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A new method of tooth mesowear and a test of it on domestic goats

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Mesowear III is a new form of mesowear that uses separately the mesial and distal surfaces of enamel band 2 of the upper second molar to differentiate between a browsing and a grazing diet. The enamel band of browsers is flat and planar, in grazers it contains gouges and sub-facets which eventually become round. Mesowear III scores these shapes on a scale 1–4; score 1 represents the typical browser and score 4 represents a typical grazer. Differences in diet can also be studied by observing the junction between the mesial and distal side of the enamel band (j point); the j point is sharper and well defined in browsers, while it is rounder or non-existent in grazers. Wild browsers, grazers, and mixed feeders separate well using mesowear III. The wild taxa data were similar to mesowear III of experimental goats that were fed a controlled grazing or browsing diet (species of plants known). In addition, the browsing *versus* the grazing goat mesowear III signal becomes more distinct from each other by 40 days of feeding. Mesowear III so far gives a finer signal than previous mesowear (I and II).

Introduction

This study is twofold. First, we introduce a new method of tooth mesowear. Second, we test this new method utilizing an experimental wear sequence from extant domestic goats whose diet and duration of feeding was controlled.

Traditional tooth mesowear has been a useful technique in deciphering the paleodiets of various ungulates (Fortelius & Solounias 2000, Mihlbachler *et al*. 2011, Solounias *et al*. 2010, 2012). Up until now, this was achieved by a scoring of the apices of molars from the buccal view. Tall sharp apices are most common in browsers whereas shortened and rounded apices are more common in grazers. At least two scales have been developed on extant species for the evaluation of mesowear. Originally, Fortelius and Solounias (2000) developed the method in which they treated the wear down of cusps as a two-variable feature: the cusps were high or low, and sharp, rounded, or blunt. The cusps could be sharp and high, round and high, round and low, and blunt and low; high and blunt teeth

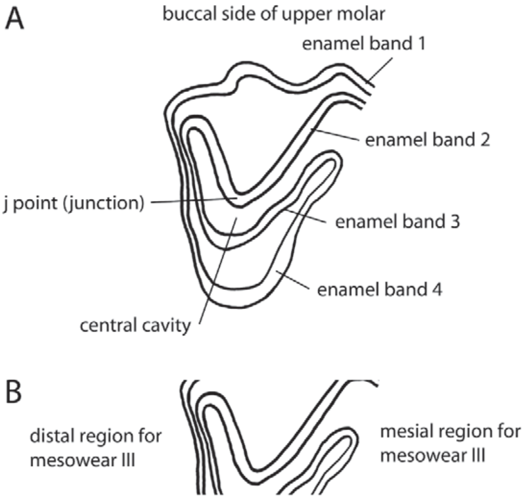

Fig. 1. The terminology associated with the upper molar. (**A**) the metacone and the hypocone of the tooth with the enamel bands, j point, and central cavity, and (B) the portion of the tooth used to analyze mesowear III.

were impossible, and low and sharp teeth were rare. One apex per individual was scored. Kaiser *et al*. (2000) and Kaiser and Solounias (2003) applied these observations to more than one cusp per individual and tested its predictability by involving more observers on the same teeth. We term this mesowear I. Mihlbachler and Solounias (2006) is the first study where a new type of mesowear was introduced and developed (mesowear type II). In this study they used a four-point scoring system for the first time. Subsequently from this four-point scale a seven-state scale was developed. Mihlbachler *et al*. (2011) used this new scoring seven-state scale where the height of the cusp was integrated with the wear of the apex into a single scale (single variable). In these two studies the paracone or the metacone of M2 was preferably used. A selection of the sharper of the two is the ideal cusp. If a cusp is broken or problematic an adjacent cusp is used in the scoring. We term this mesowear II. There are several studies utilizing these methods (Eronen *et al*. 2009, Clauss *et al*. 2007, DeMiguel *et al*. 2008).

Throughout our studies and by examining thousands of teeth, additional significant mesowear differences, which have previously not been addressed, were noted. For example, regions other than the buccal apex contained information that appeared to be significant. In a ruminant or equid tooth, four primary bands of enamel are present. The buccal band is presently termed band 1. This band was used in mesowear I and II. Going lingually, the next is band 2, followed by band 3. The innermost band is band 4. The paracone and the metacone are constructed by enamel bands 1 and 2, and the two bands are separated by layers of dentine. Similarly, the protocone and the hypocone are composed of enamel bands 3 and 4, which are also separated by layers of dentine. The central cavities are between bands 2 and 3 (Fig. 1). Observations (by NS) carried out for many years suggested that band 2 of the upper second molar contained better mesowear information than band 1. Band 2 shows mesowear with interesting variations, which could be used in differentiating the diet of the individual. In addition, the occlusal view of the mesial part of band 2 gave different mesowear from the distal.

We devised two experiments using modern goats to see if the patterns observed in the wild were reflected in an experimental setting. Goats were fed selected types of browsing and grazing plants at different time intervals, and the teeth were examined and compared with those of wild ruminants. Experiments with mesowear are rarely done, and our collaboration with the Government College University Lahore allowed us to investigate the dietary implications of mesowear II and III.

Methods

Description of the new method of mesowear: Mesowear type III

So far we investigated in detail mesowear of band 2 of the upper second molar, hoping to achieve better resolution than that from band 1 (mesowears I and II). In order to do this properly, we evaluated band 2 from an occlusal view of extant wild specimens of known browsers, grazers and mixed feeders. We developed a new scoring scale of only 4 wear states (Fig. 2). We scored the occlusal mesial part of band 2 separately from the distal and subsequently averaged the two. We evaluated mesowear of all specimens qualitatively by observation with a stereomicroscope.

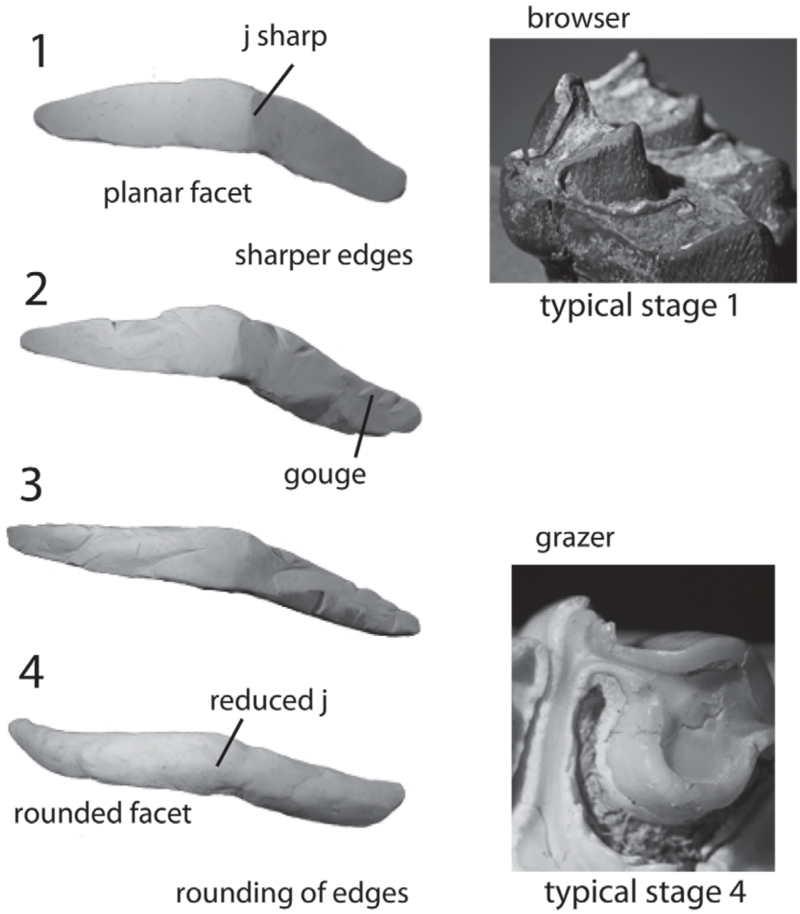

Fig. 2. A model of the stages of mesowear III, using a clay representation of each wear state. Photographs of a molar with a mesowear III score of 1 (an ideal browser), and a molar with a mesowear III score of 4 (an ideal grazer) are also included.

From observation of the teeth of known wild extant grazers and browsers, we developed a method using enamel band 2 to interpret the diet of the individual. Here, we describe a morphocline sequence going from the ideal browser to the ideal grazer. This morphocline has been divided into 4 scores, although we are aware that the actual wear of the teeth is a continuum. We believe that one can use these 4 scores to extrapolate the diet of an individual.

For band 2, there are two sides (the mesial side and the distal side), separated by a midpoint (termed j for junction). The mesial side has different mesowear from the distal side using mesowear III (to be described).

The contraction of the muscles during occlusion and the cutting of vegetation form the mesial side and the distal side on the enamel band of both the paracone and the metacone. The mesial side is the leading edge, considering the direction of the bite force. At the mesial side, there is higher force because the mandible is pulled back against the upper mesial side, which is tilted. The leading side is often a flatter edge and is subjected to more attrition. The distal side trails during the cut and most likely has a weaker attritional contact. Due to this difference in forces during mastication, the mesial side and the distal side often have different mesowear III and are therefore scored separately. The two scores are averaged to form a collective evaluation for the entire band. (*See* Table 1 for a complete description on the scoring using mesowear III.)

The two sides of the cusp have a mid-point where they merge. We name the mid-point junction or j point. We scored the wear of the j point separately from the wear of the sides. When the j point is very sharp and well defined, we score it as 1. When the j point is completely absent and the mesial and distal sides of the enamel band form one continuous surface, we score it as 4. Scores 2 and 3 are intermediate stages. A j with score 2 still has the sharp edge but contains one or two gouges and sometimes contains a small facet. A j with score 3 is more rounded but is still visible.

We used band 2 of the metacone of the upper second molar when it was available, but when that region was damaged, we used band 2 of the paracone.

Wild ruminants used in the study

For the extant ruminants, we used specimens from the American Museum of Natural History. The teeth were molded and cast. The browsing animals we used were: *Okapia johnstoni* (n = 11), *Giraffa camelopardalis* (n = 15), and *Alces alces* (n = 13). The grazing animals we used were: *Ourebia ourebi* (n = 7), *Kobus ellipsiprymnus* (n = 10), and *Chonnochaetes taurinus* (n = 12). The mixed feeders we used were: *Cervus canadensis* (n = 4) and *Grazella granti* (n = 17).

Dietary experiment using goats

We conducted two experiments using adult goats (*Capra hircus)*. Before the experiment, the goats population most likely fed on leaves of *Malia azedarach* (dharek tree), and *Morus alba* (mulberry tree) and grasses such as *Cynodon dactylon* (khabal grass), *Sorghum helepense* (Johnson grass), and *Echinochloa colona* (jungle rice). These plants make up the natural diet of goats in that region.

In the first experiment, five goats were placed in an enclosure and fed browsing plants (the leaves of *Malia azedarach*, *Zizyphus jujuba* and *Morus alba*) for 18–20 days. Five different goats were kept under the same conditions and fed grazing plants (*Cynodon dactylon*, *Sorghum helepense* and *Echinochloa colona*).

In the second experiment, four goats were subjected to browsing. In the course of the experiment we sacrificed one goat on days 10, 20, 30 and 40. The skull and jaw of each goat was skeletonized. The same procedure was repeated for four goats that were subjected to grazing, keeping all other conditions the same. The browsing goats were fed the leaves of *Malia azedarach*, *Morus alba* and *Zizyphus jujuba*. The grazing goats were fed *Cynodon dactylon*, *Sorghum helepense*, *Dichanthium annulatum*, *Digitaria violascens* and *Echinocloa colona*.

We received permission from the Department of Zoology GCU in Lahore which manages farms with goats and other livestock, to use the goats for the dietary experiment. They were treated well with food and water, and they were kept in an outdoor enclosure in their natural habitat. The goats were anesthetized intramuscularly and were euthanized by exsanguination.

Results

The separation between the mesowear III scores of extant browsing and grazing rumi-

Table 1. Description of the scoring for mesowear III.

Score	Description
1	The ideal browser morphology for the mesial or the distal side of the cusp. The facet is one large, planar surface. The edge of the surface is relatively sharp, with no gouges.
2	The surface is no longer one large, continuous unit, but is broken up into two or more smaller sub-facets. The edge is not as sharp as in score 1, and it has gouges, primarily on the buccal side of the enamel band.
3	The surface is similar to score 3, but it is broken up into more sub-facets and has more gouges. There is also rounding between the sub-facets and on the edges.
4	The ideal grazer morphology for the mesial or distal side of the cusp. The edges of the enamel band are rounded instead of sharp, with no gouges or sub-facets, and the surface forms a uniform arch.

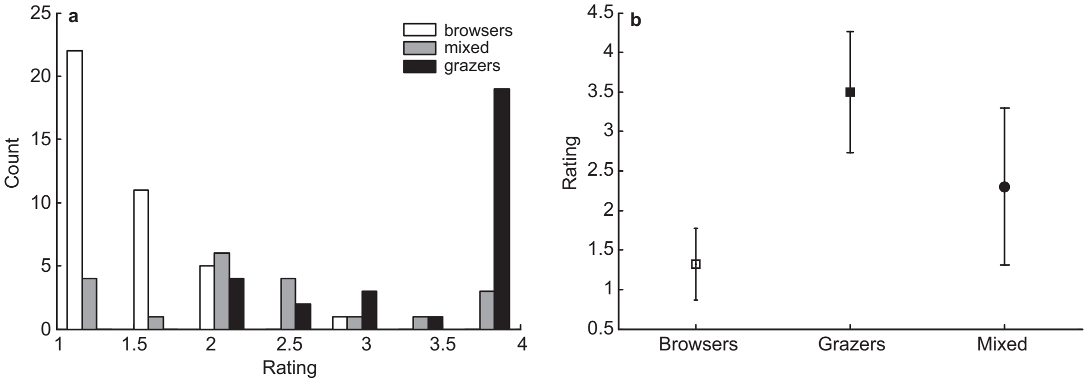

Fig. 3. (**a**) The distribution of mesowear III rankings for 39 browsers, 29 grazers, 21 mixed feeders. (**b**) The mean ± SD mesowear III scores for the known browsers, grazers, and mixed feeders. The browsers and grazers differed significantly in this respect (Mann-Whitney-Wilcoxon test: $U = 768.5$, df = 39,29, $p << 0.001$).

nants was statistically significant, as determined by the Mann-Whitney-Wilcoxon test, which was performed in Matlab (Mathworks, Inc.). As expected, the mixed feeders overlapped with the grazers and the browsers (Fig. 3). The results of mesowear III are better than those of mesowear II (Table 2); they show finer discrimination.

The results for the goat experiment are preliminary as the sample was small (*see* Fig. 4). Before the experiment, the goats started off with a more browsing diet, as reflected by the mesowear III average of 1.75. Mesowear of the pre-experiment goats was obtained by analyzing mesowear III of five goats which fed on their natural diets in the same location as that of the experiment. In the four goats that shifted from natural food to browsing, the teeth became incrementally flatter during the period of 30–40 days of browsing (Fig. 4). In the four goats that shifted from natural food to grazing, the teeth became incrementally rounder for the first 30 days of grazing, and did not change during the last 10 days (Fig. 4).

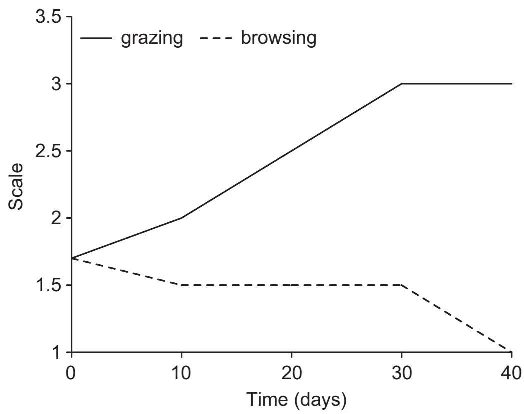

Fig. 4. Results for the goat experiment. The lines show a progression of the mesowear for the four goats that shifted from the natural diet to browsing or grazing. The mesowear III of the goats at the begining of the experiment ranged from 1 to 2.5.

Discussion

The dietary adaptation of the species we chose to represent the browsers, grazers, and mixed feeders is based on previous studies (Hoffman & Stewart 1972, Chapman & Feldhamer 1982). The data of the extant species studied, strongly correlate with the known diets in the wild (Fig. 3 and Table 2); these results confirm the validity of mesowear III. Our findings are also in line with previous studies on tooth microwear and mesowear I and II which were done on the majority of the same species (Fortelius & Solounias 2000, Solounias & Semprebon 2002). Premaxillary studies of the same species have also confirmed their diets, independent of the tooth data (Solounias *et al*. 1988, Solounias & Moelleken 1993).

The mixed feeders vary their diet between grazing and browsing and our results indicate that their mesowear III falls between that of the browsers and grazers.

The species we chose to represent the grazers and browsers are from the extreme ends of the ruminant dietary continuum. These species are ideal grazers and browsers. Such a selection was necessary in order to develop a new method for describing ruminant diet. In nature, however, many of the species fall more towards the middle of the range; their diets are less specialized. More data are needed, but eventually the overlap will become a problem in effective discrimination of browsers *versus* grazers.

The wild animals are again ideal for establishing the new method of mesowear. It is known that in nature, herbivores feed on hundreds of plant species (Labâo-Tello & Van Gelder 1975). Precise relationships of plants to dietary adaptations are impossible to resolve and animals will be broadly adapted.

In the experiments of controlled diet in captivity presented here we intended to further elucidate what is observed in wild individuals. In the first experiment, we tested if the mesowear of goats that were fed a controlled browsing or grazing diet matched the mesowear of the wild animals. In the second experiment, we investigated how fast the teeth were altered, and if there were progressions (morphoclines) in the mesowear. We found that the mesowear III patterns in the controlled conditions were the same as those found in wild animals, and that indeed there was a progression (Fig. 4). We, however, recognize that our setting has several weaknesses. Before the experiment, the goats had a browsing diet, which was confirmed by mesowear III of five individuals tested. Also the sample sizes were small.

Mesowear I and II are good in separating populations from each other from a dietary point of view. For example, *Pachytragus laticeps* and *Merychippus insignis* from different quarries produced slightly different results, when using mesowear I and II (Fortelius & Solounias 2000, Solounias & Semprebon 2002). Mihlbachler *et al*. (2011) developed a new scale of 7 scores, the most common score in many studies being 2 (mesowear II). Although dietary differences among ruminants are detectable, there is a large cluster of species that do not differ in this respect because they all have the mesowear II value centered around 2. It is evident (*see* Table 2) that the

Table 2. Data for mesowear II and III for extant ruminants.

Species	*n*	Days	Mesowear II	Mesowear III	Anterior band 2	Posterior band 2	Junction point "j"
Browsers							
Okapia johnstoni	11		0.8	1.4	1.5	1.3	1.5
Giraffa camelopardalis	15		1.3	1.3	1.3	1.6	1.6
Alces alces	13		1.1	1.2	1.2	1.3	1.6
Grazers							
Ourebia ourebi	7		2.2	3.5	3.6	3.4	3.8
Kobus ellipsiprymnus	10		3	3.1	3.1	3.1	3.5
Chonnochaetes taurinus	12		4	3.8	3.8	3.8	3.7
Mixed feeders							
Cervus canadensis	4		1	1.6	1.6	1.6	1.5
Grazella granti	17		2.2	2.5	2.3	2.6	2.9
First experiment							
Capra hircus browsing	5	18–20	1.8	1.7	1.3	2.2	2.5
Capra hircus grazing	5	18–20	1.9	2.6	2.5	2.8	2.8
Second experiment							
Capra hircus browsing	1	10	1.5	1.5	1	2	1
Capra hircus browsing	1	20	1.5	1.5	1	2	1
Capra hircus browsing	1	30	1.5	1.5	1	2	1
Capra hircus browsing	1	40	1	1	1	1	1
Capra hircus grazing	1	10	0	2	2	2	1
Capra hircus grazing	1	20	1	2.5	2	3	2
Capra hircus grazing	1	30	1	3	3	3	2
Capra hircus grazing	1	40	1.5	3	3	3	2

majority of the species in this study also have the score of 2 using mesowear II. The results of the present study show a finer resolution of the diets using mesowear III. Therefore, enamel band 2 is the best band so far to differentiate between ruminant species based on their diet.

Figure 2 shows four idealized stages of wear, which in nature is a continuum. In a browser, the vegetation in the diet is relatively unabrasive, and is presumably cut more thoroughly, which allows the lower enamel surface to contact the upper enamel surface more as they slide past each other repetitively. Over time, this creates a flat surface on band 2. If the animal browses throughout its lifetime, the flat surface is retained. In a grazer, the situation is different. The vegetation is much more abrasive and contains microscopic silica phytoliths. Grasses also are known to be sprinkled with grit and dust, as they are close to the ground. As the lower teeth push against the upper teeth, enamel edge fractures form what we have termed "gouges." Over time, the number of gouges increases, and large flakes of enamel break off, creating a complex surface with more than one facet per side. Eventually, the wear progresses further, forming a rounded surface on the edges. Finally, the center of the enamel is rounded off as well. The j becomes very indistinct, and the mesial and distal facets of band 2 merge into one.

The *temporalis* muscle pulls the jaw backwards during occlusion, causing the mesial side of the cusps of the upper teeth to be subjected to more attrition than the distal sides. Therefore, the mesial side is the leading edge, and the distal side is the trailing edge. In browsers, one would expect the mesial side to have a lower score (ideally a 1) using mesowear III, indicating that it is flatter than the distal side; while in grazers, one would expect the mesial side to have a higher score (ideally a 4), indicating that it is rounder than the distal side. In both cases, the distal side is less worn than the mesial side because the force of attrition is weaker.

Mesowear III is best used with adult teeth, similar to previous methods and for the same reasons. The young teeth are not yet worn enough to give a good indication of the diet of the animal, and they are also supplementing their diets with milk. In old individuals, most of the crown of the teeth is worn off. The part of the internal structure closest to the roots is simplified, and bands 2 and 3 are often no longer present at that depth of the tooth. This makes mesowear of the tooth unreliable. Also, chemical erosion, enzymatic reactions, bacterial activity, and other factors could contribute to the eventual wear of the teeth.

We anticipate that mesowear III will be a useful proxy to use in combination with mesowear I or II and light microscope microwear for better resolving diet in extant and fossil ungulates.

Conclusions

Most studies on dietary adaptations do not include experiments that show the progression of tooth wear. Our study incorporates the data from controlled feeding conditions, and compares them with the data from wild species. Mesowears III of the experimental animals and the extant wild species not only correlated with each other, but also with the known diets determined by previous studies and mesowear I and II. Our conclusions are based on the observation that a browsing diet creates a flat and planar surface on band 2 of enamel on the upper second molar, and that a grazing diet creates a more rounded surface on the same enamel band. This is due to the consistency of the vegetation; browsing plants are less abrasive, and allow for greater contact between the upper and lower enamel surfaces. Grazing plants are more abrasive and contain microscopic silica phytoliths that initially create gouges and sub-facets on the enamel band, which progressively become rounder as the individual continues to feed on a grazing diet. In addition to the enamel band itself, the junction between the mesial and distal sides of the band (termed j point) is influenced by the grazing or browsing diet: browsers tend to have a sharper and more defined j point, whereas grazers tend to have a j point that is rounder or sometimes absent. Mixed feeders include both browsing and grazing vegetation in their diet, and consequently, their mesowear III falls between those of grazers and browsers. While previous mesowear studies (I and II) are useful in deciphering dietary adaptations of different ruminants, mesowear III shows finer resolution between the browsing and grazing diets. We anticipate that mesowear III will be useful, in conjunction with mesowears I and II, for studying paleodiets of extant and fossil ungulates.

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