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Uneven distribution of enamel in the tooth crown of the hypsodont Plains Zebra *Equus quagga*

Daniela E Winkler, Thomas M Kaiser

Unworn teeth of herbivorous mammals are not immediately functional. They have to get in wear to expose enamel ridges which can then act as shear-cutting blades to disintegrate the food. We use the Plains Zebra (*Equus quagga*) as a hypsodont, herbivorous model organism to investigate how initial wear of the tooth crown is controlled by underlying structures. We find that the enamel proportion is smaller at the apical part of the tooth crown in all upper tooth positions. Measurements of enamel thickness on the first molar show that the outer enamel band is widest in the lower half of the tooth crown, where enamel content is also highest. We therefore find evidence that the distribution of enamel within the tooth crown is uneven and lower enamel content at the apex promotes early wear. This gradient in enamel distribution is less pronounced in the last molar (txM3), which has also a higher overall enamel content. The M3 is thus hypothesised to have a slightly different functional trait in mastication, resisting highest bite forces along the tooth row and maintaining functionality while anterior teeth are already worn down.

1 **Uneven distribution of enamel in the tooth crown of the hypsodont Plains**
2 **Zebra *Equus quagga***

3 Daniela E. Winkler^{1,3} and Thomas M. Kaiser^{2,3}

4 ¹Corresponding Author, daniela.winkler@uni-hamburg.de; phone: +49 40
5 428386231

6 ²thomas.kaiser@uni-hamburg.de

7 ³Center of natural History (CeNak), University of Hamburg, Martin-Luther-King-Platz
8 3, 20146 Hamburg, Germany

9 Introduction

10 Hypsodonty is a common evolutionary strategy by herbivorous mammals to counter
11 large abrasive loads in the food consumed, which result in high dental wear.
12 Hypsodonty can be easily achieved in all tooth positions by extending specific
13 ontogenetic phases during tooth development (von Koenigswald, 2011). Newly
14 erupted hypsodont cheek teeth share a feature between all taxa: they are not
15 immediately functional. To disintegrate tough plant matter, the relatively rounded
16 apex of the (pre)molar tooth crowns has to wear down slightly, exposing the enamel
17 ridges which may then act as shearing blades during mastication. The rapid wear of
18 the topmost tooth crown has been noted in selenodont molars (Osborn and
19 Lumsden, 1978) and several authors have hypothesised how this initial wear is
20 facilitated. One theory is that empty chewing movements (thegosis) sharpen teeth
21 in adults and helps to bring the teeth in wear in young due to pure attritional
22 contacts (Every, 1972; Every et al. 1998). More often, however, such empty chewing
23 is considered a behavioural anomaly (termed bruxism or pathological thegosis)
24 which appears in livestock, other domestic or captive animals (eg. Murray et al.,
25 1998; Troxler, 2007; 2012) and also in man.

26 We propose that the top of the tooth crown should be less resistant to both
27 attritional and abrasional contacts in order to promote early wear and hence expose
28 functional enamel ridges quickly. This could be accomplished by either building the
29 top of the tooth crown from less and/or thinner enamel or by building a less resistant
30 enamel microstructure. Both hypotheses suggest that the top of the tooth crown is
31 structurally different from the rest of the tooth. Analysis of enamel microstructure at
32 different tooth crown heights is a destructive and time consuming method, therefore
33 we chose to study enamel distribution within the tooth crown of a subadult Plains
34 Zebra (*Equus quagga* sp.) using micro CT-scanning. The Plains Zebra is an ideal
35 model organism for large, hypsodont herbivores, because it is adapted to grazing in
36 both arid and savannah climates and therefore needs to have a high tolerance of
37 abrasional tooth wear. Amongst extant large herbivore species, the Equidae exhibit
38 the highest degree of hypsodonty, only equalled by a few Bovidae like *Bison bison*
39 (compare hypsodonty indices in Janis, 1988).

40 Material and Methods

41 The selected individual is a loan from Museum für Naturkunde (Berlin). It shows very
42 low or no wear on the premolar and molar teeth and is therefore in the optimal
43 stage to investigate enamel distribution within all tooth positions of the same
44 individual. The tooth eruption sequence for upper permanent teeth in *Equus*
45 *burchelli* (which is synonymous to *E. quagga*) is M1, M2, I1, P2, P3, P4, I2, C, M3, I3
46 (Erz, 1964). Hence we see small amounts of material loss in the earlier erupting
47 teeth M1, M2, P2, and P3 compared to the unworn P4 and M3. However, we chose
48 not to use unworn premolars and molars of several individuals in order to exclude
49 inter-individual variation in enamel distribution. We focus on the upper permanent
50 dentition, because upper teeth are employed as the standard in studying dental
51 characteristics (Fortelius and Solounias, 2000; Solounias and Semprebon, 2002;
52 Archer and Sanson, 2002) and functional traits should be more pronounced as
53 compared to lower teeth (Kaiser and Fortelius, 2003) due to the lack of gravity
54 impact. High resolution computed tomography (microCT) scans with an x-y-z
55 resolution between 0.075 and 1.0 mm were obtained at Steinmann-Institut für
56 Geologie, Mineralogie und Paläontologie (Universität Bonn, Germany) on the CT
57 scanner v|tome|x s (GE phoenix|x-ray). The software VG StudioMax 2.1 (Volume
58 Graphics, Heidelberg) was used for reconstruction of virtual models and further
59 processing. First, each tooth was recreated with all dental tissues (enamel, dentin
60 and cementum) as a voxel model using manual and automatic segmentation tools.
61 Next the mineralised enamel was selected and pure enamel voxel models were
62 created (Fig.1). We then cut both, the enamel and the full tooth model at approx.
63 75%, 50% and 25% of the initial crown height and created individual models of four
64 tooth sections: Section 1 from 100-75% crown height, Section 2 from 75-50% crown
65 height, Section 3 from 50-25% crown height and Section 4 from 25% down to the
66 base of the crown (Fig.2). Volumes of the enamel sections and full tooth sections
67 were taken directly from these models using VG StudioMax. We further measured
68 thickness of enamel ridges on virtual cross sections through txM1. Measurements
69 were taken at approximately the same position at the metacone for the outer
70 enamel ridge and the inner enamel ridge (Fig.3.A) at the apical and basal part of
71 each section. The approximate height of measurements is indicated in Fig.4.B.

72 Results

73 Data on enamel content are given in Fig.4. Though distribution of enamel content
74 per section was variable between teeth, it was consistently smallest in Section 1
75 (the most apical section) for all tooth positions. Section 2 contained 2.5-5.5% more
76 enamel than Section 1, Section 3 even 4.5 -9.0% more enamel. The largest
77 ontogenetic increase in enamel content was found for txM1 and txM2, where Section
78 4 contained more than 9.6% more enamel. The highest enamel contents were found
79 either in Section 3 (txP2, txP4, txM3) or Section 4 (txP3, txM1, txM2). It is notable

80 that txM3 was composed of more enamel than all other tooth positions and also
81 showed the lowest differences in enamel content between sections. Results for
82 enamel thickness measurements are given in Table 1. The thickness of the inner
83 enamel ridge is largest in Section 2, but very similar in all other sections. The outer
84 enamel ridge is getting thinner from the apical part of Section 1 to the apical part of
85 Section 2, but then increases in thickness from the basal part of Section 2 down to
86 the crown base (compare Fig.3.A and 3.B for location of measurements).

87 Discussion

88 The results of this study support our hypothesis that the top of the tooth crown is
89 structurally different from the remainder of the tooth. We have shown that the
90 overall enamel content is lowest at the crown top and highest in the lower half of the
91 crown. Our measurements of enamel thickness indicate that both thickness and
92 distribution of enamel vary along the tooth crown. The thinnest enamel ridges were
93 not found at the top of the crown; however the overall amount of enamel was lowest
94 at this level.

95 There are relatively more soft dental tissues (dentin and cementum) at the top of
96 the crown and therefore this part of the tooth is prone to fast wear. We further note
97 that the base of each tooth seems to be structurally “enhanced”, as the larger
98 content of enamel should strengthen it and help resist high pressure and stress
99 loads. This interpretation is consistent with our enamel thickness measurements at
100 the base of the crown (Section 4 basal). There the greatest thickness of the outer
101 enamel ridge is recorded, but the inner enamel bands are no longer present,
102 because the two fossettes are worn out.

103 In *Equus quagga*, we find the third upper molar to be structurally different from all
104 other upper teeth, as it has the highest proportion of enamel and the least variation
105 of enamel distribution along the tooth crown. We relate this phenomenon to
106 adaptive pressures related to generally two phenomena:

- 107 1. Mechanical constraint: As the upper M3 is closest to the
108 temporomandibular joint, the highest masticatory forces can be generated
109 here (Greaves, 2012). The high enamel content will then prevent excessive
110 wear and maintain chewing evenly distributed forces induced.
- 111 2. Biogenetic constraints: The M3 is the last tooth to erupt in most mammals,
112 so in the Zebra. Therefore it is also the tooth position maintaining function
113 when anterior teeth have already been worn out..

114 In general, by being more resistant to wear, txM3 can thus compensate for the
115 functional loss of anterior teeth. Because it comes in occlusion while shear-cutting
116 functionality in anterior teeth is well established, there is no need for a weakened
117 crown top as in other cheek teeth.

118 Though *Equus quagga* is an appropriate model organism, these observations are still
 119 singular and restricted to this very taxon. They can, however, help us to understand
 120 how mechanical and ontogenetic constraints of wear and resistance may be solved
 121 in a biological system, by slight modifications of common structures. The findings
 122 also illustrate, that at least the Zebra as a hypsodont herbivore has undergone
 123 severe need of optimisation of its chewing system and that the acquisition of
 124 hypsodonty does not mean, that basic constraints are rendered insignificant in
 125 terms of functional optimisation. As these constraints are universal for all mammals
 126 feeding on abrasive diets, we expect to find similar adaptations in other herbivorous
 127 species, including bovids.

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 131 Oxford) for her suggestions to improve the language. This research is publication no.
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 133 mammalian dentition—phylogenetic and ontogenetic impact on the masticatory
 134 apparatus".

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175 Figures and Tables

- 176 Fig.1.A. Cross section through a full virtual tooth model with all dental tissues. B. As
177 Fig. 1.A., enamel only. Scale bar 50mm.
- 178 Fig.2. Virtual 3D-model of txP3 with all dental tissues. The tooth is separated in four
179 sections, which are slightly separated from each other for better illustration.
- 180 Fig. 3.A. Cross section through the virtual model of txM1. Black bars indicate the
181 approximate position where measurements of enamel thickness were taken on the
182 outer and inner enamel ridge. Scale bar is 50mm. B. Dashed lines show approximate
183 heights where thickness measurements were taken. Solid lines represent borders of
184 each section. The tooth is in anatomically correct position with the top of the crown
185 facing the bottom of the image. Hence, the lower dashed line within one section
186 marks the positions referred to as “apical”, the upper dashed line as “basal” in
187 Tab.1.

188 Fig.4. Enamel content per section and tooth position. Each bar represents 100%
189 enamel content per tooth position and shows relative enamel content per section.
190 Percentages above bars give the relative enamel content per tooth position.

191 Tab.1. Measurements of enamel thickness for the outer and inner buccal enamel
192 ridge at two positions ("apical", "basal") of each section. For the basal part of
193 Section 4 no thickness could be measured for the inner enamel ridge as it has
194 already ended at another height.

Table 1(on next page)

Tab.1

Tab.1. Measurements of enamel thickness for the outer and inner buccal enamel ridge at two positions (“apical”, “basal”) of each section. For the basal part of Section 4 no thickness could be measured for the inner enamel ridge as it has already ended at another height.

Tab.1. Measurements of enamel thickness for the outer and inner buccal enamel ridge at two positions (“apical”, “basal”) of each section. For the basal part of Section 4 no thickness could be measured for the inner enamel ridge as it has already ended at another height.

txM1	Position of measurement	
	Outer enamel ridge [mm]	Inner enamel ridge [mm]
Section 1 apical	12	10
Section 1 basal	11	10
Section 2 apical	11	13
Section 2 basal	14	13
Section 3 apical	14	11
Section 3 basal	13	10
Section 4 apical	15	11
Section 4 basal	16	-

Fig.1

Fig.1.A. Cross section through a full virtual tooth model with all dental tissues. B. As Fig. 1.A., enamel only. Scale bar 50mm.

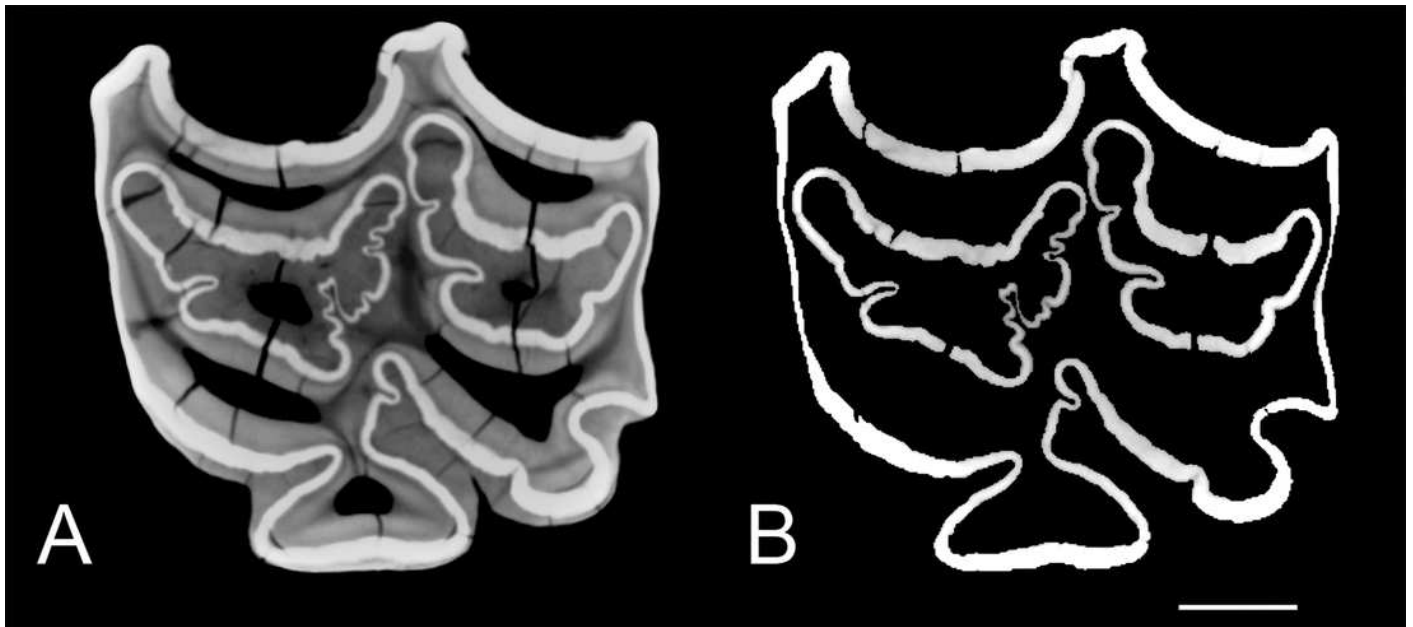
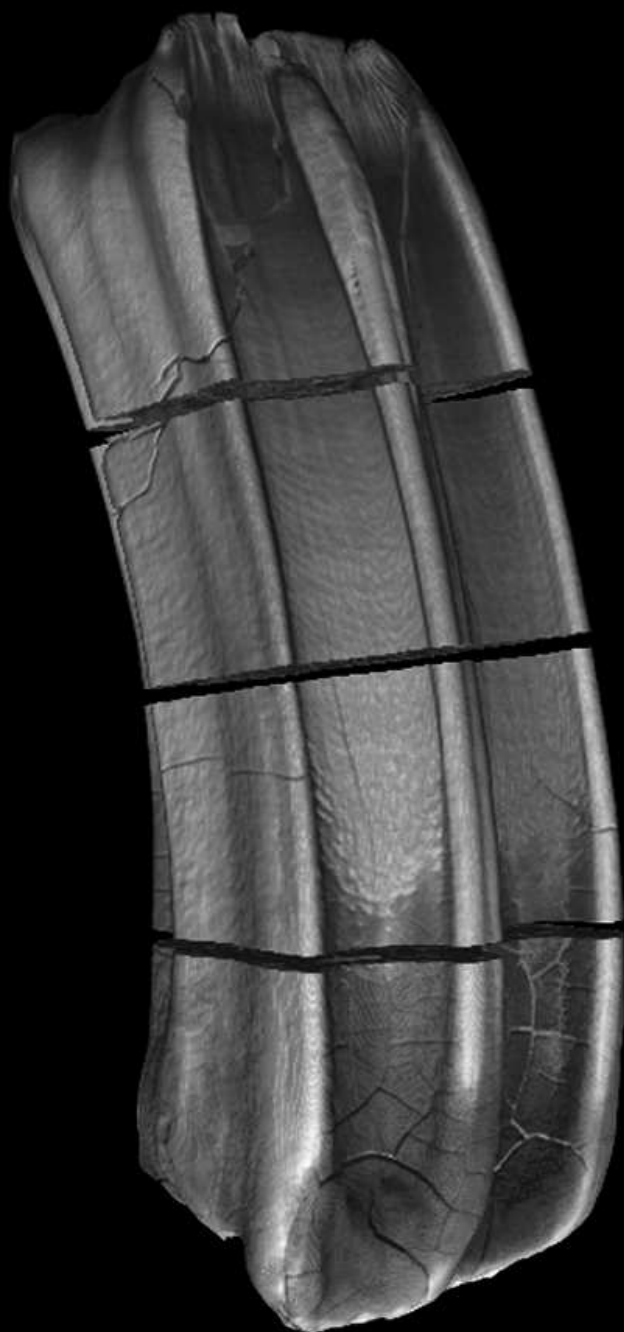


Fig.2

Fig.2. Virtual 3D-model of txP3 with all dental tissues. The tooth is separated in four sections, which are slightly separated from each other for better illustration.



Section 4

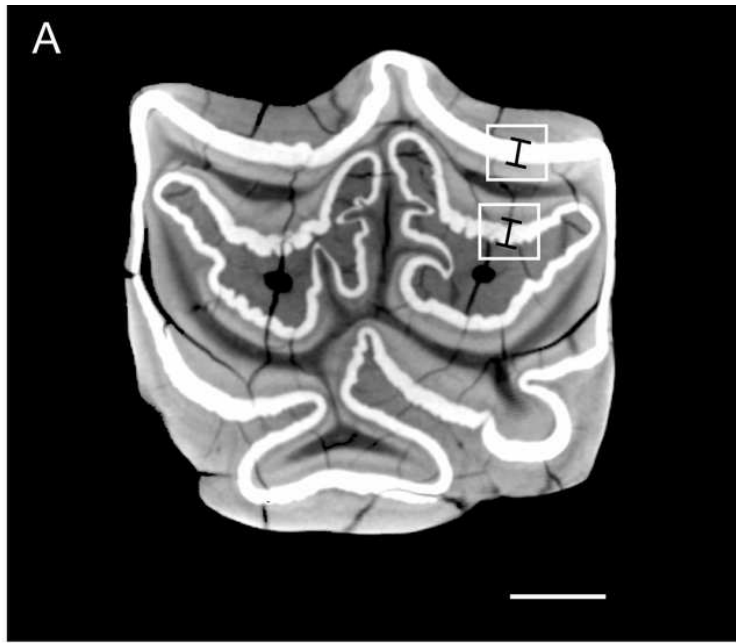
Section 3

Section 2

Section 1

Fig. 3

Fig. 3.A. Cross section through the virtual model of txM1. Black bars indicate the approximate position where measurements of enamel thickness were taken on the outer and inner enamel ridge. Scale bar is 50mm. B. Dashed lines show approximate heights where thickness measurements were taken. Solid lines represent borders of each section. The tooth is in anatomically correct position with the top of the crown facing the bottom of the image. Hence, the lower dashed line within one section marks the positions referred to as “apical”, the upper dashed line as “basal” in Tab.1.



B

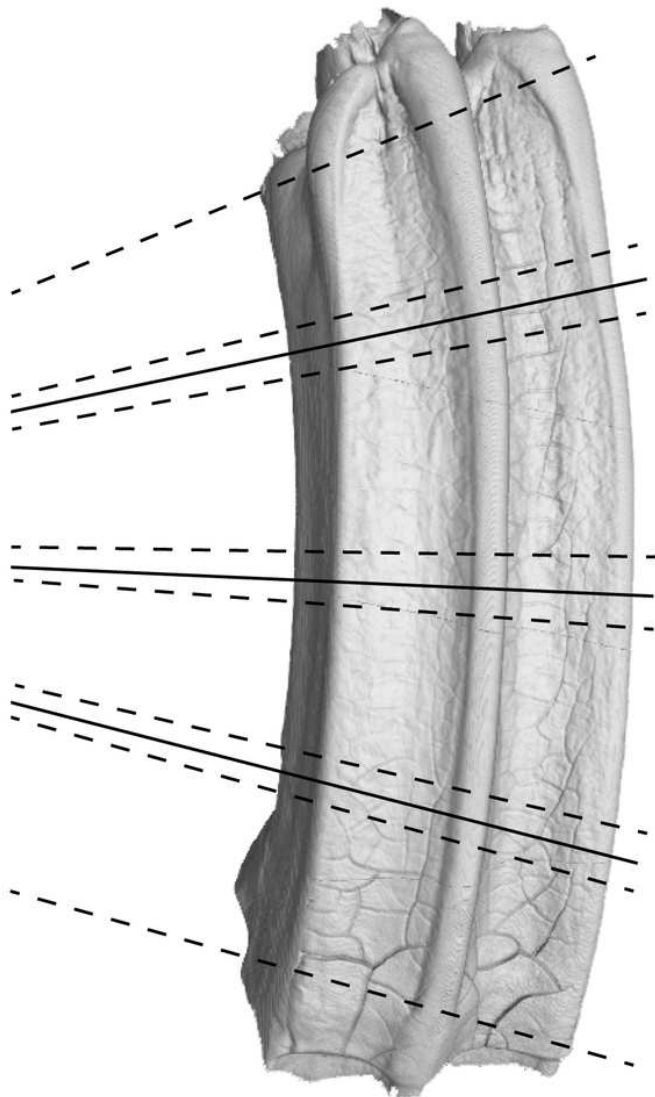


Fig.4

Fig.4. Enamel content per section and tooth position. Each bar represents 100% enamel content per tooth position and shows relative enamel content per section. Percentages above bars give the relative enamel content per tooth position.

