



Research paper

Impact of wear and diet on molar row geometry and topography in the house mouse

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ARTICLE INFO

Keywords:

Geometric morphometrics
Dental complexity
Food consistency
Mus musculus

ABSTRACT

Objectives: Dental evolution affects the geometry of the tooth, but the adaptive relevance of these changes is related to tooth sharpness, complexity, and relief (topography). On a set of laboratory mice, we assessed how wear related to age and food consistency affected molar geometry and topography.

Design: Three groups of laboratory inbred mice (C57BL/6J strain) were considered: Four week old mice close to weaning, six month old mice fed on regular rodent pellets, and six month old mice fed on rodent pellets that were powdered and served as jelly. Their upper and lower molar rows were imaged in 3D. The geometry of the surfaces was quantified using a template describing the whole surface of the rows. Topographic indices were estimated on the same surfaces.

Results: The geometry of the molar rows was heavily affected by age-related wear. Food consistency affected mostly the upper molar row, which was more worn and less helical in soft food eaters. Tooth sharpness and relief decreased with age-related wear. Tooth relief was lower in soft food eaters, but only on the upper molar row. Tooth complexity was insensitive to wear.

Conclusion: The primary factor affecting tooth geometry and topography is age-related wear, as wear erodes the molar surfaces. Tooth complexity, however, appears to be insensitive to wear, making this index relevant for comparison of tooth morphology among wild mice of unknown age. Soft food eaters displayed more worn teeth, with less helical molar row occlusal surface, possibly because behavior and jaw morphology were disturbed due to this unusual food resource.

1. Introduction

Efficient food processing is of prime importance for the fitness of an individual and, among the components constituting the feeding apparatus, the dentition plays a key role in achieving the comminution of food particles. Adaptive diversification of tooth geometry into complex multicuspid shapes is thought to be one of the key innovations that led to the mammalian radiation (Wilson et al., 2012). However, different tooth geometry can have similar functional performance (Wainwright, Alfaro, Bolnic, & Hulsey, 2005), especially related to the complexity of the tooth crown (Wilson et al., 2012). Nowadays, geometric morphometrics (Adams, Rohlf, & Slice, 2013) allows the quantification, in 2D or in 3D, of the tooth geometry, providing an insight into its diversification. Additionally, proxies of functional significance, such as tooth relief or complexity, have been developed from 3D data that describe tooth surface (Boyer, 2008; Bunn et al., 2011; Evans, Wilson, Fortelius, & Jernvall, 2007; Santana, Strait, & Dumont, 2011). Tooth complexity appears to be adapted to the mechanical properties of the

food preferentially consumed by a species (Boyer, 2008; Santana et al., 2011). Thus, tooth complexity estimates may provide hints about the diet of a species (Boyer, Evans, & Jernvall, 2010). However, these two types of information – geometry and complexity of the tooth – are rarely considered together, despite the fact that they may shed light on the adaptive role of shape differences and how they evolved.

Rodents, and specifically murine rodents (Old World rats and mice) constitute a highly variable group. A part of this success is related to the diversification of their dentition (Misonne, 1969) and how occlusion is achieved (Lazzari, Tafforeau, Aguilar, & Michaux, 2008), which allows them to exploit very different food resources. Diversification of the dentition can even occur within species at the micro-evolutionary level, as has been observed in the house mouse *Mus musculus domesticus* (Ledevin et al., 2016; Renaud, Dufour, Hardouin, Ledevin, & Auffray, 2015). It is tempting to investigate the functional role of such shape differences to provide insight into both their potential adaptive role and how microevolution can shape this intraspecific variation. However, at the scale of population-level variation, the changes caused by wear on

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tooth geometry may be of the same order of magnitude as, or even be the first order signal overwhelming, any other evolutionary differences (Ledevin et al., 2016). Thus, a prerequisite for applying functional proxies to the mouse tooth would be to better understand the effect of wear on these estimates and on the tooth geometry. However, such studies are primarily conducted in primates (Dennis, Ungar, Teaford, & Glander, 2004; Pampush, Spradley et al., 2016). Furthermore, most studies regard interspecific differences (Godfrey, Winchester, King, Boyer, & Jernvall, 2012; Winchester et al., 2014), rather than addressing whether intraspecific tooth shape variation may be of functional significance in microevolutionary processes.

Thus, we propose a pilot study on the house mouse to assess the effect of wear on tooth geometry and functionality in controlled laboratory conditions. Differences in wear stage related to age were investigated by comparing young and old mice. The potential for specific food material properties to cause differential wear was assessed by comparing mice reared on diets of different consistencies. For these three groups of mice, the geometry of the upper and lower molar rows was quantified using 3D geometric morphometrics (Ledevin et al., 2016). On a larger sample of the same groups, the shape of the first upper molar (UM1) was quantified using an outline analysis (Renaud, Auffray, & Michaux, 2006). Furthermore, the topographic characteristics of the molar rows, presumed to be better estimates of functional performance than shape (Godfrey et al., 2012; Ungar, 2004; Winchester et al., 2014), were estimated on the same molar rows. This allowed us to address the following questions. (1) What is the relative impact of age and food treatment on wear as traced by tooth geometry? (2) Do these geometric changes correspond to differences in tooth topography? (3) What are the perspectives of applications to trace dietary differences in natural populations?

2. Material and methods

2.1. Samples

Female mice from the inbred strain C57BL/6J were ordered from the Charles River Laboratory (Lyon, France). They were three weeks of age when obtained. A cohort of eight mice was sacrificed at four weeks (young group, Y-B6). The other individuals were reared at the PBES (Ecole Normale Supérieure de Lyon, France) until the age of six months. Half were fed a standard hard pellet diet (hard food group, HF). For the other half of the mice, the same pellets were ground to powder and mixed with agar-agar. This mixture was hydrated when given to the

mice so that the consistency would correspond to a jelly (soft food group, SF). This resulted in 19 HF and 20 SF.

The mice were sacrificed according to the directive 2010/63/UE of the European Parliament on the protection of animals used for scientific purposes. Breeding conditions in the PBES have the agreement B 69 123 0303–17/02/2009 of the French Ministère de l'Agriculture.

2.2. 2D tooth shape

The first upper molar (UM1) was pictured in 2D, with the occlusal surface manually oriented to the horizontal plane. Then, the outline of the occlusal surface was manually delineated using 64 points at curvilinear equidistance along the outline. This series of points was analysed using a Fourier analysis, which decomposes the outline into successive harmonics that describe the outline in increasing details, after an initial standardisation of the starting point along the long axis of the outline (Renaud et al., 2006; Renaud, Pantalacci, & Auffray, 2011). Each harmonic was described by two Fourier coefficients (FCs). Retaining seven harmonics resulted in an acceptable compromise between information content added by a harmonic and measurement error on this harmonic (Renaud et al., 2011). FCs were standardised by the size of the outline ('zeroth' harmonic), retaining shape information only. The set of 14 FCs (seven harmonics per two coefficients) constituted the set of 2D shape variables to be considered in subsequent multivariate analyses.

2.3. 3D characterization of the upper and lower molar rows

For a subset of mice (three Y-B6, four HF, and four SF), skulls were scanned at a cubic voxel resolution of 20 μm using a microtomograph (μCT) Locus GE at Voxcan (Marcy l'Etoile, France).

The right upper molar row (UMR) was delimited on each slice using a threshold method in Avizo (v. 7.1–Visualization Science Group, FEI Company). Connections with bone were manually closed and the surface generated. Mouse molars are composed of transverse enamel ridges, the cusps of which align to form longitudinal rows that direct the propalinal (antero-posterior) movement during chewing. The upper molars show central, labial, and lingual rows and the lower molars labial and lingual rows (Fig. 1). Describing this complex geometry is challenging because it appears difficult to locate reliable landmarks on such molars and to delineate curves along the ridges (Skinner & Gunz, 2010). Thus, we developed a method of shape description that entirely relies on the description of the tooth surface. On the first mouse of the

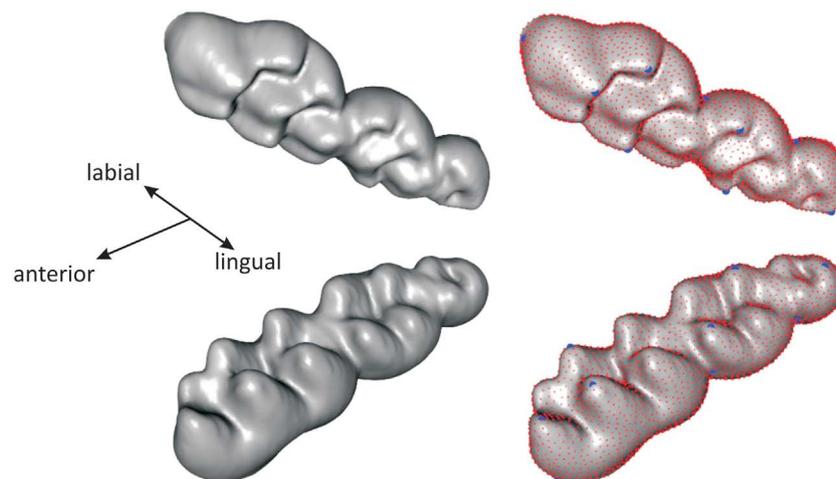


Fig. 1. Upper and lower molar rows of a young C57BL/6J mouse (specimen Y-01) and the associated template with landmarks serving for the initial superimposition of the different specimens. Fourteen fixed landmarks (blue dots) were positioned on each upper molar rows as guide during the registration process of the template, which was composed of 2194 sliding semi-landmarks (red dots) that were analysed using a Procrustes procedure. A similar method was used for the description of the lower molar row, with 14 fixed landmarks and 2213 semi-landmarks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

young group, a template was designed to describe the entire erupted crown. This template was defined by 2194 equally-spaced sliding semi-landmarks anchored by 14 fixed landmarks (Fig. 1). These fixed landmarks were defined on all specimens and were only used as priors to guide the registration process. The template was deformed in order to match the original surface of each tooth row (Ledevin et al., 2016). An iterative procedure was used to slide points along tangent planes according to the minimum bending energy criterion until convergence (Gunz, Mitteroecker, & Bookstein, 2005). Then the resulting sliding semi-landmarks were adjusted for scaling, translation, and rotation according to a Procrustes superimposition. These procedures were performed using the packages 'Morpho' (Schlager, 2014a) and 'mesheR' (Schlager, 2015). Procrustes coordinates, i.e. coordinates of the sliding semi-landmarks after Procrustes superimposition, constituted the shape variables describing the shape of the tooth row.

The same procedure was applied to the lower molar row (LMR), with a template of 2213 sliding semi-landmarks and 14 fixed landmarks (Fig. 1).

2.4. Multivariate analyses

The shape variables were first reduced using a principal component analysis (PCA). It was performed on the variance-covariance matrix of the FCs for the 2D analysis and on the variance-covariance matrix of the Procrustes coordinates for the 3D analyses. PCAs allow for a reduction of dimensionality (Sheets et al., 2006), by retaining for subsequent statistical analyses only the axes explaining most of the total observed variance.

2.5. Topographic analysis of the molar rows

Three parameters were used to characterize the topographic properties of the molar rows. Dirichlet Normal Energy (DNE) assesses tooth sharpness by measuring curvature and undulation of the surface (Bunn et al., 2011). Orientation Patch Count (OPC) estimates the number of separately oriented facets on a tooth surface (Evans et al., 2007) and is considered a proxy for dental complexity (Santana et al., 2011). It is measured by dividing a tooth surface into contiguous patches that share an orientation and then summing the number of such patches. Because the count may vary depending on the orientation of the occlusal surface, we used a variant of the method [Orientation Patch Count Rotated (OPCR)] (Evans & Jernvall, 2009). It is measured by starting from the initial orientation, rotating the surface by 5.625° a total of eight times around the Z-axis, and averaging the resulting OPC estimates to provide the OPCR value. The third parameter, Relief Index (RFI), corresponds to the log ratio between the surface area of a tooth's crown (Area3D) and the area of the tooth's planometric footprint (Area2D) (Boyer, 2008).

The three parameters were computed from the templates of the upper and lower molar rows used for the geometric morphometric assessment of their shape using the R package molaR (Pampush, Winchester et al., 2016). The templates were used to discard the root portions of the original surface that would otherwise cause errors during computation of the complexity parameters.

The surface of each dental row was manually oriented using MeshTools (Lebrun, 2014) so that the occlusal surface would be aligned with the X and Y planes, lying face-up, and oriented orthogonal to the Z-axis.

The resulting surfaces were simplified to 10,000 polygonal faces (Pampush, Winchester et al., 2016) using the R package Rvcg (Schlager, 2014b). The resulting surfaces were used for estimations of DNE, OPCR, and RFI. To evaluate the sensitivity of the parameters to the initial manual orientation of the occlusal surface, the sets of upper and lower molar rows were oriented three times, twice by one of the authors (SR) and one time by the other (RL).

To circumvent the error in the estimation of the complexity

parameters related to inconsistencies in the manual orientation of the molar rows, a four-step semi-automatic procedure was developed. (1) A surface of reference was randomly chosen and manually aligned in space using the Meshtools software, as was done for the manually-oriented datasets. (2) The auto3dgm R package (Boyer et al., 2015) was used to automatically align all remaining dental surfaces and the reference surface using a Procrustes superimposition. (3) All aligned surfaces were loaded into Avizo (v 9.1) and the difference in position between the reference surface before and after the Procrustes superimposition was calculated. The same transformation was applied to the remaining dental row surfaces with the goal to orient them exactly as the reference surface, with the occlusal surface facing up, while retaining the Procrustes superimposition. (4) These surfaces were analysed in the same manner as the manually-oriented ones (i.e. being simplified to 10,000 polygonal faces before the computation of DNE, OPCR and RFI). Using this procedure provides consistent estimates of the topographic parameters because all surfaces are oriented in the same way, even though the reference surface must still be manually oriented.

2.6. Statistical analyses

Given that this research incorporates inbred laboratory mice of controlled age, intra-group variance was expected to be reduced compared to inter-group differences. Consequently, significant differences might be expected despite the small sample sizes of the 3D subsamples. Differences in topographic parameters were investigated using *t*-tests, that perform well even with very small sample sizes (de Winter, 2013).

Shape differences between groups were tested using multivariate analyses of variance (MANOVA) and an associated pairwise Hotelling's *T*-squared test. Variables were the first PCs calculated from (1) the set of Fourier coefficients for the 2D outline analysis of the first upper molar, and (2) the Procrustes coordinates for the 3D analysis of the upper and lower molar rows.

General linear models were used to assess the percentage of variance and the associated probability related to the operator (SR vs. RL collecting data), age (young vs old mice) and food nested within age (until weaning, hard food, soft food) on the resulting parameters (DNE, OPC, OPCR, and RFI).

The relationships among different parameters were investigated using linear regressions, providing the R^2 value and the associated probability.

Multivariate analyses were performed using the R package ade4 (Dray & Dufour, 2007). Statistics were performed using Past (Hammer, Harper, & Ryan, 2001) and the R package fmanova (Langsrud & Mevik, 2012).

3. Results

3.1. Upper and lower molar rows: 3D characterization

Upper molar rows of young mice strongly differed from those of mice aged six months (Fig. 2A). This constituted the first order signal along the first axis of the PCA of the 3D Procrustes coordinates (71.8% of total variance). The PC1 axis corresponds to progressive abrasion of the teeth (Figs. 2 C, 3), which is more pronounced at the distal end of the molar row. SF mice are extreme along PC1, having more worn teeth. HF and SF tooth rows are also segregated along PC2. From negative (SF-like values) to positive (HF-like values), PC2 corresponds to a more helical molar row, with the third molar more worn on the labial side and the first molar more worn on the lingual side.

The increased abrasion of the old mice is obvious when comparing mean shapes (Fig. 3). The difference between HF and SF molar rows is subtler. SF teeth seem to be slightly more worn than HF, but more importantly, the wear is not balanced in the same way between the

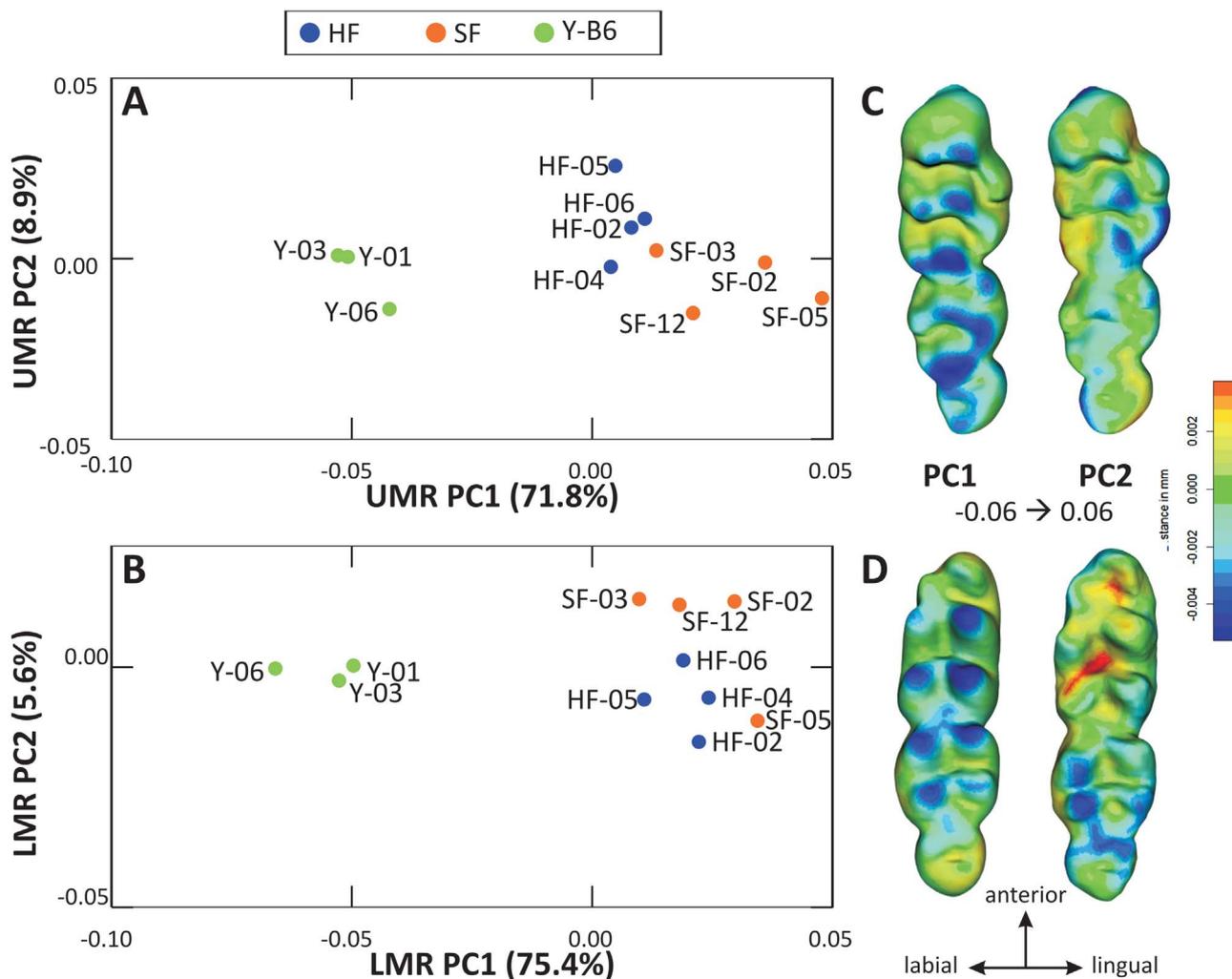


Fig. 2. Three-dimensional geometric morphometric description of the upper (A) and lower (B) molar rows. The axes correspond to the first and second axes of a PCA on the shape variables (Procrustes coordinates of the 3D semi-landmarks composing the template). (C, D) Reconstruction of the shape changes along the axes, from scores = -0.06 to scores = +0.06. In red extension, in blue contraction. (C) Upper molar row. (D) Lower molar row. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

lingual and labial side (Fig. 3). This should result in SF mice having more planar occlusal surface than HF mice, which display helical ones.

A similar pattern was found for the lower molar row (Fig. 2B), with PC1 explaining a similar amount of variance (75.4%) as in the analysis of the upper molar row. PC1 describes variation corresponding to abrasion of the molar row, especially of the second molar and the rear of the first molar (Fig. 2D). SF mice are scattered around HF mice, being more extreme along PC1 (for specimen SF-05) and PC2. As for the upper molars, the second axis corresponds to a difference in abrasion between the lingual and labial side. HF individuals are generally characterized by negative PC2 values and SF by positive ones, a pattern resulting from an increased abrasion on the labial side at the rear of the molar row. The high abrasion of the second molar may be due to the fact that the lower molar row is slightly arch-shaped, leading to a more pronounced contact during occlusion at the level of the second lower molar than at the level of the first or third molars.

The PCA of the upper molar row (UMR) Procrustes coordinates resulted in three axes that explain more than 5% of the total variance (71.8%, 8.9%, 5.6% respectively). Differences between groups were significant (MANOVA: $P = 0.0010$). HF and SF molar rows were significantly different from the young group (HF: $P = 0.002$; SF: $P = 0.011$). HF and SF were not significantly different ($P = 0.155$). The non-significance of this test may be related to the very small sample

size. To increase the power of the tests, the number of variables was reduced to the first two axes. The between-group differences were still significantly different (MANOVA: $P = 0.0004$). The young and old mice were better differentiated (HF: $P = 0.005$; SF: $P = 0.002$) and the difference between HF and SF was close to the significance threshold ($P = 0.055$).

The shape of the lower molar row (LMR) could be summarized by two axes explaining > 5% of the total variance (75.4%, 5.6% respectively). The difference among groups was significant (MANOVA: $P = 0.0008$), specifically that of the young group and the others (Y-B6 vs. HF: $P = 0.001$; Y-B6 vs. SF: $P = 0.001$). HF and SF were not significantly different from one another ($P = 0.089$).

The first axis of each analysis, which summarizes the geometric variation of the UMR and LMR respectively, appear to be highly related ($R^2 = 0.888$, $P < 0.0001$).

3.2. First upper molar: quantification of the 2D outline

Young mice differed from old ones, as in 3D analysis of the molar tooth row, but the difference between young and old mice was less marked (Fig. 4). As in 3D, HF mice were intermediate between Y-B6 and SF along the first axis (57.0% of total variance). Differences between mean outlines of the three groups were subtle. The anterior

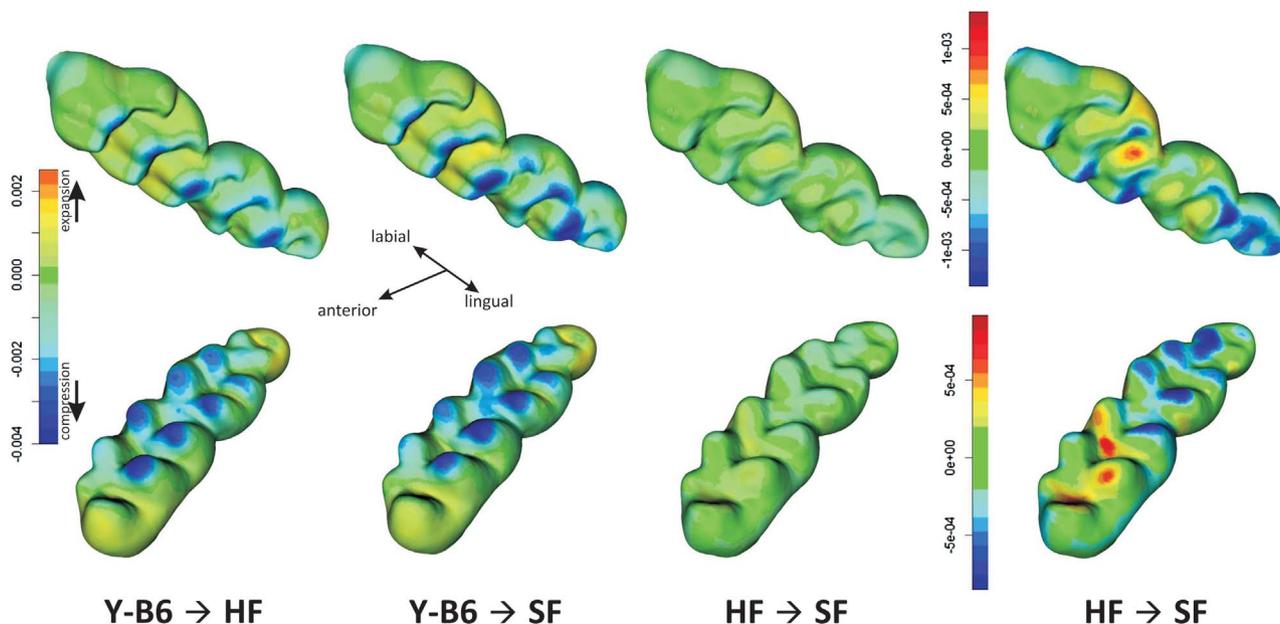


Fig. 3. Visualization of the 3D shape changes between group means. The deformation of the template between the average shape of young mice (Y-B6) vs. mice fed hard food (HF) and soft food (SF) is represented. Left, all deformations to the same scale. Right: local scale emphasizing minor differences between hard and soft food eaters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

part was not affected, but the posterior part was slenderer in young than in old mice, and in HF than in SF mice, although this difference was minor.

Four axes explained > 5% of variance (57.0%, 16.4%, 6.3%, 5.7%). On these four axes, all groups were significantly different from each other (MANOVA and associated pairwise tests: P = 0.0001).

3.3. Molar row topography

3.3.1. Measurement error related to manual orientation

General linear models were used to investigate the relative importance of the manual alignment (operator) of the occlusal surface, age, and food on the complexity parameters of the molar rows. The operator effect was never found significant (Table 1), no constant shift

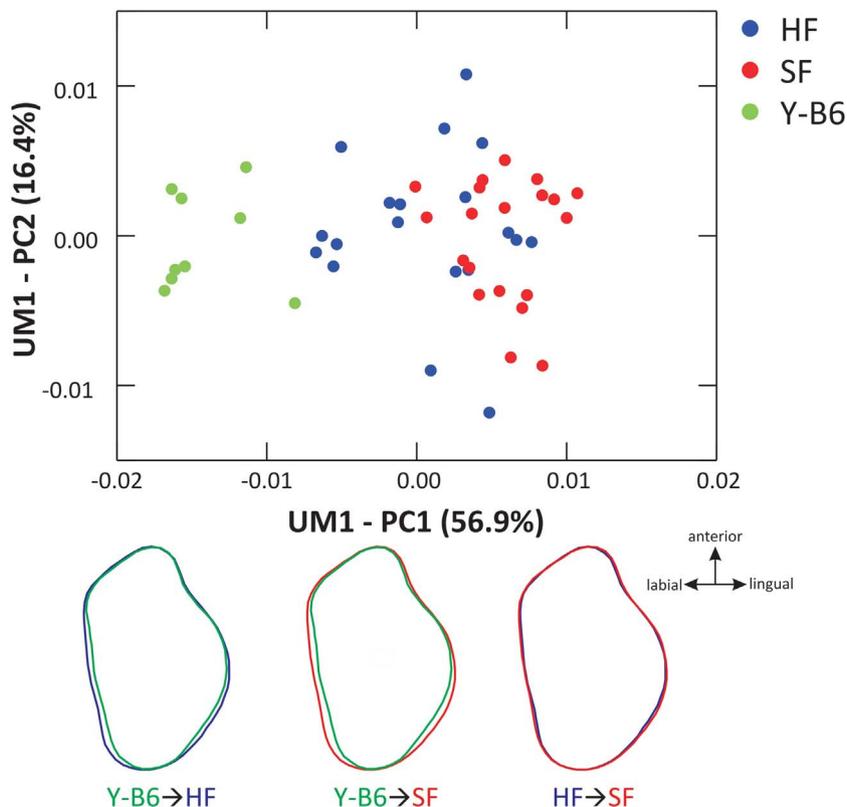


Fig. 4. Morphospace of the first upper molar, described in 2D. The axes correspond to the first and second axes of a PCA of the Fourier coefficients describing the 2D outline of the tooth. More specimens are included in this 2D analysis than in the 3D analysis. Below, visualisation of the shape changes between the mean of the three groups: young mice (Y-B6), and mice of six months fed hard food (HF) and soft food (SF). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Effect of the error related to orientation of the molar row (operator), of the age (young vs old) and food nested within age (young, hard food and soft food), for the topographic descriptors of the upper (UMR) and lower (LMR) molar tooth rows. The % of variance related to each effect has been estimated, as well as the significance of this effect. In bold: $P < 0.05$.

		% variance			Probability		
		Operator	Age	Age/Food	Operator	Age	Age/Food
UMR	DNE	0.02	53.8	0.01	0.993	< 0.001	0.936
	OPCR	0.6	45.3	0.5	0.852	< 0.001	0.622
	RFI	1.6	36.6	20.4	0.579	< 0.001	0.001
LMR	DNE	0.00	83.8	1.5	1.000	< 0.001	0.107
	OPCR	3.4	2.2	10.8	0.577	0.400	0.068
	RFI	1.0	81.8	1.9	0.397	< 0.001	0.074

having been observed between operators (See Supp. Fig. S1 in the online version at DOI: <http://dx.doi.org/10.1016/j.archoralbio.2017.04.028>). Operator effect is almost absent for DNE. An important variation related to the manual alignment affected OPC, which the OPCR procedure reduced, but did not eliminate entirely. Among-operator variation was more important for the lower than for the upper molar rows. This is possibly due to the arched, helical shape of the lower occlusal surface, making its consistent orientation across specimens difficult. RFI was consistently estimated among operators, except for a few molar rows, for which some estimates strongly departed from the others (Supp. Fig. S1).

Age significantly influences all complexity parameters, except for lower molar OPCR. Food is never a significant factor, except for upper molar RFI.

3.3.2. Complexity parameters on the semi-automatically adjusted molar rows

DNE measurements of the upper and lower molar rows were in similar ranges (Fig. 5), meaning that cusps were equally sharp on both molar rows. Young animals displayed molar rows with higher DNE (Table 2). One mouse (Y-03) displayed an extremely high upper molar DNE. Food consistency had no impact on this estimate.

OPCR was higher for the upper than for the lower molar row, corresponding to a higher geometric complexity of the upper molar row: the first upper molar has three longitudinal rows of cusps instead of two, as in the lower molar (Fig. 1). Neither age nor food consistency had a consistent effect on OPCR (Table 2). Two young animals and one SF (SF-05) displayed extremely high OPCR values for either the lower molar row or both rows (Fig. 5) but the other mice appear to share similar OPCR values (Fig. 6).

RFI was higher for the lower than for the upper molar rows, due to a greater 2D area for the upper molar row but a greater 3D area for the lower molar row. RFI of both upper and lower molar rows were affected by age, leading to a decrease in RFI (Fig. 5; Table 2): abrasion decreased the 3D area of the tooth without greatly affecting the 2D area. RFI was affected by food consistency, but only for the upper molar row (Table 2).

The same trends emerge when comparing the results obtained using the manually oriented surfaces (Table 1) and the semi-automated procedure (Table 2). The sole parameter sensitive to food consistency was upper molars RFI. However, the semi-automated procedure failed to detect an effect of wear on the upper molar OPCR. Furthermore, the effect of age on DNE was only marginally significant using the semi-automated procedure (Table 2). This may be due to the combination of a very small group sample size and the occurrence of an outlier in the young group.

Comparing the upper and lower molar rows, DNE and RFI were both positively and significantly related (Table 3; Fig. 5). OPCR of the upper

and lower molar rows were not significantly related. The different response of RFI to food consistency in the upper and lower molar rows led to a significant regression, but with a clear shift between HF and SF mice around the general trend (Fig. 5).

3.3.3. Shape and topography of the molar rows

The geometry of each molar row is approximated by the scores of the individuals along the first axis of each PCA, as this axis summarizes most of the variance. This estimate of the molar row geometry was compared to the calculated estimates of tooth complexity (Table 3).

OPCR was not related to shape for either the upper or the lower molar rows. In contrast, DNE and RFI were correlated to the molar row geometry, although more strongly for the lower than for the upper molar row. RFI was the parameter most strongly related to shape. The weak relationship between shape and DNE for the upper molar row may be related to Y-03 being an outlier for DNE but not in shape (Fig. 2). Discarding this specimen from the regression increased the R^2 value and the probability ($R^2 = 0.720$; $P = 0.002$). This change in model statistics following omission of this specimen indicates that the presence of sharp, unused cusp morphology does impact DNE estimates, although the geometric effect is minor.

4. Discussion

4.1. Geometric signature of wear on the molar rows

This study demonstrates the impact of wear on the cusp geometry of the two occluding molar rows. The effect related to age is the primary source of variation, corresponding to the abrasion of the cusps with increasing wear. We also observed a weak effect of diet on the cusp geometry of the upper molar row. Mice fed soft food display teeth that are worn more and differently from hard food eaters. Despite the limited sample size for the 3D analysis, this result is supported by complementary results in the 2D analysis, which was based on a more extensive sampling protocol. This result is counter-intuitive as mice fed soft food presumably use their molars less because they have no hard food items to chew.

One possible explanation is that chewing behaviour is independent of diet; both HF and SF mice had similar jaw movements and contact between molar rows. The absence of hard items to be processed would have led to increased tooth-tooth attrition in soft food eaters. This contrasts with results obtained from monkeys, where hard diet eaters show more rapid tooth wear than do animals raised on a soft diet (Teaford & Oyen, 1989). Possibly increasing this effect of tooth-tooth attrition, the absence of hard items may have deprived the SF mice of the sense of satiety. This supposition is supported by the observation that the mice fed the soft food diet weighed more than the hard food eaters at the end of the treatment, despite a similar energetic value of the two foods (Renaud & Auffray, 2010), which suggests that they ate more food overall.

A second explanation is related to the fact that the lower molars are located on the mandibular jaw, which was shown to respond to food consistency itself (Renaud & Auffray, 2010; Renaud, Auffray, & de La Porte, 2010). Previous research demonstrated that bone remodelling occurring in the absence of the stimulation related to the consumption of hard food resources caused morphological differences between HF and SF mice. Soft food mandibles display an uplift of the zone where the lower molars are inserted (Anderson, Renaud, & Rayfield, 2014; Renaud et al., 2010), thus changing the shape of the mandibular arch. Consequently, this may alter the alignment of the two molar rows while in occlusion and thus, change the tooth wear pattern (Molnar & Molnar, 1990).

During mastication in murine rodents, the lower jaw effects a mostly propalinal movement from the distal bottom to the anterior (mesial) top (Hiimeä & Ardran, 1968; Lazzari et al., 2008; Weijts, 1975). The movement, however, also includes a rotational component because of

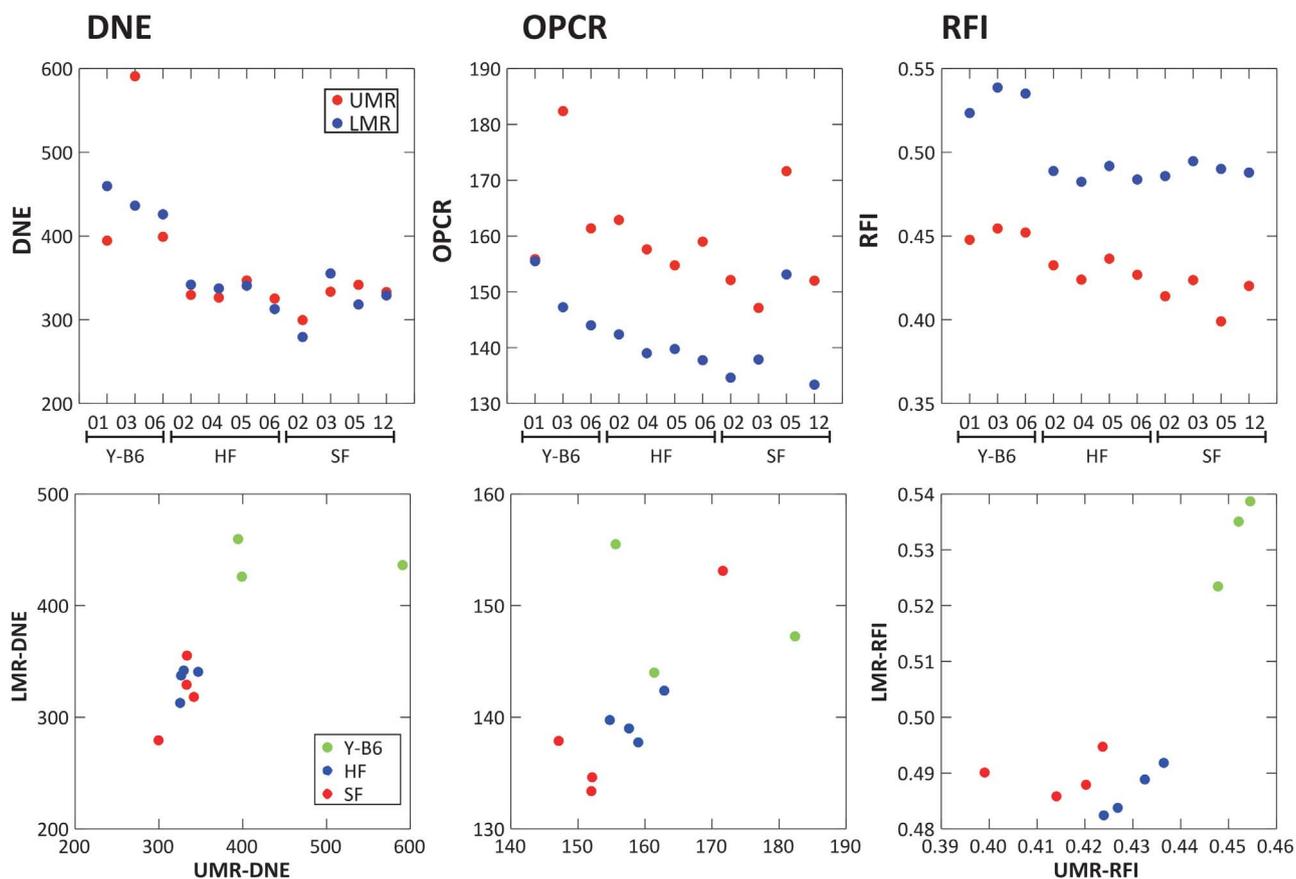


Fig. 5. Dental topographic variables based on a semi-automatic procedure of alignment of the molar rows. Upper panels: tooth curvature (DNE), complexity (OPCR), and relief (RFI) of the upper and lower molar rows. Bottom panels: topographic estimates of the upper molars vs. the lower molars. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Differences between groups (Y-B6, HF, SF) in topographic variables characterizing the upper (UMR) and lower (LMR) molar row, based on the semi-automatic adjustment of the molar rows. Probabilities of *t*-tests are given. In bold significant probabilities at $P \leq 0.05$.

		Y-B6 vs HF	Y-B6 vs SF	HF vs SF
UMR	DNE	0.064	0.059	0.646
	OPCR	0.316	0.303	0.646
	RFI	0.002	0.003	0.043
LMR	DNE	< 0.001	0.002	0.487
	OPCR	0.031	0.194	0.995
	RFI	< 0.001	< 0.001	0.354

elasticity of the symphysis (Weijjs, 1975). The angle between the two hemimandibles decreases during the forward shift of the mandibles, apparently occurring symmetrically about the sagittal plane in the rat (Weijjs, 1975), and presumably in the mouse as well. This may explain the helical occlusal surface of the molar rows, occlusion starting towards the back of the molar row on the labial side, and finishing with the first lower molar in front of the first upper one, but shifted on the lingual side.

The helical shape of the molar rows is a part of the difference between hard and soft food eaters. This suggests that the response of the symphysis, and thus possibly the pressure exerted during chewing, might have varied between the two groups. In some SF specimens, this led to relatively planar occlusal surface, in contrast with the usual helical shape helping to direct the rotational movement of the mandible. The elasticity of the symphysis may be less solicited in soft food eaters, due to less strength exerted during chewing, leading to a reduced rotational component in the masticatory movement.

Rather than being more worn, the lower molar row may thus be more variable in soft food eaters, as a consequence of the changed jaw morphology, reduced morphological integration (Anderson et al., 2014) and possibly, disturbed feeding behaviour. This would explain the greater variation in geometry and topography observed among mice fed soft food.

The two explanations are not mutually exclusive and, in fact, may have reinforced each other. Our results underline the importance of behaviour in the response of animals confronted with unusual feeding habits. Mastication is a complex behaviour that starts to develop prenatally (Turman, 2007) and is thereafter permanently adjusted to the texture of the food (Foster, Woda, & Peyron, 2006). Our results further underline that the feeding structure of mice evolved as an integrated system in the context of diversified but relatively hard items, such as seeds and grains. Departing from this diet affects the whole system by releasing the usual constraints. The decreased morphological integration observed in the jaw in such instances (Anderson et al., 2014) may affect the molar rows as well.

4.2. Importance of wear in natural populations

The importance of wear regarding the 3D morphology echoes the fact that cusp abrasion was found to be the first order signal when investigating the diversification of the first upper molar across natural populations of house mice in various continental and insular settings (Ledevin et al., 2016). In contrast, the 2D outline of the tooth was shown to be relatively invariant with wear in another murine species, the wood mouse *Apodemus sylvaticus* (Renaud, 2005). The 2D outline is measured relatively low on the crown and is thus impacted by wear relatively late during the animal's life. However, the same study

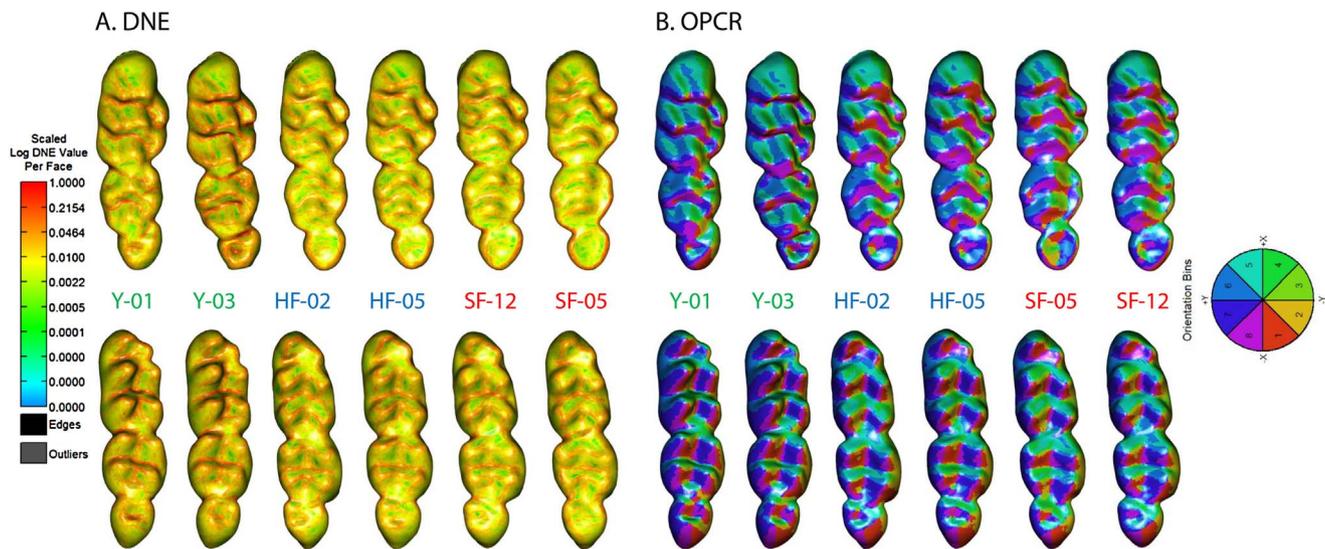


Fig. 6. Visualization of the dental topographic characteristics for three mice exemplifying each of the three groups (Y-B6, HF, and SF). A. DNE. B. OPC. Upper panel: upper molar row. Bottom panel: lower molar row. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3

Relationship between topographic parameters of the upper and molar rows, and between geometric and topographic parameters for both molar rows. R² and the probability of the linear model between both variables are given, as well as the slope relating the same parameter for the upper and lower molar rows. In bold significant probabilities at P ≤ 0.05.

		R ²	P	Slope
UMR vs LMR	DNE	0.553	0.011	0.52 ± 0.16
	OPCR	0.307	0.077	0.40 ± 0.20
	RFI	0.655	0.003	1.02 ± 0.25
UMR	Shape (PC1)	DNE	0.539	0.010
		OPCR	0.099	0.347
		RFI	0.909	< 0.001
LMR	Shape (PC1)	DNE	0.879	< 0.001
		OPCR	0.234	0.132
		RFI	0.943	< 0.001

demonstrated that even the 2D outline of the tooth varied when considering very young or old animals. Because of the sloped morphology at the rear of the first upper molar, wear reduces its overhanging imprint on the 2D outline, a signature evidenced in our 2D analysis.

The present study was disposed to display such an effect. It included both very young, sub-adult mice just at weaning age, and six month old mice. Although it is difficult to assess the life expectancy of a house mouse in the wild, a generation time of three to four generations per year is considered probable. Thus, six months would be a relatively old age. As wild house mice reproduce all year round when food is available (Pocock, Searle, & White, 2004; Rowe, Swinney, & Quay, 1983), natural populations are likely to be dominantly composed of relatively young mice. Thus, in regard to our 2D morphometric analysis, any observed wear effects should remain limited compared to inter-population differentiation in natural samples. A similar pattern was detected for sexual dimorphism, which was found to be a significant source of mandible shape variation in inbred lab mice of controlled food and age (Renaud et al., 2010), even though this signal is repeatedly lost in wild populations because it is diluted by more important sources of variation (Renaud, Hardouin, Pisanu, & Chapuis, 2013).

4.3. Topographic signature of wear

Teeth get flatter with wear in all species examined so far (Ungar,

2015). The decreasing relief (RFI) observed in our mice from weaning to six months of age fits that pattern. Since our quantification of tooth relief is based on the surface area of the entire crown, from the junction with the root, its relief includes a quantification of crown height (Boyer et al., 2010) that will inevitably decrease with wear.

The decrease in tooth curvature (DNE) with wear observed in the mouse sample is less trivial. It is in agreement with several studies showing that the sharpness of the crown tends to decrease with increasing wear (Dennis et al., 2004; Ungar, 2015). Regarding the mouse tooth row, it corresponds to abrasion that will tend to round the enamel edges, which are pronounced in young, unworn molars. However, it contrasts with observed increases in DNE with wear in monkey species (Pampush, Spradley et al., 2016). This signal was interpreted as molar sculpting for safeguarding against dental senescence: the molar shape evolved to maintain morphology that performs equally well when unworn and worn. Apparently, murine rodents did not develop such a strategy, possibly because of their relatively short life spans. The teeth of our 6 month old animals are far from being worn down, which suggest that the strategy for avoiding dental senescence in these short-lived rodents may predominantly rely on the initial relief index and a relatively high DNE associated with enamel edges that surround places where dentine shows on the surface (Lazzari et al., 2008). The young Y-03 mouse exemplifies such sharp, unworn teeth.

A contrasting pattern was provided by the dental complexity, estimated by OPCR. This estimate was the less sensitive to wear, as suggested by its weak relationship with relief index and the geometry of the tooth row. These results are in agreement with former studies on other mammalian models, which suggest that OPCR was only poorly related to the other topographic estimates [e.g. (Godfrey et al., 2012)], and that OPCR was not related clearly to wear (Pampush, Spradley et al., 2016).

4.4. Relationship between geometry and topography of the molar rows

An aspect of the present study that makes it unique is that we assess both 3D geometric shape and crown topography for all the teeth in the same molar rows. Geometry was evidenced to be very tightly related to relief in this context, and the first-order signal corresponds to increasing wear. Curvature (DNE) was also correlated with tooth shape, but to a lesser degree. In contrast, complexity (OPCR) was not related to the geometry of the tooth rows. This suggests that the mouse dentition maintains its complexity, despite changes in the shape of the cusps occurring with wear, or at least, the cusps maintain a more or less

constant complexity after a sharp decrease around weaning age. Such an effect may also exist for curvature (DNE). One of the young mice (Y-03) displayed an extreme value for the upper molar DNE, while the two other young mice displayed values more similar to the values of older mice. Molar rows may rapidly converge to a sharpness that will thereafter maintain most of their topographic characteristics and hence presumably their functionality throughout the animal's life.

4.5. Comparison between tooth rows

Few studies have assessed characteristics of both upper and lower molar topography and morphometry (Pampush, Spradley et al., 2016), because traditionally, topographic studies have focused on the lower molar row, particularly the second lower molar [e.g. (Boyer et al., 2010; Boyer, 2008; Godfrey et al., 2012; Ungar, 2004; Winchester et al., 2014)]. However, it has been advocated that, from a functional point of view, considering the complexity and topographic properties of the whole molar row would make more sense (Evans et al., 2007).

In our mouse sample, the lower molar row tends to display higher relief than its upper counterpart. However, they were abraded at the same rate, as both are correlated with a slope of one. Upper and lower molars tend to have similar indices of curvature (DNE) as well. Our findings of higher relief for the lower molars as compared to the upper molars and a consistency in both DNE and RFI estimates between the upper and lower molar rows are similar to results obtained from studies of primate dentition (Pampush, Spradley et al., 2016).

Complexity (OPCR) of the upper molar row is slightly higher than that of its lower counterpart. This makes sense in the light of the fact that the upper molar, which is composed of more cusps, appears more complex from a qualitative point of view. In contrast to what is observed for DNE and RFI, the two occluding molar rows do not share a common pattern of wear-related variation in complexity, which may be explained by one of a few different reasons. First, complexity may converge rapidly after weaning towards a range of values common to all mice. In support of this argument is the observation that complexity is the only topographic parameter not related to wear. The limited range of morphological variation may hinder the detection of any relationship between upper and lower molar complexity. Second, even OPCR appears to be sensitive to the step of manual alignment of the occlusal surface. Therefore, inconsistencies in orientation may further obscure any relationship. Finally, other morphological aspects, such as the shape of the mandibular arch (Molnar & Molnar, 1990), or behavioral components of mastication, as suggested by the results observed for the soft food mice, may influence the manner in which wear occurs and, consequently, the resulting tooth complexity. Regardless of the underlying explanation, this lack of a shared variance pattern means that the signal for wear-related complexity changes is too limited to overwhelm other sources of variation.

4.6. Perspective of applications

Dental topography is associated with dietary specializations across taxa [e.g. (Bunn et al., 2011; Godfrey et al., 2012; Ungar, 2004)]. The resistance of a food material to crack propagation depends on its elasticity and fracture toughness (Popowics & Fortelius, 1997). Species consuming tough food items tend to possess molars with high, sharp cusps for puncturing and shearing these materials, whereas species consuming fruits tend to have molars with low cusps and open basins for gripping and crushing these foods (Evans & Sanson, 2003; Winchester et al., 2014). At the interspecific level, differences due to wear seem to be of secondary importance (Bunn et al., 2011; Ungar, 2004). The variation of topographic proxies with wear may be more problematic in the perspective of investigating whether variations in tooth shape could be of functional importance at a micro-evolutionary scale (i.e. within a species). Even when excluding heavily worn teeth, wear appears to be the first order signal in a morphometric analysis of

tooth shape across a range of natural house mouse populations (Ledevin et al., 2016). The strong relationship evidenced in our data between relief index and wear demonstrates that this parameter is of little value for reconstructing dietary preferences on such a small evolutionary scale. However, as it is the only parameter to vary between hard and soft food eaters, tooth relief may provide an insight into the texture of the food the mice were exposed to, but only on the upper molar row. Comparing the relief of the upper and lower molar rows may provide a way to discriminate between mouse populations eating food of different textures, even without knowing the age of wild-trapped animals.

Dental curvature (DNE) indices seem to rapidly converge on rather constant values, at least for the upper molars. Consequently, DNE may be of higher relevance for inferring possible functional variation among populations. Complexity (OPCR) seems even less directly related to wear, but it is also the least accurate proxy for dietary inferences among closely related species (Winchester et al., 2014), even though it bears some ecological signature when considering groups that are as different as carnivores and rodents (Evans et al., 2007). The insensitivity of the OPCR index to wear may be of advantage for comparing wild-trapped mice of unknown age, but its potential to trace ecological differences remains unanswered.

Overall, our pilot study suggests that the results that have been obtained thus far, mostly from primates studies, may extrapolate well to other groups, such as murine rodents. Applications across taxa may be rather straightforward, but our results from experiments of known-age mice demonstrate that interpretations about intraspecific variation in tooth shape will have to take into account the relative importance of both wear and variation in the process of chewing in shaping the teeth.

Conflict of interests

No conflict of interest.

Funding

ANR Bigtooth (ANR-11-BSV7-008), LabEx Sciences Archéologiques de Bordeaux (ANR-10-LABX-52).

Ethical approval

Breeding of the mice was done at the PBES under the agreement B 69 123 0303–17/02/2009 of the French Ministère de l'Agriculture.

Acknowledgments

We thank Emmanuel Chereul (Voxcan) for his contribution while scanning the animals, and Marie Texeira (PBES Lyon) for her help in organizing the experiments. Doug Boyer greatly helped us in elaborating methodological protocols. We also would like to thank Jessica Joganic for helpful comments on final drafts of the manuscript, as well as the constructive comments of two anonymous reviewers. This study was supported by the ANR Bigtooth (ANR-11-BSV7-008) and by the LabEx Sciences Archéologiques de Bordeaux (ANR-10-LABX-52).

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