New methodology to reconstruct in 2D the cuspal enamel of modern human lower molars

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Abstract

Objectives

In the last years different methodologies have been developed to reconstruct worn teeth. In this article we propose a new 2D methodology to reconstruct the worn enamel of lower molars. Our main goals are to reconstruct molars with a high level of accuracy when measuring relevant histological variables and to validate the methodology calculating the errors associated with the measurements.

Methods

This methodology is based on polynomial regression equations, and has been validated using two different dental variables: cuspal enamel thickness and crown height of the protoconid. In order to perform the validation process, simulated worn modern human molars were employed. The associated errors of the measurements were also estimated applying methodologies previously proposed by other authors.

Results

The mean percentage error estimated in reconstructed molars for these two variables in comparison with their own real values is -2.17% for the cuspal enamel thickness of the protoconid and -3.18% for the crown height of the protoconid. This error significantly improves the results of other methodologies, both in the interobserver error and in the accuracy of the measurements.

Conclusions

The new methodology based on polynomial regressions can be confidently applied to the reconstruction of cuspal enamel of lower molars, as it improves the accuracy of the measurements and reduce the interobserver error. The present study shows that it is important to validate all methodologies in order to know the associated errors. This new methodology can be easily

exportable to other modern human populations, the human fossil record and forensic sciences.

Introduction

Unworn teeth are important to assess crown formation times and enamel extension rates with a high degree of accuracy. Especially, an intact protoconid is of particular interest in developmental studies because it is the first cusp to start forming and the cusp that normally takes the longest time to finish its growth in molars (Mahoney, 2008). Unfortunately, the presence of unworn teeth in the fossil record is relatively scarce. The present study proposes a new methodology to reconstruct the protoconid of slightly worn lower molars, in order to increase of the number of samples where developmental variables could be evaluated. We employed the computerized microtomography (microCT) technique, which has enabled the acquisition of many high-resolution virtual sections of fossil teeth. Variables that were previously inaccessible are now available, and the accuracy of the measurements has been significantly improved (Kono, 2004; Grine, 2005; Kono and Suwa, 2005; Macchiarelli et al., 2006; Olejniczak and Grine, 2006; Olejniczak et al., 2007, 2008a; b; Benazzi et al., 2014; Martínez de Pinillos et al., 2014; Xing et al., 2015).

Cuspal enamel thickness and crown height are two variables that are particularly affected by wear, and they are commonly employed, among others, to estimate enamel formation times. Thus, the obtention of a statistically-controlled methodology to reconstruct the enamel would significantly help in estimating enamel formation times.

The enamel of teeth covers the crown from the cusp to the cervix, and has previously been divided into two continuous regions depending on whether long-period incremental lines within the enamel reach the surface (lateral enamel) or not (cuspal enamel) (Hillson, 1996; Nanci, 2007; FitzGerald and Rose, 2008). Crown formation time can therefore be calculated by summing cuspal enamel formation time with lateral enamel formation time. When this method of estimating total crown formation is adopted, different methodologies are required to estimate cuspal and lateral enamel formation times.

Cuspal enamel thickness is one useful measurement that has been employed to estimate cuspal enamel formation times (Dean et al., 2001; Mahoney, 2008). It has been defined as the linear distance between the tip of the dentine horn and the outer enamel surface at the point coincident with the first perikyma (Reid and Dean, 2006; Reid et al., 2008, Smith et al., 2010). Estimating the time and rate of lateral enamel formation can be done using total counts of perikymata on the tooth surface or of long period incremental markings within the enamel from histological sections of teeth. In studies that compare teeth within and between individuals, it has become customary to divide the crown height into equal proportions or quantiles (usually deciles) to scale for differences in absolute crown height. Crown height of the protoconid is defined as the linear distance between the cusp tip of the enamel to the cemento-enamel junction (Reid and Dean, 2006). The number of perikymata can be counted and compared among deciles of crown height from cusp to cervix. The relative change in perikymata spacing, or packing pattern, among deciles can provide some kind of estimate of enamel extension rates (the rate at which ameloblasts differentiate along the enamel dentine junction during tooth formation). When periodicity (the number of days of enamel formation between perikymata) is known, the total number of perikymata can be used to estimate lateral enamel formation times (Guatelli-Steinberg and Reid, 2008).

In order to maximise available samples to make comparisons between species and populations, different methods of reconstructing worn molars have been described in the last few years. On the one hand, a reconstruction of worn surfaces has been conducted by following the contour of each side of the tooth cusp and projecting them towards the cusp tip until both sides meet (Guatelli-Steinberg and Reid, 2008). Here we refer to this method as Method 1. On the other hand, reconstructions of worn teeth have been based on the profiles of unworn ones of the same type (Smith et al., 2012). We refer to this method as Method 2. However, these methodologies have not been thoroughly described, thus the exact protocols remain unknown. Moreover, neither of these methods has been tested and/or validated, and so any errors in estimating cuspal formation times or enamel:dentine ratios from utilizing these reconstructions protocols is unknown. Some authors have criticized these studies that have not validated their methods (Benazzi et al., 2014), and others have emphasised the importance of validating the methodologies (Saunders et al., 2007). The only author who developed and validated a reconstruction methodology was Saunders et al. (2007).

The main goal of this study was to present a new methodology to reconstruct slightly worn first and second lower permanent molars cusp (protoconid) by means of microCT images. A validation of this new methodology is also presented, as well as a comparative study applying the previous techniques (Method 1 and Method 2).

Materials and methods

Materials

A total of 26 lower molars (M_1=10, M_2=16) were included in this study, belonging to different historic and archaeological modern human populations from the Iberian Peninsula (Table 1; Fig. 1): 14 individuals from the medieval churchyard of San Pablo (Burgos), 4 from the archaeological sites of Maltravieso Cave and 1 from Santa Ana Cave (both in Cáceres), 4 individuals of Galls Carboners Cave (Tarragona), 2 from Guineu Cave (Barcelona) and 1 from El Mirador Cave (Burgos, Spain). None of these teeth displayed any evidence of wear (category of wear stage 1, according to Molnar (1971)).

The comparative sample from San Pablo (Burgos) consists of a medieval churchyard and archaeological collection (XII-XIV) from the Dominican monastery of San Pablo housed in the Laboratory of Human Evolution at the University of Burgos (Spain).

Maltravieso Cave is located in Cáceres (Extremadura, Spain), in the southwestern part of the Iberian Peninsula. The cave was accidentally discovered in 1951 in a limestone quarry. The affected area was called Sala del Descubrimiento. In this room a thousand ceramic and human remains that were part of a collective grave were uncovered (Callejo Serrano, 1958). The few pottery fragments available indicate that the room was used as a burial cave at least in the half of the second millennium BC (Cerrillo Cuenca and González Cordero, 2007). In 2002, various mechanical and manual test pits were performed in the area originally occupied by the Sala del Descubrimiento, uncovering new 172 remains belonging to *Homo sapiens* and several new pottery fragments (Muñoz and Canals, 2008). All of this new remains were assigned to be part of the assemblage discovered in the 50's.

Santa Ana Cave presents several stratigraphical units that correspond to the Pleistocene (Carbonell et al., 2005). All the remains from the Pleistocene sediments were unconvered in a calcified breccia. However, sediments from historical ages have also been found, including Ancient Rome evidences. Although the exact historical period of the molar remains unknown, its attribution to *Homo sapiens* is unquestioned.

Galls Carboners Cave is located in the Prades Mountains (Tarragona, Spain). A collective burial was excavated in different periods, the first in 1970's and then again in 2009 and 2010. Along with human remains (an NMI of 16 individuals) some lithic tools were recovered, as well as some ceramic fragments and faunal remains. The dating of a human remain places this site in 3,310±30BP (Cal BP 3,620-3,460).

Guineu Cave is located in Font-Rubí (Barcelona, Spain). In this site, a long sequence with occupations ranging from hunter-gatherer to Bronze Age populations has been documented. In the 4th and 3rd millineum BC the cave was used as a burial place (Morales et al., 2013). The teeth used in this paper belong to this period. Some dated human remains shows an age about 2,871-3,353 Cal BC.

El Mirador Cave is located on the southern side of the Sierra de Atapuerca (Burgos, Spain). The human assemblage where this tooth belongs to is a collective burial found in an about 14 m^2 natural chamber located in the NE corner of the cave. Although there are some individuals in their original anatomical position, the superficial remains were mixed and disturbed by the actions of the clandestine excavators in the 1980s. Up to now, there are a minimum number of 22 individuals of different sexes and ages (Gómez-Sánchez et al., 2014). All of these human remains belong to the Chalcolithic period and have been dated to 4.760-4.200 years cal. BP.

(Here come Table 1 and Figure 1)

For our study, one antimere per individual was selected. The inclusion criteria were the presence of the complete crown, good preservation and the absence of fractures or dental pathologies. The teeth were microCT using a Phoenix v/tome/x s of GE Measurement system, housed at the National Human Evolution Research Centre (CENIEH, Burgos, Spain) with the following scan settings: voltage 100 kV, 100 mA, 0.02 cooper filter and resultant isometric voxel size ranging from 18 (isolated dental remains) to 75 (mandibles) microns. MicroCT images were processed employing the software AMIRA 6.0.0 (Visage Imaging, Inc.).

Description of the new methodology

Reference plane

The plane of reference used to reconstruct the lower molars has been defined by Benazzi et al. (2014). Briefly summarized, the cervical plane is obtained as the best-fit plane among 50 equidistant landmarks located on the cemento-enamel junction. Then, the reference plane is created perpendiculary to the cervical plane, crossing through the two mesial dentine horns. The protoconid was situated to the right of the reference plane in order to standardize all of them. An example of the reference plane of one molar used in this study can be seen in Fig. 2a.

(Here comes Figure 2)

Outline of enamel and relative coordinates

We used the open-source software Inkscape 0.91 to convert the plane of reference into a vector graphic, using the raster-to-vector conversion (Supplementary Online Material [SOM] Text S1). In this way, the outline of the enamel was traced (Fig. 2b).

We are interested in one particular area of the outline of the enamel, which was used to reconstruct the protoconid and is named here as POL-PR, which means POLynomial of the PRotoconid (Fig. 2c). The outlines of the protoconids were used to generate a polynomial regression equation based on the relative coordinates of their points (from 0 to 100). The specific description of how to obtain these points and their coordinates in each area is described below.

The cartesian coordinate system is defined by three landmarks (green dots in Fig. 2c). The first landmark is the origin and is placed at the top of the dentine horn tip of the protoconid. The relative coordinates of the first landmark are (0,0). The second landmark is placed at the point where a horizontal line from the origin crosses the buccal aspect of the enamel of the protoconid. The coordinates of this second landmark are (100,0) and the distance between both landmarks is named *Xprotoconid*. The third landmark is located at the point where a horizontal line drawn from the highest point of the enamel cusp tip crosses the vertical line traced from the origin. The coordinates of this third landmark are (0,100) and the distance with the origin is named *Yprotoconid.* We placed 51 equidistant points over the outline of the protoconid between the second and third landmark (SOM Text S2), and calculated their relative coordinates (SOM Text S3).

Polynomial regression analysis

Polynomial regressions with 99% confidence intervals were fit through these coordinates for the protoconid (POL-PR). These regressions and their correlations were performed in R Commander, by running the package *ggplot2* and exporting the figures in a vector format (.pdf). The final number of degrees of the polynomial regression was established when four decimals of the adjusted R-squared start repeating when the degrees of the polynomial regression are progressively increased.

Validation of the new methodology

Subsamples

The sample is composed of 26 lower molars. They were randomly divided in 2 subsamples using the function *sample* available in R. The validation subsample consisted of 5 molars (2 M1s and 3 M2s) as it is recommended this subsample be comprised of 10% of the parent sample (Alreck and Settle, 2003). The second subsample (n=21) was used to generate the polynomial regression. POL-PR was therefore analyzed using 1071 points (21 molars x 51 points/molar). We also evaluated whether n=21 molars was sufficiently high to get a polynomial regression that resists the addition/removal of molars without modifying its curvature.

Digital wear simulation and reconstruction

The validation subsample of 5 molars was digitally worn using the open-source software GIMP 2.8. The simulated wear consisted of removing all the enamel that was above the horizontal line that crosses over the dentine horn of the protoconid.

Each molar was reconstructed using the polynomial regressionPOL-PR. A detailed description of how to automatically place the regression plot over the worn tooth can be read in SOM Text S4, but see also SOM Video S1. Protoconid occlusal outlines were also reconstructed following the instructions in Guatelli-Steinberg and Reid (2008) and Smith et al. (2003, 2006a, 2009, 2012) (Methods 1 and 2, respectively) The former study follows the contour of each side of the tooth cusp, projecting them proximally until both sides met, while the latter studies reconstruct the molars based on the profiles of unworn teeth. Concerning Method 2, as they reconstruct worn teeth using an unworn tooth of the same tooth type, we selected one random M1 and one random M2 from the subsample of 21 molars to reconstruct either the M1s or the M2s that were digitally worn.

Variables and interobserver error

We measured the cuspal enamel thickness of the protoconid (CET) and the crown height of the protoconid (CH) in the reconstructed teeth. Then, we compared the real value of the original image of the teeth with the values obtained after using the different reconstruction methods (POL-PR, Method 1 and Method 2).

For CET, we estimated the location of the first perikyma in 9 molars of this study. The first perikyma was placed on average at 9.43° from the cusp tip of the protoconid distally (towards the cervix) (SOM Text S5; SOM Fig. S1).

The validation process was carried out in each tooth by four coauthors of this study (CG, LMF, YQ, RG), who measured the two variables (CET, CH) in the validation subsample of five molars using the three methods of reconstruction (POL-PR, Method 1, Method 2) and they also measured the original variables in the unworn microCT planes. Interobserver error was also evaluated.

Comparison of the polynomial regressions of M1+M2 vs. M1/M2

One important question is whether or not the polynomial regression of both lower molars (M1+M2) is more accurate when estimating CET and CH, instead of performing separate regressions for each tooth type. For this purpose, we divided the two subsamples per molar type. On the one hand, we obtained the 8 M1s to perform the polynomial regression that was employed to reconstruct the protoconid of the 2 remaining M1s of the validation sample. On the other hand, 13 M2s were used to get the polynomial regression that will be used to reconstruct the remaining 3 M2s. Two coauthors measured the estimated and real values of CET and CH (MM-M, CG).

Results

The subsample of n=21 molars was used to calculate the polynomial regression POL-PR. This is a 4th degree polynomial regression, with $p<0.0000$ and adjusted R-squared = 0.9784) (SOM Table S1; Fig. 3). Fig. 3 represents the change of the adjusted R-squared depending of the degrees of the polynomial regression.

(Here comes Figure 3)

To evaluate whether the subsample of 21 molars comprises a sufficiently high number of molars to generate a polynomial regression that remain unmodified with the inclusion/removal of new molars, we randomly assigned one number (1-21) to each molar. We then generated 21 polynomial regressions, consecutively adding one molar per regression and observing its effect on the curves. We repeated this process for the 8 M1s and the 13 M2s separately. All these regressions are represented in Fig. 4. A common pattern observed in all regressions in that the curves tend to stabilize as the number of molars in the polynomial regressions increases. In the case of the M1s, after the 6th molar the curve tends to be stable at the points where CH and CET are measured. This occurs after the 9th molar in the M2s and after the 16th molar in the mixed sample. Looking at the last three polynomial regressions (the ones that represent 19, 20 and 21 molars), we observe that their patterns of curvature are identical and indistinguishable. This means that POL-PR is resistant to significant changes of its curvature with n=21 molars, which implies that it can be used as a model to test its usefulness when reconstructing the morphology of the protoconid.

(Here comes Figure 4)

Table 2 shows the descriptive statistics of the interobserver error of both CET and CH variables, including means and standard deviations of estimated and real values per molar. This data evidences the differences that are present when comparing different reconstruction methodologies.

POL-PR presents less standard deviation values than the other two methodologies in both CET and CH, with the exception of CET (M3) and CH (M1). In these two cases the standard deviations between POL-PR and the closest methodology are very narrow. The differences between the mean of the real values and the means of the reconstruction methodologies exhibit that POL-PR best reconstruct three out of five molars for both CET and CH variables.

(Here comes Table 2)

Shapiro-Wilk tests were performed to test normality distribution for both variables (CET and CH) and molars (Table2). Except for POL-PR (M2) and Real (M1) that were non-normal distributed ($p<0.05$), the remaining variables and molars resulted to be normal distributed. We also tested equality of variances by performing Fisher tests two by two (Table 3). These tests demonstrate that POL-PR values compared to their real ones present the same variance ($p>0.05$) in all molars. This fact also occurs for the Method 2 but it is not the case for Method 1, where some comparisons don't have the same variance as CET (M1, M2, M4) and CH (M2).

The interobserver error and the average percentage error between the real/estimated values of the variables (CET and CH) are represented in Fig. 5. Percentage error has been calculated by applying this formula: [(mean_Method_X – mean_Real)*100 / mean_Real]. Depending on the results of previous statistical tests (Shapiro-Wilk and Fisher), new statistical tests were carried out to compare means / medians in all molars between real and estimated values in both CET and CH variables. Thus, Welch tests, Mann-Whitney tests and t-tests were applied accordingly (Table 3). All reconstruction methodologies display significant statistical differences in some variables and molars when they are compared to the real values. Due to POL-PR is characterized by narrower standard deviations respect to the other methods, it is understandable that slightly over- and underestimates would imply statistical significant differences in their means / medians. Moreover, the probability of finding statistically non-significant differences increases when the variance is wider (as is the case of Method 1 and 2).

(Here comes Table 3 and Figure 5)

Combining all five molars of the validation subsample, the average percentage error with its ±95% confidence interval for the methods based on the polynomial regression (POL-PR) is shown in Table 4. CET tends to be -2.17 underestimated using POL-PR, with a 95% confidence interval of -4.60% and 0.74% . CH tends to be underestimated by -3.18% using POL-PR, with a confidence interval of -3.77% and -2.54%.CETCH

(Here comes Table 4)

The polynomial regressions and associated prediction intervals for M1s and M2s can be seen in Fig. 6. There is a high degree of overlap between molar positions. M2s acquire its maximum crown height in a slightly more buccal position compared to the same point in M1s. Crown heights and cuspal enamel thicknesses of the validation subsample have been measured depending on their molar position. Percentage errors between real and estimated measurements of CET and CH can be seen in Table 5. Applying the polynomial regression of the M1 to the two M1s of the validation subsample, the mean percentage error in respect of their real values in CET is -9.48%. Applying the same protocol to the M2s yields a value of +4.11%. For crown heights, these values are --3.88% and -2.52% for the M1s and M2s, respectively.

(Here comes Fig. 6 and Table 5)

Our results indicate that molar-specific polynomial regressions applied separately to M1s and M2s don't considerably improve the estimates obtained from the regression equation made by combining M1s and M2s. For instance, mean percentage error using the latter regression for CET is

-2.17%, which is a lower percentage error than the values from the molar-specific regressions. In CH these differences are minimal, as the mean percentage error is -3.18% using the polynomial regression of both molars, and -3.88 and -2.52 using the regression equation of the M1s and M2s, respectively. We have therefore considered the regression that is formed by merging both molars as the best proxy to estimate CET and CH in both M1s and M2s.

Discussion

In paleoanthropology, fossil teeth with complete and unworn crowns are relatively scarce. However, these teeth are extremely valuable for studies of either the external morphology (Martinón-Torres et al., 2006; Gómez-Robles et al., 2008, 2011a; b; Martinón-Torres et al., 2012) or the internal morphology of molar teeth (Martínez de Pinillos et al., 2014; Martinón-Torres et al., 2014). Non-destructive methods, either synchrotron or micro-computed tomography are capable of taking very accurate 2D and 3D measurements of teeth in comparison to physical sections (Kono, 2004; Olejniczak et al., 2008b).

In order to measure many dental variables in worn teeth (such as cuspal enamel thickness, crown height, relative enamel thickness, etc) a reconstruction of the missing parts is required. Therefore, the accuracy of the method employed to make the reconstructions is very important as it can over- or underestimate the measurements, which can in turn lead to taxonomic, phylogenetic, physiological and age-at-death misclassifications (Martin, 1983, 1985; Dean and Reid, 2001; Suwa and Kono, 2005; Lacruz and Bromage, 2006; Lacruz et al., 2006; Smith et al., 2006a; b, 2010; Guatelli-Steinberg et al., 2007; Guatelli-Steinberg and Reid, 2008; Reid et al., 2008).

Here we have developed a new methodology based on polynomial regression equations to accurately reconstruct worn molars cusps. The polynomial regression to reconstruct the protoconid of lower molars, POL-PR, is employed to estimate cuspal enamel thickness and crown height of the protoconid. Not only are these variables more accurately measured, but also other variables that depend on them might be improved, such as enamel formation times and enamel extension rates (Dean and Reid, 2001; Reid and Dean, 2006; Lacruz, 2007; Guatelli-Steinberg and Reid, 2008; Lacruz et al., 2008; Dean, 2009; Smith et al., 2010; Guatelli-Steinberg et al., 2012). In order to evaluate the accuracy of our methodology, we also reconstructed the same teeth applying the methods previously described by other authors.

The results demonstrate that the new methodology described here shows less interobserver variation than the two previously described methods, whose procedures remain unvalidated (Reid and Dean, 2006; Guatelli-Steinberg and Reid, 2008; Smith et al., 2012).

The methodology that displays the highest degree of interobserver variation is that described as Method 1. The fact that these values are so different from what is termed by us as Method 2 is because the latter involves the real contour of a tooth and the former does not. However, both of these methods involve high degrees of subjectivity. In some particular cases we observed that the measurements obtained through these methodologies fit better with the real value compared with those obtained using the polynomial regression equations. However, the interobserver variation in these methodologies is very wide, and so obtaining such apparently good estimates might well be explained by random and arbitrary effects. It is highly likely that the dispersion and variance of the measurements were different with the inclusion of new observers in the estimation of these variables. Compared to the interobserver error apparent when using the methodologies described by Method 1 and Method 2, our own methodology shows the lowest variation and the results also fall closer to the known values. The polynomial regression equations generated here are an attempt to establish for the first time a new methodology where the procedure for reconstructing worn tooth is standardized and comparable.

A further point to note is that the interobserver variation documented using the polynomial regression equation described here is not statistically different to the interobserver variation of the real measurements made from the unworn teeth. This suggests that the new methodology is highly uniform and homogeneous, and is easily reproducible by researchers/observers. When reconstructing these dental variables using this new methodology, we recommend also calculating the 95% confidence intervals and/or prediction intervals. Hence, in each of these ways we feel our method significantly improves upon previous methods when reconstructing specific important dental variables affected by cuspal wear.

Although the estimations of CET and CH using POL-PR are valuable, we acknowledge that more *H. sapiens* teeth are necessary to extend its validity. Furthermore, it has also been stated that differences exist in the enamel thickness in different populations from all around the globe and among hominin species (Grine, 2005; Suwa and Kono, 2005; Olejniczak et al., 2008a; Smith et al., 2012). To what extent the inclusion of different populations of modern humans affects the polynomial regression remains unknown. More studies should be focused on this issue, as the knowledge of the protoconid morphology and how it varies from one population/species to another that together offer an unique opportunity to improve this methodology.

Reconstructing crown heights accurately might be useful to estimate the percentage of enamel that has been lost. Their respective worn deciles and perikymata number can therefore be evaluated. As the first deciles of the crown height, which correspond to the ones that are closer to the cusp tip of the enamel, present lower number of perikymata counts compared to the most cervical deciles (Dean and Reid, 2001; Reid and Dean, 2006; Guatelli-Steinberg and Reid, 2008), we would expect this new methodology to be sufficiently capable of significantly reducing the range of variation of crown formation times in slightly worn molars. However, we acknowledge that its validity in assessing the total number of perikymata and crown formation times must be quantified in future studies.

In conclusion, this new methodology designed to reconstruct slightly worn lower permanent molars has clear advantages over other methods. First, it is easily reproducible, allowing an increasing numbers of teeth to be included in studies of dental development with greater confidence. Second, it shows a high degree of accuracy when reconstructing the contours of worn cuspal enamel. Third, associated estimation errors can be determined. Fourth, it can be used for both M1 and M2 lower molars of *Homo sapiens*. Fifth, the new estimates would be comparable among different authors following this methodology. Sixth, it uses mainly open-source software. Seventh, it greatly reduces the operator-dependent procedures in order to drastically reduce the bias.

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Supplementary Online Material (SOM)

SOM Text S1. How to do a raster-to-vector conversion in Inkscape 0.91. Preparation of the image

The objective is to get something similar to Fig. 2b. This process has 8 steps:

1. Before opening the image in Inkscape, it is recommended to increase the contrast in order to ease the raster-to-vector conversion. This can be changed in any raster editor, such as GIMP 2.8 or Photoshop.
2. Open the image in Inkscape and click on **Path** / **Trace Bitmap**.
3. In the **Mode tab**, we modify the threshold to either **Quantisation** or **Brightness cutoff**. If the result is not satisfactory, we can also modify the number of **Colors** of the **Color quantization** option. The image is prepared when we see the area of the enamel in black.
4. Delete all nodes and areas that are not part of the enamel.
5. Click on the area of the enamel and reduce the number of nodes that define its contour by pressing on Ctrl+L. Avoid oversimplification readapting the contour of the line to the real contour of the enamel.
6. Click on **Object / Fill and Stroke**. In the Fill tab, select **No paint**. In the **Stroke paint tab**, click on **Flat colour**. We can modify the line width in the **Stroke style tab**.
7. Click on **Edit paths by nodes** (F2). Select one node on each side of the dentine horn tips (4 in total) and one node at the midpart of the enamel outer surface on the lingual and buccal sides (2 in total). Now, click on **Break path at selected nodes**.
8. Click on **Path / Break Apart**. Delete all the new elements that do not include either dentine horns or enamel cusps.

SOM Text S2. Contour of the protoconid and division in 50 equal segments

The objective is to get something similar to Fig. 2c. This process has 5 steps:

1. Click on **Draw freehand lines** and trace a horizontal line from one side to the other of the dentine horn and over the tip of the protoconid. Use the tool **Object / Align and Distribute** to correctly position this line.
2. Create a new node at the highest point of the enamel tip by selecting the two closer nodes around this position and clicking on **Insert new nodes into selected segments / Insert node at max Y**. Copy the value of X of this new node because it will be used in the next step.
3. Trace a vertical line covering the enamel thickness from the tip of the dentine horn to the highest point of the enamel cusp. To position this line accurately, select both nodes that define the line itself and paste the X value from the previous step.
4. At the outer contour of the protoconid where the two created lines, horizontal and vertical, create two new nodes (similar process as step 2 in S2 Text). The nodes that remain out of this area are deleted.
5. Repeat the steps 3-8 from S2 Text. The only difference is that the distance of the contour of the protoconid must be divided by 50, and this value must be introduced in the option **Major length** of the Ruler.

SOM Text S3. Getting the XY coordinates of the points in Inkscape

This process has 4 steps:

1. Download the Inkscape extension **ExportXY** and install it. It can be downloaded here: https://drive.google.com/file/d/0B4W8BlPWjgqURUFOYnU0YVBFeHM/view?usp=sharing
2. Once the extension is installed, reopen Inkspace and open the figure we just created.
3. Click on **Extensions / Export / ExportXY**.
4. The coordinates are duplicated and inverted. In any spreadsheet software, remove duplicated coordinates (which might be organized in rows). The fact that coordinates are inverted is because de origin point in Inkscape is placed at the top left corner. To correct this inversion, multiply the Y coordinates by (-1).
5. Convert the absolute coordinates into relative coordinates (0,100).

SOM Text S4. Placing automatically the vector graphic over the worn molar in Inkscape 0.91

This process has 5 steps:

1. A spreadsheet has been developed in order to calculate the coordinates and measures of the vector graphic (SOM Excel S1).
2. In the same canvas of Inkscape, drag and drop both the worn tooth slice and the vector graphic of the polynomial regression POL-PR (PDF) (SOM Fig S2). Be sure to work in the area of the canvas where the X and Y values are positive.
3. POL-PR (SOM Fig. S2): click on **Draw freehand lines** and trace a horizontal line. This line must cross over the dentine horn tip of the protoconid. The two nodes of the line must be positioned at the dentine horn tip and the buccal border of the outer enamel (SOM Video S1).
4. Copy from the Inkscape toolbar the XY coordinates of the nodes that define the horizontal line and paste them into the spreadsheet.
5. In the spreadsheet copy the values of XYWH (XY coordinates, Width and Height). Select now the vector graphic (PDF) in Inkscape and paste these values in the toolbar.

SOM Text S5. Estimation of the position of the first perikyma in the cuspal enamel

The Isosurface tool in the Amira 6.0.0 was used to visualize the 3D model of the tooth. It was rotated in order to identify the first-formed perikyma. Once this perikyma is located in the 3D model, we accurately localized its position in the plane of reference (see main text for further details concerning the plane of reference) (SOM Fig. S1).

We measured the angle which is comprised by two lines. The first line goes from the dentine horn tip to the enamel cusp tip. The second line goes from the dentine horn tip to the position of the first-formed perikyma.

Only in 9 out of 25 molars was possible to undoubtedly identify the first perikyma. In these molars, the angle was measured. It was not possible to measure this angle in the remaining teeth because they were scanned at lower resolution (most of them because they were within their crypts) or because there were some doubts identifying the first perikyma.

SOM Table S1. Regression equations based on 21 molars to reconstruct the upper part of the enamel (POL-PR).

	Estimate	0.50%	99.50%
Intercept	8.70E+01	8.54E+01	8.87E+01
X	1.42E+00	1.18E+00	1.66E+00
X^2	-5.36E-02	-6.33E-02	-4.40E-02
X^3	5.00E-04	3.55E-04	6.44E-04
X^4	-1.93E-06	-2.64E-06	-1.22E-06

SOM Figure captions

SOM Figure S1. (Above) Isosurface of one molar (6.0.0). The horizontal green line represents the plane of reference (see main text for further details). The red vertical line indicates the position of the first perikyma. (Below) Graphic definition of the angle between the enamel tip, the first perikyma (red point) and dentine horn tip.

SOM Figure S2. Polynomial regression and 99% confidence interval of POL-PR. It is based on the contour of 21 molars. Vertical blue line represents maximum heights. Relative coordinates are represented in their own corners.