Effects of dietary differences between sympatric Japanese serow and sika deer on environmental reconstruction by means of mesowear analysis

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Diet reconstruction using mesowear analysis has mainly been applied to extinct species and their paleoenvironments. Little is known regarding the effects of dietary differences on sympatric environments using this analysis and the limited existing knowledge from extant species may introduce errors when applied to fossil assemblages. I aimed to determine the sensitivity of mesowear analysis using extant ungulates with known diets. An interspecific comparison was conducted using wild populations of Japanese serow (*Capricornis crispus*, *n* = 37) and sika deer (*Cervus nippon*, *n* = 55) living in deciduous broad-leaved forest of the Nikko National Park, central Japan. One of the mesowear variables differed significantly between the two species (Fisher's exact test: *p* < 0.05). According to hierarchical cluster and principal component analyses, Japanese serow were classified as browsers, while sika deer as mixed feeders. Previous studies support these results; therefore, mesowear analysis can be used to detect dietary differences in sympatric species.

Introduction

Paleontologists often refer to guilds of herbivorous mammals to reconstruct paleoenvironments since their feeding preferences reflect the vegetation resources available in specific environments (Vrba 1985, Plummer & Bishop 1994, Fortelius *et al*. 1996). The relationship between food habits and environment of herbivore ungulates has been determined as follows: grazers indicate the presence of either open areas or open patches in closed woody areas, while browsers indicate the presence of more forested environments or bushy/shrubby patches in open landscapes.

Intermediate feeders have fewer environmental constraints with regard to habitat preferences (Kingdon 1997).

The physical properties of food habits are also reflected in tooth wear. Some very general habitat-driven mechanisms have increased the abrasiveness of food available to herbivores; for example, more grit and dust indicates coarser vegetation in drier environments due to increased water stress on plants (Kaiser & Rössner 2007). Mesowear analysis is a method of dietary reconstruction based on occlusal wear of cheek teeth facets of extant species with known diet and habitat preferences (Fortelius

& Solounias 2000). This method is based on visual observation and considers a large number of fossil teeth specimens over a short period of time. For hypsodont and mesodont species, mesowear appears to be stable throughout much of the adult life (Fortelius & Solounias 2000, Rivals *et al*. 2007). In addition, Yamada (2012) showed that teeth have consistent diet-specific mesowear patterns. Therefore, mesowear analysis has been applied as a tool to reconstruct the paleodiet of herbivorous mammals from several localities, from which the type of forage available could be inferred, and indirectly, the paleoenvironment of the locality (Kaiser 2003).

Mesowear analysis indicates diet based on two morphological variables of the buccal cusps of the upper second molar (M2): occlusal relief, and cusp shape. Occlusal relief is classified as "high" or "low" depending on how high the buccal cusps are raised above the valley between them. Occlusal relief measurements are conducted as follows: First, the vertical distance between a line connecting two adjacent cusp tips and the valley bottom between them is measured (Fig. 1). Second, the distance is divided by the entire tooth length. The cut-off point for the selenodont molar is 0.1 (low < 0.1, high ≥ 0.1, Fortelius & Solounias 2000). Relatively "high" occlusal relief is typical for taxa that browse more, whereas "low" occlusal relief is typical for taxa that tend to graze more (Fortelius & Solounias 2000). Cusp shape is classified as "sharp," "round," or "blunt." A sharp cusp has practically
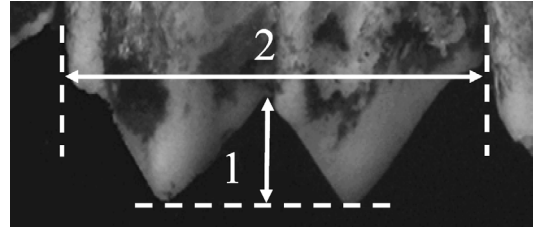

Fig. 1. Occlusal relief was measured as the vertical distance between a line connecting two adjacent cusp tips and the valley bottom between them (marked 1 in the figure) divided by the length of the whole tooth (marked 2 in the figure).

no rounded area between the mesial and distal facets. A round cusp has a distinct round tip without planar facet wear but with retained facets on the lower slopes (Fig. 2). A blunt cusp lacks distinct facets altogether (Fortelius & Solounias 2000). Browsers tend to have a sharp cusp, and grazers, which consume a more abrasive diet, tend to have rounded or blunted cusps.

However, this relatively new assessment method has a few problems, one of which is the interpretation of results for the large mammal fossil assemblage horizon. Several studies on extinct sympatric species (MacFadden 2000, Calandra *et al*. 2008) suggested they have different diet. For example, Blondel *et al*. (2010) applied mesowear analysis and reported that fossil bovids from the late Miocene sediments of Toros-Menalla (Chad) had different mesowear patterns although this mammalian assemblage inhabited
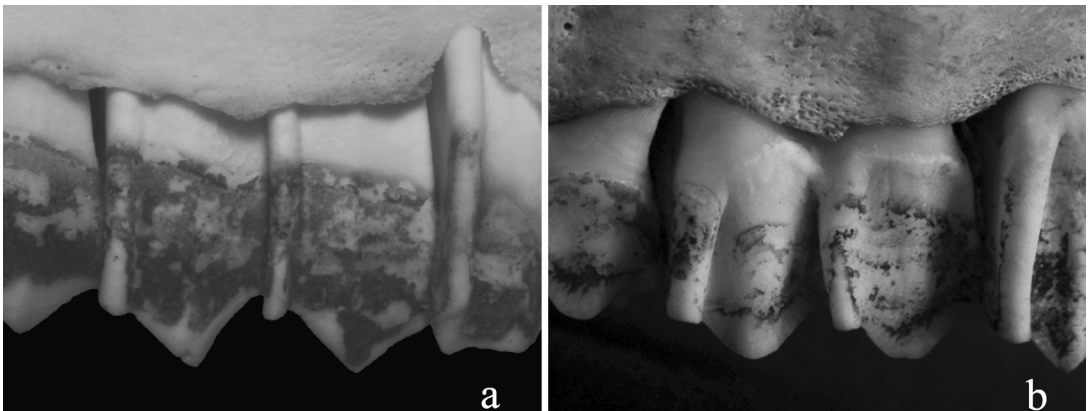

Fig. 2. Molars of two ungulates (buccal view of left M2, teeth not to scale). (**a**) Japanese serow (high occlusal relief and sharp cusp); (**b**) sika deer (high occlusal relief and round cusp).
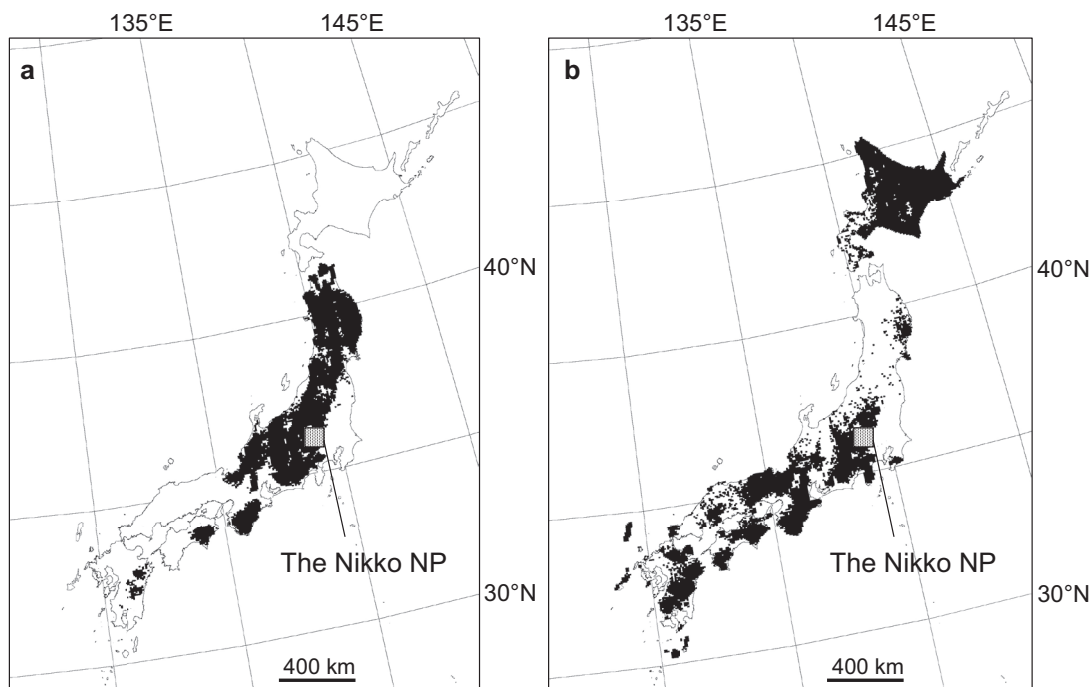
Fig. 3. Distribution of (**a**) Japanese serow and (**b**) sika deer between 1978 and 2003 (modified from Biodiversity Center of Japan 2004), and the locality of the Nikko National Park (NP, Tochigi Pref, Japan).

the same area (Le Fur *et al.* 2009). For investigating the paleoenvironment, it is necessary to determine how mesowear exactly reflects the dietary segregation. Although Fortelius and Solounias (2000) showed that mesowear replicates the known local sequence of the Serengeti feeding succession, their samples were drawn from a variety of locations. Therefore, mesowear data from sympatric ungulates with known food habits are strongly recommended.

This study was focused on the dietary segregation of two extant ungulates in Japan: the Japanese serow (*Capricornis crispus*, Bovidae) and the sika deer (*Cervus nippon*, Cervidae). In general, these two species are not sympatric in most of Japan (Fig. 3). The Japanese serow lives in higher mountains, whereas sika deer usually inhabit mountains and lowlands and tend to prefer flat habitats (Takatsuki *et al.* 2010). However, in the Nikko National Park (NP, central Japan, Fig. 3), previous ecological studies have shown that these two ungulates exist almost sympatrically despite having contrasting food habits.

As Nowicki and Koganezawa (2001) described, the park is a mountainous area with

many peaks over 2000 m (the highest is 2578 m) a.s.l. The timberline is from 2400 to 2500 m a.s.l. The areas above 1500–1600 m a.s.l. are covered by sub-alpine coniferous forests. Broad-leaved forests grow below 1600 m a.s.l. In many areas, up to 1800 m a.s.l. natural forests have been replaced by plantations of Japanese cedar (*Cryptomeria japonica*) (Nowicki & Koganezawa 2001, Takatsuki 1983). The mean annual precipitation and temperature at the Nikko Weather Station (1292 m a.s.l.) in the southern part of the park are 2230 mm and 6.0 °C, respectively (Nowicki & Koganezawa 2001).

In the park, Japanese serow are territorial and solitary. In contrast, sika deer are not territorial and occur in herds. The deer scatter widely over the mountains in summer, while they concentrate in mid-winter (Takatsuki 1983). Their food habits are also contrasting. The Japanese serow is a browser species (Takatsuki and Suzuki 1984, Takatsuki *et al.* 1988, Takatsuki *et al.* 1995, Ochiai 1999, Jiang *et al.* 2008), whereas sika deer have more flexible food habits (reviewed in Takatsuki 2009). In the park, Japanese serow feed on the leaves of deciduous trees and her-

baceous shrubs during the summer, while in winter they feed mainly on needles of coniferous trees (Koganezawa 1999). In contrast, graminoids (i.e., Gramineae, Cyperaceae and Juncaceae), which dominate the forest floor, are an important food for sika deer (Takatsuki 1983). Koganezawa (1999) suggested that the diets of the two species overlap in winter, although Japanese serow rarely fed on graminoids whereas sika deer consumed them at a high rate throughout the year. Nowicki and Koganezawa (2002) also reported no evidence of food competition between these species.

In contrast to previous studies using mesowear analysis, this study attempted a strict comparison of two extant ungulates — the sika deer and the Japanese serow — living in the same locality (Nikko National Park, central Japan) but having contrasting food habits. Outside the park, the Japanese serow feeds mainly on tree leaves (Takatsuki & Suzuki 1984) and shows 100% high occlusal relief, 66.7% sharp cusp and 33.3% round cusp (n = 30; Yamada unpubl. data), whereas several sika deer populations show significantly different mesowear patterns corresponding to their dietary differences (Yamada 2012).

Material and methods

Both of the two ungulates have brachydont molar (Fig. 2). Skull specimens of Japanese serow (n = 37) and sika deer (n = 55) from the Nikko NP used in this study were stored at the Tochigi Prefectural Museum (Japan), and the University Museum, University of Tokyo (Japan) from 1984 to 2005.

Mesowear variables

Teeth were examined visually or using low magnification (10×) magnifying glass. Paracone and metacone were scored for sharper left M2 buccal cusps. Fortelius and Solounias (2000) found an increase in abrasive wear with increasing age in individual extant ungulates. This study used tooth specimens from full eruption of dentitions to loss of the inner profile of the first

molar. In addition, cusps that were damaged were not scored, in accordance with the methods of Fortelius and Solounias (2000).

Reference mesowear data of extant ruminants

Although Fortelius and Solounias (2000) provided reference data for 64 extant ungulates, they selected 27 to form a subset of species for which satisfactory dietary data were available and interpretation was uncontroversial. Their dataset of "typical" species form a good basis for comparison with fossil forms, an was used in most previous studies. In addition, morphologic differences of mammalian teeth could bias the method of wear (Fortelius & Solounias 2000, Blondel *et al.* 2010); therefore, this study only referenced the data from 21 ruminants of the "typical" dataset that share common tooth morphology (selenodont forms) with Japanese serow and sika deer. These ruminants were classified into the following three broad dietary categories as: browsers, consuming < 10% grass; grazers, consuming no browse; and mixed feeders, which fall between these two groups.

Statistical analysis

The frequencies of occlusal relief (high or low) and cusp shape (sharp or round) were compared between populations using Fisher's exact test. The percentages of high occlusal relief, sharp cusp, and round cusp in the sika deer population were then used for comparison with extant ruminants in hierarchical cluster analysis (HCA) with complete linkage (furthest neighbors) based on Euclidian distance. Euclidian distance measures the similarity between each dataset; thus, the closer the data, the smaller the Euclidian distance at the branching points. However, the results of each dataset do not directly indicate the sequential difference because the clusters may flip (Kaiser 2009).

Although previous studies commonly used HCA for reconstruction based on mesowear analysis, hierarchical classification is an exploratory method. Inserting extra samples alters the

morphology of the dendrogram and may possibly yield biased clusters. Therefore, principal component analysis (PCA) of five variables for comprehensive evaluation was also performed in this study.

All statistical analyses were carried out using R ver. 2.11.0 (R Development Core Team 2005).

Results

Comparisons between the Japanese serow and the sika deer populations

The Japanese serow population showed 100% high occlusal relief, while the sika deer population showed only 4.8% low occlusal relief (Table 1). The sharp-cusp frequency was 70.3% in the Japanese serow population but only 50% in the sika deer population. None of the specimens in this study had a blunt cusp. Cusp-shape (sharp and round) frequencies were significantly different between the two populations (Fisher's exact test: $p < 0.05$), whereas no significant difference was found with respect to occlusal relief (high and low; Fisher's exact test: $p = 0.08$).

Comparisons with extant ruminants

Two multivariate analyses were performed using the data from other extant ruminants to investigate which food habits the mesowear variables of Japanese serow and sika deer in the Nikko NP resembled most closely.

Four main clusters were produced from HCA of the typical ruminant dataset: two grazers, one mixed feeder with two subcluster (mixed feeder and mixed feeder with browser), and one browser (Fig. 4). The sika deer population clustered with Grant's gazelle (*Nanger granti*), a mixed feeder. The Japanese serow population clustered with mule deer (*Odocoileus hemionus*) and giraffe (*Giraffa camelopardalis*), both browsers, and their subcluster was connected to mixed feeders.
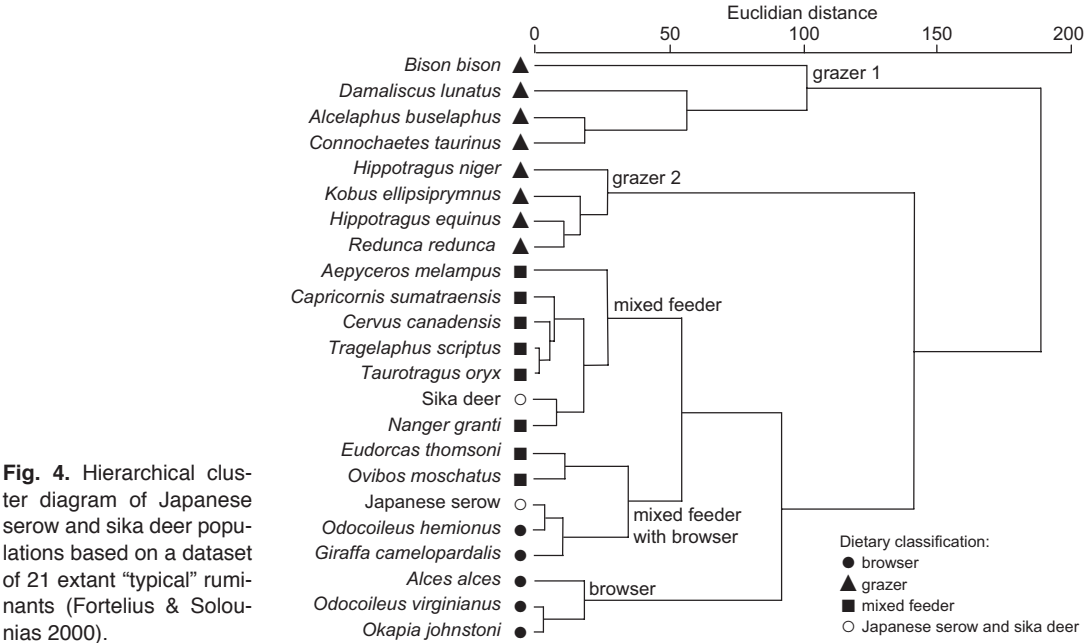
A plot of the first and second principal components (PC1 and PC2) showed good separation of three dietary categories along the PC1 axis but little separation along the PC2 axis (Fig. 5). The proportions of variance for PC1 and PC2 were 66.2% and 30.7%, respectively (Table 2). The percentages of low/blunt cusps and percentages of high/sharp cusps had a great influence on PC1 whereas the percentages of sharp and round cusps had a great influence on PC2 (Fig. 5). The Japanese serow was plotted among browsers, whereas the sika deer population was plotted among mixed feeders.

Discussion

The frequencies of cusp shapes were significantly different between the Japanese serow and the sika deer populations from the Nikko NP. As compared with the sika deer population, the Japanese serow population had a higher proportion of high, sharp cusps, which indicates a browsing diet. In the sika deer population, the percentage

Table 1. Mesowear variables of the two extant ungulates. %L = percentage of low occlusal relief, %H = percentage of high occlusal relief, %S = percentage of sharp cusp, %R = percentage of round cusp, %B = percentage of blunt cusp.

	Number of specimens	Low	High	Sharp	Round	Blunt	%L	%H	%S	%R	%B
Japanese serow											
male	23	0	23	18	5	0	0	100	78.3	21.7	0
female	14	0	14	8	6	0	0	100	57.1	42.9	0
total	37	0	37	26	11	0	0	100	70.3	29.7	0
Sika deer											
male	13	3	10	4	9	0	23.1	76.9	30.8	69.2	0
female	42	2	40	21	21	0	9.1	90.9	45.5	54.5	0
total	55	5	50	25	30	0	4.8	95.2	50	50	0

Fig. 4. Hierarchical cluster diagram of Japanese serow and sika deer populations based on a dataset of 21 extant "typical" ruminants (Fortelius & Solounias 2000).

of low occlusal relief associated with abrasive food habits was 9.1%. However, the deer showed significantly higher percentage of round cusp than the serow. These results suggest that these two ungulates differ in their tooth-wear regimes and this difference correlates with their food habits, even though they were sympatric.

A dietary reconstruction based on HCA with other extant ruminants classified the Japanese serow as a browser and the sika deer as a mixed feeder. Although Japanese serow were classified into the "mixed feeder with browser" subcluster (Fig. 4), PCA of the same dataset also showed the two ungulates to have different dietary patterns. As the mesowear analysis results corresponded

well with the ecology of the two species in the Nikko NP, a valid inference can be made that their mesowear signatures were a result of their different dietary strategy. Results of this study suggest the following two explanations for the outcomes of a heterogeneous dietary reconstruction of fossil assemblages: (1) If mesowear signatures of several species occupying the same area differ from each other but the species are classified as having similar food habits, these differences result most likely from dietary differences. For example, the two ungulates in the Nikko NP inhabit same vegetation (deciduous broad-leaved forest). Their food habits were, however, different although there was an overlap in winter

Table 2. Eigenvectors of the PC1 and PC2 axes for the ruminant dataset, the Japanese serow population, and the sika deer population.

Character	PC1 Eigenvector (factor loading)	PC2 Eigenvector (factor loading)
Percentage of high occlusal relief	−0.521 (−0.948)	−0.218 (−0.270)
Percentage of low occlusal relief	0.521 (0.948)	0.218 (0.271)
Percentage of sharp cusp	−0.421 (−0.766)	0.513 (0.635)
Percentage of round cusp	0.180 (0.327)	−0.761 (−0.943)
Percentage of blunt cusp	0.498 (0.905)	0.252 (0.312)
Eigenvalue	3.312	1.535
Proportion of variance	0.662	0.307

Fig. 5. PCA plot of Japanese serow and sika deer populations based on a dataset of 21 extant "typical" ruminants (Fortelius & Solounias 2000). ● = browser, ▲ = grazer, ■ = mixed feeder. AA: *Alces alces*, GC: *Giraffa camelopardalis*, OH: *Odocoileus hemionus*, OV: *Odocoileus virginianus*, OJ: *Okapia johnstoni*, Me: *Aepyceros melampus*, Cs: *Capricornis sumatraensis*, Cc: *Cervus canadensis*, Gg: *Nanger granti,* Gt: *Eudorcas thomsoni,* Om: *Ovibos moschatus*, To: *Taurotragus oryx*, Ts: *Tragelaphus scriptus*, ab: *Alcelaphus buselaphus*, bb: *Bison bison*, ct: *Connochaetes taurinus*, dl: *Damaliscus lunatus*, he: *Hippotragus equinus*, hn: *Hippotragus niger*, ke: *Kobus ellipsiprymnus*, and rr: *Redunca redunca*. %H = percentage of high occlusal relief, %L = percentage of low occlusal relief, %S = percentage of sharp cusp, %R = percentage of round cusp, %B = percentage of blunt cusp.

(Koganezawa 1999). It is reasonable to assume that the same pattern would apply to extinct species. (2) If mesowear analysis indicates differing food habits, these differences reflect environmental conditions. Yamada (2012) showed that mesowear reflects differences in vegetation. In case several mesowear patterns are detected, a mosaic habitat (i.e., open savanna with closed forest) is most likely to exist. In addition, catastrophic assemblages may contain animals from different habitats. Different habitat fossil assemblages may contain animals from different habitats because of drought or flood. Thus, a taphonomic approach is strongly demanded for reconstructing an ancient environment by mesowear analysis.

The mesowear signal of co-occurring herbivores should not immediately be assumed to reflect differences in forage availability in local areas. Dietary reconstructive methods based on fossilized-teeth wear will not distinguish the cause of wear as to whether it results from environmental differences or dietary segregation of sympatric species. For example, the amount of exogenous grit or dust on leaf surfaces and soil ingested during foraging should be greater in an open and dry habitat. Kaiser *et al*. (2013) presented a hypothesis that those abrasives of very fine particle size were the main factor in tooth wear. Therefore, care should be taken when using this approach as the only pathway to reconstruct habitat change. Stable isotope analysis may be a powerful tool to examine the effect

of these ecological factors on mesowear results. Louys *et al*. (2012) reported that dietary differences suggested by mesowear analysis are also indicated in the stable isotope ratios of individual hair of 16 species of African antelopes.

Based on the results of this study, mesowear analysis of two sympatric ungulates can be used to reconstruct several different food habits. From this perspective, diverse mesowear results of fossil assemblages reflect dietary signals, not the environment.

Acknowledgements

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