Evolutionary Systematics 2 2018,55–63 | DOI 10.3897/evolsyst.2.22678

PENSOFT

EVOLUTIONARY SYSTEMATICS

Nano-indentation of native phytoliths and dental tissues: implications for herbivore-plant combat and dental wear proxies

Thomas M. Kaiser[1], Caroline Braune[1], Gerhard Kalinka[2], Ellen Schulz-Kornas[1,3]

1 *Center of Natural History (CeNak), University of Hamburg, Martin-Luther-King-Platz 3, D-20146 Hamburg, Germany*

2 *Federal Institute for Materials Research and Testing, Unter den Eichen 87, D-12209 Berlin, Germany*

3 *Max Planck Weizmann Center for Integrative Archaeology and Anthropology, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany*

http://zoobank.org/5C7DBB2B-B27D-4CE6-9656-33C4A0DA0F39

Corresponding author: *Thomas M. Kaiser* (thomas.kaiser@uni-hamburg.de)

Received 30 November 2017
Accepted 23 February 2018
Published 4 June 2018

Academic editor:
Andreas Schmidt-Rhaesa

Key Words

indentation hardness
phytolith
enamel
dentine
tooth wear

Abstract

Tooth wear induced by abrasive particles is a key process affecting dental function and life expectancy in mammals. Abrasive particles may be plant endogenous opal phytoliths, exogene wind-blown quartz dust or rain borne mineral particles ingested by mammals. Nano-indentation hardness of abrasive particles and dental tissues is a significant yet not fully established parameter of this tribological system. We provide consistent nano-indentation hardness data for some of the major antagonists in the dental tribosystem (tooth enamel, tooth dentine and opaline phytoliths from silica controlled cultivation). All indentation data were gathered from native tissues under stable and controlled conditions and thus maximize comparability to natural systems. Here we show that native (hydrated) wild boar enamel exceeds any hardness measures known for dry herbivore tooth enamel by at least 3 GPa. The native tooth enamel is not necessarily softer then environmental quartz grit, although there is little overlap. The native hardness of the tooth enamel exceeds that of any silica phytolith hardness recently published. Further, we find that native reed phytoliths equal native suine dentine in hardness, but does not exceed native suine enamel. We also find that native suine enamel is significantly harder than dry enamel and dry phytoliths are harder than native phytoliths. Our data challenge the claim that the culprit of tooth wear may be the food we chew, but suggest instead that wear may relates more to exogenous than endogenous abrasives.

Introduction

Teeth wear, because they contact each other or are abraded by particles ingested during feeding. Because wear cannot be avoided, abrasive particles ingested, largely impact tooth function and life expectancy (Carranza et al. 2004, Carranza et al. 2008, Ozaki et al. 2009, Skogland 1988), and thus overall energy intake and chewing efficiency (e.g. Clauss et al. (2008), Fritz et al. (2009), Kaiser et al. (2010), Schwarm et al. (2009)). Reducing particle size of ingesta by mastication is considered a key adaptation in mammals (Clauss and Hummel 2005, Reilly et al. 2001). Chewing efficiency is supposed to be compensated by different food intake rates and/or chewing durations (Logan 2003, Pérez-Barbería and Gordon 1998). There are three general sources of particles that putatively are important tooth wear agents: 1. endogenous plant opal phytoliths (so far thought to consist of amorphous, variably hydrated and porous silica (Baker et al. 1959, Ciochon et al. 1990, Gügel et al. 2001, Rabenold and Pearson 2011), 2. abrasives covering ingesta (e.g. wind or rain borne minerals, mostly quartz particles from the environment), and 3. tooth tissue chips (e.g. enamel, dentin, cementum). While phytoliths immediately relate to the plant species eaten, dust and grit reflects the environment and its soil minerals as well as to climate driven atmospheric dust

transport mechanisms. The major mechanical property controlling three-body abrasiveness of particles are hardness, particle size and geometry (Williams 2005). Based on hardness estimates of tooth enamel, tooth dentine, opal phytoliths, and environmental dust particles a debate has emerged regarding the contribution of abrasives from different sources to dental wear in mammals (Damuth and Janis 2011, Lucas et al. 2013, Mainland 2003, Müller et al. 2014, 2015, Sanson et al. 2007, Schulz et al. 2013). Abrasiveness of dietary particles has major implications for our understanding of the causal agents of dental wear, because tooth wear markers (Fortelius and Solounias 2000, Scott et al. 2005, Ungar et al. 2008) are some of the very few dietary and environmental proxy systems available to anthropological, archaeological and palaeobiological research questions. They are used as tools to assess short and long-term dietary traits, seasonality in feeding, resource availability and partitioning in extant and fossil vertebrates, including ancient and extant hominins (for review see Rivals et al. (2009)). In particular, in terrestrial C3 environments, dental wear markers are often the only proxy system bridging extant biomes and the fossil record.

Hardness measurements (Lucas et al. 2013, Sanson et al. 2007) suggest, that the contribution of opal phytoliths to dental wear is far less than previously reported (Baker et al. 1959) and recently again proposed by Merceron et al. (2016), and that exogenous particles, not opal phytoliths, are the major source of enamel wear in the plant component of a mammal's ingesta. Lucas et al. (2014) argue that phytoliths are mimicking grit without actually wearing teeth. If these data were confirmed, some widely accepted paradigms of plant-animal interaction would have to be reconsidered. For example, one would have to re-think the scenario of phytoliths production as predation defence (Massey et al. 2007, Massey et al. 2009) and the evolution of hypsodonty (Damuth and Janis 2011) as a response to grassland expansion (Janis 1984, 1993, Osborn 1910, Scott 1937, Simpson 1944, Stirton 1947, Webb 1977, 1983, Webb and Opdyke 1995). Moreover the independent acquisition of high-crowned cheek teeth in several ungulate lineages (e.g., camels, equids, and rhinoceroses) in the early to middle Miocene of North America has classically been used as an indication that savannah vegetation spread during this time. The acquisition also called "The Great Transformation" has long been regarded as the classic story of adaptation to a changing environment (Gould 2002, Huxley 1953, MacFadden 2005, Matthew 1926, Mayr 1963, Osborn 1910). Contrasting in the South American grazer –type herbivore linages Strömberg et al. (2013) proposed that hypsodonty was more likely a response to external grit from abundant ash in the context of subtropical forests.

This discussion clearly indicates that we still have far too little knowledge on the range and variability of material properties of enamel in various mammalian species, internal abrasives of plants (e.g. phytoliths) and still there is only one study reporting on the material properties of dentine (Baker et al. 1959).

Nanoindentation became a promising approach measuring mechanical properties of human enamel with high precision and resolution on a very small, sub-micrometre scale (Ang et al. 2010, Braly et al. 2007, Cuy et al. 2002, Saber-Samandari and Gross 2009). Here we employ a similar nano-indentation approach (according to ISO 14577-1 (2015)) for measuring the hardness of tooth enamel, dentine of wild boar and add measurements of the abrasive plant agent (phytoliths) under native conditions. An intrinsic problem of nano-indentation on small particles (e.g. phytoliths) embedded in a relatively soft matrix is that not only the indenter is pressed against the particle, but the particle distributes the indentation force to its surrounding matrix and may cause its plastic or elastic deformation. Because of the crucial parameters of measuring hardness on phytoliths by indentation, we review previous measurements for their integrity and comparability. Baker et al. (1959) used a Knoop indenter in order to compare hardness of tooth enamel and phytoliths. Unfortunately, they do not provide details of both, the phytolith preparation and the test procedure. Therefore we recommend considering data given by Baker et al. (1959) with care. Sanson et al. (2007) found that phytoliths they measured have about half of the Vickers hardness of sheep tooth enamel. However, the number of tested phytoliths was lower than 10 in every case, the scatter were large and the potential influence of matrix deformation was not discussed. Sanson et al. (2007) pointed out that with using the indentation force Baker et al. (1959) reported, the resulting imprints would be larger than even very large phytoliths. Baker et al. (1959) performed their indentation tests with a 3-side diamond Berkovich indenter under a 5 mN max. load. SEM imaging of a prepared and tested particle shows, that the phytoliths surfaces were smoothly polished and the imprint of the indentation lay clearly inside the polished area of the particles. Vickers hardness as measured by Sanson et al. (2007) is defined as the relation between the maximum load the indenter introduces to the area of contact between indenter and substrate. This area can be calculated either from the size of the remaining imprint after unloading or from the total indentation depth relative to the first contact to the surface:

$$HV = F_{max} / As$$

F_{max}: maximal load; As: Surface area of the imprint after unloading. For a perfect Berkovich indenter $As=26.43\ h^2$

With classical Vickers hardness, the size of the remaining imprint is determined using a microscope. However, with using an instrumented nano-indentation device, the Vickers hardness is calculated based on the indentation depth and "h". Since "As" is proportional to the square of "h", only small deviations may lead to a large deviation in the calculated Vickers hardness without accounting for the potentially occurring additional plastic deformation of the embedding matrix, which may significantly increase "h". This in turn would result in a systematic error, which would lead to significantly lower hardness

values if matrix is deformed. Although the SEM micrograph in Sanson et al. (2007) does not appear to suggest plastic matrix deformation (because the edges of matrix and phytolith polished face are at the same level) but based on these data one cannot definitively demonstrate the complete absence of such deformation. In order to be comparable to data of this study, hardness values given by Sanson et al. (2007) were converted into indentation hardness following the protocol suggested by Chudoba and Griepentrog (2005).

Similar data are reported by Lucas et al. (2013). They used a load range between 2 and 4 mN for indenting individual phytoliths. Similar to Sanson et al. (2007), the particles were polished before testing. However, in contrast to the work mentioned before, the indentation hardness H_{IT} was calculated which is defined slightly different from the Vickers hardness HV. Both measures, HV und H_{IT} refers to the plastic deformation under stress:

$$H_{IT} = F_{max} / A_p(h_c) \text{ with } h_c = h_{max} - F_{max} / (dF/dh)_{h=hmax}$$

A_p: projected area of contact between indenter and substrate; h_c: depth from the deepest point of the indent tip to the indenters contact with the particles unaltered surface. h_c is estimated from the slope of the unloading part of the indentation load/displacement function near F_{max}. A_p is calibrated using fused silica (quartz standard) as reference.

Lucas et al. (2013) found the indentation hardness of enamel to be about 5 GPa, but significantly lower values were given for phytolits (0.9 GPa squash, 2.5 GPa grass), higher values for quartz dust (Fig. 2). Again, the potential influence of the softer embedding matrix surrounding the particles while being measured is not discussed by Lucas et al. (2013), although we would consider this effect critical. Furthermore, no information is given on the number of measurements taken on a single phytolith and the variance of those measurements. Both, Sanson et al. (2007) and Lucas et al. (2013) do not give reference to the indentation depth "h", a parameter crucial to our understanding of indentation and subsequent hardness calculation, although, "h" could be reconstructed based on the information given. All these are not necessarily shortcomings of previous studies, but rather reflect the specific study designs and independent approaches. Nevertheless, the lack of consistency in the design of studies and methodological improvements addressing the question has led to results that have caused controversial interpretations in studies on dietary reconstruction (e.g. see Merceron et al. (2007)) when it comes to the basic question: What are the most important agents of tooth wear and how do microwear and texture signatures relate to ingesta and environment? Although in Lucas et al. (2013) and Sanson et al. (2007) the same basic pattern was obtained (enamel is harder than phytoliths), the range of the sheep enamel measured by Sanson et al. (2007) is within the range of the phytoliths measured by Lucas et al. (2013).

In order to implement more consistency and reproducibility we undertook a survey of methods available. We came to the conclusion that recording nano-indentation hardness of both, particles and dental tissues involved in the dental tribological system would provide the most reliable measure of indentation hardness, which makes up one of the most critical variables (Kaiser et al. 2016) to understand the wear effect of particles (Williams 2005). In the present study, we strictly employ nano-indentation and measure native (hydrated) materials and also distinguish dry and native tissues and particles. We also avoid the possibly distorting effect of the embedding matrix by applying a new protocol of sample preparation for indentation measurements, which involves immediate contact of the phytolith measured and an underlying slide surface. We further give indentation depth and perform multiple measurements on single phytoliths grown under strictly controlled conditions. In addition we compare indentation hardness data of native phytoliths and phytoliths prepared by dry ashing according to Piperno (2006). We further supply indentation hardness data of native and dry tooth enamel and dentine, measured with the same protocol as the phytoliths. We thus for the first time provide consistent data of the three major antagonists in the tooth-phytolith tribosystem: Silica phytolith – tooth dentine – tooth enamel.

Material and methods

Phragmites australis (Cav.) Steud. were grown on cocos fibers in a greenhouse for eight weeks in the summer season of Hamburg, Germany. Seedlings were grown using seeds provided by Jelitto (Staudensamen GmbH, Schwarmstedt, Germany). The plants were manually watered twice a week with 10 ml of stock solution diluted in 1 L desalinated water as described by Braune et al. (2012). Silica, [SiO_2:Na_2O (100 mg l^{-1})] and iron [EDTA ferric sodium salt (36.7 mg l^{-1})] were added to the working solution. A solution with *Bacillus thuringiensis israelensis* (1 ml l^{-1}) (Neudomück, W. Neudorff GmbH KG, Emmerthal, Germany) was used to protect the plant roots against larvae of different gnat species. Finally, the pH of the solution was adjusted to 5.8–6.0 with hydrochloric acid and potassium hydroxide. Phytoliths were extracted according to. Braune et al. (2012) and after Piperno (2006). The leaves and stems were cut into 5 mm pieces. Desalinated water (100 ml) was added to 5 g of frozen plant material, and the mixture heated for 4.5 h in a kitchen microwave oven at 900 W (Sharp, Osaka, Japan). Desalinated water was added to the samples every 30 min. The samples were cooled and homogenised with a Grindomix (Retsch, Haan, Germany) at 10.000 rpm for 5 s. Afterwards they were heated again for 1.5 h and strained through a plankton sieve (80 µm mesh). The samples were reduced with a centrifuge at 2000 rpm for 5 min. The dry-ashing method (Piperno 2006) was exercised using 1 g aliquots of frozen leave material of *Phragmites australis*. Tin foil was used instead of porcelain crucibles. Afterwards, the samples were washed with 10% hydrochloric acid and finally with desalinated water. For conservation, sodium azide was

Table 1. Descriptive statistics of the nano-indentation hardness values H_{IT} (GPa) given for the materials analysed (phytoliths = *Phragmites australis*, enamel and dentine = wild boar (*Sus scrofa*), N indents = number of indents measured, min = minimum value, max = maximum value, 1Q = first quartile, 3QR = third quartile, VAR = variance, SD =standard deviation, CV = coefficient of variation, SE = standard error, h = indentation depth [nm]).

material	N	mean	median	min	max	1QR	3QR	VAR	SD	CV	SE	h
phytolith [native]	17	1.51	1.64	0.75	2.38	1.251	1.759	0.244	0.494	0.244	0.12	100–200
phytolith [dry]	24	1.89	1.93	0.75	3.58	1.548	2.245	0.682	0.826	0.435	0.169	100–200
epoxy resin	19	0.22	0.22	0.01	0.39	0.19	0.25	0.005	0.07	0.318	0.016	250–500
enamel [native]	8	6.49	6.45	5.01	7.73	6.04	7.09	0.726	0.852	0.131	0.301	800–1800
enamel [dry]	17	4.16	4.35	3.26	4.65	4.02	4.43	0.167	0.409	0.098	0.099	1200–1500
dentine [native]	24	1.71	1.47	1.16	2.83	1.34	2.03	0.261	0.511	0.298	0.104	800–1800
dentine [dry]	40	0.91	0.89	0.76	1.2	0.81	1	0.014	0.117	0.128	0.019	1200–1500

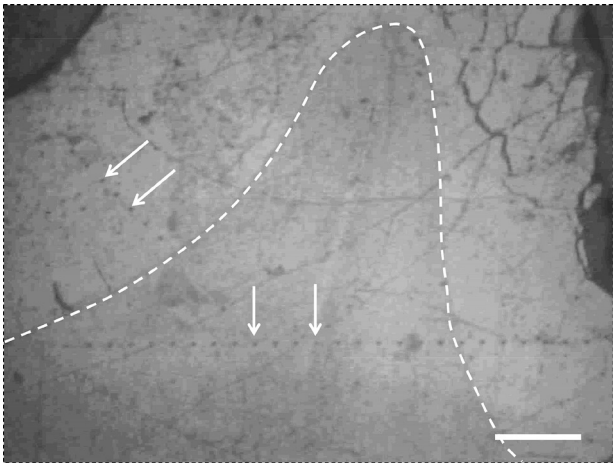

Figure 1. Polished slices of wild boar tooth tissue embedded in epoxy resin. The enamel-dentine junction (EDJ) is indicated by a dotted line. Some of the several marks of nano-indentation are indicated by arrows (scale bar = 200 µm).

added to a final concentration of 2% (w/v) to all samples. Extracted phytoliths were embedded in an approximately 10 µm thin layer of epoxy resin (Technovit EPOX, Heraeus Kulzer GmbH, Hanau, Germany). The thickness of the layer equalled the depth of the phytolith, hence the phytolith was in contact with the slide. The upper side was polished and subsequently measured. Using this arrangement we tried to reduce the likelihood of indentation forces displacing the phytolith to all directions (e. g. pushing it into the resin matrix or the slide). In using the CMS-Method for indentation measurement, we further reduce such critical force and displacement responses, since CMS oscillations have small amplitudes and movements of the phytoliths would be easily detected by sudden changes (instability) in the stiffness signal. In order to be on the safe side, we discarded those measurements, in which sudden shifts in stiffness occurred, although this rarely happened.

Three upper check teeth of a semi-adult wild boar (*Sus scrofa*) were extracted under frozen conditions. The wild boar was selected, because it is an omnivore consuming a variety of vegetable food (up to 99%) like green plant matter, roots, agricultural crops, mast (including acorn, beechnuts, chestnuts) as well as animal foods including vertebrate and invertebrates (for a review see Schely and Roper (2003)). Its diet is highly seasonal, interannual and regional and thus similar to early modern human diet.

The upper second, third and fourth unworn premolars (P2, P3, P4) were defrosted in water and embedded in methacrylat 1 (P2, Technovit 4002, Heraeus Kulzer GmbH, Hanau, Germany), methacrylat 2 (P3, Technovit 4071, Heraeus Kulzer GmbH, Hanau, Germany) and epoxy resin EP (P4, Reckli GmbH, Herne, Germany). Subsequently the specimen was cut in 3 mm slices mesio-distally in parallel with the occlusal surface. In order to establish a consistent procedure, we tested for embedding in methacrylate as well as for epoxy resin (methacrylate 1 P2, Technovit 4002, Heraeus Kulzer GmbH, Germany; methacrylat 2 P3, Technovit 4071, Heraeus Kulzer GmbH, Germany, and epoxy resin EP P4, Reckli, Germany) and used both methods.

Extracted phytoliths and dental tissues were manually ground and polished using descending grades of silicon carbide paper. Parts of the selected phytoliths were fine-polished using 1 µm diamond paper. All procedures were applied in liquid phase and samples were kept hydrated during all steps of preparation. The following figures give the number of phytoliths measured and the number of indents measured (in brackets). Indentation was measured on the polished phytolith surface ($n_{\text{phytolith native}} = 7(17)$, $n_{\text{phytolith dry}} = 8(24)$) while ensuring that the entire process of preparation and measuring was undertaken under hydrated conditions. Measuring locations on samples of dental tissues were placed in a central position of the enamel and dentin area respectively ($n_{\text{enamel native indents}} = 3(8)$, $n_{\text{dentine native}} = 3(24)$; $n_{\text{enamel dry}} = 2(17)$, $n_{\text{dentine dry}} = 2(40)$, see Table 1).

Nano-indentation measurements were carried out with three different Berkovich-diamond-indenter systems according to ISO 14577-1 (2015). In order to be comparable to data of this study hardness values given by Sanson et al. (2007) were converted into indentation hardness following the protocol suggested by Chudoba and Griepentrog (2005). In contrast to former studies, the indentation hardness H_{IT} (according to ISO 14577-1 (2015)) was calculated as follows:

$$H_{IT} = F_{max} / A_p(h_c) \text{ with } h_c = h_{\max} - \varepsilon F_{max} / S$$

F_{max}: maximal load; A_p: projected area of contact between indenter and substrate; h_c: depth from the deepest

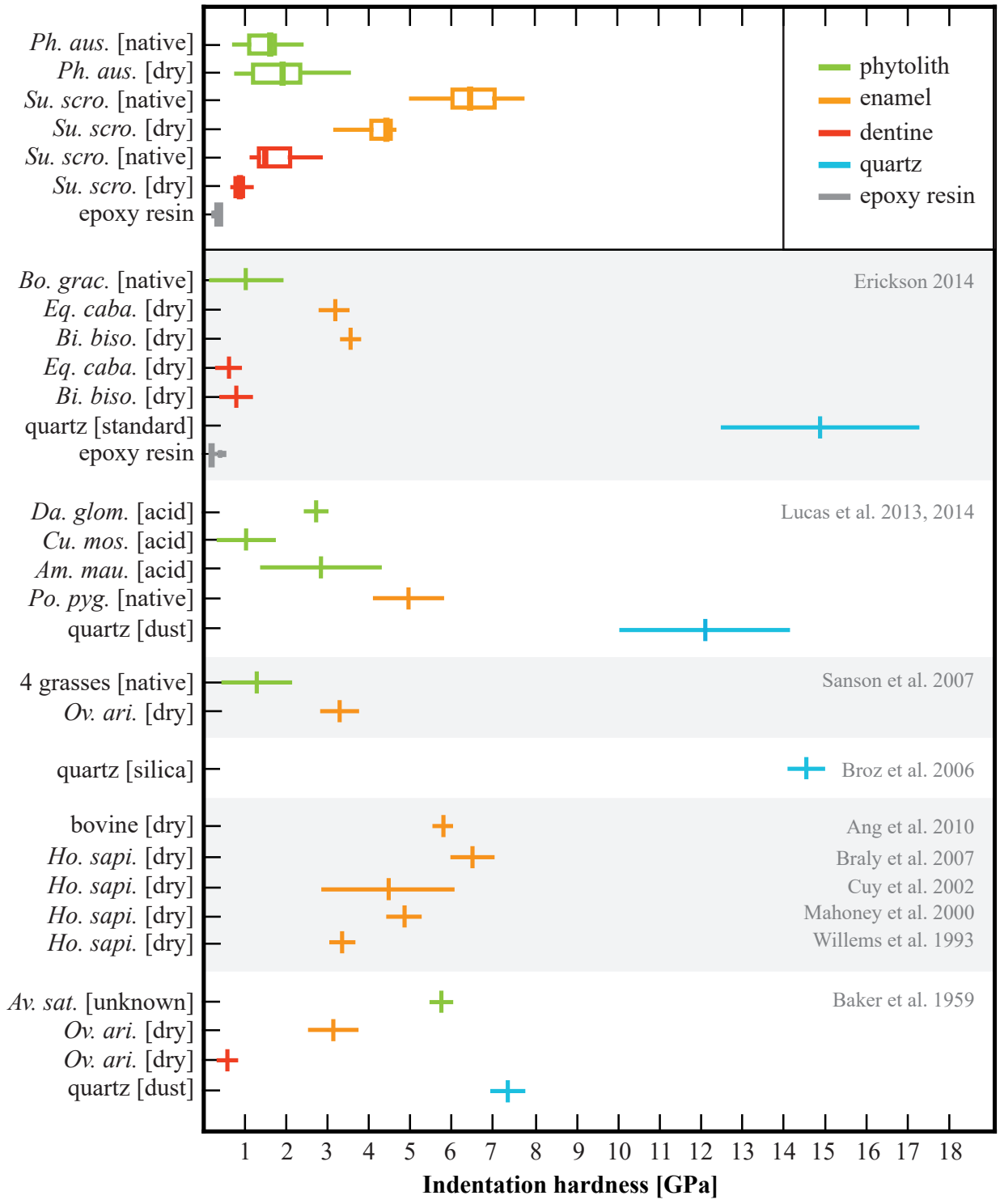

Figure 2. Indentation hardness of phytoliths, dental tissues, environmental dust and quartz particles. Upper section: nano-indentation data presented here, band inside the box plots = median, box = 1Q = first quartile, 3QR = third quartile, end of the whiskers = minimum and maximum values; epoxy resin values used for embedding given as comparative. Lower section: published comparative data by Ang et al. (2010), Baker et al. (1959), Broz et al. (2006), Cuy et al. (2002), Erickson (2014), Lucas et al. (2013), Lucas et al. (2014), Mahoney et al. (2000), Sanson et al. (2007), Willems et al. (1993) converted following Chudoba and Griepentrog (2005), only mean, and minimum to maximum values were available; plant phytoliths: *Am. mau.* = *Ampelodesmos mauritanicus*, *Av. sat.* = *Avena sativa, Bo. grac.* = *Bouteloua gracilis*, *Cu. mos.* = *Cucurbita moschata, Da. glom* =*Dactylis glomerata, Ph. aus.* = *Phragmites australis*; dental tissues: *Bi. biso.* = *Bison bison, Eq. caba.* = *Equus caballus*, *Ho. sapi.* = *Homo sapiens*, *Ov. ari.* = *Ovis aries, Po. pyg.* = *Pongo pygmaeus*, *Su. scro.* = *Sus scrofa*.

point of the indent tip to the indenters contact with the particles unaltered surface. h_c is estimated from the slope of the unloading part of the indentation load/displacement function near F_{max}; ε: geometry factor of the indenter tip; S: contact stiffness; A_p is calibrated with using indents on a reference material.

The Nanoindeter XP (Agilent Technologies, Santa Clara, USA) was employed using the continuous stiffness measurement (CSM) option (Li and Bhushan 2002). With this option, a small additional oscillating force is superimposed to the main load ramp. Due to the separation of in-phase and out-of-phase components of the load-displacement hysteresis, the initial contact between tip and surface can be determined accurately. Furthermore, since the contact stiffness is determined continuously during the main loading ramp, any change of stiffness during the complete test can be easily detected. Thus, the force range for stiffness evaluation of an indented particle below the force where sink-in occurs can be determined for each indent individually. The force has to be high enough for the signal-to-noise ratio to be as high as possible, but low enough to prevent sink-in. It was found that an indentation between 200 and 350 nm fulfils these conditions for most of the particles. Dry phytoliths and dry dental tissues were measured using the Nanoindenter XP at an indentation rate of max. 1500 nm for dental tissues and max 200 nm for silica phytoliths (Table. 1). We also use the nanomechanical triboscope (Hysitron Inc., Eden Prairie, Minneapolis, USA) for hardness tests on enamel.

Results

Our nano-indentation values (minimum-maximum values see Table 1, box plots in Figure 2) for dry dental enamel of wild boar (3.26–4.65 GPa) are compared to the micro-hardness values of dry dental sheep enamel by Baker et al. (1959) (5.5–3.7 GPa), Sanson et al. (2007) (2.93 and 3.57 GPa). In general our values of native (5.01–7.73 GPa) and dry wild boar enamel (3.26–4.65 GPa) and dry (0.76–1.20 GPa) and native wild boar dentine (1.16–2.83 GPa) are partly within the ranges reported in past (for an overview see Figure 2). As expected, dentine values were found to be consistently softer than any tooth enamel hardness ever reported. Dry wild boar dentine was in the range of dry sheep (Baker et al. 1959), bison or horse dentine (Erickson 2014), while native wild boar dentine was significantly harder.

The mean value given for wild boar enamel (6.5 GPa) rivals that for outer enamel in humans (3.2–3.6 GPa in (Willems et al. 1993); 3–6 GPa in Cuy et al. (2002); 4.5–5.2 GPa in Mahoney et al. (2000); 6–7 GPa in Braly et al. (2007), Figure 2) and bovine enamel (5.7 GPa in Ang et al. (2010)), and may come close to values for single hydroxyapatite (HAP) crystals (6.4–7.1 GPa, dependent on orientation in Saber-Samandari and Gross (2009); although 10 GPa is given in Ang et al. (2010)). If this level of hardness pervades wild boar enamel, it would be required to be almost solid HAP (though this would be incompatible with the finding that dry pig enamel has only two-thirds the hardness of wet enamel). Such a heavy mineral content all through the enamel layer would influence the way that wild boar teeth fail.

We consistently found native dental tissues to be harder than dry dental tissues and there is no overlap between dentine and enamel. While dry wild boar enamel is within the range of published hardness data of sheep enamel (Baker et al. 1959, Sanson et al. 2007), native wild boar enamel exceeds any hardness measures published for dry herbivore ungulate tooth enamel by at least 1 GPa (Table 1). In fact, data given by Lucas et al. (2013) for *Pongo* overlap between 5–6 GPa. A small increase in hardness is reported for dry phytoliths over wet, but statistical significance seems unlikely given the variation (Table 1). There is just a small overlap of 0.71 GPa between the nano-indentation hardness of native phytoliths (0.75–2.38 GPa) and the hardness of native dentine (1.16–2.83 GPa). We find native as well as dry phytoliths to consistently be softer than native as well as dry tooth enamel (minimum >3.26 GPa), and environmental quartz dust particles (values from Lucas et al. (2013)), but being slightly harder than both, native and dry dentine (Figure 2).

Discussion

Nano-indentation and dental wear proxies

When a rigid particle hits enamel, the latter can either be abraded by elastic/plastic chipping or displaced by a 'standing wave' moving ahead of the particle (Lucas et al. 2013). These alternatives depend on particle geometry, friction, the shear stress (represented by indentation hardness) and fracture toughness. Since native and dry phytoliths and native and dry dental tissues are shown to be highly variable in hardness and even geometry depending on the extraction method, studies aiming for animal-plant interaction at the abrasion interface of the dentition should be more critical at this point. If the properties of dental tissues are investigated, preferably native tissues should preferably be considered. For the first time phytoliths are analysed from plants cultivated on strictly silica-controlled media and nano-indentation hardness values are gained based on two different phytolith extraction methods. Although intuitively dry biomass is widely considered more abrasive then fresh, the extraction via dry ashing appears to increase the hardness of the opal phytoliths (Figure 2). There are no empirical data yet available that allow inference on abrasiveness, however, one would tentatively assume higher abrasiveness, because 1. high temperatures used in the dry ashing process are proposed to harden structure due to the loss of water and 2. the dry opal phytoliths are proposed to be less elastic and thus more likely to fracture and form sharp edged bodies.

Our phytolith sample derives from plants cultivated on strictly silica-controlled media, and it displays the largest variability in hardness values of silica phytoliths ever reported. Data indicate, that the average native phytoliths

hardness (1.64 GPa) is by 4.8 GPa lower than the average native enamel hardness, and only slightly harder than native dentine (1.47 GPa). Therefore, our data support the idea that phytoliths are softer than enamel (Lucas et al. 2013, Sanson et al. 2007), but also give new evidence that phytoliths are only slightly harder than dentine. The hardness ranges further indicate, that phytoliths relate to a large variety of wear textures. It has to be considered that even though phytoliths are softer than enamel some dental wear is caused as indicated by feeding experiments (Müller et al. 2014, 2015). This is no surprise from a tribologic point of view, since it is possible for softer materials to abrade relatively harder materials under the right conditions (Richardson 1968). Lucas et al. (2013) suggest that the wear caused by phytoliths may be due to repeated plastic deformation of the surface enamel crystals rather than direct abrasion. But until now there is no direct evidence from feeding experiments to document the formation of surface textures and quantify wear by contacts between occluding teeth in a completely grit-free environment.

Longstanding micro-hardness estimates of 7 GPa for quartz and 5 GPa for phytoliths (Baker et al. 1959) have clearly been misleading, owing to the use of large indentations in obtaining those data. Now knowing more about the internal structure of opal phytoliths Schulz-Kornas et al. (2018) one would assume, that large indenters were more likely to "miss" individual silica aggregates and rather displace them instead of indenting them. The micro-scale of indentation selected in most previous studies must therefore be critical for both, phytolith particles and enamel apatite crystallites because indenters should be much smaller than particle/crystallite/microsphere dimensions (Hill 1950, Samuels and Mulhearn 1957). Moreover, prior studies have examined dry rather than native tissues, and our data suggest that these conditions should produce different hardness values. In particular, dental tissues measured dry underestimate hardness. Native phytoliths may easily indent native dentine, and will certainly contribute to the scouring of dentin. In nature the effects of internal abrasives often act together with the effect of external abrasives, particularly in the context of grazing, with grasses often containing high levels of phytoliths and being prone to grit contamination as well (Damuth and Janis 2011). We raise the question if in fact phytoliths from living cells act as inefficient wear agents and grit has higher potential as an agent of tooth wear. However, material properties of dead and dry biomass may differ significantly from living biomass. As a yet untested hypothesis, we would expect internal abrasives (e.g. phytoliths) to more firmly attach to "soft" structures in dry biomass, even increasing its abrasive effect on dental tissues. This hypothesis will be subject to future testing. We further propose the idea that phytoliths impose a higher selective pressure on dentine as abrasives while in herbivorous mammals enamel ridges would play a role in biomechanically stabilizing dentin basins, which otherwise would become too deep to maintain structural integrity of the occlusal surface. Hypotheses relating the evolution of hypsodonty to increased roughage feeding, as frequently assumed (Damuth and Janis 2011, Strömberg 2006) are challenged by our findings. Also, we support the claim that phytoliths from living biomass may in fact play a subordinate role in tooth enamel wear, while no information is yet available if this holds true for dead (dry) biomass. The impact of grit and dust (including its morphology and abundance) is still widely enigmatic. The debate therefore remains wide open.

Acknowledgements

This research was supported by the "Deutsche Forschungsgemeinschaft" (DFG, German Research Foundation, KA 1525 / 8-1, 8-2) and is publication no. 97 of the DFG Research unit 771. We thank Michael Griepentrog, Bundesanstalt für Materialforschung und - Prüfung, Berlin, Germany, for contributing measurements on the Nanoindenter-XP system. Klaus Zwonarz, Anna Maria Vogt, Martina Bistritz, Rüdiger Sernow are acknowledged for their assistance with preparation and handling samples.

References

Ang SF, Bortel EL, Swain MV, Klocke A, Schneider GA (2010) Size-dependent elastic/inelastic behavior of enamel over millimeter and nanometer length scales. Biomaterials 31: 1955–1963. https://doi.org/10.1016/j.biomaterials.2009.11.045

Baker G, Jones LHP, Wardrop ID (1959) Cause of wear in sheep's teeth. Nature 184: 1583–1584. https://doi.org/10.1038/1841583b0

Braly A, Darnell LA, Manna AB, Teaford MF, Weihs TP (2007) The effect of prism orientation on the indentation testing of human molar enamel. Archives of Oral Biology 52: 856–860. https://doi.org/10.1016/j.archoralbio.2007.03.005

Braune C, Lieberei R, Steinmacher D, Kaiser TM (2012) A simple microwave extraction method for the isolation and identification of plant opal phytoliths. Biologia Section Botany 67: 927–930. https://doi.org/10.2478/s11756-012-0074-1

Broz ME, Cook RF, Whitney DL (2006) Microhardness, toughness, and modulus of Mohs scale minerals. American Mineralogist 91: 135–142. https://doi.org/10.2138/am.2006.1844

Carranza J, Alarcos C, Sanchez-Prieto JV, Mateos C (2004) Disposable-soma senescence mediated by sexual selection in an ungulate. Nature 432: 215–218. https://doi.org/10.1038/nature03004

Carranza J, Mateos C, Alarcos S, Sánchez-Prieto CB, Valencia J (2008) Sex-specific strategies of dentine depletion in red deer. Biological Journal of the Linnean Society 93: 487–497. https://doi.org/10.1111/j.1095-8312.2007.00903.x

Chudoba T, Griepentrog M (2005) Comparison between conventional Vickers hardness and indentation hardness obtained with different instruments. Zeitschrift für Materialkunde 96: 1242–1246. https://doi.org/10.3139/146.101168

Ciochon RL, Piperno DR, Thompson RG (1990) Opal phytoliths found on the teeth of the extinct ape *Gigantopithecus blacki*: implications for paleodietary studies. Proceedings of the National Academy of Sciences 87: 8120–8124. https://doi.org/10.1073/pnas.87.20.8120

Clauss M, Hummel J (2005) The digestive performance of mammalian herbivores: why big may not be that much better. Mammal Review 35: 174–187. https://doi.org/10.1111/j.1365-2907.2005.00062.x

Clauss M, Kaiser TM, Hummel J (2008) The morphophysiological adaptations of browsing and grazing mammals. In: Gordon IJ, Prins HHT (Eds) The Ecology of Browsing and Grazing. Springer, Berlin, Heidelberg 47–88. https://doi.org/10.1007/978-3-540-72422-3_3

Cuy JL, Manna AB, Livi KJ, Teaford MF, Weihs TP (2002) Nanoindentation mapping of the mechanical properties of human molar tooth enamel. Archives of Oral Biology 47: 281–291. https://doi.org/10.1016/S0003-9969(02)00006-7

Damuth J, Janis CM (2011) On the relationship between hypsodonty and feeding ecology in ungulate mammals, and its utility in palaeoecology. Biological Reviews of the Cambridge Philosophical Society 86: 733–758. https://doi.org/10.1111/j.1469-185X.2011.00176.x

Erickson KL (2014) Prairie grass phytolith hardness and the evolition of ungulate hypsodonty. Historical Biology 26: 737–744. https://doi.org/10.1080/08912963.2013.841155

Fortelius M, Solounias N (2000) Functional characterization of ungulate molars using the abrasion-attrition wear gradient: A new method for reconstructing palaeodiets. American Museum Novitates 3301: 1–36. https://doi.org/10.1206/0003-0082(2000)301<0001:FCOUMU>2.0.CO;2

Fritz J, Hummel J, Kienzle E, Arnold C, Nunn C, Clauss M (2009) Comparative chewing efficiency in mammalian herbivores. Oikos 118: 1623–1632. https://doi.org/10.1111/j.1600-0706.2009.17807.x

Gould SJ (2002) The structure of evolutionary theory. Belknap Press of Harvard University Press, Cambridge, 1433 pp.

Gügel IL, Grupe G, Kunzelmann K-H (2001) Simulation of dental microwear: characteristic traces by opal phytoliths give clues to ancient human dietary behavior. American Journal of Physical Anthropology 114: 124–138. https://doi.org/10.1002/1096-8644

Hill R (1950) A theoretical investigation of the effect of specimen size in the measurement of hardness. Philosophical Magazine and Journal of Science: Series 7 41: 745–753. https://doi.org/10.1080/14786445008561007

Huxley J (1953) Evolution in action. Harper, London, 182 pp.

ISO 14577-1 (2015) Metallic materials - Instrumented indentation test for hardness and materials parameters Part 1: Test method. International Organization for Standardization, Geneva, 46 pp. https://www.iso.org/standard/56626.html [accessed 2015-07-08]

Janis CM (1984) The use of fossil ungulate communities as indicators of climate and environment. In: Brenchley P (Ed.) Fossils and climate. Wiley, London, 85–104.

Janis CM (1993) Tertiary mammal evolution in the context of changing climates, vegetation, and tectonic events. Annual Review of Ecology and Systematics 24: 467–500. https://doi.org/10.1146/annurev.es.24.110193.002343

Kaiser TM, Clauss M, Schulz-Kornas E (2016) A set of hypotheses on tribology of mammalian herbivore teeth. Surface Topography: Metrology and Properties 4: 014003. https://doi.org/10.1088/2051-672X/4/1/014003

Kaiser TM, Fickel J, Streich WJ, Hummel J, Clauss M (2010) Enamel ridge alignment in upper molars of ruminants in relation to their natural diet. Journal of Zoology 281: 12–25. https://doi.org/10.1111/j.1469-7998.2009.00674.x

Li X, Bhushan B (2002) A review of nanoindentation continuous stiffness measurement technique and its applications. Materials Characterization 48: 11–36. https://doi.org/10.1016/S1044-5803(02)00192-4

Logan M (2003) Effect of tooth wear on the rumination-like behavior, or merycism, of free-ranging koalas (*Phascolarctos cinereus*). Journal of Mammalogy 84: 897–902. https://doi.org/10.1644/BBa-002

Lucas PW, Omar R, Al-Fadhalah K, Almusallam AS, Henry AG, Michael S, Thai LA, Watzke J, Strait DS, Atkins AG (2013) Mechanisms and causes of wear in tooth enamel: implications for hominin diets. Journal of The Royal Society Interface 10. https://doi.org/10.1098/rsif.2012.0923

Lucas PW, van Casteren A, Al-Fadhalah K, Almusallam AS, Henry AG, Michael S, Watzke J, Reed DA, Diekwisch TGH, Strait DS, Atkins AG (2014) The role of dust, grit and phytoliths in tooth wear. Annales Zoologici Fennici 51: 143–152. https://doi.org/10.5735/086.051.0215

MacFadden BJ (2005) Fossil horses - evidence for evolution. Science 307: 1728–1230. https://doi.org/10.1126/science.1105458

Mahoney E, Holt A, Swain M, Kilpatrick N (2000) The hardness and modulus of elasticity of primary molar teeth: an ultra-micro-indentation study. Journal of dentistry 28: 589–594. https://doi.org/10.1016/S0300-5712(00)00043-9

Mainland IL (2003) Dental microwear in grazing and browsing Gotland sheep (*Ovis aries*) and its implications for dietary reconstruction. Journal of Archaeological Science 30: 1513–1527. https://doi.org/10.1016/S0305-4403(03)00055-4

Massey FP, Ennos AR, Hartley SE (2007) Herbivore specific induction of silica-based plant defences. Oecologia 152: 677–683. https://doi.org/10.1007/s00442-007-0703-5

Massey FP, Massey K, Roland Ennos A, Hartley SE (2009) Impacts of silica-based defences in grasses on the feeding preferences of sheep. Basic and Applied Ecology 10: 622–630. https://doi.org/10.1016/j.baae.2009.04.004

Matthew WD (1926) The evolution of the horse: a record and its interpretation. Quarterly Review of Biology 1: 139–185. https://doi.org/10.1086/394242

Mayr E (1963) Animal species and evolution. Harvard University Press, Cambridge, 797 pp. https://doi.org/10.4159/harvard.9780674865327

Merceron G, Ramdarshan A, Blondel C, Boisserie JR, Brunetiere N, Francisco A, Gautier D, Milhet X, Novello A, Pret D (2016) Untangling the environmental from the dietary: dust does not matter. Proceedings of the Royal Society B 283: 20161032. https://doi.org/10.1098/rspb.2016.1032

Merceron G, Schulz E, Kordos L, Kaiser TM (2007) Paleoenvironment of *Dryopithecus brancoi* at Rudabánya, Hungary:evidence from dental meso- and micro-wear analyses of large vegetarian mammals. Journal of Human Evolution 53: 331–349. https://doi.org/10.1016/j.jhevol.2007.04.008

Müller J, Clauss M, Codron D, Schulz E, Hummel J, Fortelius M, Kircher P, Hatt J-M (2014) Growth and wear of incisor and cheek teeth in domestic rabbits (*Oryctolagus cuniculus*) fed diets of different abrasives. Journal of Experimental Zoology A 327: 283–298. https://doi.org/10.1002/jez.1864

Müller J, Clauss M, Codron D, Schulz E, Hummel J, Fortelius M, Kircher P, Hatt J-M (2015) Tooth length and incisal growth and wear in guinea pigs (*Cavia porcellus*) fed diets of different abrasiveness. Journal of Animal Physiology and Animal Nutrition 99: 591–604. https://doi.org/10.1111/jpn.12226

Osborn HF (1910) The age of mammals in Europe, Asia and North America. Macmillan, New York, 634 pp. https://doi.org/10.5962/bhl.title.102077

Ozaki M, Kaji K, Matsuda N, Ochiai K, Asada M, Ohba T, Hosoi E, Tado H, Koizumi T, Suwa G, Takatsuki S (2009) The relationship between food habits, molar wear and life expectancy in wild sika deer populations. Journal of Zoology 280: 202–212. https://doi.org/10.1111/j.1469-7998.2009.00653.x

Pérez-Barbería FJ, Gordon IJ (1998) Factors affecting food comminution during chewing in ruminants: A review. Biological Journal of the Linnean Society 63: 233–256. https://doi.org/10.1111/j.1095-8312.1998.tb01516.x

Piperno DR (2006) Phytoliths. A comprehensive guide for archaeologists and paleoecologists. Altamira Press, Oxford, 238 pp.

Rabenold D, Pearson OM (2011) Abrasive, silica phytoliths and the evolution of thick molar enamel in primates, with implications for the diet of *Paranthropus boisei*. PLoS ONE 6: e28379. https://doi.org/10.1371/journal.pone.0028379

Reilly SM, McBrayer LD, White TD (2001) Prey processing in amniotes: Biomechanical and behavioral patterns of food reduction. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 128: 397–415. https://doi.org/10.1016/S1095-6433(00)00326-3

Richardson RCD (1968) The wear of metals by relatively soft abrasives. Wear 11: 245–275. https://doi.org/10.1016/0043-1648(68)90175-0

Rivals F, Schulz E, Kaiser TM (2009) A new application for dental wear analyses: estimation of duration of hominid occupations in archaeological localities Journal of Human Evolution 56: 329–339. https://doi.org/10.1016/j.jhevol.2008.11.005

Saber-Samandari S, Gross KA (2009) Micromechanical properties of single crystal hydroxyapatite by nanoindentation. Acta Biomaterialia 5: 2206–2212. https://doi.org/10.1016/j.actbio.2009.02.009

Samuels LE, Mulhearn TO (1957) An experimental investigation of the deformed zone associated with indentation hardness impressions. Journal of Mechanics and Physics of Solids 5: 125–134. https://doi.org/10.1016/0022-5096(57)90056-X

Sanson GD, Kerr SA, Gross KA (2007) Do silica phytoliths really wear mammalian teeth? Journal of Archaeological Science 34: 526–531. https://doi.org/10.1016/j.jas.2006.06.009

Schely L, Roper TJ (2003) Diet of wild boar *Sus scrofa* in Western Europe, with particular reference to consumption of agricultural crops. Mammal Review 33: 43–56. https://doi.org/10.1046/j.1365-2907.2003.00010.x

Schulz E, Piotrowski V, Clauss M, Mau M, Merceron G, Kaiser TM (2013) Dietary abrasiveness is associated with variability of microwear and dental surface texture in rabbits. PLoS ONE 8: e56167. https://doi.org/10.1371/journal.pone.0056167

Schulz-Kornas E, Braune C, Winkler DE, Kaiser TM (2018) Does silica concentration and phytolith ultrastructure relate to phytolith hardness? Biosurface and Biotribology https://doi.org/10.1016/j.bsbt.2017.12.004

Schwarm A, Ortmann S, Wolf C, Streich WJ, Clauss M (2009) More efficient mastication allows increasing intake without compromising digestibility or necessitating a larger gut: Comparative feeding trials in banteng (*Bos javanicus*) and pygmy hippopotamus (*Hexaprotodon liberiensis*). Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 152: 504–512. https://doi.org/10.1016/j.cbpa.2008.12.006

Scott RS, Ungar PS, Bergstrom TS, Brown CA, Grine FE, Teaford MF, Walker A (2005) Dental microwear texture analysis shows within-species diet variability in fossil hominins. Nature 436: 693–695. https://doi.org/10.1038/nature03822

Scott WB (1937) A history of land mammals in the western hemisphere. Macmillan, New York, 786 pp.

Simpson GG (1944) Tempo and mode in evolution. Columbia University Press, New York, 237 pp.

Skogland T (1988) Tooth wear by food limitation and its life history consequences in wild reindeer. Oikis 51: 238–242. https://doi.org/10.2307/3565648

Stirton RA (1947) Observations on evolutionary rates in hypsodonty. Evolution 1: 34–41. https://doi.org/10.1111/j.1558-5646.1947.tb02711.x

Strömberg CAE (2006) Evolution of hypsodonty in equids: testing a hypothesis of adaptation. Paleobiology 32: 236–258. https://doi.org/10.1666/0094-8373

Strömberg CAE, Dunn RE, Madden RH, Kohn MJ, Carlini AA (2013) Decoupling the spread of grasslands from the evolution of grazer-type herbivores in South America. Nature Communications 4: 1–8. https://doi.org/10.1038/ncomms2508

Ungar PS, Grine FE, Teaford MF (2008) Dental microwear and diet of the Plio-Pleistocene hominin *Paranthropus boisei*. PLoS ONE 3: e2044. https://doi.org/10.1371/journal.pone.0002044

Webb SD (1977) A history of savanna vertebrates in the New World. Annual Review of Ecology and Systematics 8: 355–380. https://doi.org/10.1146/annurev.es.08.110177.002035

Webb SD (1983) The rise and fall of the late Miocene ungulate fauna in North America. In: Nitecki MH (Ed.) Coevolution. University of Chicago Press, Chicago, 267–306.

Webb SD, Opdyke ND (1995) Global climatic influence on Cenozoic land mammal faunas. In: Kennett J, Stanley S (Eds) Effects of past global change on life. National Academy Press, Washington, 184–208.

Willems G, Celis JP, Lambrechts P, Braem M, Vanherle G (1993) Hardness and Young's modulus determined by nanoindentation technique of filler particles of dental restorative materials compared with human enamel. Journal of Biomedical Materials Research 27: 747–755. https://doi.org/10.1002/jbm.820270607

Williams J (2005) Engineering Tribology. Cambridge University Press, Cambridge, 488 pp. https://doi.org/10.1017/CBO9780511805905