

A NEW TOOTH WEAR–BASED DIETARY ANALYSIS METHOD FOR PROBOSCIDEA (MAMMALIA)

JUHA SAARINEN, ALEKSIS KARME, THURE CERLING, KEVIN UNO, LAURA SÄILÄ, SAMUEL KASIKI, SHADRACK NGENE, THADEUS OBARI,
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A NEW TOOTH WEAR-BASED DIETARY ANALYSIS METHOD FOR PROBOSCIDEA (MAMMALIA)

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ABSTRACT—Dietary analyses of herbivorous mammals are important for paleoecological reconstruction. Several methods applicable for fossil teeth have been developed lately. The mesowear method based on wear-induced occlusal shape and relief of ungulate molars has proven to be a robust method for dietary analysis. In its original form it can only be used for selenodont, plagiolophodont, and ectolophodont ungulate molars, but the principle can be extended to other kinds of tooth morphology. We introduce a new method of dietary analysis for proboscideans similar to the mesowear method, based on angle measurements from worn dentin valleys reflecting the relief of enamel ridges. The enamel ridges should be heavily worn when the abrasiveness of diet increases, resulting in lower occlusal relief and larger angles. For testing this, we compared the mesowear angles with stable carbon isotope values from dental enamel from populations of extant and fossil species from localities from Kenya and India. This enables us to compare diet and tooth wear in proboscideans, because the stable carbon isotope ratios in tropical environments provide a reliable standard for assessing the relative amounts of C4 and C3 plants in diet, and most of the C4 plants are grasses, which should be reflected in the mesowear signal.

SUPPLEMENTAL DATA—Supplemental materials are available for this article for free at www.tandfonline.com/UJVP

INTRODUCTION

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Several methods for paleodietary analyses applicable for fossil mammal teeth have been developed during the last decades (Walker et al., 1978; Lee-Thorp and van der Merwe, 1987; Cerling et al., 1997; Fortelius and Solounias, 2000; Ungar et al., 2003; Evans et al., 2007). Mammal teeth are abundant in the fossil record, and they form the basis of research concerning mammal paleocommunities. Tooth morphology of herbivorous mammals, both primary morphology shaped by evolutionary history and secondary morphology caused by tooth wear, reflects diet and thus vegetation, environmental conditions, and ultimately climate. Analyzing paleodiets is important because it provides fundamental information about evolution and paleoecology of mammals but also serves as a proxy for vegetation and large-scale regional climate changes.

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A method called ‘mesowear analysis’ introduced by Fortelius and Solounias (2000) is an easy-to-do and robust method for analyzing the average dietary signal of herbivorous mammals. In its original form, the mesowear method is based on simple scoring of molar tooth cusp height and sharpness: low-abrasive browsing diet allows attrition (tooth-to-tooth wear) to dominate, causing high and sharp-cusped occlusal relief to develop, whereas abrasive plant material (like grass) causes the cusps to wear down lower and rounder, eventually ending up very low and blunt in

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specialized grazing mammals. Mesowear analysis has been extensively used by several researchers in dietary analysis of fossil mammal herbivores ranging from late Miocene hipparionine horses (Kaiser, 2004) to South American endemic ungulates (Croft and Weinstein, 2008) and Pleistocene rhinoceroses (Kahlke and Kaiser, 2010). Mesowear analysis of ungulate faunas offers us a powerful tool for reconstructing paleoenvironments (Kaiser and Schulz, 2006). Mesowear scoring is easy and quick to do, and it enables large, statistically significant samples to be studied, but in its original form it can only be applied to ungulates with selenodont (molars with anteroposteriorly elongated, crescent-shaped cusps, like those of ruminants) or ectolophodont (molars with anteroposterior buccal loph, like those of rhinoceroses) tooth morphologies, because they have buccal ridges showing wear facets in their molars. It is, however, possible to extend the method for herbivorous mammals with other kinds of tooth morphology. We propose here a new method similar in principle to the original mesowear method, but applicable to proboscidean molars for which the traditional mesowear method is not applicable as such.

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We measured ‘mesowear angles’ from molar teeth of several extinct species of lamellar-toothed proboscideans from East Africa, the modern African elephant from Tsavo East National Park, and the modern Asian elephant from India and compared them with stable carbon isotope results from the same populations in order to develop a new tooth wear-based method for dietary analysis applicable also for fossilized proboscidean molars.

The idea for the new dietary analysis method is similar in principle to the traditional mesowear analysis introduced by

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Fortelius and Solounias (2000). The traditional mesowear method cannot be used as such for the molar teeth of elephants because, due to the specialized fore-aft chewing and thus the oblique rubbing of the enamel lamellae against each other, easily measurable facets do not develop on the occlusal surface. However, the principles of tooth wear in proboscideans are expected to be similar to other ungulates, so the hypothesis is that abrasive plant material (such as grass) accelerates and equalizes the wear rates of enamel and dentin, causing a flatter occlusal relief, whereas nonabrasive material (soft dicotyledonous plants) enables tooth-against-tooth wear (attrition) to dominate so that the more wear-resistant enamel ridges scour deeper valleys into the softer dentin, thus maintaining a higher relief. The mechanisms of tooth wear are not yet fully understood; for example, it still remains to be confirmed whether it is exogenous quartz dust or phytoliths that make grass particularly abrasive on dental enamel (Lucas and Omar, 2012). Nevertheless, there is extensive empirical evidence that grass causes abrasion on dental enamel, and it is reflected in the mesowear signal of ungulate molars (Fortelius and Solounias, 2000; Louys et al., 2012). A simple analysis method similar to mesowear analysis can be developed for elephants by measuring angles from the bottoms of dentin valleys when the sides of the angle are placed as tangents to the top of the enamel ridges. The bigger the angles are, the flatter the occlusal surface is, which we hypothesize would indicate more abrasive components in the diet.

Stable isotope ratios in tooth enamel offer proxy methods for dietary analyses of several mammal groups. Values of $\delta^{13}\text{C}$ differ in C3 and C4 photosynthesizing plants, so they impart different isotopic signatures in dental tissue of herbivorous mammals consuming them (Lee-Thorp and van der Merwe, 1987; Cerling et al., 1997). Thus, the diets of herbivorous mammals eating primarily C3 plants can be separated from those specialized in utilizing C4 plants, most of which are grasses, by analyzing isotope ratios in tooth enamel. This methodology can of course only be used for comparing the fractions of C3 and C4 plants in the diets, but it is useful because a strong C4 signal reflects diets based on grasses where C4 grasses dominate (Cerling et al., 1997).

It is essential to test the method with proboscidean populations for which diet has been previously analyzed. This is also necessary for determining boundary values of mean mesowear angles of the molars separating browse-dominated and graze-dominated diets in elephants. Values of $\delta^{13}\text{C}$ in the tooth enamel reflect the $\delta^{13}\text{C}$ in the diet. Tropical grasses (except bamboos) are mostly C4 photosynthesizing, so the amount of grass in the diet of herbivorous mammals in tropical African savannah can be roughly estimated from $\delta^{13}\text{C}$ in tooth enamel. This is also the case in tropical Asia. We compared the mesowear angles with $\delta^{13}\text{C}$ values analyzed from the same populations and individual tooth specimens of tropical East African and Asian proboscideans in order to connect the mesowear angles with diet. Comparing traditional mesowear signals and stable isotope records of African antelopes has shown that there is good correlation between them, which gives support to our approach (Louys et al., 2012).

MATERIALS AND METHODS

Stable isotope analyses had already been undertaken for some of the teeth from the African elephant molar collection of the Tsavo Research Station, Tsavo East National Park, Kenya, and for fossil proboscideans from Kenyan late Neogene localities stored in the collections of the National Museum of Kenya (Cerling et al., 1999, 2004), so the material for this study comes primarily from those collections. A few specimens of modern Asian elephants from India and some African elephant specimens from various locations stored at the collections of the American Museum of Natural History were included as well. Tropical East

African and Asian proboscideans were deliberately chosen as the study material because $\delta^{13}\text{C}$ values in their tooth enamel can be used as a proxy for the proportion of C4 grass in their diet. Values of $\delta^{13}\text{C}$ give estimates of the significance of C4 grasses in diets from the tropics since the late Miocene, which is the age of the oldest proboscidean samples analyzed in this study (Cerling et al., 1999). C4 photosynthesizing plants have different $\delta^{13}\text{C}$ values (-10‰ to -14‰) than C3 photosynthesizing plants (-22‰ to -35‰) (Cerling et al., 1997). These values of $\delta^{13}\text{C}$ are fractionated in tooth enamel consistently and differ in mesic and xeric environments and in different groups of herbivorous mammals (Supplementary Data, Table S1; see also Cerling et al., 1997). Large ruminants have ca. 14.5‰ fractionation and small non-ruminants ca. 12‰ fractionation of $\delta^{13}\text{C}$ values in enamel. We used the 14.5‰ fractionation of the large ruminants (because they are large herbivores like the proboscideans) for determining which $\delta^{13}\text{C}$ values correspond with >70% of C3 and C4 and >90% of C3 and C4 in the diet. These values were averaged from the values in mesic and xeric environments, because proboscideans occupy both kinds of environments and the environment type of the fossil taxa are often not well defined (Supplementary Data, Table S1). The averaged values were used as ‘threshold’ values for C3-dominated (>70%) and pure C3 (>90%) diets and C4-dominated (>70%) and pure C4 (>90%) diets. Values of $\delta^{13}\text{C}$ enable us to test the hypothesis that flat occlusal wear angles reflect strong abrasion caused by grazing, because $\delta^{13}\text{C}$ values should reflect the proportion of C4 grasses in proboscidean diets.

Columbian mammoth (*Mammuthus columbi*) from Rancho La Brea, California, was chosen as a case study for testing the new method in assessing the diet of an extinct elephant species for which stable isotope ratios do not give results directly comparable to those of the species from tropical Africa and Asia, because Rancho La Brea is outside the tropical zone where grasses are mostly C4 photosynthesizing and where the $\delta^{13}\text{C}$ values directly reflect the amount of grasses in the diet. The Columbian mammoth molars studied here are stored at the Page Museum in Los Angeles, California, U.S.A.

Most of the specimens studied here are last lower molars (m3) of old proboscidean individuals, but specimens representing all the teeth (except dp2 and dp3 milk molars of very young individuals) were included. The reason for choosing mainly lower molars and premolars is purely practical: there were more specimens available. However, upper molars were also included, and we consider this justified because the mesowear angles do not differ significantly from those of the lower molars (Supplementary Data, Table S2; see also Kaiser and Fortelius, 2003).

The method requires measuring angles from the bottom of the dentin valleys to the top of enamel lamellae on the occlusal surface of elephant molars representing moderate states of wear (Fig. 1). These ‘mesowear angles’ record the relief on the occlusal surface of the molars and should reflect the abrasiveness of the diet. We used the dentin valleys within the lamellae instead of the cement valleys between the lamellae for measuring the mesowear angles. The reason for this is that it is primarily the lamellae, not the cement between the lamellae, that shear against each other during the mastication of elephants, and the dentin valleys developing within the lamellae should thus record the dietary signal more consistently than the wear in the cement valleys. The angles were always measured from the center of the lamellae or from the widest part of the worn dentin valley, but in case of the genus *Loxodonta*, not from the especially widened central ‘loop’ of the lamellae, which is a special feature lacking from other genera (Fig. 2D). The angles were measured for all of the dentin valleys preserved on the occlusal surface. Mean mesowear angle was calculated for each molar specimen as the mean of the angles from three central lamellae of the molar. In cases where the number of lamellae was even, the three lamellae

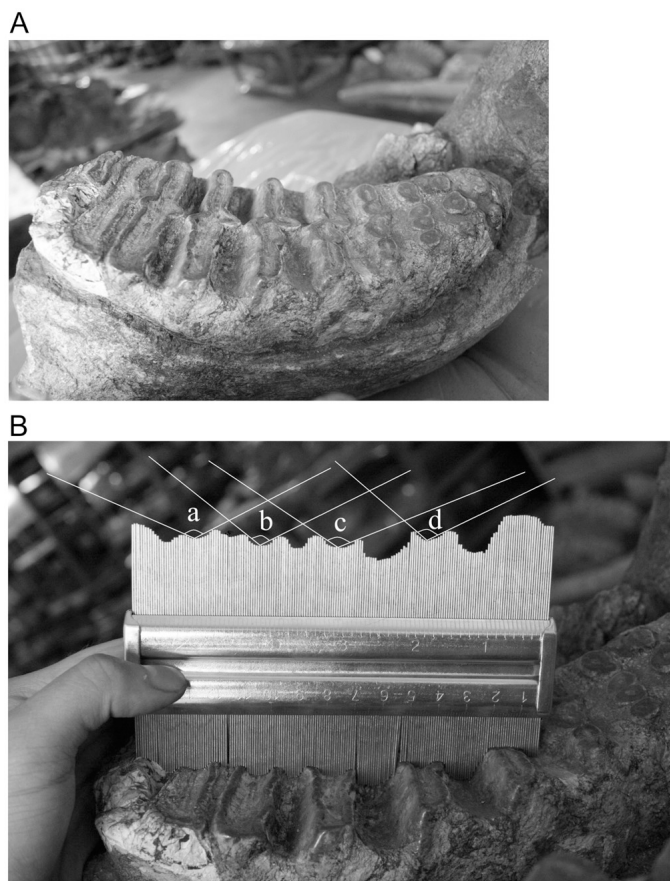


FIGURE 1. Mesowear angles. **A**, a lower third molar of *Loxodonta adaurora* (KNM-KP 385) from Kanapoi, Kenya; the anterior lamellae are in wear and dentin valleys surrounded by enamel ridges have developed as a result of wear; **B**, a contour gauge is used here for illustrating the mesowear angles measured from the dentin valleys (angles a-d).

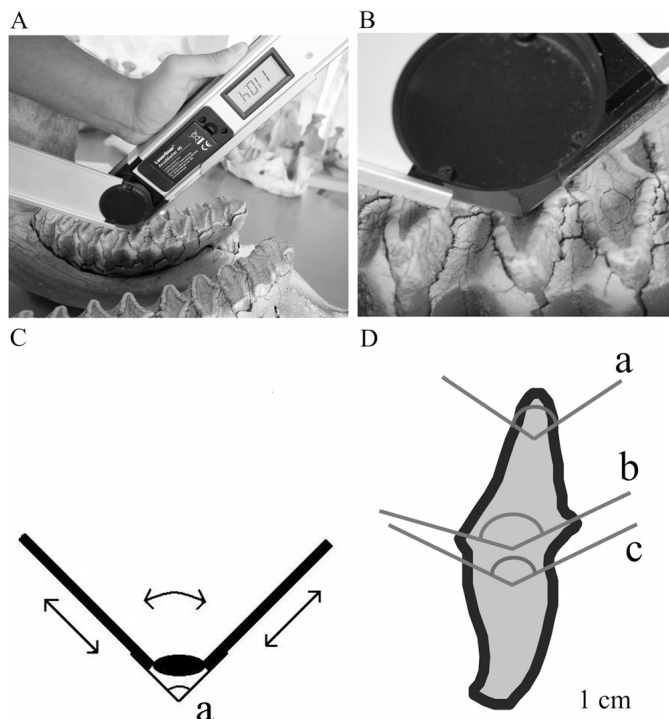


FIGURE 2. The measuring method. **A**, a digital angle meter was used for measuring the mesowear angles from the bottom of the dentin valleys; a lower third molar of an African elephant from the collections of the Tsavo East Research Station (specimen nr. 828) is shown here; **B**, a close-up of the thin metal plates attached to the movable arms of the angle meter to enable the precise fitting of the angle at the bottom of the dentin valley; **C**, a schematic picture of the angle meter: the angle is measured by rotating the two arms of the device, and both of the arms can be extended or retracted so that any angle can be fitted exactly at the point where the thin metal plates attached to the proximal end of the movable arms meet (angle a); **D**, occlusal view of enamel (black line) and dentin valley (gray area) of a central lamella from a lower *Loxodonta africana* molar; the mesowear angle is measured from the center of the lamellae where lamellar width is relatively consistent between species (position c) rather than from the narrower sides (e.g., position a), or in case of the genus *Loxodonta*, from the especially widened center ‘loop’ of the lamellae (position b); note that angle b > angle c > angle a.

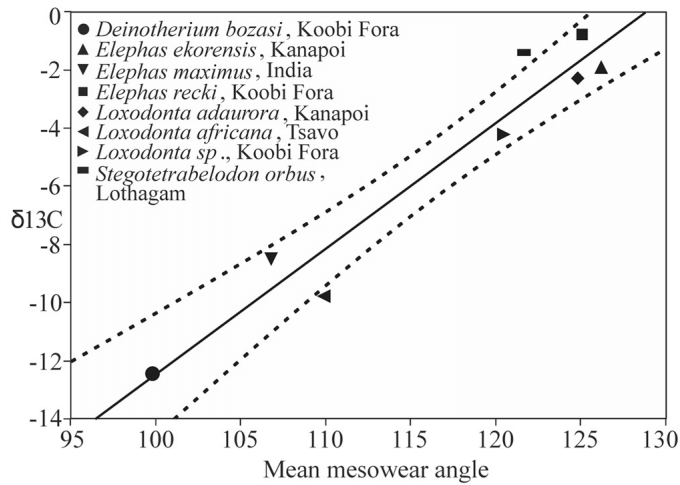
used for calculating the mean mesowear angle were chosen so that one of the lamellae is anterior to the center of the tooth and two are posterior to it. This method of choosing the lamellae for the mean mesowear angle is based on our observations of proboscidean tooth wear: the anterior-most lamellae are subject to very heavy wear and the lamellae are often worn flat, whereas the posterior-most lamellae have not erupted or are not subject to wear. The central lamellae are subject to medium wear and are likely to record the dietary signal most consistently.

The measurements were taken by a digital angle meter with 0.1° precision (Fig. 2), and a contour gauge was used for photographic documentation of the occlusal relief. After collecting the mesowear angle data, mean mesowear angles of three central

lamellae in occlusion were calculated. These mean mesowear angles were then compared with average $\delta^{13}\text{C}$ values sampled from the same population or fossil population of the species for each locality studied. Mean mesowear angles and $\delta^{13}\text{C}$ values were also compared for individual specimens wherever this was possible (the specimens used for $\delta^{13}\text{C}$ sampling are often too fragmentary for obtaining mesowear angles, and the $\delta^{13}\text{C}$ sampling is often not done for the better preserved specimens from

TABLE 1. Mean $\delta^{13}\text{C}$ and mean mesowear angles of the populations with sample sizes (n) and standard errors (SE) of means.

Population	Mean $\delta^{13}\text{C}$ value (‰)	SE ($\delta^{13}\text{C}$)	n	Mean mesowear angle (°)	SE (mesowear angle)	n
<i>Loxodonta africana</i> , Tsavo	-9.8	0.3	18	109.9	1.0	68
<i>Elephas maximus</i> , India	-8.5	0.1	2	106.8	2.2	4
<i>Deinotherium bozasi</i> , Koobi Fora	-12.5	0.3	8	99.8	5.3	3
<i>Elephas recki</i> , Koobi Fora	-0.8	0.3	16	125.0	3.2	13
<i>Loxodonta</i> sp., Koobi Fora	-4.2	2.4	6	120.4	3.7	8
<i>Elephas ekorensis</i> , Kanapoi	-1.9	0.3	3	126.2	3.4	5
<i>Loxodonta adaurora</i> , Kanapoi	-2.3	0.6	3	124.8	4.9	10
<i>Stegotetrabelodon orbus</i> , Lothagam	-1.4	0.5	11	121.7	2.2	8
<i>Mammuthus columbi</i> , Rancho La Brea				135.5	1.7	25



Linear Fit

$$\delta^{13}C = -55.73136 + 0.4328813 * \text{Mean meso wear angle}$$

Summary of Fit

RSquare	0.94
RSquare Adj	0.93
Root Mean Square Error	1.18
Mean of Response	-5.16
Observations (or Sum Wgts)	8

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	130.31	130.31	93.83
Error	6	8.33	1.39	Prob > F
C. Total	7	138.64		<0.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-55.73	5.24	-10.64	<0.0001*
Mean mesowear angle	0.43	0.04	9.69	<0.0001*

FIGURE 3. Linear regression of mean $\delta^{13}C$ values from tooth enamel and mean mesowear angles in the molars sampled from proboscidean populations. The 95% confidence curves are shown as dashed lines. $R^2 = 0.94$, $P < 0.001$.

which mesowear angles could be measured). Boundary $\delta^{13}C$ values separating grass-dominated and browse-dominated diets were used for estimating similar boundary values for mesowear angles. Finally, we compared the means of mesowear angles in all the proboscidean species we studied here. In the population-level comparisons, some but not all the $\delta^{13}C$ values come from the same specimens as the mesowear angle values.

RESULTS AND DISCUSSION

Mean $\delta^{13}C$ values and mean mesowear angles of the proboscidean populations and fossil assemblages examined in this study, with sample sizes and standard deviations, are shown in Table 1, and the values for all the proboscidean specimens used in the specimen-level comparisons are listed in Supplementary Data (Table S2). Linear regression between the mean mesowear angle

of three central lamellae and mean $\delta^{13}C$ of the extant populations and fossil assemblages of East African proboscideans and extant Asian elephant shows a strong and statistically significant positive correlation (Fig. 3). This demonstrates that the angle measurements can be used as a proxy of the proportion of C4 grass in the diet of proboscideans, if the abrasion on dental enamel is mostly caused by C4 grass, which is largely the case in tropical East Africa and Asia since late Miocene, the age of the oldest samples studied here (Cerling et al., 1999). Because grass has been observed to be the main abrasive component in herbivore diets, we suggest that the mesowear angle method can be used for roughly assigning proboscidean diets as grass- or browse-dominated also outside of tropical areas. The great benefit of this method is that it indicates directly and robustly the amount of abrasive vs. nonabrasive material in proboscidean diets independently of plant photosynthesis mechanisms. This allows us to detect C3 grazing, which the $\delta^{13}C$ values do not show. Moreover, the mesowear method is fast, easy-to-do, and globally applicable. The isotopic method is relatively laborious compared with the mesowear method, it requires minor damaging of the studied specimens for extracting enamel material for the analysis, and it may contain a risk of contamination.

We calculated, using the linear regression, the boundary values of mean mesowear angles corresponding to the averaged boundary values in $\delta^{13}C$ for >70% and >90% of C3 and C4 plants consumed in the diet (Fig. 4). There is a lot of variation in the $\delta^{13}C$ values in vegetation depending on environment and plant physiology. For example, the $\delta^{13}C$ boundary value of ca. -7‰ can correspond with a proportion of 70–100% of C3 plants in diet (Supplementary Data, Table S1), so the boundary values are not to be interpreted as absolute measures of the ratio of C3/C4 plants in the diet, but rather as thresholds marking the limit beyond which the values indicate C3 or C4 dominant and essentially pure C3 or C4 diets.

The enamel $\delta^{13}C$ cutoff value of ca. -7‰, which separates C3-dominated diet from diets with significant C4 component, corresponds with the mean mesowear angle of ca. 113°. Below this value there can be 70–100% of C3 plants in the diet depending on the environment, but above it there has to be C4 plants included. Below ca. -10‰ and the corresponding mesowear angle of 106°, the diet consists almost purely of C3 plants (90–100% C3) and would roughly correspond with the traditional dietary class of ‘browsers.’ C4-dominated diets (70–100% C4) are marked by a $\delta^{13}C$ threshold value of -2 and a corresponding mean mesowear angle of 124°. Above ca. 0‰ and the corresponding mesowear angle of 130°, the diet consists almost purely of C4 plants (90–100% C4), corresponding roughly with the traditional dietary class of ‘grazers.’ The C3 ‘end member’ value of ca. -21‰ in enamel corresponds with a mean mesowear angle of 80°, and the ‘end member’ C4 value of ca. 4‰ corresponds with the mean mesowear angle of 138°.

Whereas the correlation of mean mesowear angle and $\delta^{13}C$ values is excellent and highly significant at the level of populations, the correlation at the level of individual specimens is weaker but still significant (Fig. 5). Note that in the specimen-level analysis, several species are included and within the species there is no significant correlation between the $\delta^{13}C$ values and the mesowear angles. There is a lot of variance in the angle measurements compared with the $\delta^{13}C$ values in individual tooth samples. There are several reasons for this. First of all, the sample size of individual specimens with both measurable mesowear angles and a corresponding $\delta^{13}C$ value is in this study probably too low for an extensive comparison. It is thus possible that this result is biased and requires more material with more variance. However, it is also possible that the correlation is not good at the individual level because the stable isotope composition forms in the enamel much earlier in an individual’s lifetime than the tooth wear. It is possible that there is such a lot of variance in the

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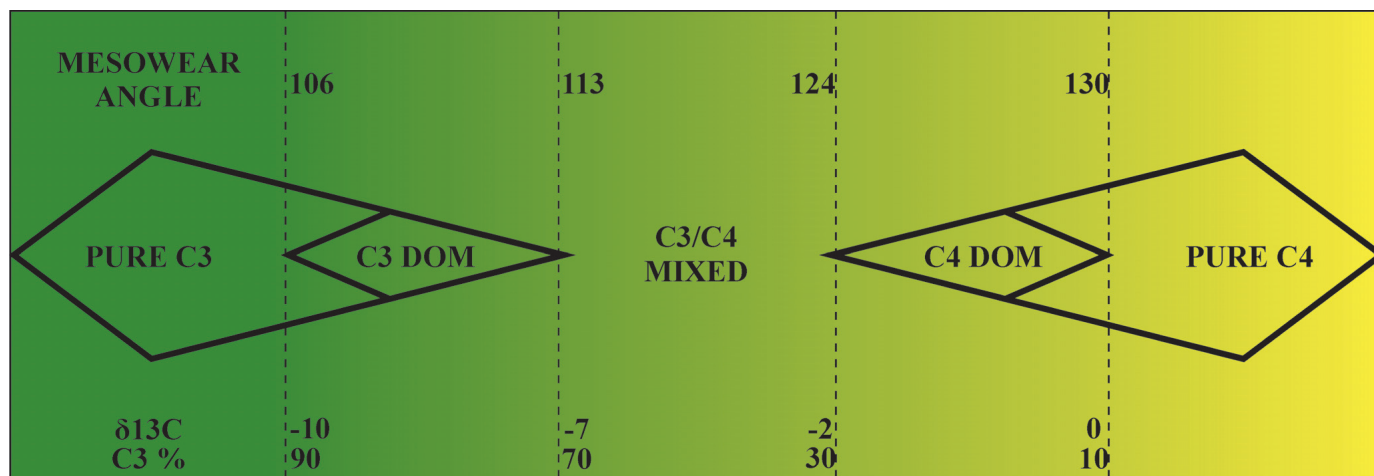
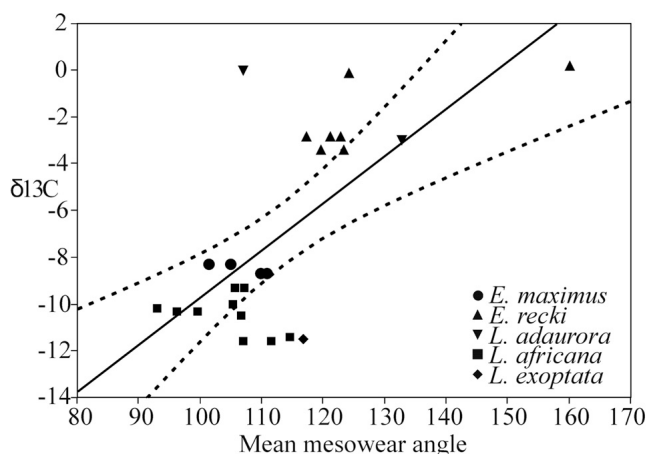


FIGURE 4. C3/C4 dietary spectrum of proboscideans from tropical Africa and Asia with boundary values of $\delta^{13}\text{C}$ and the corresponding (mean) mesowear angles. The $\delta^{13}\text{C}$ boundary values are based on the averaged values for mesic and xeric environments from Table S1 in Supplementary Data. Under the $\delta^{13}\text{C}$ value of ca. -7‰ and the corresponding mesowear angle of 113° , the diet is C3-dominated ($>70\%$ C3 plants, browse-dominated), and under the $\delta^{13}\text{C}$ value of ca. -10‰ and the corresponding mesowear angle of 106° , the diet comprises on average $>90\%$ C3 plants (browsing). Above the $\delta^{13}\text{C}$ value of ca. -2‰ and mesowear angle of 124° , the diet is C4-dominated ($>70\%$ C4 plants, grass-dominated), and above the $\delta^{13}\text{C}$ value of ca. 0‰ and mesowear angle of 130° , the diet comprises on average $>90\%$ C4 plants (grazing).



Linear Fit

$$\delta^{13}\text{C} = -29.88455 + 0.2019723 * \text{Mean mesowear angle}$$

Summary of Fit

RSquare	0.45
RSquare Adj	0.43
Root Mean Square Error	3.15
Mean of Response	-7.01
Observations (or Sum Wgts)	24.00

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	180.18	180.18	18.13
Error	22	218.64	9.94	Prob > F
C. Total	23	398.82		0.0003*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-29.88	5.41	-5.52	<0.0001*
Mean mesowear angle	0.20	0.05	4.26	0.0003*

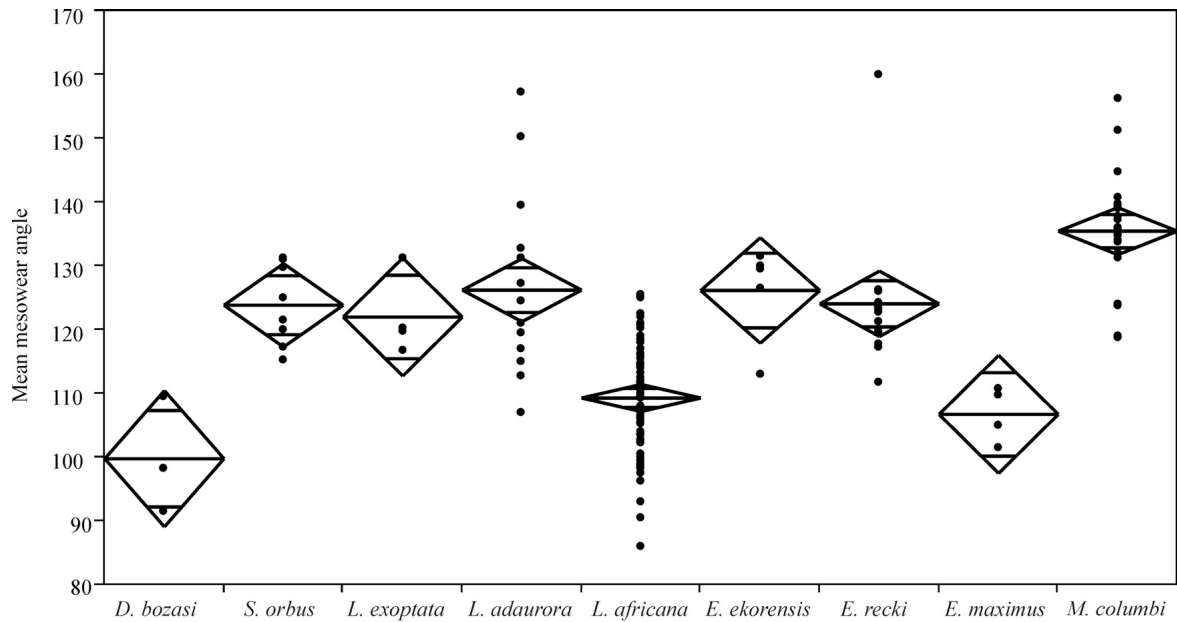
FIGURE 5. Linear regression of $\delta^{13}\text{C}$ values from tooth enamel and mesowear angles of individual molar specimens. The 95% confidence curves are shown as dashed lines. $R^2 = 0.45$, $P = 0.0003$.

dietary preferences of proboscidean individuals during their lifetime that $\delta^{13}\text{C}$ values and mesowear actually do not record the same dietary signal for an individual even if the variables correlate well at the level of populations. Several studies have shown that there is significant seasonal variation in the diets of the modern African and Asian elephants (Sukumar and Ramesh, 1995; Cerling et al., 2006, 2009; Rivals et al., 2012). We also noticed that sometimes there is quite a remarkable difference in the mesowear angles of the left and right sides of an individual jaw (but always less than 10°), which is probably due to uneven use of the jaw halves in mastication. Based on all these observations, we conclude that the tooth wear-based diet analysis method for proboscideans should only be used at the level of populations or species, not individual dental specimens, and sufficient sample sizes (more than five, preferably more than 10) are strongly recommended in any study using this method.

Both the modern African elephant and the Asian elephant are mixed-feeders, and several studies have shown that their diet is C3-dominated (Sukumar and Ramesh, 1995; Cerling et al., 1999). Although the diet of modern elephants is on average C3-dominated, some individuals may have remarkable C4 component in their diet. This has been noticed especially in the elephant populations living in open, grass-dominated environments such as the Tsavo East African elephant population in Kenya (Cerling et al., 1999, 2004). Our results support this: the particularly large sample from Tsavo East shows a lot of variance in the mean mesowear angle (up to ca. 120° , suggesting a significant C4 component in diet), whereas the average value is ca. 110° , suggesting on average C3-dominated diet.

Our comparison of the means of mesowear angles using pairwise Student's t-tests (Fig. 6) of the species supports the results from earlier studies (Sukumar and Ramesh, 1995; Cerling et al., 1999, 2004). The modern African elephant (*Loxodonta africana*) and Asian elephant (*Elephas maximus*) populations have significantly smaller mean mesowear angles than all the extinct elephant species and are in this respect closer to the deinotherium *Deinotherium bozasi*. The mean mesowear angles of the African elephants of Tsavo East and the small sample of Asian elephant from India indicate browse-dominated diet, and the small sample of *D. bozasi* from Koobi Fora, Kenya, has even smaller mesowear angles, indicating pure browsing diet. All the East African fossil elephant species (*Stegotetrabelodon orbis*, *Elephas*

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Species	- Species	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
<i>M. columbi</i>	<i>D. bozasi</i>	35.68	5.72	24.38	46.99	<0.0001*
<i>M. columbi</i>	<i>E. maximus</i>	28.73	5.04	18.77	38.70	<0.0001*
<i>L. adaurora</i>	<i>D. bozasi</i>	26.44	5.95	14.67	38.21	<0.0001*
<i>E. ekorensis</i>	<i>D. bozasi</i>	26.38	6.83	12.87	39.89	0.0002*
<i>M. columbi</i>	<i>L. africana</i>	26.16	2.16	21.89	30.42	<0.0001*
<i>E. recki</i>	<i>D. bozasi</i>	24.29	5.99	12.44	36.14	<0.0001*
<i>S. orbus</i>	<i>D. bozasi</i>	24.06	6.34	11.54	36.59	0.0002*
<i>L. exoptata</i>	<i>D. bozasi</i>	22.23	7.15	8.10	36.35	0.0023*
<i>L. adaurora</i>	<i>E. maximus</i>	19.49	5.31	9.00	29.98	0.0003*
<i>E. ekorensis</i>	<i>E. maximus</i>	19.43	6.28	7.02	31.84	0.0024*
<i>E. recki</i>	<i>E. maximus</i>	17.34	5.35	6.76	27.92	0.0015*
<i>S. orbus</i>	<i>E. maximus</i>	17.11	5.73	5.78	28.44	0.0033*
<i>L. adaurora</i>	<i>L. africana</i>	16.91	2.72	11.53	22.29	<0.0001*
<i>E. ekorensis</i>	<i>L. africana</i>	16.85	4.32	8.31	25.40	0.0001*
<i>L. exoptata</i>	<i>E. maximus</i>	15.28	6.62	2.19	28.36	0.0224*
<i>E. recki</i>	<i>L. africana</i>	14.76	2.81	9.21	20.32	<0.0001*
<i>S. orbus</i>	<i>L. africana</i>	14.54	3.48	7.66	21.41	<0.0001*
<i>M. columbi</i>	<i>L. exoptata</i>	13.46	5.04	3.50	23.42	0.0085*
<i>L. exoptata</i>	<i>L. africana</i>	12.70	4.80	3.21	22.19	0.0091*
<i>M. columbi</i>	<i>S. orbus</i>	11.62	3.80	4.11	19.14	0.0027*
<i>M. columbi</i>	<i>E. recki</i>	11.39	3.20	5.07	17.72	0.0005*
<i>L. africana</i>	<i>D. bozasi</i>	9.53	5.51	-1.36	20.42	0.0859
<i>M. columbi</i>	<i>E. ekorensis</i>	9.30	4.59	0.24	18.37	0.0443*
<i>M. columbi</i>	<i>L. adaurora</i>	9.24	3.12	3.07	15.42	0.0036*
<i>E. maximus</i>	<i>D. bozasi</i>	6.95	7.15	-7.18	21.08	0.3326
<i>L. adaurora</i>	<i>L. exoptata</i>	4.22	5.31	-6.27	14.70	0.4282
<i>E. ekorensis</i>	<i>L. exoptata</i>	4.15	6.28	-8.26	16.57	0.5092
<i>L. africana</i>	<i>E. maximus</i>	2.58	4.80	-6.91	12.07	0.5924
<i>L. adaurora</i>	<i>S. orbus</i>	2.38	4.15	-5.82	10.58	0.5674
<i>E. ekorensis</i>	<i>S. orbus</i>	2.32	5.34	-8.23	12.86	0.6647
<i>L. adaurora</i>	<i>E. recki</i>	2.15	3.60	-4.97	9.28	0.5517
<i>E. ekorensis</i>	<i>E. recki</i>	2.09	4.93	-7.65	11.83	0.6719
<i>E. recki</i>	<i>L. exoptata</i>	2.06	5.35	-8.51	12.64	0.7002
<i>S. orbus</i>	<i>L. exoptata</i>	1.84	5.73	-9.49	13.17	0.7490
<i>E. recki</i>	<i>S. orbus</i>	0.23	4.21	-8.09	8.54	0.9570
<i>L. adaurora</i>	<i>E. ekorensis</i>	0.06	4.88	-9.58	9.70	0.9901

FIGURE 6. Comparison of the means of mean mesowear angles (°) in the proboscidean species studied here by pairwise Student's t-tests. Significantly different species pairs are marked in the table by boldface. The modern African and Asian elephants together with the extinct deinotheres (*Deinotherium bozasi*) have significantly smaller mean mesowear angles than the fossil elephant species, indicating a much less abrasive diet. As a preliminary case study, the mean mesowear angles of *Mammuthus columbi* from Rancho La Brea (late Pleistocene, California) were analyzed together with the species used for the comparisons with isotope data. *M. columbi* has significantly larger mean mesowear angles than any of the other species, which indicates that it had a remarkably abrasive diet, and it was likely a specialized grazer.

ekorensis, *E. recki*, *Loxodonta exoptata*, and *L. adaurora*) are similar to each other in having significantly larger mean angles than the extant African and Asian elephants, indicating on average grass-dominated diets. These results correspond perfectly with the results from the carbon isotope studies (Sukumar and Ramesh, 1995; Cerling et al., 1999). Standard errors of the mean mesowear angles of each proboscidean species are given in Supplementary Data (Table S3). The Columbian mammoth (*Mammuthus columbi*) from the late Pleistocene of Rancho la Brea, used here as a pilot case for using the tooth wear–based dietary analysis method outside tropical areas, shows significantly larger mean mesowear angles (135.5°) than any of the other species studied, exceeding the ‘pure grazer’ threshold value of 130° (Table 1, Fig. 6). Thus, we conclude that *Mammuthus columbi* was likely a specialized grazer, and certainly at least a grass-dominated feeder. This result agrees with studies of preserved gut contents and dung of *M. columbi* from Wasatch Mountains (Gillette and Madsen, 1993), Utah, and Bechan and Cowboy Caves (Mead et al., 1986; Hansen, 1980) from Colorado Plateau, Utah, which show that the diet was clearly dominated by grasses and sedges, although woody plants were taken as well.

CONCLUSIONS

Angle measurements can be used as a measure of occlusal relief in Proboscidea. These mesowear angles correlate well with stable carbon isotope signals from tooth enamel at the population level and thus reflect the proportion of C4 plants in the diet of tropical proboscideans and, more generally, the proportion of abrasive foods (principally grass) in their diet. We used the mesowear angle method to investigate the diet of the extinct Columbian mammoth (*Mammuthus columbi*) population from the late Pleistocene locality of Rancho la Brea, California, and the results indicate grazing diet.

The important scientific contribution and methodological benefits of the mesowear angle method can be listed here as follows:

1. This is the first easy-and-quick method for proboscidean diet analysis, because it requires only measuring angles from the bottom of dentin valleys between the enamel lamellae of the tooth surface, thus depending on the relative height of the lamellae. It can be efficiently done for relatively large samples of teeth.
2. It gives a robust signal of the average diets in populations.
3. It is readily applicable for fossil teeth, and it enables for the first time extensive dietary analyses of the large samples of fossil proboscidean teeth stored in museum collections all over the world.
4. It can be easily used for relatively well-preserved fossilized teeth without risk of biases or contamination caused by diagenesis.
5. It will not damage the tooth samples in any way because it only requires measuring.



ACKNOWLEDGMENTS

We owe great thanks to the Kenya Wildlife Service, National Museums of Kenya (Nairobi), Page Museum (Los Angeles), and American Museum of Natural History (New York) for providing the material for this study. We thank M. Muungu from the National Museums of Kenya, J. Meng, J. Galkin, R. McPhee, and E. Westwig from the American Museum of Natural History, and J. Harris and S. Cox from the Page Museum for providing access to the collections and helping with logistics. J. Saarinen was working as a research associate of the University of Nairobi,

Kenya, while conducting the research in the Tsavo East African elephant collections of the Tsavo East Research Station of the Kenya Wildlife Service. Research cooperation with the National Museums of Kenya was made possible by a Memorandum of Understanding between the Finnish Museum of Natural History and the National Museums of Kenya. We thank F. Karanja, E. Mitema, and D. Kiarie from the University of Nairobi for their help in arranging the research affiliation, and Deputy Vice-Chancellor L. Irungu and the Dean of the Faculty of Veterinary Medicine, N. Munene J., for accepting the research affiliation with the University of Nairobi. We thank the reviewers A. Lister and G. Semprebon and the editors J. Harris and A. Goswami for helpful comments and suggestions that helped us improve this article. The work was funded by the Finnish Graduate School of Geology, Nordenskiöld-samfundet, and the Finnish Cultural Foundation.

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- Submitted July 31, 2013; revisions received April 3, 2014; accepted April 13, 2014.
Handling editor: Anjali Goswami.
- Citation for this article: Saarinen, J., A. Karme, T. Cerling, K. Uno, L. Sällä, S. Kasiki, S. Ngene, T. Obari, E. Mbu, F. K. Manthi, and M. Fortelius. 2015. A new tooth wear-based dietary analysis method for Proboscidea (Mammalia). *Journal of Vertebrate Paleontology*. DOI: 10.1080/02724634.2014.918546 520