Stable isotopes provide independent support for the use of mesowear variables for inferring diets in African antelopes

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We examine the relationship between mesowear variables and carbon and nitrogen isotopes in 16 species of African antelope (Mammalia: Bovidae). We show significant differences in carbon and nitrogen isotope values between individuals exhibiting sharp versus round cusps, and high versus low occlusal relief. We show significant correlations between mesowear variables and both carbon and nitrogen isotopes. We find significant correlations between mesowear score and nitrogen, but not carbon isotopes. Finally, we find no significant correlations between hypsodonty index and either isotope examined. Our results provide strong support for the use of mesowear variables in palaeodietary reconstructions of antelopes. Our results further suggest that for the antelopes examined here, mesowear signals are a direct result of diet, while hypsodonty may be the result of phylogenetic legacy.

Keywords: mesowear; palaeodiet; stable isotope; nitrogen; carbon

1. INTRODUCTION

Mesowear—the macroscopic wear observed on teeth—is now a common means of inferring the dietary preferences of modern and extinct mammals [1–3]. It relies on the hypothesis that the appearance of wear facets on the molar teeth of herbivores will correlate with the level of abrasiveness in a species’ diet. Two types of tooth wear (attrition and abrasion) have been observed to affect teeth in quantifiably different ways [4,5]. Attrition refers to the formation of wear facets through tooth-on-tooth contact, and is thought to lead to sharper cusps and higher occlusal relief. This type of wear is most common in browsers (defined as species that include at least 70% dicots in their diet [6]). Abrasive wear occurs through tooth-on-food contact, and generally creates more rounded cusps and lower occlusal relief owing to the dietary presence of intrinsic abrasive particles such as phytoliths or forage qualities such as toughness or nutritive value [5]. Hence, original mesowear analyses examined the relative proportion of two variables (sharpness of cusps and occlusal relief) summarized in a categorical manner across a wide range of species (figure 1). The use of these variables allows fossil species of unknown diet to be classified to one of several broad dietary categories [4]. Later, refinement of the mesowear technique led to the conversion of the mesowear signal into a single, continuous variable, referred to as the mesowear score (MS) [3,7]. Components of the MS have been correlated with biotic and abiotic variables, including precipitation, hypsodonty index (HI) and microwear, as well as broad categorical dietary classes [5,8].

Another way of inferring diets is through stable isotope analysis. Carbon and nitrogen isotopes found in an animal’s body tissue, including bones, teeth and hair, are derived from dietary carbon and nitrogen [9], and in the case of hair particularly reflect the protein component of the diet [10]. Carbon isotopes in body tissues of herbivores reflect the proportion of C3/C4 plants in their diet. C3 plants (largely browse) have δ¹³C values ranging from −32‰ to −9‰ in closed understory canopy conditions to −21‰ in more open environments [11], whereas C4 plants (including many tropical grasses) can range from −21‰ to −9‰. Nitrogen isotopes will vary with trophic level, although other variables such as soil, climatic and inter- and intraspecific physiological differences can produce significant shifts in δ¹⁵N values [9]. Isotopes found in mammal hair preserve the record of the diet spanning months to years [12]. Similarly, mesowear is reported to record dental wear over the last few months or years of an animal’s life. Therefore, a significant correlation between isotopes found in hair and mesowear variables should be expected. However, this relationship has never before been tested. Here, we test such a correlation by comparing mesowear variables with carbon (δ¹³C) and nitrogen (δ¹⁵N) stable isotopes in 16 species of African antelopes (Mammalia: Bovidae).

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2. MATERIAL AND METHODS

A total of 104 wild-caught antelope specimens representing 16 species from the Koninklijk Museum voor Midden-Afrika (Tervuren, Belgium) were sampled for both mesowear variables and carbon and nitrogen stable isotopic composition. Linear measurements (occlusal length, ORL; occlusal height, ORH; crown height, CH; bucco-lingual width, BLW) were taken using digital callipers from both upper and lower molars (figure 2a). These measurements were considered because they enable both occlusal relief and HI to be quantified. Mesowear variables for the upper second molars of each specimen were measured following

sharp cusps  
high occlusal relief  

round cusps  
high occlusal relief  

blunt cusps  
low occlusal relief

Figure 1. Stylized antelope teeth showing typical mesowear variables.

Figure 2. Measurements and correlations for hypsodonty index (HI) and mesowear score (MS). (a) Stylized antelope tooth and measurements taken: ORL, occlusal length; ORH, occlusal height; CH, crown height; BLW, bucco-lingual width. (b–d) Correlations (b) between stable isotopes and HI, (c) between HI and MS, and (d) between stable isotopes and MS: dotted line and circles, δ¹³C; solid line and squares, δ¹⁵N.
Table 1. Correlation between individual measurements and carbon and nitrogen isotope values assessed using Kendall’s τ. Uncorrelated variables indicated in bold. ORL, occlusal length; ORH, occlusal height; CH, crown height; BLW, buccolingual width.

<table>
<thead>
<tr>
<th>tooth</th>
<th>isotope</th>
<th>ORL</th>
<th>ORH</th>
<th>CH</th>
<th>BLW</th>
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<tr>
<td>M1</td>
<td>δ¹³C</td>
<td>0.22534</td>
<td>0.194</td>
<td>0.053735</td>
<td>0.13781</td>
</tr>
<tr>
<td></td>
<td>δ¹⁵N</td>
<td>0.000744</td>
<td>0.014474</td>
<td>0.46383</td>
<td>0.042196</td>
</tr>
<tr>
<td>M2</td>
<td>δ¹³C</td>
<td>0.22076</td>
<td>0.221</td>
<td>0.039181</td>
<td>0.059504</td>
</tr>
<tr>
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<td>δ¹⁵N</td>
<td>0.000952</td>
<td>0.002296</td>
<td>0.6366</td>
<td>0.3755</td>
</tr>
<tr>
<td>M3</td>
<td>δ¹³C</td>
<td>0.16858</td>
<td>0.11695</td>
<td>-0.09266</td>
<td>0.40692</td>
</tr>
<tr>
<td></td>
<td>δ¹⁵N</td>
<td>0.012049</td>
<td>0.089678</td>
<td>0.39972</td>
<td>0.54859</td>
</tr>
<tr>
<td>m1</td>
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<td>0.13439</td>
<td>0.26321</td>
<td>0.031797</td>
<td>0.15428</td>
</tr>
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<td>0.002723</td>
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<tr>
<td>m2</td>
<td>δ¹³C</td>
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<td>0.18058</td>
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<td>0.079732</td>
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<td>δ¹⁵N</td>
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<td>0.017025</td>
<td>0.25142</td>
<td>0.26029</td>
</tr>
<tr>
<td>m3</td>
<td>δ¹³C</td>
<td>0.12206</td>
<td>0.15074</td>
<td>-0.36673</td>
<td>0.00307</td>
</tr>
<tr>
<td></td>
<td>δ¹⁵N</td>
<td>0.086597</td>
<td>0.045004</td>
<td>0.002289</td>
<td>0.96602</td>
</tr>
</tbody>
</table>

Louys et al. [13]: Sharpness was determined by examining the sharpest cusp and scored as sharp, round or blunt, whereas occlusal relief was determined by dividing ORH by ORL, although in cases where the cusps were chipped this was determined visually. HI was calculated by dividing CH by BLW for the lower third molar. For each specimen yielding MSs, at least 1 g of hair was extracted from the pelt of the same specimen. Hair samples were cleaned by wiping acetone to remove any adhering dirt and grease as described by Cerling et al. [14]. Sub-samples of the clean hair were weighed into tin capsules and analysed for their nitrogen and carbon stable isotopic composition by continuous flow isotope ratio mass spectrometry using a Sercon 20/22 IRMS coupled to a Sercon GSL sample combustion device using helium carrier gas. Sample data are reported in standard delta per mil notation (δ ‰) relative to V-PDB and AIR international standards for carbon and nitrogen, respectively.

Mann–Whitney U-tests were used to determine whether specimens having high or low occlusal relief showed significant differences in δ¹³C and δ¹⁵N values, whereas the Kruskal–Wallis test with post hoc Mann–Whitney U-test was used to assess these differences in specimens having sharp, round or blunt cusps. Correlation between raw molar measurements of MS or HI and δ¹³C and δ¹⁵N was assessed using Kendall’s τ, and significance was assessed at α = 0.05. The correlation between δ¹³C and δ¹⁵N values and sharpness (scored as 0, sharp; 1, round; 2, blunt), occlusal relief (0, high; 1, low) and MS (0, high relief and sharp cusps; 1, high relief and round cusps; 2, low relief and sharp cusps; 3, low relief and round cusps; 4, low relief and blunt cusps) was assessed by both Kendall’s τ and polars analysis, with significance assessed at α = 0.05 (it is noted that the δ¹³C are not normally distributed and hence the results from the polynomial analysis should be viewed with caution).

For species with a sample size greater than or equal to 5, average MS and the proportion of specimens having sharp, round and blunt cusps and high occlusal relief were calculated. The correlation between these and average δ¹³C and δ¹⁵N was again assessed using Kendall’s τ at α = 0.05. All analyses were run on PAST v. 2.14 [15], with any broken or unattainable measurements scored as missing values.

3. RESULTS

The tooth measurement and mesowear measurement results as well as the stable isotopic results are given in the electronic supplementary material, tables S1 and S2.

Both δ¹³C and δ¹⁵N show significant correlations with almost all molar metrics across all teeth examined (table 1). However, no correlation is found between crown height and δ¹³C in all teeth save the lower third molar, and in all first molars, there is no significant correlation between occlusal breadth and δ¹³C. The upper second molar has the highest average correlation with δ¹³C in all measurements. Our findings therefore support the use of this tooth preferentially for mesowear analyses (following Fortelius & Solounias [4]), and forms the basis of all other analyses conducted herein.

A Kruskal–Wallis test indicates that there are significant differences between δ¹³C values in antelopes exhibiting different cusp sharpness (H = 7.465, p = 0.02393). Mann–Whitney pairwise comparisons indicate that the only significant differences are between antelopes with sharp and rounded cusps (p = 0.01084), with no
significant differences observed between blunt cusps and either sharp \( (p = 0.8673) \) or round \( (p = 0.215) \) cusps. No significant differences occur between \( \delta^{15}N \) values in antelopes exhibiting different cusp sharpness \((H = 3.534, p = 0.1708)\). Significant differences between \( \delta^{13}C \) and \( \delta^{15}N \) values in antelopes exhibiting different occlusal heights are observed \( (p = 0.0449 \text{ and } p < 0.001, \text{ respectively}) \).

Our analyses show no significant correlation between HI and either carbon or nitrogen isotopes (figure 2b). We do find a significant correlation between MS and HI (figure 2c) and \( \delta^{15}N \) (figure 2d, solid line), but not \( \delta^{13}C \) (figure 2d, dotted line). There were significant correlations between \( \delta^{13}C \) and both cusp sharpness (figure 3a) and occlusal height (figure 3b). While a significant correlation was also found between \( \delta^{15}N \) and occlusal height (figure 3d), no such result was observed with cusp sharpness (figure 3c).

Only 10 of the 16 species examined had sufficient specimens to be included in our species-averaged analysis: the bay duiker \((Cephalophus dorsalis)\), the white-bellied duiker \((Cephalophus leucogaster)\), the black-fronted duiker \((Cephalophus nigrifrons)\), the yellow-backed duiker \((Cephalophus silvicultor)\), the Weyn’s duiker \((Cephalophus weynsi)\), the waterbuck \((Kobus ellipsiprymnus)\), the blue duiker \((Philantomba monticola)\), the reedbuck \((Redunca redunca)\), the common duiker \((Sylvicapra grimmia)\) and the bushbuck \((Tragelaphus scriptus)\). Our species-averaged analysis is summarized in table 2. The only significant correlation at \( \alpha = 0.05 \) is between the proportion of rounded cusps and average \( \delta^{13}C \).

4. DISCUSSION
To our knowledge, this is the first study examining correlations between mesowear variables, HI and stable isotopes in any extant taxa. Our analyses show that traditional mesowear variables are highly informative about antelope diet. In particular, there are significant relationships between \( \delta^{13}C \) and both sharpness of cusps and occlusal height. The positive correlation between sharpness and \( \delta^{13}C \) is consistent with the underlying rationale for mesowear analyses: as the amount of C4 (and hence tropical grass) in the diet increases, cusps decrease in sharpness. An opposite and more unexpected pattern was found in occlusal relief—as the amount of C4 decreased, low relief increased. This may be due to the high number of frugivorous species in our analysis, whose mesowear patterns can act more like those of grazers than browsers [13].
There were significant differences between specimens exhibiting high and low occlusal reliefs for both δ¹³C and δ¹⁵N. Significant differences in δ¹³C are also found between specimens exhibiting sharp versus round cusps. That no significant differences were found between blunt cusped specimens and other specimens confirms that blunt cusps are not diagnostic for distinguishing antelope diets [13]. MS is also found to correlate significantly with δ¹⁵N, although not with δ¹³C, which may be a result of the high number of frugivorous species examined.

Surprisingly, compared with the specimen-by-specimen analysis, the species-averaged analyses did not correlate with mesowear variables as robustly. Finding both undamaged second molars and associated pelts for a great number of specimens per species proved difficult, and these results probably reflect the decreased sample sizes in the specimen-based analyses. However, they do indicate that a specimen-by-specimen approach may more accurately predict diet than a broad species-averaged approach. Our results suggest that in future, mesowear analyses could be used on individual specimens using an ecomorphological taxon-free approach. This study has implications for understanding the relationship between diet and dental wear. Interestingly, we found very little correlation between HI and either δ¹⁵N or δ¹³C. This suggests that the abundance of C4 foods in a mammal’s diet may bear no relation to their HI, and that blunt cusps are not diagnostic for distinguishing antelope diets [13]. MS is also found to correlate significantly with δ¹⁵N, although not with δ¹³C, which may be a result of the high number of frugivorous species examined.

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Table 2. Correlation between species-averaged mesowear variables and carbon and nitrogen isotope values assessed using Kendall’s τ. Correlated variables indicated in bold. MS, mesowear score.

<table>
<thead>
<tr>
<th>Variable</th>
<th>% sharp</th>
<th>% round</th>
<th>% blunt</th>
<th>% high</th>
<th>average MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ¹³C</td>
<td>Kendall’s τ</td>
<td>-0.2046</td>
<td>0.49441</td>
<td>-0.42061</td>
<td>0.23002</td>
</tr>
<tr>
<td></td>
<td>p (uncorr.)</td>
<td>0.41023</td>
<td>0.046594</td>
<td>0.038411</td>
<td>0.35454</td>
</tr>
<tr>
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<td>Kendall’s τ</td>
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<td>-0.22473</td>
<td>0.48086</td>
<td>-0.36803</td>
</tr>
<tr>
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<td>0.050044</td>
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REFERENCES


