

RESEARCH ARTICLE

Dental microwear texture gradients in guinea pigs reveal that material properties of the diet affect chewing behaviour

Daniela E. Winkler^{1,2,3,*}, Marcus Clauss⁴, Maximilian Rölle¹, Ellen Schulz-Kornas^{3,5,6}, Daryl Codron⁷, Thomas M. Kaiser³ and Thomas Tütken¹

ABSTRACT

Dental microwear texture analysis (DMTA) is widely used for diet inferences in extant and extinct vertebrates. Often, a reference tooth position is analysed in extant specimens, while isolated teeth are lumped together in fossil datasets. It is therefore important to test whether dental microwear texture (DMT) is tooth position specific and, if so, what causes the differences in wear. Here, we present results from controlled feeding experiments with 72 guinea pigs, which received either fresh or dried natural plant diets of different phytolith content (lucerne, grass, bamboo) or pelleted diets with and without mineral abrasives (frequently encountered by herbivorous mammals in natural habitats). We tested for gradients in dental microwear texture along the upper cheek tooth row. Regardless of abrasive content, guinea pigs on pelleted diets displayed an increase in surface roughness along the tooth row, indicating that posterior tooth positions experience more wear compared with anterior teeth. Guinea pigs feedings on plants of low phytolith content and low abrasiveness (fresh and dry lucerne, fresh grass) showed almost no DMT differences between tooth positions, while individuals feeding on more abrasive plants (dry grass, fresh and dry bamboo) showed a gradient of decreasing surface roughness along the tooth row. We suggest that plant feeding involves continuous intake and comminution by grinding, resulting in posterior tooth positions mainly processing food already partly comminuted and moistened. Pelleted diets require crushing, which exerts higher loads, especially on posterior tooth positions, where bite forces are highest. These differences in chewing behaviour result in opposing wear gradients for plant versus pelleted diets.

KEY WORDS: Mastication, Bite force, Tooth wear, Abrasion

INTRODUCTION

Ingesta processing during chewing generates considerable mechanical stress on teeth and thus modifies the occlusal surface during an individual's life. Food material properties are one

important factor amongst many others to consider when trying to understand the dental wear processes that affect the occlusal surface (for a review, see Schulz-Kornas et al., 2020). During one chewing sequence, the food particles break down and a bolus is formed (Stokes et al., 2013). It is known that external bolus properties as well as food material properties change gradually during that sequence (Foster et al., 2006; Woda et al., 2006; Witt and Stokes, 2015). This in turn could lead to gradual changes of the dental wear along the tooth row. Additionally, in many large mammals (i.e. ungulates), the macroscopic morphology of the molars differs systematically with position in the tooth row (Winkler and Kaiser, 2015a,b) and could further influence gradual changes depending on the size and shape of the molar occlusal area and the force of the power stroke, which both vary between teeth (Pérez-Barbería and Gordon, 1998, 1999). Macroscopic wear gradients with the first molar showing the most and the third molar the least wear have been reported for several ungulates (Fandos et al., 1993; Clauss et al., 2007; Taylor et al., 2016) and for primates (Teaford, 1982, 1983). However, only a few studies have focused on microscopic tooth wear analysis along the tooth row (Schulz et al., 2010; Ramdarshan et al., 2017; Arman et al., 2019; Ackermans et al., 2020).


More often, microscopic wear analyses are applied to successfully differentiate general feeding habits. A common dental wear analysis for diet inferences of extant and fossil mammals, including hominins (for a review, see Ungar, 2015, 2019), is dental microwear texture analysis (DMTA) (Ungar et al., 2003; Scott et al., 2005, 2006; Schulz et al., 2010, 2013a). It has advantages compared with 2D microwear analysis (stereomicroscopic scanning electron microscopy and low-magnification light microscopy; DeSantis, 2016), because it is less observer biased and captures the whole 3D topography of an enamel surface (Scott et al., 2005; DeSantis et al., 2013; Kubo et al., 2017), not only relying on singular wear features (i.e. pits and scratches).

DMTA has been applied to herbivorous ungulates (Ungar et al., 2007; Merceron et al., 2010; Schulz et al., 2010, 2013a; Scott, 2012; Winkler et al., 2013), primates (Calandra et al., 2012; Schulz-Kornas et al., 2019) and carnivores with varying levels of bone consumption (Ungar et al., 2010; DeSantis et al., 2012, 2013), but also to infer diet in non-mammalian taxa such as fish (Purnell and Darras, 2016) and reptiles (Winkler et al., 2019a; Bestwick et al., 2019, 2020).

Beyond the application of DMTA to detect dietary differences between species or populations, it is of importance to understand how the microscopic wear features form, and what diet properties cause which kind of microwear texture. For example, high surface complexity in hominins such as *Paranthropus robustus* was ascribed to hard object feeding (Scott et al., 2005), but such hypotheses need to be validated in extant taxa, preferably under controlled experimental conditions. However, results from *in vitro* indentation experiments recently questioned this correlation

¹Applied and Analytical Palaeontology, Institute of Geosciences, Johannes Gutenberg University, J.-J.-Becher-Weg 21, 55128 Mainz, Germany. ²Department of Natural Environmental Studies, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8563, Japan. ³Center of Natural History, University of Hamburg, 20146 Hamburg, Germany. ⁴Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland. ⁵Department of Cariology, Endodontology and Periodontology, University of Leipzig, 04103 Leipzig, Germany. ⁶Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, 04103 Leipzig, Germany. ⁷Department of Zoology and Entomology, University of the Free State, Bloemfontein 9300, South Africa.

*Author for correspondence (daniela.eileen.winkler@k.u-tokyo.ac.jp)

 D.E.W., 0000-0001-7501-2506; M.C., 0000-0003-3841-6207; M.R., 0000-0003-3708-8432; E.S., 0000-0003-1657-8256; D.C., 0000-0001-5223-9513; T.M.K., 0000-0002-8154-1751

(van Casteren et al., 2020), as they could not produce high surface complexity on enamel through contact with hard foods such as seeds. Feeding experiments with large and small mammals have shed light on the long-standing debate about which factors contribute more to dental (micro)wear formation – internal (i.e. phytoliths) or external abrasives (i.e. mineral dust) (see Winkler et al., 2019b, 2020a, and references within). For sheep, there is evidence that under low grit contamination (~1%), the effect of quartz abrasives ingested with clover or grass silage is negligible, and textural differences are predominantly due to mechanical properties of the plants, and thus most likely their phytolith content (Merceron et al., 2016). However, 4–8% of fine silt-sized (~4 µm) and fine sand-sized (~150 µm) quartz abrasives added to pelleted diets resulted in either distinctly flatter or rougher microwear textures compared with an abrasive-free control diet (Ackermans et al., 2020). For small mammals, there is abundant evidence that both internal and external abrasives contribute substantially to dental wear (Schulz et al., 2013b; Müller et al., 2014, 2015; Martin et al., 2019), but in different ways. Notably, external abrasives result in microwear textures with significantly higher roughness, and deeper and more complex wear features compared with mineral abrasive-free plant feeds of high phytolith content (Winkler et al., 2019b, 2020a).

If differences in (microscopic) wear pattern (microwear texture) along the tooth row were just an effect of the tooth eruption sequence and duration of exposure to wear, systematic variation in DMTA would not be expected, as the same patterns would emerge on teeth as they erupt, soon reaching an equilibrium. Experiments with small mammals have shown that the turnover time of microwear texture is 2–3 weeks (Winkler et al., 2020b). If, however, specifics of dental morphology or chewing behaviour correspond to position in the tooth row, then systematic variation along the row would be predicted. Schulz et al. (2010) reported no significant microwear texture inter-tooth differences in wild zebra (*Equus grevyi*) and wildebeest (*Connochaetes taurinus*); however, they detected specific gradients along the maxillary tooth row. In *E. grevyi*, distal teeth showed lower textural fill volume (a measure of lesions on the enamel surface), probably attributed to distal teeth encountering reduced abrasive particle sizes. In *C. taurinus*, such an effect was absent, which may be attributed to differences in digestive strategy of ruminants versus hindgut fermenters such as zebra. In the former, there is a ‘rumen washing mechanism’ (Hatt et al., 2019), leading to less abrasive cud being chewed during rumination and possibly levelling texture gradients caused on first ingestion. Studies of other ruminants, i.e. sheep, in controlled feeding experiments support this assertion: Ramdarshan et al. (2017) found no significant wear differences between mandibular teeth, and Ackermans et al. (2020) came to a similar conclusion for the maxillary dentition, although they also highlighted that the upper second molar (M2), which is often employed as the reference tooth position in large herbivorous mammals, had the lowest discriminatory power between experimental diets of varying abrasiveness. Ackermans et al. (2020) further found the strongest differentiation between diet groups when lumping measurements of all upper molars together. This is probably due to the increased number of measurements per feeding group. In macropods, tooth position influenced surface complexity (area-scale fractal complexity, *Asfc*), with the highest inter-tooth variation being seen between the fourth molar and all other teeth (Arman et al., 2019). In summary, there might be inter-tooth differences in dental wear patterns of large mammals, or even gradients, but evidence is scarce.

For small mammals, inter-tooth differences in microwear texture have not yet been reported. Amongst several other rodents, guinea pigs (*Cavia porcellus*) possess dentition with similar morphology and occlusal area for each tooth. Their occlusal surface is characterized by a rasp-facet, in which the functional enamel crests form a grinding surface (von Koenigswald, 2018). The permanent dentition erupts *in utero* (Teaford and Walker, 1983) and is composed of one premolar and three molars (Berkovitz, 1972), all rootless and ever-growing (euhyposodont). Therefore, unlike in ungulates (von Koenigswald, 2018), their molar teeth are always in the same wear stage and show no macroscopic wear differences, and do not require consideration of eruption sequence. Guinea pigs are hindgut fermenters and have a unidirectional food passage, so that chewing of less abrasive cud as in ruminants will not affect any potential surface texture gradients along the tooth row. Therefore, guinea pigs are an ideal model organism to assess the effect of tooth position on dental microwear texture (DMT) formation. Moreover, finite element analysis predicted increasing bite forces for guinea pigs from the fourth premolar (P4) to the third molar (M3), with bite forces at M3 exceeding those of P4 by a factor of 1.8 (Cox et al., 2012). We expect that bite force differences are a key masticatory parameter influencing DMT formation along the tooth row (Kaiser et al., 2016).

To test for a gradient in microwear texture formation in guinea pigs, we analysed all teeth of one side of the upper cheek dentition in 72 individuals. The animals received either a plant diet (lucerne, grass, bamboo) free of adherent mineral abrasives (Winkler et al., 2019b) or a pelleted diet with admixed mineral abrasives of different sizes, shapes and quantities (Winkler et al., 2020a) for 3–5 weeks. Plant diets were chosen to represent different phytolith content (lucerne<grass<bamboo) and fed fresh or as hay. We put forward the following hypotheses. (1) A microwear texture gradient along the tooth row in guinea pigs is detectable, showing increasing surface roughness from the fourth premolar (P4) towards the third molar (M3), due to the increase of chewing forces in the distal direction. (2) Abrasive-free natural plant feeds leave a less pronounced wear gradient compared with diets with external mineral abrasives.

If gradients are indeed detectable, using random tooth positions for dietary inference is expected to reduce the resolution of dietary differences. Moreover, such a result would mean that the common practice of lumping isolated teeth of different or unknown tooth positions from fossil species into a single dataset for diet analysis might be problematic. However, in fossil samples, often only isolated teeth are preserved, and different tooth positions are lumped together in order to increase sample sizes. To test which DMTA parameters still have adequate dietary resolution using randomized tooth positions, we tested correct dietary categorization when either a random subset of 25% or 75% of all teeth was subjected to the analysis.

MATERIALS AND METHODS

Animal housing and diets

The feeding experiment was performed during September and October 2017 at the Vetsuisse Faculty, University of Zurich, with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment licence no. ZH135/16). All animals in this experiment were adult female Dunkin Hartley (HsdDhl:DH) guinea pigs (3–4 weeks old at the start of the experiment). Guinea pigs belong to the family Caviidae and probably descended from wild *Cavia tschudii* Fitzinger 1867 (Spotorno et al., 2004), which are adapted to feeding on grasses in their natural habitat of

grasslands in South America (Dunnum, 2015; Lacher, 2016). Animal housing conditions and diets are described in detail in Winkler et al. (2019b, 2020a). Animals received their designated diets for a duration of 3–5 weeks, except for the IsoL group, which was kept on the diet for 8 weeks (Table 1). This time span has been found to be sufficient to form dietary informative wear (Winkler et al., 2020b). Guinea pigs that received plant diets (mean±s.d. initial body mass 263±14 g) were housed in six groups of six individuals ($n=36$ total) in sheltered outdoor stables (2.25 m² each), protected from direct sunlight and rain, and equipped with an infrared lamp, a thick layer of sawdust on the floor, two large wooden shelters, two plastic tubes, two open dishes and two nipple drinkers. Stables were sheltered on the sides by canvas to prevent airborne dust from contaminating the forages, as was the stable floor below the sawdust. Guinea pigs kept on pelleted diets (initial body mass 249.4±12.5 g) were housed in groups of six or three (six individuals per diet, $n=36$ total) in indoor stables (0.58 m² each), equipped with an infrared lamp, a thick layer of sawdust on the floor, a large shelter, two plastic tubes, one open dish for water and food, and one nipple drinker. All groups were provided with water (supplemented with vitamin C at 200 mg l⁻¹) and food for *ad libitum* consumption and received no extra gnawing material.

DMTA

After the feeding periods, animals were killed by exposure to CO₂, and after maceration of the skulls in an enzyme bath, their teeth were moulded for surface texture measurements. These were acquired using the high-resolution confocal disc-scanning surface measuring system μ surf custom (NanoFocus AG, Oberhausen, Germany) with a blue LED (470 nm) and high-speed progressive-scan digital camera (984×984 pixels), set to a 100× long distance objective (resolution in $x,y=0.16$ μ m, step size in $z=0.06$ μ m, numerical aperture NA=0.8), and processed with MountainsMap Premium v.7.4.9175 Software (DigitalSurf, Besançon, France; www.digitalsurf.com). Following Winkler et al. (2019a, 2020a), we measured the anterior enamel band on all cheek teeth of one maxillary tooth row, obtaining 3–4 non-overlapping scans per tooth (see Fig. 1 for example 3D images for each dietary group). If the anterior enamel band was damaged on both sides, we switched to the third enamel band (99 out of 1033 scans). Forty-six surface texture parameters were quantified using the International Organization for Standardization (ISO) 25178 (roughness), motif, furrow, isotropy, ISO 12871 (flatness) and scale-sensitive fractal

analysis (SSFA) implemented in MountainsMap Premium (Table S1). Parameters were grouped according to their main characterizing feature in the following categories: area, complexity, density, direction, height, peak sharpness, plateau size, slope and volume. Processing followed the protocol described by Schulz et al. (2010, 2013a) and applied in Winkler et al. (2019b, 2020a), except for the flatness parameters FLT_p, FLT_q, FLT_t and FLT_v (ISO 12871), where we adjusted the S-filter (λ_s) and used a Gaussian filter of 0.0025 mm instead of the default value of 0.025 mm, because the default filter resulted in NA-values for several individual surfaces. Results for these parameters therefore deviate from those published in Winkler et al. (2019b, 2020a).

DMTA parameters

The vast majority of the DMTA parameters are field parameters (Blateyron, 2013) using every data point of the tooth surface (field or area) measured, i.e. characterizing surface height, slope, complexity and wavelength content. In addition, there are feature parameters that consider specific points, lines or areas of the measured surface. For roughness (ISO 25178), parameters were grouped according to five main algorithm groups used for the calculation (Table S1). For example, it applies to the group of height parameters using the statistical distribution of the height along the z -axis; the spatial parameters using the spatial periodicity of the surface; the hybrid parameters using the spatial shape; function and related parameters calculated from the material ratio curve; and feature parameters derived from segmentation of the surface into motifs (dales and hills) according to the watershed method. In addition to 30 ISO 25178 parameters, we used 3 motif, 3 furrow, 4 isotropy, 4 ISO 12871 (flatness) and 2 SSFA parameters. In sum, this results in 46 parameters analysed. For clarity, we sorted these parameters into nine parameter groups consisting of similar parameters as follows.

Area

Parameters describing overall surface area, or area of specific features (hills, dales) are summarized here. Diets of higher abrasiveness generally result in larger values of the area parameters.

Complexity

Surface complexity refers to the distribution of wear features, with DMT characterized by differently sized features, which overlap each other, having high complexity. Hard-object feeding and more

Table 1. Overview of characteristics of natural and pelleted diets fed to guinea pigs, with experiment duration

Diet	Abbreviation	Experiment duration (days)	ADIA content (% DM)	Particle size (μ m)
Natural diets				
Lucerne fresh	Lf	21	0.47	–
Lucerne dry	Ld	21	0.39	–
Grass fresh	Gf	21	0.58	–
Grass dry	Gd	21	0.67	–
Bamboo fresh	Bf	21	3.21	–
Bamboo dry	Bd	21	3.25	–
Pelleted diets				
Lucerne pellet	IsoL	59	–	–
Control	C	35	–	–
4% Small quartz	4sS	35	–	4.41±1.39
8% Small quartz	8sS	35	–	4.41±1.39
4% Large quartz	4lS	35	–	166.01±15.47
4% Large volcanic ash	4lVA	35	–	96.44±9.03

Acid detergent-insoluble ash (ADIA) data (as percentage dry mass, DM) are from Winkler et al. (2019b). Particle size data (means±s.d.) are from Winkler et al. (2020a).

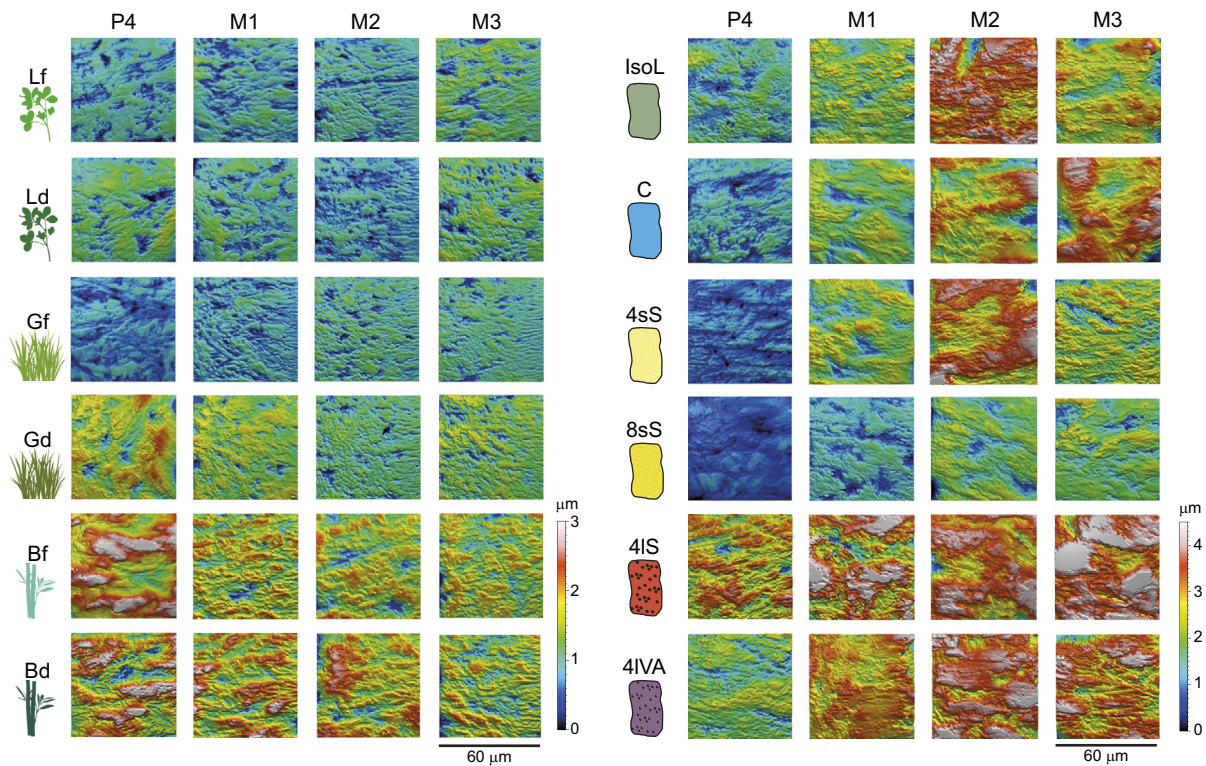


Fig. 1. Example 3D images of dental microwear texture for each tooth position (P4 to M3) from one guinea pig per diet group. Left, natural diets; right, pelleted diets. Note that all scans are scaled to the same vertical scale given within each plate. Blue and green colours refer to low topographic profile height, while yellow and red colours relate to greater height; white areas represent the greatest elevation of microwear texture profiles. Note that profile height was similar between tooth positions, or slightly decreased along the tooth row, for natural diets (left), while height increased along the tooth row for all pelleted diets (right). Lf, lucerne fresh; Ld, lucerne dry; Gf, grass fresh; Gd, grass dry; Bf, bamboo fresh; Bd, bamboo dry; IsoL, lucerne meal-based pellet type 1; C, lucerne meal-based pellet type 2; 4sS, 4% small quartz (~4.4 µm); 8sS, 8% small quartz (~4.4 µm); 4IS, 4% large quartz (~166.0 µm); 4IVA, 4% large volcanic ash (~96.4 µm).

abrasive diets generally result in greater complexity, except for the parameter nMotif (number of motifs).

Density

Features of the surface such as peaks or furrows can be characterized according to their mean density. Peak density is often larger in soft diets, while furrow density is higher with higher loads of external abrasives.

Direction

Both parameters indicating periodic motifs of the surface in the x and y direction, and dominant direction of wear features are included in this category. The directionality can be in one or several directions (e.g. having a predominant texture direction, 'lay') for anisotropic surfaces or being without predominant texture direction (isotropic). Anisotropy is larger in individuals feeding on diets with high quantities of internal and/or external abrasives (grazers).

Height

All parameters that refer to either overall (mean) height differences or height measures between specific features of the surface (e.g. depth of furrows, maximum peak height) are summarized in this category. Diets of higher abrasiveness generally result in greater height differences on the dental surface topography.

Peak sharpness

There is only one parameter referring to the form of peaks, being either sharper or rounded.

Plateau size

Bearing ratio parameters Smr and Smc are aimed at characterizing functional behaviour of the surface, such as wear and lubrication. The areal material ratio Smr is used to set the amount of bearing area (plateau) remaining after a certain depth of material is removed. The inverse areal material ratio Smc can be used to describe an optimum crevice volume necessary for a sealing surface to allow for some lubrication entrapment to flush out wear particles.

Slope

Completely flat surfaces have no slope; thus, less abrasive diets resulting in flat surface texture profiles have lower slope values.

Volume

Parameters referring to volume measures of the complete surface, or particular features, are summarized in this category. As large volumes could be achieved by a deepening of surface features, there is a strong dependency to height parameters; thus, abrasive diets lead to overall larger volume parameters.

Statistics

For each of the 46 DMT variables, the effects of diet and tooth position were evaluated using linear models with the variables Diet (6 levels) and Tooth (4 levels), as well as the interaction between them, as fixed effects. Tukey's HSD *post hoc* test for multiple pairwise comparisons was applied where necessary (α -level=0.05 for all tests; Table S2, Table S3). To account for non-independence incurred by multiple measurements of the same set of individuals,

we included Individual as a random factor (Kristensen and Hansen, 2004). Normality and heteroscedasticity of residuals was tested using Shapiro–Wilk’s and Levene’s tests, respectively. If these assumptions were not met, data were ln-transformed and, if normality was not achieved by data transformation, ranked data were used. Analyses were carried out in the software R v.3.5.2 (<http://www.R-project.org/>), with the package lmerTest used for mixed effects linear models (Kuznetsova et al., 2017). Data from natural and pelleted diets were analysed separately.

If wear responses do differ across tooth positions, the common practice in palaeoecological research of lumping isolated teeth to improve sample size is likely to inflate Type II errors in particular, i.e. reducing the probability of detecting significant diet differences across specimens. To test for this effect, we used a randomized numerical experiment that simulated the ‘lumping’ approach, in order to determine the likelihood that any set of isolated teeth would reflect the overall expected trend. Here, we ranked the six diet treatments per experiment from 1 to 6 according to the means for each DMTA variable, and then evaluated correlations (using Spearman’s rank correlations) between overall rankings with rankings obtained from random subsets of data, i.e. subsets containing a randomized assortment of tooth positions. Randomized subsets included first 25% and then 75% of the total data, sampled without replacement. Sub-sampling was repeated over 10^4 iterations, but correlations were only performed when all six diets were represented in the sub-sample. The mean and standard deviation (s.d.) of the R_s coefficient were determined, as well as the proportion of significant ($P < 0.05$) correlations, for each variable. These simulations were carried out in the software R v.3.5.2, again repeating the analyses for natural and pelleted diets separately (Table S4, Table S5).

Principal component analysis (PCA) including 10 DMTA parameters (employing median parameter values per specimen) was conducted in JMP Pro v.14.2.0 using varimax rotation and covariances rather than correlations, separately for the natural and pelleted diets (Fig. 2). Selection of parameters was based on the PCA included in Winkler et al. (2020a).

Terminology

When referring to ‘chewing behaviour’, we incorporate several aspects in this term. We refer to how much forage is taken into the oral cavity and loaded on parts of the dentition, where the processing is happening (i.e. anteriorly or posteriorly), jaw motion and how much force is exerted during mastication. Note that most inferences on chewing behaviour of our experimental animals are drawn indirectly from the observed DMT pattern, with the exception of the interpretation of Movie 1.

When using the term ‘abrasion’, we are referring to mechanical processes occurring during food–tooth contacts described in dental literature (Mair, 1992; Eisenburger and Addy, 2002a,b; Grippo et al., 2004) as friction between a tooth and exogenous agents, i.e. food itself, or ingested (mineral) particles such as grit and dust. It results in material deformation (plastically deformed enamel) and/or displacement, which may result in visible wear features (furrows, dales, peaks) and/or tissue loss.

RESULTS

Natural diets

Guinea pigs feeding on natural plant diets (Table 1) were observed to continuously ingest plant matter (Movie 1) in what we describe as a ‘conveyor belt’ feeding strategy.

In DMTA, strong gradients of increasing or decreasing parameter values along the tooth row were mostly seen in the diet groups Gd (grass dry), Bf (bamboo fresh) and Bd (bamboo dry) but not on other diets (Fig. 1, Fig. 3; see Fig. S1, available from University of Hamburg open-source data repository: doi:10.25592/uhhfdm.9163). Therefore, the interaction diet×tooth was often significant (Table S2).

Area

All area parameters showed decreasing values along the tooth row towards the posterior end for all diets. Both diet and tooth position significantly affected area parameter values, but only for Sda (closed dale area) was a significant interaction of diet×tooth found (Table S2). The P4 had significantly larger values than

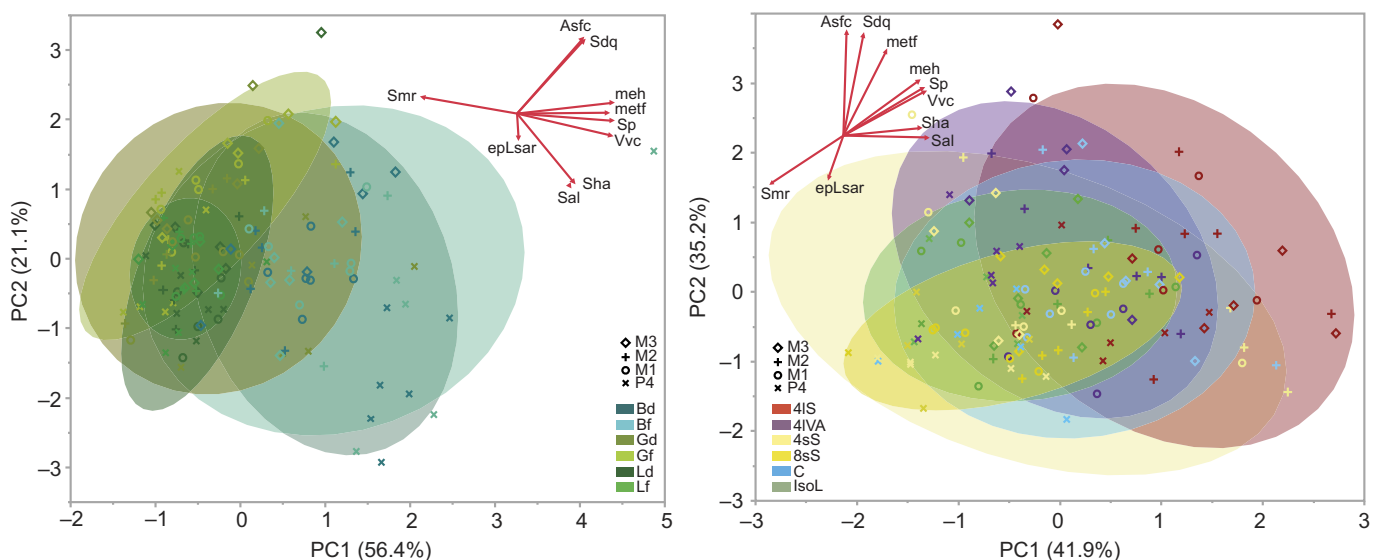


Fig. 2. Principal component analysis for all guinea pigs fed natural (left) and pelleted diets (right). Ellipses account for 90% of variation and depict dietary space. Tooth positions are indicated using different symbols. Note that overlap of dietary space is largest in guinea pigs receiving pelleted diets. DMTA parameters are defined in Table S1.

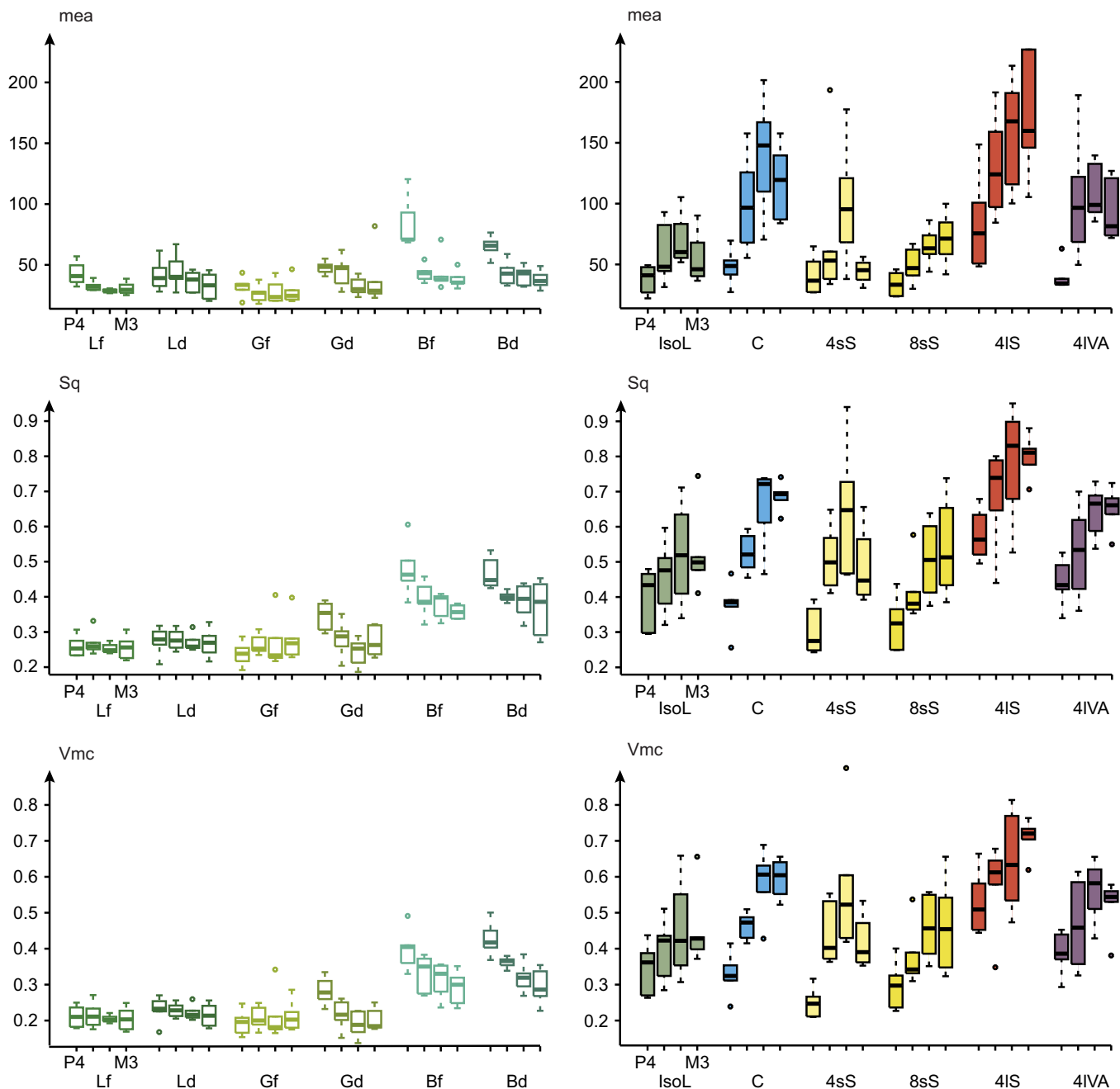


Fig. 3. Representative dental microwear texture analysis (DMTA) parameters from area, height and volume categories. Left, natural plant diets; right, pelleted diets; P4–M3 are shown for each group ($n=6$). mea, mean area; Sq, RMS surface roughness; Vmc, material volume of the core. Note the higher parameter values for pelleted diets compared with plant diets as well as the stronger gradients for all parameters in pelleted diets.

other tooth positions, but for the Bf group more variance was also found (Fig. 3; Table S2; see Fig. S1: doi:10.25592/uhhfdm.9163).

Complexity

Asfc and Sdr (developed interfacial area ratio) increased along the tooth row for the Ld (Lucerne dry), Gf (grass fresh) and Bd groups (see Fig. S1: doi:10.25592/uhhfdm.9163). Guinea pigs on both bamboo diets and Gf showed significantly larger values compared with those fed both lucerne diets for both parameters. nMotif (number of motifs), in contrast, was smaller in both bamboo diet groups than in the lucerne and grass diet groups, and a significant

diet \times tooth interaction was found (Table S2). nMotif also increased from P4 to M3 for the Lf (Lucerne fresh), Gd, Bf and Bd groups, while for the Ld and Gf groups, the parameter values were more variable, and no clear trend was visible. The P4 generally showed significantly lower complexity values compared with those of all molar teeth.

Density

Spd (peak density) and medf (mean density of furrows) increased along the tooth row, regardless of diet. This trend was less expressed in the Ld and Gf groups because of the higher variability especially in M2 and M3 (see Fig. S1: doi:10.25592/uhhfdm.9163). Overall,

both bamboo diet groups showed lower Spd and medf than all other diet groups. For Sal (auto-correlation length), a decreasing trend was seen in Gd and Bd groups; the other diet groups were more variable. Here, a significant diet×tooth interaction was found (Table S2).

Direction

There was no clear pattern in parameters relating to the main texture direction: Str (texture aspect ratio), Sdr and Tr1R–Tr3R (first, second and third direction). Concerning the overall orientation of features, IsT (texture isotropy) decreased along the tooth row in guinea pigs fed the grass and lucerne diets, while it increased for those on the Bd diet. In contrast, epLsar (anisotropy) showed the opposite pattern, with an increase (or no gradient) in guinea pigs fed the lucerne and grass diets, and a decrease in those on the Bd diet. The Bf group showed high variability for P4 and no clear gradient (see Fig. S1: doi:10.25592/uhhfdm.9163). The interaction diet×tooth was significant for Str and IsT (Table S2).

Height

Sixteen out of 18 height parameters [with the exception of madf (maximum depth of furrows) and FLTv (reference to valley flatness deviation)] showed a significant diet×tooth interaction (Table S2). There were two distinct patterns, with the Lf, Ld and Gf diet groups showing no or only a minor gradient between tooth positions, while the Gd, Bf and Bd groups showed a significant decrease in parameter values posteriorly along the tooth row (Fig. 3; see Fig. S1: doi:10.25592/uhhfdm.9163).

Peak sharpness

No significant effect of tooth position was found for Spc (peak sharpness), but the diet×tooth interaction was significant (Table S2). The Lf group had significantly smaller values than the other diet groups. For the Ld, Gf and Bd groups, P4 had smaller values than the other tooth positions, while for the Bf group, P4 was larger (see Fig. S1: doi:10.25592/uhhfdm.9163).

Plateau size

There was a significant diet×tooth interaction for Smc (inverse areal material ratio) and Smr (areal material ratio) (Table S2). The lucerne diet groups showed no gradient along the tooth row, values for the Gf group slightly increased (Smc) or decreased (Smr), while for the bamboo diet groups, P4 had significantly larger (Smc) or smaller values compared with the other molar tooth positions (see Fig. S1: doi:10.25592/uhhfdm.9163).

Slope

The Ld, Gf and Bd groups showed a gradient of increasing Sdq (root mean square gradient) along the tooth row (see Fig. S1: doi:10.25592/uhhfdm.9163). For the Lf, Gd and Bf groups, no clear trend was visible. Generally, P4 showed significantly lower values compared with M1 and M3, except in the Bf group.

Volume

Volume parameters indicated two distinct patterns, with the Lf, Ld and Gf diet groups showing no distinct change along the tooth row, while for the Gd, Bf and Bd groups, values significantly decreased from P4 to M1, and in several instances along the complete tooth row (Fig. 3; see Fig. S1: doi:10.25592/uhhfdm.9163). All volume parameters except for Shv (closed hill volume) and Vvv (void volume of valleys) showed a significant diet×tooth interaction (Table S2).

Pelleted diets

During the experiment, guinea pigs feeding on pelleted diets were not captured on video while feeding. All pelleted (Table 1) diets caused strong gradients in microwear texture along the tooth row (Fig. 1) of either increasing or decreasing parameter values (Fig. 3; see Fig. S1: doi:10.25592/uhhfdm.9163). Therefore, almost no significant diet×tooth interaction was found (Table S3).

Area

Diet and tooth individually had significant effects on all area parameters (Table S3). Parameter values increased along the tooth row, with a less expressed gradient in the 4sS (4% small quartz) and 4IVA (4% large volcanic ash) groups (Fig. 3; see Fig. S1: doi:10.25592/uhhfdm.9163).

Complexity

Diet and tooth individually had significant effects on all complexity parameters (Table S3). Asfc and Sdr increased along the tooth row for all diets. Only for 4IVA did M1 not follow that pattern, showing lower values compared with those for P4, M2 and M3. The IsoL (lucerne meal-based pellet type 1) and 4sS diet groups showed less pronounced gradients (see Fig. S1: doi:10.25592/uhhfdm.9163). For nMotif, there was a significant decrease in parameter values from P4 to M1, and a tendency for decreasing parameter values along the whole tooth row.

Density

All density parameters showed a significant effect of diet and tooth individually (Table S3). Sal increased along the tooth row for all diet groups, but the M3s for the 4sS and 4IVA groups did not follow the general pattern and had lower values compared with the M2s. Spd and medf decreased from P4 to M2, with similar or higher values for M3 compared with M2 (see Fig. S1: doi:10.25592/uhhfdm.9163).

Direction

There was no clear pattern and high variability in parameters relating to the main texture direction (Str, Sdr, Tr1R, Tr2R, Tr3R) and orientation of wear features (IsT, epLsar) (see Fig. S1: doi:10.25592/uhhfdm.9163). Diet had a significant effect on IsT and epLsar, with the 4sS and 8sS (8% small quartz) groups showing significantly higher epLsar than the C (control group, abrasive-free pellet), 4IS (4% large quartz) and 4IVA groups, and 4sS and 8sS groups showing significantly smaller IsT compared with the C and 4IS groups (Table S3).

Height

All height parameters except Sku (kurtosis of the height distribution) displayed a distinct, significant gradient of increasing parameter values along the tooth row for almost all diets (Fig. 3; see Fig. S1: doi:10.25592/uhhfdm.9163). Only for the 4sS group were M1–M3 very similar, while P4 still was distinctly smaller.

Peak sharpness

Peak sharpness (Spc) displayed a significant diet×tooth interaction (Table S3). Values generally increased along the tooth row for the IsoL, 8sS, 4IS and 4IVA groups, slightly increased for the 4sS group, and were similar between tooth positions for the C group (see Fig. S1: doi:10.25592/uhhfdm.9163).

Plateau size

Both plateau size parameters showed a significant effect of diet and tooth individually (Table S3). Smr showed distinct gradients for

decreasing parameter values along the tooth row, while Smc increased (see Fig. S1: doi:10.25592/uhhfdm.9163). For the IsoL and 4sS groups, the gradient was less expressed, but still P4 was significantly different to the other teeth.

Slope

Sdq either increased along the tooth row, or was significantly smaller for P4 than for M2 and M3 (Table S3; see Fig. S1: doi:10.25592/uhhfdm.9163).

Volume

All volume parameters showed a significant effect of diet and tooth individually (Table S3). Volume parameters displayed significant gradients of increasing values along the complete tooth row, or from P4 to M2 (Fig. 3; see Fig. S1: doi:10.25592/uhhfdm.9163). For the IsoL, C, 4sS and 4IVA diet groups, several parameter values were lower for M3 compared with M2, but still higher than for M1.

PCA

Principal components (PC) 1 and 2 accounted for more than 75% of the variation observed for guinea pigs receiving either natural or pelleted diets when all teeth were included in the analysis (Fig. 2). PC1 mainly reflects increasing surface height and volume, while surface complexity increases along PC2. The dietary spaces of guinea pigs receiving natural diets were separated between diets of lower abrasiveness (Lf, Ld, Gf) and diets of higher abrasiveness (Bf, Bd) mainly along PC1, with Gd lying in between. For the Lf, Ld and Gf diet groups, all tooth positions clustered together, while for the Bf and Bd groups, P4 was separated along PC1.

Dietary spaces of guinea pigs receiving pelleted diets hugely overlapped. Within each diet group, a separation of P4/M1 and M2/M3 was observed along PC1, and for the 4IVA group also along PC2.

Randomized tooth sample (simulated fossil assemblage)

For natural diets, correlations between diet group rankings based on observed versus randomly subsampled data were significant on <70% of occasions for all but 16 parameters: Sal, medf, Sda, mea (mean area), nMotif, S5v (five-point valley height), S10z (ten-point height), Sq (standard deviation of the height distribution), Sv (maximum pit height), Sxp (peak extreme height difference), Sz (maximum height), meh (mean height), FLTq (root mean square flatness deviation), Sdv (closed dale volume), Vmc (material volume of the core) and Vvv (Table S4). This result was consistent for both 25% and 75% subsets. The best correlation between diet and DMTA parameter was found for Sdv (mean $R_s > 0.91$), followed by S10z (mean $R_s > 0.89$) and FLTq (mean $R_s > 0.88$). For pelleted diets, only mea (mean $R_s > 0.85$) and Sdv (mean $R_s > 0.84$) resulted in significant correlations between observed and randomized subsets with >70% of correctly ranked diets for draws comprising either 25% or 75% of all teeth (Table S5).

DISCUSSION

DMTA showed that enamel surface roughness of guinea pigs feeding on pelleted diets generally exceeded that of animals on natural plant diets. These guinea pigs showed larger wear features (dales, furrows, plateaus) as compared with those feeding on plant diets. The least abrasion was observed in both lucerne diet groups (Lf, Ld) and the fresh grass group (Gf), while the dry grass group (Gd) and both bamboo groups (Bf, Bd) displayed DMT patterns with overall greater roughness, indicating that these diets were more abrasive (Winkler et al., 2019b). Amongst the pelleted diets, those

containing 4% fine sand-sized quartz (4IS) and volcanic ash (4IVA) stood out with the greatest height (for 4IS) and complexity (for 4IVA) values. Large mineral abrasives have been found to result in rougher surfaces, signified by larger height and volume parameters, while smaller mineral abrasives display a surface polishing effect (Winkler et al., 2020a). Furthermore, large angular mineral particles resulted in more complex DMT patterns (Winkler et al., 2020a). For a more detailed discussion of the implications of these results, see Winkler et al. (2020a). In the following discussion, we will focus solely on the different dental wear gradients observed between pelleted and natural diets; the details of how material properties of plants or mineral abrasives affect DMT formation are beyond the scope of the present study and are discussed in Winkler et al. (2020a).

Different chewing behaviour on pellets and plants

We observed a strong gradient of increasing surface roughness along the maxillary tooth row in the guinea pigs fed pelleted diets (Fig. 1, Fig. 3). The gradient was similar regardless of whether guinea pigs received an abrasive-free pelleted diet or a pelleted diet containing different concentrations and sizes of external mineral abrasives. For individuals receiving 4% or 8% fine silt-sized quartz abrasives (mean grain size 4.4 μm , 4sS and 8sS) concentration or 4% volcanic ash (mean grain size 96 μm , 4IVA), the gradient was often restricted to P4–M2, with M3 showing values closer to M1. This increased roughness was most pronounced in area, height and volume parameters, indicating larger wear features on the posterior tooth positions (Fig. 3). In earlier studies, we found that in ungulates from the wild (Schulz et al., 2013a) as well as guinea pigs feeding on natural diets (Winkler et al., 2019b), large area, height and volume parameter values were characteristic of diets of high abrasiveness. However, because of the experimental setting in our current study, the increasing parameter values along the tooth row in guinea pigs fed five of the six pelleted diets indicate that in addition to dietary abrasiveness, other masticatory parameters (i.e. bite force, distribution of food within the oral cavity, mechanical food properties) influenced the wear pattern. This pattern of increasing surface roughness along the tooth row was consistent, which is also evident from the mostly not significant diet \times tooth interaction for pelleted diets (Table S3).

Surprisingly, the guinea pigs feeding on natural plant diets not only showed a less pronounced wear gradient but also often showed an opposite trend to the guinea pigs receiving pelleted diets (Fig. 1; Fig. S3). While the low abrasive lucerne diets (Lf, Ld) and fresh grass diet (Gf) showed almost no differences in DMTA between tooth positions, the more abrasive dry grass diet (Gd) and bamboo diets (Bf, Bd) exhibited distinct decreases in surface roughness along the tooth row. Therefore, chewing behaviour of guinea pigs feeding on pelleted diets and guinea pigs feeding on plant diets seems to be different, which is probably the result of differences in food ingestion and distribution within the oral cavity.

Guinea pigs have a grinding dentition with serial enamel ridges forming an occlusal surface of rasp-facets (Berkovitz and Shellis, 2018), that is adapted to feeding on grasses, which consist of slender leaves with blades and stems. Cylindrical compressed pelleted diets have a very different geometry from leafy grass blades, and we propose that pelleted diets require being fractured by crushing rather than reduced by grinding. Typically, crushing dentitions differ in morphology from grinding dentitions. Teeth adapted for crushing typically are more pestle and mortar shaped (Ungar, 2015), bunodont and covered by a thick enamel cap. The thin enamel ridges and narrow teeth of guinea pigs are not well suited for

crushing and might even be susceptible to damage if used in this manner. Cox et al. (2012) calculated that bite forces increase along the tooth row in guinea pigs, with forces being 1.8× higher on the M3 as compared with the P4. In combination with an unusual chewing mode, the processing of pelleted diets is apparently prone to wear posterior teeth more in comparison to anterior teeth. In contrast to natural diets, pellets also seem not to be reduced initially by gnawing using the incisors. When feeding guinea pigs with pellets and pellet-sized pieces of carrot, Byrd (1981) only recorded gnawing cycles for carrot feeding. He further recorded longer activity of the deep masseter, superficial masseter and medial pterygoid when guinea pigs chewed pellets versus carrots. These muscles are most important during mandibular elevation, which indicates that pellets were chewed with greater force. We therefore hypothesize that pelleted diets are processed by loading all teeth simultaneously with a new ‘haul’ of food (implying that with increasing chewing force, posterior teeth experience higher loads and thus more wear). Alternatively, pelleted diets are processed to a higher degree by the rear dentition, in order to exert higher forces for easier fracture of the diet. Both scenarios would explain how higher forces at the rear of the dentition result in higher abrasion, expressed in higher surface roughness.

When feeding on plants, guinea pigs continuously ingest leaves in a ‘conveyer belt’ feeding strategy, where material is gnawed with the incisors and pushed backwards to the cheek teeth (Movie 1). The plant matter is successively reduced in size while it is shifted further back into the oral cavity. Therefore, less actual processing of plant matter occurs on the posterior part of the dentition than at the front, as material arriving at M3 has already been comminuted to some extent by P3–M2 and, hence, despite the different forces, the M3 is more likely to meet comminuted material. We also suggest that the already size-reduced plant parts are more easily immersed in saliva, which additionally softens and hydrates them. The processed plant matter thus probably quickly becomes softer and less abrasive. Most abrasion by ingested plant matter can therefore be expected at the anterior part of the dentition, when leaves are still larger and drier. This would explain why we observed decreasing surface roughness along the tooth row for the more abrasive plant feeds (Fig. 3; Fig. S1).

In contrast, pellets are not easily drenched in saliva; they need longer to fracture in small parts that can be soaked and become softer. This results in pelleted diets being more abrasive and leading to more tooth wear, which in combination with possibly higher bite forces leads to increased wear at the rear of the dentition. We therefore partly accept hypotheses 1 and 2, but note that guinea pigs fed plant diets not only show a less expressed wear gradient along the dentition but also have a different wear pattern from those fed pelleted diets, which is probably attributed to different chewing behaviour on pelleted and natural plant feeds.

Randomized tooth sample

Analysis of the randomized tooth sample (simulating a fossil assemblage) indicated that with more expressed DMT gradients along the tooth row, and with a less distinct DMTA dichotomy in the possible diets, ranking of diets using random tooth positions becomes less correct. For pelleted diets, only Sdv and mea had a high frequency (>70%) of correct diet rankings regardless of tooth composition of the sample (Table S5). Guinea pigs feeding on natural plant diets, in contrast, had a less pronounced gradient along the tooth row for the low-abrasion diets (Lf, Ld, Gf), and only a distinct gradient for the high-abrasion diets (Gd, Bf, Bd) (Fig. 3; see Fig. S1: doi:10.25592/uhhfdm.9163), and generally showed a

dichotomy of the three low-abrasion and three high-abrasion diets irrespective of tooth position. This led to a greater degree of variation across diets, which accounts for the more significant diet rankings overall (Table S4), and to a higher number of DMTA parameters (16 out of 46) that discriminated well between diet extremes in the randomized tooth sample (Sal, medf, Sda, mea, nMotif, S5v, S10z, Sq, Sv, Sxp, Sz, meh, FLTq, Sdv, Vmc, Vvv). Most DMTA measures significantly reflect diet rankings, but the postulated differences in chewing behaviour mean that signals were not consistent for all teeth, or at least signals for each tooth differed in intensity.

In general, these results imply that differences in most DMT variables across individuals are strongly affected by the specific teeth (representing different tooth positions) in the sample, and that ‘lumping’ approaches should consequently proceed with caution. Therefore, we note that accounting for tooth position is necessary when evaluating DMT within and across samples, either by keeping specific teeth separate or by including this as a factor in statistical analyses. That said, DMT parameters such as Sdv and mea appear to retain good resolution of diet-related wear differences across the different natural and pelleted diets and could still be used as focal measures for samples that, by necessity, comprise teeth from multiple positions. Overall, height, volume, area and density parameters performed best for dietary classification for all diets tested in this study, which suggests that these are the most stable parameters that most reliably record dietary differences.

Conclusions

Guinea pigs fed with natural plant foods show different DMT gradients along the tooth row to guinea pigs fed with pelleted diets. We attribute this observation to combined effects of different ingestion and food processing behaviour together with bite force gradients along the tooth row. While plant diets are continuously comminuted, employing the whole length of the dentition, pelleted diets are probably crushed using the posterior molars, where highest bite forces are exerted. The guinea pig dentition is not adapted for such chewing behaviour, which probably results in more dental wear observed on posterior molars when chewing pellets. Wear gradients can therefore affect diet reconstruction based on DMTA when only isolated teeth are available. Even though correct diet ranking in a randomized tooth sample was possible for natural plant diets, we advise only comparing teeth from the same positions, if possible. In diets of naturally low inherent abrasiveness (browse), and without contamination of external mineral abrasives, inter-tooth differences might be negligible. Otherwise, the parameters Sdv and mea displayed the best dietary discrimination and could be employed for reliable dietary discrimination in samples that are composed of teeth from different positions, for example in fossil assemblages.

Acknowledgements

We thank Anja Tschudin for support with pelleted diet formulation, and Louise Martin, Nicole Schmid, Sandra Heldstab, Kathrin Zbinden, Katrin Weber and Annelies De Cuyper for support with animal husbandry.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: D.E.W., M.C., D.C., T.T.; Methodology: D.E.W., M.C., M.R.; Validation: D.E.W.; Formal analysis: D.E.W., D.C.; Investigation: D.E.W., M.R.; Resources: T.M.K.; Writing - original draft: D.E.W., M.C.; Writing - review & editing: M.C., E.S., D.C., T.M.K., T.T.; Visualization: D.E.W.; Project administration: T.T.; Funding acquisition: T.T.

Funding

This project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement 681450) (ERC Consolidator Grant Agreement to T.T.).

Data availability

All original, unfiltered surface texture scans used in this study are stored at the database of the public natural history museum (University of Hamburg, Center of Natural History Hamburg, mammal collection); these and Fig. S1 can be accessed via the open-source data repository of the University of Hamburg: doi:10.25592/uhhfdm.9163.

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