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Dental microwear texture analysis of extant sika deer with considerations on inter-microscope variability and surface preparation protocols

Mugino O. Kubo[a,*], Eisuke Yamada[b,c], Tai Kubo[c], Naoki Kohno[d,e]

[a]Department of Natural Environmental Studies, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwanoha 5-1-5, Kashiwa, Chiba 277-8563, Japan

[b]Department of Evolutionary Studies of Biosystems, School of Advanced Sciences, SOKENDAI (The Graduate University for Advanced Studies), Shonan Village, Hayama, Kanagawa 240-0193, Japan

[c]The University Museum, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan

[d]Department of Geology and Paleontology, National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan

[e]Graduate School of Life and Environmental Sciences, University of Tsukuba, Ten'nodai 1-1-1, Tsukuba, Ibaraki 305-8752, Japan

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Abstract

Recently, dental microwear texture analysis (DMTA) has been applied to various kinds of vertebrates to infer dietary preference. More data are needed on inter-microscope variability to assure the objectivity and repeatability of this method. In the present paper, we investigated inter-microscope variability between two confocal laser microscopes with different specifications, as well as variability due to different protocols applied to the obtained surface before DMTA. We used two different methods of DMTA: the scale sensitive fractal analysis (SSFA) and the surface texture analysis (STA). We collected DMTA data of extant Japanese sika deer populations with varying diets. We found that different protocols produced significantly different results for both SSFA and STA, whereas nearly two thirds of the parameters were not significantly different between the machines when applying the same pre-analysis protocol. Finally, we analyzed DMTA data of 244 sika deer from 11 populations. Twenty nine parameters were significantly different among the populations. Tooth enamel surface of grazing sika deer is dominated by a number of well-aligned scratches, whereas that of browsing deer is more flattened and characterized by more sporadic pits. Therefore, DMTA can identify intraspecific variation in diets, which is smaller than the interspecific dietary variation in extant ruminants.
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Keywords: Confocal microscope; diet; Scale sensitive fractal analysis; ISO25178; *Cervus nippon*

1. Introduction

Dental microwear analysis focuses on microscopic scars preserved on tooth surface, which are reflecting property of food consumed by the animal. This analysis became one of the frequently applied methods for dietary reconstruction for extinct taxa, because of the non-destructive manner of the analysis [1–8]. Quantification of microwear using scanning electron microscopes (SEM) were prevailed in 1990s [1–5,9,10]. In these studies, individual microwear features, such as pits and scratches, were measured and counted within the SEM microphotographs. However, the analysis were afflicted by significant inter- and intra-observer error during identification and measurement of the microwear [11]. It is also indicated that 2D microwear analysis has lost height data of scar depth, which is rather important considering that it could be tightly connected with food property and mastication manner. To overcome these problems, dental microwear texture analysis (DMTA) were developed [12–14]. Instead of SEM, DMTA uses optical profilometry for collecting 3D surface data of tooth, and are advantageous due to its objectivity and repeatability of data collection. Two different methods of DMTA are devised: the scale sensitive fractal

*Corresponding author.
E-mail address: mugino@k.u-tokyo.ac.jp (M.O. Kubo).
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analysis (SSFA) and the surface texture analysis (STA) with ISO 25178 [12–17]. Both of the analytical methods yield quantitative measurements characterizing dental surface texture, and recently have been applied to various kinds of vertebrates, both extant and extinct [17–26]. Such DMTA data have been rapidly accumulated and will be available for those who are interested in comparing the data with their own. Establishment of data archive and utilization of it can accelerate research. For sharing the DMTA data, however, it is cautioned that different optical profilometers do not always produce similar data of the same tooth surface. Recently Arman et al. [27] vigorously tested for inter-microscope variability using five Sensofar Plμ and two Plympus LEXT microscopes. It was shown that inter-microscope differences are present for two SSFA parameters, though the differences can be minimized by applying automated treatments to remove measurement noise. In addition to the difference of measurement instruments, they pointed out that different manipulation of obtained surface data before calculation of DMTA parameters, such as filter application, produced considerably different results of SSFA, and offered a pre-analysis protocol that maximizes the data compatibility. So far, variation resulted from using different microscopes has not been reported yet for STA.

In 2014, we started DMTA of extant herbivores with known diet using a confocal laser microscope, VK-8500 manufactured by Keyence, Japan. We collected DMTA data of over 200 individuals of wild sika deer in Japan with known diet, varying from grazing to browsing according to their habitat environments, and conducted STA using VK-8500 [28]. It was found that there seemed to be a trend that several ISO parameters had negative or positive relationship with proportion of graminoids in sika deer diet, though neither of the correlation was statistically significant. Lately, in 2016, we additionally introduced a newer machine (VK-9700) and started to use SSFA parameters. Therefore, we have a chance to evaluate the inter-microscope variability in both SSFA and STA.

In the present study, to test the microscope difference, we collected data by VK-9700 and compared with those collected by VK-8500. The objectives of the current study are: 1) to investigate inter-microscope variability in DMTA parameters, for both SSFA and STA, and identify the most appropriate pre-analysis protocol to minimize the variability, 2) to reanalyze the published data of extant sika deer and test correlation between DMTA parameters and graminoid consumption after the updated pre-analysis protocol is applied.

2. Materials and methods

2.1. Extant sika deer

The sika deer (*Cervus nippon*) inhabit in wide range of the Japanese archipelago, from the northern island (Hokkaido, 43°N) to the southern islands (Kerama Islands, 26°N). Therefore, their habitat environment varies from subarctic coniferous forests to subtropical evergreen broadleaved forests. According to the habitat environments, food habits of sika deer shows considerable variation among local populations [29]. The sika deer inhabiting the subarctic coniferous forests or cool temperate deciduous forests subsist on graminoids, particularly dwarf bamboo, which are abundant in the forest understories. On the other hand, the sika deer inhabiting the temperate broadleaved forests primary consume leaves, fruits and seeds of woody plants (Table 1). This north-south variation in diet are shown to be associated with their dentognathic morphology [30], molar wear rate [31,32] and mesowear [32], the latter is known as one of the common proxy of dietary preference in paleoecological research. Consequently, one would expect that microwear characteristics is also reflecting this dietary variation among populations.

2.2. Microscopes and data acquisition

We used a confocal laser microscope, VK-9700 (Keyence, Japan) in order to acquire 3D data that can be compared with those acquired by VK-8500 (Keyence, Japan) [28]. The specifications of the machine were appeared in Table 2, together with other representative 3D profilometers used in DMTA. VK-8500 was equipped by 50× long distance objective lens. On the other hand, VK-9700 was equipped by both 50× and 100× long distance lens.

Table 1
Ecological characteristics of the studied sika deer populations, together with the number of specimens analyzed for microwear texture analysis.

Population	Major vegetation	Graminoids in diet (%)[a]	Number of specimen
1. Eastern Hokkaido	Deciduous broad-leaved and mixed forest	46.3	21
2. Nikko	Deciduous broad-leaved forest	73.9	22
3. Okutama	Deciduous broad-leaved forest	32.1	17
4. Izu Peninsula	Deciduous broad-leaved and evergreen broad-leaved forest	30.5	35
5. Yamanashi	Deciduous broad-leaved forest	42.2	16
6. Nara Park	Open grassland with planted conifers and deciduous trees	81.4	32
7. Shimane	Evergreen broad-leaved forest	38.1	28
8. Yamaguchi	Evergreen broad-leaved forest	6.5	8
9. Fukuoka	Evergreen broad-leaved forest	7.2	19
10. Tsushima Islands	Evergreen broad-leaved forest	3.4	40
11. Yakushima Island	Evergreen broad-leaved forest	4.4	6

[a]Percentage of graminoids in diet is based on rumen content or feces analyses and shown in annual mean. The data were from Kubo and Yamada [32].

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