial phase of root development than modern teeth, but that later development is more rapid so that it completes development at the same time as modern teeth. Their findings, based on examination of incremental lines in the dentin of ground sections, provide independent confirmation of our analyses that suggest marked differences between Neandertal and *Homo sapiens sapiens* in the partitioning (timing and amount of growth) of successive developmental stages within the tooth germ.

Interpretation

We propose that the Neandertal molar complex comprising large teeth with a small occlusal area, a relatively small entoconid and hypoconulid, thin enamel and long taurodont roots demonstrates the presence of unique growth trajectories in Neandertal teeth. All of these dental features may be attributed to changes in signaling pathways that regulate the epithelial-mesenchymal interactions that form the teeth. These may specifically affect the epithelial component, since this gives rise to the enamel knots that define the timing and location of cusp initiation as well as the inner enamel epithelium that differentiates into enamel forming ameloblasts and Hertwig's epithelial root sheath. There is good evidence to show that many of the active signaling pathways affecting tooth formation are common to other organs dependent on epithelial-mesenchymal interactions, such as hair, sweat glands and lungs (Plikus et al. 2005). The changes seen in the Neandertal teeth may constitute one component of a broader distinctive pattern of development affecting the form and function of other organs in which epithelial derived tissues play a major role. Thus it may be time to evaluate the selective significance of the dental changes seen in the Neandertals, within a wider perspective than that of dental function.

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