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Effects of Molar Crown Orientation to Measures of Lateral Enamel Thickness in the Mesial Cusp Section

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Abstract Four definitions of enamel thickness measurement of the lateral crown face in the mesial cusp section were compared using sixty molars (ten each for six molar types) of *Homo sapiens* recovered from the Libben site. In these definitions, a reference line tangent to the lowest point of the enamel-dentine junction (EDJ) of the occlusal basin is drawn parallel to the two dentine cusp tips, the two enamel cusp tips, or the two cervical margins, or perpendicular to the vertical axis of the tooth. Enamel thickness is then measured perpendicular to the EDJ at the two intersecting points of the reference line with the lateral EDJ contour. While measurements based on the four definitions have previously been uncritically compared the present study demonstrates that the four reference orientations differ significantly from each other. These orientational differences result in alternative measures of enamel thickness of the buccal and lingual crown faces. Consequently, the observed pattern of buccolingual gradient in thickness is highly dependent on measurement definition. Although lateral enamel thickness has been highlighted in functional interpretations of enamel distribution patterns, the present results indicate that buccolingual differences of mid-lateral enamel thickness do not exclusively manifest a functionally significant profile, but rather include a large variance component based on artifact of orientation method.
Key words : Lateral enamel thickness, Human molar, Mesial cusp section, Crown orientation

Introduction

The section which passes through the tips of the two mesial cusps (mesial cusp section, hereafter MCS) has been most frequently used in measuring linear enamel thickness (Gantt, 1977; Martin, 1983; Grine and Martin, 1988; Macho and Thackeray, 1992; Macho and Berner, 1993, 1994; Schwartz, 1997, 2000a, 2000b; Beynon *et al*., 1998; Schwartz *et al*., 1998; Ulhaas *et al*., 1999; Shimizu, 2002; Grine, 2002, 2004, 2005; Dean and Schrenk, 2003; Suwa and Kono, 2005; Grine *et al*., 2005). In most of these studies, one aim was to evaluate linear enamel thickness of the lateral crown face. Such a measure of regional enamel thickness has been interpreted in a functional context (*e.g.*, Macho and Berner, 1993, 1994; Schwartz, 1997; Grine, 2005). The expectancy is that enamel is thicker in the functionally important regions within the molar crown, such as those areas that are subject to more attrition and/or higher loads. The difference between buccal and lingual enamel thickness observed in the MCS has played a major role in the discussion of the functional significance of enamel distribution patterns (*e.g.*, Macho and Berner, 1993, 1994; Spears and Macho, 1998; Schwartz, 1997, 2000a; Macho and Spears, 1999; Ulhaas *et al*., 1999; Grine, 2005; Suwa and Kono, 2005).

Several different definitions have been introduced so far for the purpose of measuring lateral enamel thickness in the MCS. A commonly applied method is to draw a horizontal reference line tangent to the lowest point of the occlusal enamel-dentine junction (EDJ), and use its two intersects with the lateral EDJ as measurement points (Gantt, 1977; Molnar and Gantt, 1977; Martin, 1983; Grine and Martin, 1988; Macho and Thackeray, 1992; Macho and Berner, 1993, 1994; Schwartz *et al*., 1998; Grine, 2002, 2005; Shimizu, 2002; Grine *et al*., 2005). Different ways of defining the reference axis of the MCS have been proposed such as: 1) align the buccal and lingual cervical margins horizontally (Macho and Thackeray, 1992; Macho and Berner, 1993, 1994; Schwartz *et al*., 1998; Ulhaas *et al*., 1999), 2) align the two dentine horns horizontally (Grine, 2002, 2005; Grine *et al*., 2005; Grine and Martin, 1988, but see below), 3) align the two enamel cusp tips horizontally (Martin, 1983), or 4) align the long axis of the tooth vertically (Gantt, 1977; Molnar and Gantt, 1977; Suwa and Kono, 2005).

In spite of the importance of evaluating lateral enamel thickness, the effect of the above methodological differences on measured thickness values has not been investigated. Moreover, there exists some confusion over the use of the different definitions of MCS orientation. Some authors considered two or more of these definitions to be identical (Schwartz, 1997, 2000a; Schwartz *et al*., 1998; Grine, 2004, 2005; Grine *et al*., 2005). Others described their adopted definition differently in successive studies that featured the same data set (Martin, 1983; Grine and Martin, 1988). The implication must be that such variation in methodology was considered to have no consequence to the results.

However, in order to accumulate reliable knowledge about lateral enamel thickness in extant and extinct primate species, it is first necessary to actually examine whether or not differences of definition affect results and interpretations. The present study attempts to address this issue, as part of a larger project concerning the evaluation of molar enamel thickness of extant and extinct hominids and hominoids with the aid of 3-dimensional imaging methodology (Kono *et al*., 2002; Kono 2004; Suwa and Kono, 2005). The aims of this paper are: 1) to examine the difference between the four orientations of the MCS, 2) to compare values of lateral enamel thickness measured in relation to these four orientations within and between tooth types, and 3) to investigate the validity of these measurements in a context of functional significance of enamel distribution pattern.

Materials and Methods

Sixty permanent molars of *Homo sapiens*, ten each for six molar types, were analyzed in this study. These specimens are derived from the Libben collection which was archaeologically excavated in Ohio, USA (Lovejoy *et al*., 1977), and are part of a larger sample under study (Suwa and Kono, 2005). All molars were directly extracted from jaws, or in fewer cases the serial position was confirmed from antimeres or neighboring teeth. Each specimen was first carefully examined under a binocular light microscope to ensure that they had no wear on their mesial cusps.

The entire crown of each molar was scanned using a micro-focal X-ray CT scanning system (TX225-ACTIS, TESCO Corporation, Tokyo). Scanning was performed with a tube voltage of 130 kV and current of 0.12 mA, with a 0.2 mm thick copper plate filter to lessen beam hardening effects. Each molar was scanned to obtain serial slices at an interval of 0.028 mm, with its vertical axis roughly perpendicular to the scanning plane and buccolingual axis parallel to the X-ray beam. Each section was reconstructed into an image of 512×512 pixels, with a pixel size of 0.028 mm. The number of reconstructed slices was ca. 300 per molar.

The serial slices of each molar were compiled 3-dimensionally into a 512×512×512 matrix of isotropic voxels. The initial 512-matrix was reduced to a 256×256×256 matrix size for ease of measurement. This was done by averaging the gray scale values of adjacent voxels. The orientation of the molar crown was systematically adjusted so that the projected occlusal surface area of the EDJ was maximized, and the dentine horns of two mesial cusps were aligned along the x-axis of the matrix (Kono *et al*., 2002; Kono, 2004; Suwa and Kono, 2005). In the case of the maxillary molars, only the trigon basin was used in determining projected occlusal area, thus excluding the influence of the hypocone from this alignment procedure. The MCS was extracted as a plane parallel to the vertical axis of the tooth as defined by the above method of orientation. We hereafter refer to this initial orientation as the "occlusal" orientation. A horizontal line perpendicular to the vertical axis of the tooth was the reference line used in defining the measurement points of lateral enamel thickness in occlusal orientation. Three-dimensional data analysis was performed using the softwares Vol-Rugle and CT-Rugle (Medic Engineering Inc., Kyoto). The rotation algorithm we used calculates the weighted gray scale value of the resulting voxel from the CT values of the surrounding original voxels. This enabled us to obtain rotated matrices without degradation that commonly occurs during rotation of digital data using conventional imaging software, especially when rotating small amounts like 5 degrees or less.

The MCS obtained by the above method was then rotated in 2-dimensions so that the two cervical margins, two dentine horns, or two enamel cusp tips became horizontal. These rotational procedures were performed in the same way as described above. For each of the four orientations, including the original occlusal orientation, the horizontal reference line was drawn tangent to lowermost occlusal EDJ, and lateral enamel thickness was measured perpendicular to EDJ at both buccal and lingual crown faces. The half-maximum-height method (Spoor *et al*., 1993; Suwa and Kono, 2005) was used in order to define the boundary between dentine and enamel, and between enamel and surrounding air, respectively. The coordinate values of the boundary was calculated to subpixel resolution (Hlusko *et al*., 2004; Suwa and Kono, 2005). The amount of rotation necessary to obtain the alternative orientation from the original occlusal orientation was recorded, as well as the buccal and lingual lateral enamel thickness values. These were compared between the four methods of orientation and between molar types. Statistical analysis was performed with the aid of SYSTAT 10.0 software.

Results

The degree of rotation necessary to obtain each of the alternative orientations is summarized in Table 1. Fig. 1 demonstrates that differences between orientations occur in each molar position. The differences are statistically significant in nearly two thirds of the combinations between any two of the four orientations of the six molar types (Table 2). Fig. 2 shows an example of how the four orientations differ in a single molar.

In the mandibular molars, the pattern of the above difference is distinctive to each tooth position (Fig. 1). Starting from the initial occlusal orientation, in order to align the two cervical margins, the MCS must be rotated on average about seven degrees lingually in the mandibular

Table 1. Magnitude of rotation required to obtain different orientations from the original occlusal orientation.

Tooth type	n	Cervical margin				Dentine tip				Enamel tip			
		x	s.d.	Min	Max	x	s.d.	Min	Max	x	s.d.	Min	Max
LM1	10	−6.79	2.45	−10.49	−2.76	0.43	1.59	−1.40	3.00	−2.30	1.38	−4.65	0.00
LM2	10	−7.29	2.24	−11.70	−4.16	−0.70	4.03	−7.46	7.68	1.54	1.82	−2.98	6.16
LM3	10	−0.86	3.86	−6.94	4.37	−1.56	3.25	−7.41	3.97	1.64	3.07	−3.18	5.26
UM1	10	−0.38	2.33	−3.73	3.40	1.73	2.04	−2.14	4.85	−5.00	2.46	−9.82	−2.64
UM2	10	−1.08	1.82	−4.76	1.46	1.02	1.55	−1.08	4.01	−3.94	1.32	−5.99	−2.03
UM3	10	−0.57	3.75	−9.02	3.18	2.09	3.28	−1.65	7.71	−2.48	3.24	−5.89	4.30

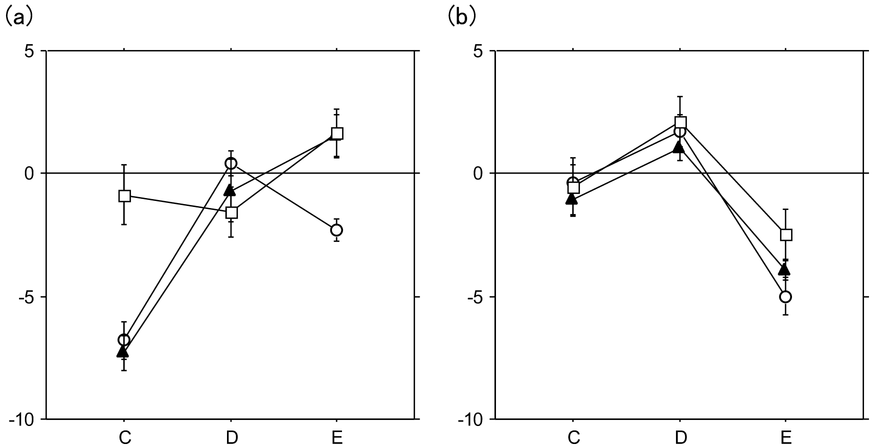

Fig. 1. Rotation required to horizontally align the two cervical margins ('C'), the two dentine tips ('D'), or the two enamel cusp tips ('E'), from the original occlusal orientation. (a) mandibular molars; (b) maxillary molars. Open circle, first molars; filled triangle, second molars; open rectangle, third molars. Y-axis in degree, with error bar indicating one standard error.

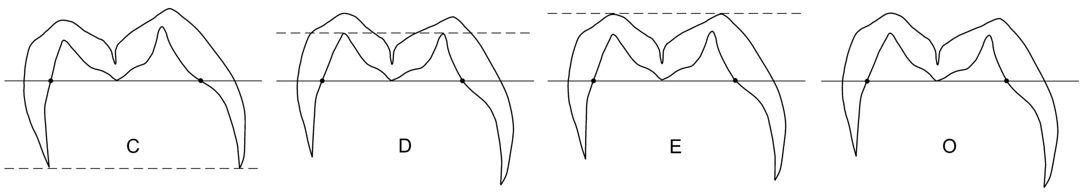

Fig. 2. An example of the four different MCS orientations of a lower first molar. 'O' is the original occlusal orientation, while in 'C', 'D', and 'E', the two cervical margins, the two dentine tips, and the two enamel cusp tips are aligned horizontally, respectively.

Table 2. Results of paired *t*-tests comparing the rotational difference between orientations.

	O-C	O-D	O-E	C-D	C-E	D-E
LM1	***	ns	**	***	***	***
LM2	***	ns	ns	***	***	*
LM3	ns	ns	ns	ns	*	**
UM1	ns	*	***	**	***	***
UM2	ns	ns	***	**	***	***
UM3	ns	ns	*	*	ns	***

Abbreviations: O, original occlusal orientation; C, cervical margin; D, dentine tip; E, enamel tip.

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant.

first and second molars, while only a minimum amount of rotation was necessary in the lower third molar (and all maxillary molars). The two enamel apices of the lower first molar were horizontally aligned by lingually rotating the MCS, while a buccalward rotation was necessary in the posterior two molars. In the maxillary molars, the difference between tooth types was less distinct.

The results of the enamel thickness measurements are summarized in Table 3. The CVs of these measurements range from around 10 to up to over 25. The buccal side of the maxillary molars and lingual side of the mandibular molars tend to be less variable than the opposite sides. No clear tendency is seen among the four orientation methods with regard to variability. Fig. 3 demonstrates that thickness values vary considerably within a single tooth type depending on definition. These differences are statistically significant in more than half of the possible combinations between two orientations in either buccal or lingual thicknesses (Table 4). Specifically, thickness measured in relation to the line parallel to

Table 3. Mid-lateral enamel thickness by four different measurement definitions and maximum lateral thickness[1].

Tooth type	n	Side	Cervical margin			Dentine tip			Enamel tip			Occlusal			Maximum		
			x	s.d.	CV	x	s.d.	CV	x	s.d.	CV	x	s.d.	CV	x	s.d.	CV
LM1	10	buccal	1.24	0.21	16.5	1.43	0.16	11.4	1.35	0.22	16.4	1.41	0.22	15.3	1.58	0.14	8.5
		lingual	1.26	0.12	9.3	1.16	0.14	11.7	1.20	0.12	9.9	1.16	0.12	10.4	1.39	0.09	6.8
LM2	10	buccal	1.30	0.25	18.9	1.70	0.23	13.5	1.77	0.25	14.1	1.75	0.23	12.9	1.93	0.17	8.8
		lingual	1.29	0.15	11.6	1.17	0.18	15.7	1.13	0.17	15.1	1.16	0.16	14.2	1.53	0.11	7.3
LM3	10	buccal	1.35	0.25	18.9	1.35	0.29	21.2	1.43	0.29	20.5	1.41	0.27	19.0	1.83	0.17	9.2
		lingual	1.21	0.16	12.9	1.23	0.19	15.4	1.16	0.20	17.1	1.20	0.22	18.2	1.52	0.09	5.6
UM1	10	buccal	1.27	0.17	13.8	1.31	0.18	13.4	1.12	0.21	18.2	1.28	0.19	14.5	1.45	0.16	11.1
		lingual	1.47	0.30	20.4	1.40	0.25	17.9	1.62	0.21	12.7	1.50	0.27	17.9	1.73	0.15	8.9
UM2	10	buccal	1.33	0.23	17.1	1.40	0.19	13.8	1.20	0.26	21.5	1.37	0.20	14.5	1.65	0.14	8.5
		lingual	1.74	0.34	19.5	1.66	0.36	21.7	1.80	0.29	15.9	1.71	0.35	20.6	1.92	0.14	7.1
UM3	10	buccal	1.24	0.28	22.2	1.37	0.27	19.6	1.12	0.25	22.5	1.29	0.26	20.5	1.72	0.10	5.8
		lingual	1.49	0.29	19.1	1.31	0.31	23.4	1.58	0.42	26.4	1.42	0.29	20.5	1.90	0.25	13.1

[1]Maximum thickness is measured at the optimal position on the buccal and lingual surface, respectively. For more detailed explanation, see Suwa and Kono (2005).

Table 4. Results of paired *t*-tests comparing differently obtained thickness values.

	O-C	O-D	O-E	C-D	C-E	D-E
Buccal						
LM1	**	ns	ns	**	**	*
LM2	***	ns	ns	***	***	*
LM3	ns	ns	ns	ns	ns	*
UM1	ns	ns	**	**	**	***
UM2	*	ns	***	*	**	**
UM3	ns	ns	ns	*	ns	**
Lingual						
LM1	***	ns	**	**	***	**
LM2	***	ns	ns	**	***	*
LM3	ns	ns	ns	ns	*	**
UM1	ns	*	ns	ns	*	**
UM2	ns	*	*	*	*	**
UM3	ns	ns	*	*	ns	**

Abbreviations: O, original occlusal orientation; C, cervical margin; D, dentine tip; E, enamel tip.

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant.

the two enamel cusp tips differed significantly from that measured with reference to the two dentine horn tips in both buccal and lingual faces in all six tooth types. In Fig. 4, the thickness values obtained in each orientation are given for three individual teeth (including the tooth depicted in Fig. 2). It can be seen that thickness differs by orientation significantly within buccal and lingual faces, and that the rank order of the four orientations is exactly opposite between buccal and lingual sides. The rank order differs from tooth to tooth, however, especially in mandibular molars (Figs. 3 and 4).

In order to investigate the validity of the use of these different measurement methods in functional interpretations, the magnitude of thickness disparity between buccal and lingual faces was evaluated (Fig. 5). While the direction of thickness gradient was in most cases consistent with the expected pattern, thicker buccally and lingually in lower and upper molars, respectively, the magnitude of buccolingual difference was highly variable among the four methods. In the most striking cases (the mandibular second molar and maxillary third molar), thickness difference varied from nearly zero to as much as 0.6 mm de-

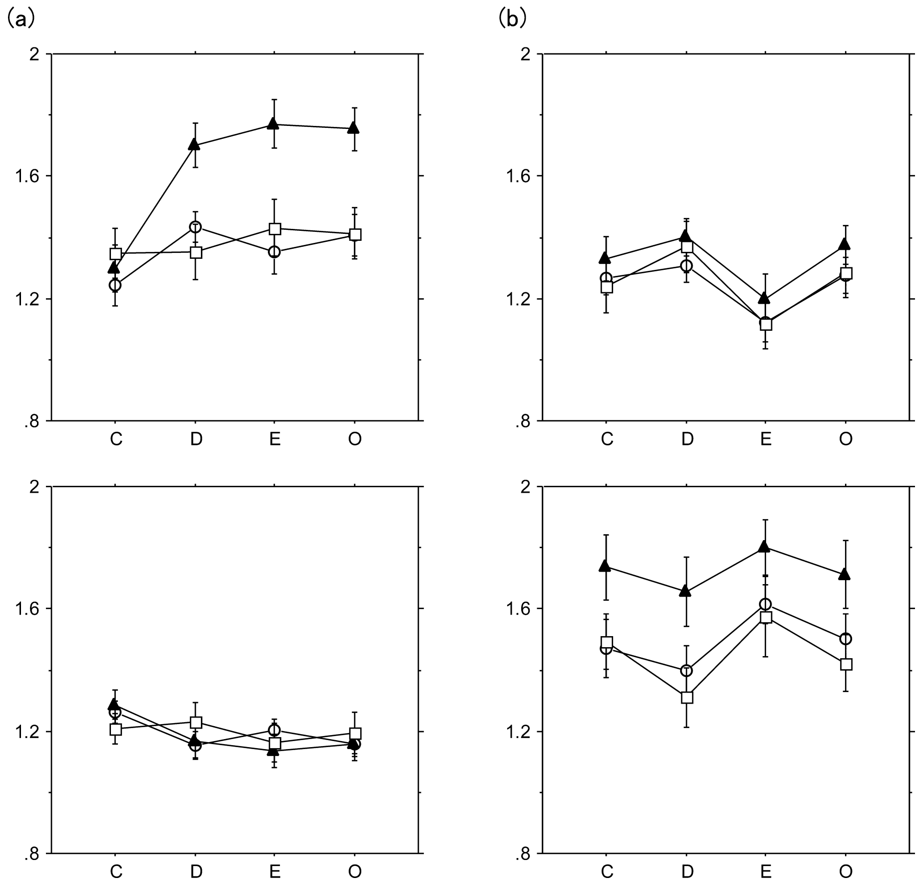

Fig. 3. Comparison of mid-lateral enamel thickness measured by four different definitions. 'O' is the original occlusal orientation, while in 'C', 'D', and 'E', the two cervical margins, the two dentine tips, and the two enamel cusp tips are aligned horizontally, respectively. (a) mandibular molars; (b) maxillary molars. Upper row, buccal side; bottom row, lingual side. Open circle, first molars; filled triangle, second molars; open rectangle, third molars. Thickness values are in mm. Error bar indicates one standard error.

pending on orientation. The results of paired *t*-tests also revealed a pattern of buccolingual disparity that differs from method to method, as well as from tooth to tooth (Table 5). For instance, in the case of the mandibular first and second molars, thickness differed significantly between faces when using dentine or enamel cusp tips for alignment, whereas it was indistinguishable when the two cervical ends were aligned. In the mandibular third molar, on the other hand, thickness difference was nearly significant in the latter orientation ($P=0.054$), but not so when the two dentine tips were aligned ($P=0.279$).

Discussion

The four orientations clearly differ from each other, and the measurements made in relation to these orientations are not identical. Among the four, the orientation using two dentine tips was closer to the initial occlusal orientation in both maxillary and mandibular molars. This implies that the two dentine horns are at approximately the same height in relation to the original orientation which was obtained by maximizing the projected area of the occlusal fovea of the EDJ.

In the maxillary molars, the orientation using the two enamel cusp tips differed the most from

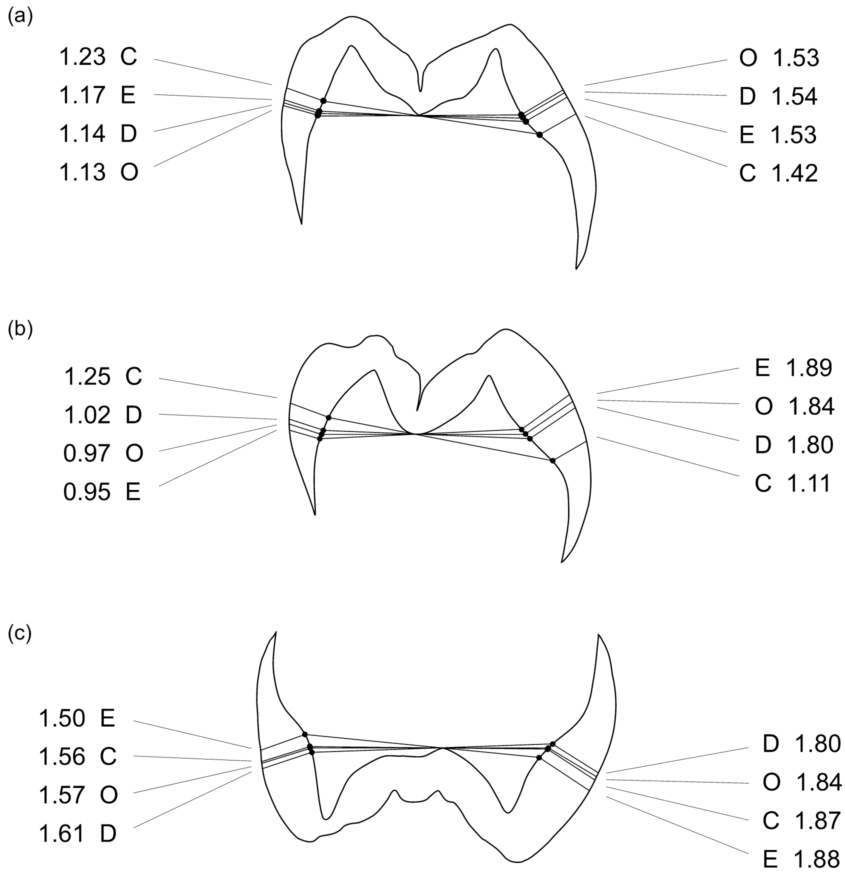

Fig. 4. Examples of mid-lateral enamel thickness measured in the MCS according to the four definitions. Each set of measurement points is plotted onto the section contour of the original occlusal orientation with the measured values of thickness (mm). 'O' is the original occlusal orientation, while in 'C', 'D', and 'E', the two cervical margins, the two dentine tips, and the two enamel cusp tips are aligned horizontally, respectively. (a) lower first molar (specimen No. 03262); (b) lower second molar (No. 05010), buccal to the right side; (c) upper first molar (No. 03277), lingual to the right side.

the other orientations. The difference was as much as five degrees between the dentine and enamel cusp tip orientations. In the mandibular molars, disparity between methods was even greater, with maximum differences approaching ten degrees. The orientation based on the two cervical margins differed the most from the other orientations. Moreover, the between-tooth difference was also very marked in this orientation. This may be explained by the different extent of cervical enamel extension of the buccal crown face. Textbook descriptions (*e.g.*, Fujita *et al.*, 1995) point out that, in the anterior mandibular molars, the enamel extends further apically and the cervical margin is situated lower on the buccal than on the lingual surface. Therefore, larger amounts of rotation are necessary in the anterior lower molars simply because of the need to align the two offset points (Fig. 4a and b). In the mandibular third molar (and the maxillary molars, Fig. 4c), the two cervical margins are positioned at about the same height relative to the vertical axis of the crown, and therefore only a little rotation is required to align them.

Another factor that markedly influence orientation of mandibular molars is enamel distribution pattern. Kono *et al.* (2002) pointed out that enamel is characteristically thin at the tip of the

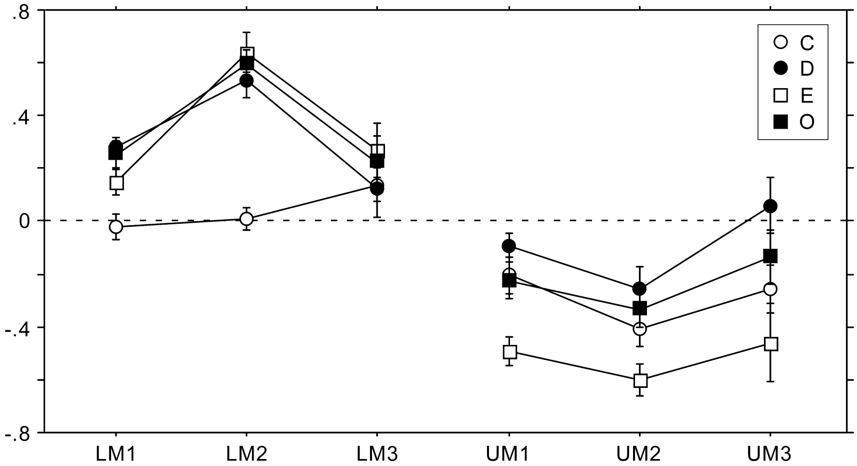

Fig. 5. Buccolingual difference of mid-lateral enamel thickness. The difference is calculated as buccal minus lingual. 'O' is the original occlusal orientation, while in 'C', 'D', and 'E', the two cervical margins, the two dentine tips, and the two enamel cusp tips are aligned horizontally, respectively. Thickness differences are in mm. The error bar indicates one standard error.

Table 5. *P*-values of paired *t*-test between buccal and lingual lateral enamel thickness.

Tooth type	Cervical margin	Dentine tip	Enamel tip	Occlusal	Maximum[1]
LM1	ns	***	*	**	***
LM2	ns	***	***	***	***
LM3	ns	ns	*	ns	***
UM1	*	ns	***	**	***
UM2	***	*	***	**	***
UM3	*	ns	*	ns	*

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant.
[1] Maximum lateral thickness is measured at the optimal position on the buccal and lingual surface, respectively. For more detailed explanation, see Suwa and Kono (2005).

mesiobuccal cusp, i.e. the protoconid, of the mandibular first molar. This was unexpected, since the buccal side of mandibular molar is functionally important (and expected to have thicker enamel), but further confirmed in subsequent studies (Kono, 2004; Grine, 2005; Suwa and Kono, 2005). In the meantime, the protoconid of the posterior mandibular molars is more thickly enameled as expected (Kono, 2004; Suwa and Kono, 2005). This may result in the difference between the mandibular first molar and the two posterior molars in the magnitude of rotation required to align the two enamel cusp tips. In the first molar, while the tip of the protoconid enamel is so low that it is necessary to rotate the MCS lingually to align this with that of the metaconid (Fig. 4a), the posterior molars do not need to be rotated lingually (but instead buccally) because they have thicker cuspal enamel on the protoconid (Fig. 4b). It must be noted that such region-specific shape differences has the potential to result in marked differences of orientation between molars. As a consequence, measurements defined in relation to such an orientation are not necessarily functionally or topographically homologous between molar types, notwithstanding the opinion of Grine (2005).

Actual enamel thickness values were shown to be highly dependent on measurement method, and therefore differed significantly from each other. It is, however, difficult to assess whether one (or more) of the four is preferable to the others. For example, they were broadly comparable regarding magnitude of variability, but all four methods resulted in greater CVs than those of maximum lateral thickness (Table 3). The latter measure was shown to be less variable than other linear measures of enamel thickness, such as occlusal or cuspal thickness in the MCS (Suwa and Kono, 2005). It can be said that all four types of mid-lateral thickness examined in the present study are less 'stable' (sensu Suwa and Kono, 2005) than maximum lateral thickness, and thus less than ideal as a representative of thickness of individual specimens.

Although the fact that the four methods differ from each other does not necessarily mean that results are not reliable nor useful (so long as they are treated separately, at least), we must be cautious especially when enamel thickness are to be evaluated in a functional context. We demonstrated above that the degree of disparity between buccal and lingual enamel thickness is highly dependent on the adopted orientation and ensuing definition of measurement points. For example, if we were to use the two cervical margins as reference points, we would find that buccolingual differences in the maxillary molars were greater than in the mandibular molars. The opposite pattern would be encountered when the reference line used was determined by the two dentine tips. We note that Grine (2005), who defined his "cervico-lateral" thickness in relation to the two dentine tips, found significant buccolingual differences only in the lower three molars. The pattern of buccolingual disparity in thickness also differs between tooth types, especially in the mandibular molars (Fig. 5). This is related to the distinctive pattern of orientational difference as outlined above. When using cervical extremities as the reference points, the lower third molar would tend to show a larger degree of buccolingual difference compared to the second molar, while the opposite would be the case in the other three orientations.

The results of the present study concerning the pattern of buccolingual thickness gradient are not entirely consistent with previously shown results (such as Macho and Berner, 1993, 1994; Grine, 2005). This is not necessarily unexpected, however, since the MCS was obtained by physical sectioning of molars in those studies. The validity of linear enamel thickness measured in a physically sectioned MCS is another matter of concern. The assumption that the cut section includes the tips of the dentine horns of the two mesial cusps is not expected to be met (*e.g.*, Smith *et al.*, 2004), and small offsets of section position have been shown to significantly affect linear enamel thickness values (Suwa and Kono, 2005). The relative positions of the landmarks used to align the MCS in the measurement of mid-lateral enamel thickness (including the lowest point of the occlusal EDJ) may also be susceptive to slight offsets of section position, so as to affect thickness values measured at the physcial cross sections.

Does the difference between methods in the magnitude of buccolingual thickness disparity, both within tooth and among tooth types, have any functional meaning? When the original buccal and lingual thickness values are reexamined in relation to orientation of the MCS, a simple pattern of correspondence can be drawn; a more buccalward rotation results in greater buccal and smaller lingual thickness values, and vice versa. This is easily understood with reference to the combination of geometric constraints of the tangent method of determining measurment points and shape of the human molar section. When the MCS is rotated buccally, the intersect positions of the reference line occur more occlusally at the buccal face and more cervically at the lingual face. The thickness value thus measured increases buccally and decreases lingually, since, in human molars, the lateral enamel generally thickens from cervix to occlusal rim. We found that the average of the buccal and lingual thicknesses is more or less consistent among the four methods (Fig. 6). This further implies that buccal and lingual thickness values change in a complementary fashion, while the MCS pivots around the

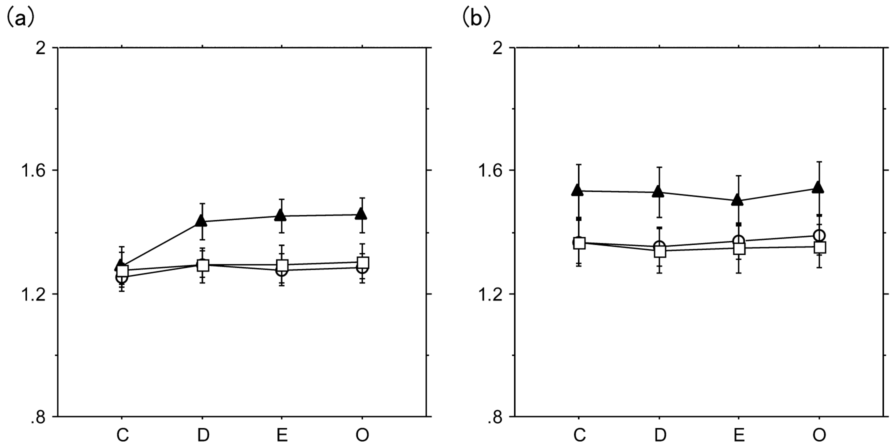

Fig. 6. Average of buccal and lingual mid-lateral enamel thickness. 'O' is the original occlusal orientation, while in 'C', 'D', and 'E', the two cervical margins, the two dentine tips, and the two enamel cusp tips are aligned horizontally, respectively. (a) mandibular molars; (b) maxillary molars. Open circle, first molars; filled triangle, second molars; open rectangle, third molars. Thickness values are in mm. Error bar indicates one standard error.

lowest point of the occlusal EDJ. However, even these average (buccal and lingual) thickness values show a higher variability (CVs range from 11 to 19, data not shown) than maximum lateral thickness. The additional variability is most likely a reflection of individual variation in height of the lowest occlusal EDJ point of the MCS with regard to lateral crown contour shape. The location of such a defined point is influenced by the relationship between the pattern of undulating occlusal EDJ surface and the position of the MCS, and does not necessarily lead to a biologically homologous set of landmarks in the consideration of the functional significance of enamel thickness.

It is thus concluded that the functional meaning of measured mid-lateral enamel thickness values is ambiguous at best. The particular degree of buccolingual thickness disparity encountered in a study may largely be an artifact of orientation method, occlusal EDJ shape, and shape of lateral crown contour. In that sense, none of the four methods may represent functional significance of thickness more appropriately than the others.

The results shown above concern the molars of *Homo sapiens*, which possess overall thicker enamel compared to those of the extant great apes (Martin, 1983; Schwartz, 1997, 2000a; Kono, 2004). Orientation difference might affect measured thickness values more prominently in the case of thicker enameled molars, because the enamel undergoes greater increase in thickness from the cervix (null) to the occlusal margin (thick). In other words, in molars which have more uniformly distributed enamel on their lateral crown faces, the effect of orientation may be smaller. Since the species characteristics of 3-dimensional enamel distribution pattern are just starting to be adequately documented (Kono, 2004), it is not advisable to use (and, moreover, to mix) measurements that contain such uncertainties. This is the case especially when examination is aimed to ascertain the functional significance of enamel distribution pattern in a phylogenetic context.

As a final remark, in the evaluation of enamel thickness of the lateral crown face, we recommend the use of a measurement definition independent of orientation, such as maximum lateral thickness (Suwa and Kono, 2005), instead of the widely used mid-lateral thickness measures. An orientation-free protocol, not tied to set anatomical landmarks, is expected to be less affected by characteristic within-tooth enamel distribution patterns and/or by related idiosyncratic factors such as shown above to have significant effects on mid-lateral thickness measurements. Our recommendation is empirically justified not only by the smaller CVs, but also by the more consistent pattern of buccolingual differences observed among both individuals and tooth type (Table 3, Table 5, and Suwa and Kono, 2005).

Conclusions

In previous studies, four different definitions have been used to measure molar enamel thickness of the mid-lateral crown of the MCS, irrespective of possible difference of meaning. In these methods, the measurement points are designated at the buccal and lingual intersects of the lateral EDJ contour and a horizontal reference line tangent to the lowest point of the occlusal EDJ, and enamel thickness is measured perpendicular to the EDJ at those locations. The reference axis of the MCS is defined as: 1) the buccal and lingual cervical margins horizontally aligned, 2) the two dentine horns horizontally aligned, 3) the two enamel cusp tips horizontally aligned, or 4) a horizontal line perpendicular to the vertical axis of the tooth. The present study revealed that the four methods result in significantly different orientations and enamel thickness values. In mandibular molars, such differences also occur between tooth type owing to regional morphological and enamel distributional characteristics. In order to align the two cervical margins, the first and second mandibular molars need to be markedly rotated because of the inferiorly extended enamel on the buccal molar surface. On the other hand, the first molar requires a lingual rotation to put the two enamel cusp tips in line because of the strikingly thin enamel at the protoconid tip. Different orientations correspondingly result in different thickness values in a predictable pattern in which a more buccal rotation results in greater buccal and smaller lingual thickness values, and vice versa. The buccolingual thickness gradient, which is the most frequently discussed aspect of enamel thickness in functional explanations, is either emphasized or minimized, while the average of thickness of both sides remains fairly constant. We conclude that 1) it is necessary to avoid mixing differently defined thickness data, and 2) it is probably not appropriate to use any of these four definitions in evaluating functional significance of enamel thickness patterns, since such apparent patterns of thickness may predominantly be based on artifact of orientation.

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