Supplementary Discussion 2:

Enamel Thickness Measurements: Methods and Results

Methods

The natural section data were taken with calipers under a low-powered binocular microscope, and cross-checked for accuracy with a scaled digital photograph of the same section. Measurements were taken only on sufficiently "radial" sections, i.e. on sections that were considered to approximately exhibit local minimal distances from the enamel-dentine junction (EDJ) to the outer enamel surface.

The micro-CT data were taken with the Tesco TX225-Actis system at the University Museum, the University of Tokyo scanning and imaging facility. Scans were taken at 130 kvp, 0.2 mA, with a copper pre-filter of 0.5 mm to minimize beam hardening artifacts. Each scan was reconstructed in 512 x 512 matrix from 900 views.

Initial micro-CT based surface rendered and cross-sectional gray-scale images of the ASI-VP-2/334 and ASI-VP-2/2 upper canines were derived as follows. 1) Initial isotropic voxel volume datasets of 42 micron size were constructed from up to 900 non-overlapping slices, each of 42 microns thickness. 2) The initial volume data set was rotated and reformatted into standard orientation with the vertical, mesiodistal, and buccolingual tooth axes determined from simultaneous examination of five orthogonal surface rendered views. 3) Further reformatting was conducted to determine the appropriate vertical section that runs through the cusp tip, and cuts the crown at a plane that runs through the convexity of the buccal crown surface. 4) Enamel thickness was measured on sections, each newly derived by re-rotating the original volume data set only once, in order to avoid data corruption from successive interpolation involved in section reformatting.

The micro-CT data sets of the ASI-VP-5/1 lower M3 and the ASI-VP-5/146 lower molar (probably M2) crown were taken as summarized above with the canines. Standard orientations of each tooth (or their approximation with the fragmentary ASI-VP-5/146) were determined as per above. The reformatted mesial cusp section (running through both protoconid and metaconid EDJ tips) of ASI-VP-5/1, and the section running through the protoconid EDJ tip of ASI-VP-5/146 are shown in Figure 3 of the text. Measurements were however taken in the 3-dimensionally "radial" sections running through each of the individual major cusps. The latter sections were determined following the methodology of reference (26) of the main text; sections that appeared to approximate a perpendicular relationship with both EDJ and outer enamel surface at the vertical levels where the measurement would be made were chosen for measurement.

Enamel boundaries were determined by the half-maximum height method with regards to the air-enamel interface, and by visual determination of closest pixel with regards to the enamel-dentine interface. General volume rendering was done with software Analyze 6.0 (Mayo Clinic, MN), and enamel thickness measurements with Vol-Rugle (Medic Engineering Inc., Kyoto). Radial thickness of the lateral crown face (reference 26) was defined as the minimum thickness from a given point of the EDJ to the outer enamel surface, and maximum radial enamel thickness refers to the maximum of such measures.

Results

Maximum radial enamel thickness values of the lateral crown face, obtained in the naturally fractured sections of ASI-VP-2/334 were 1.7 mm at the M1 metacone, 1.9 mm at the M2 paracone, 2.0 mm at the M2 metacone, 1.9 mm at M2 distal hypocone face, 1.8 mm at the M3 metacone, 2.3 (+) mm at the M3 protocone, and 2.1 mm at mid-distal M3 crown face.

Maximum radial enamel thickness values of the lateral crown face measured in the micro-CT sections opposite the main cusps were as follows. In ASI-VP-5/1, the values reported here are minimal values due to surface enamel damage, and 1.8 (+) mm at the protoconid and hypoconid, 1.7 (+) mm at the hypoconulid, 1.6 (+) mm at the metaconid, and 1.3 (+) mm at the entoconid. The corresponding thickness values of ASI-VP-5/146 were 2.08 mm at the protoconid and 2.32 mm at the hypoconid.