Sika deer fecal pellets of extremely high grit content

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We collected sika deer (Cervus nippon) fecal pellets very atypical in color and surface texture in Ashio, Tochigi prefecture, central Japan on October 25, 1999. The collection site was about 900 m above sea level in the foothills of Mt. Jizo (1489 m). The terrain was covered with pebbles and scattered patches of grass and shrubs. All the fecal pellets having the unusual appearance (30) were collected for analyses. They were considered to have been from a single pellet group because they were within 50 cm of each other. We searched nearby for pellet groups of the same appearance, but found none. In this note we describe the characteristics of the fecal pellets and compare them with other reports in the literature.

Materials and methods

The fecal pellets were stored in a plastic bag for a day, air-dried, and stored in a sealed container at room temperature until analyses were conducted. Individual pellets were weighed to the nearest 0.001 g. Their length, maximum diameter, and minimum diameter were measured with vernier calipers to the nearest 0.1 mm.

Because the Ashio area is inhabited by both deer and Japanese serow (Capricornis crispus), and fecal pellets of these two ruminants are difficult to distinguish in the field or with optical microscopes, we assessed species of the ungulate (described as Ashio1) using analysis of the mitochondrial DNA control region from genetic material collected from the pellets. The surface of one pellet was scraped to collect epithelial cells that had been sloughed off from the colon wall. The sample was suspended in ATL buffer of the QIAmp™ stool mini kit (Qiagen, Valencia, CA, USA) and spun for three minutes with 0.5 ml of Zirconia/Silica Beads 1.0 mm (Biospec Products, Inc., Bartlesville, OK, USA) to disrupt cells. DNA extraction proceeded as outlined in the QIAmp™ stool mini kit protocol. One negative control was included in the extraction to monitor contamination. A small portion of the mitochondrial control region of the sample, about 140 base pairs bound by primers LD15 (Nagata et al. 1998) and CervH3 (C. Cook, personal comm.), universal primers for Japanese sika deer and Japanese serow, was amplified by PCR in 35 cycles (94°C for 30 s, 55°C for 45 s, and 72°C for 1 min, with a final extension of 72°C for 10 min) after an initial denaturation step conducted at 94°C for 2 min. The PCR mix consisted of 0.5 μM of each primer, 0.2 mM dNTPs, 1 × KOD plus buffer, 1 mM MgSO₄, and 0.6 units of KOD plus (TOYOBO) in a 30 μl reaction volume with 3.0 μl of DNA extract. Cycle sequencing was performed using the ABI BigDye Terminator Kit v3.1 (Applied Biosystems). Sequences of both strands were obtained using an ABI 3100 automated sequencer according to the manufacturer’s protocol. PCR and sequencing were replicated once independently to test for accuracy. Sequences were analyzed using the program ChromasPro v.1.12 (Technelysium Pty. Ltd.). In order to identify the species, we performed a BLAST homology search (Altschul et al. 1990) on the DDBJ website (http://www.ddbj.nig.ac.jp). Sequence differences between Ashio1 and the Japanese serow were confirmed by alignment of the experimental sequence with the highest scoring sequence (an accession number of GenBank AB012374) from the BLAST search, and a representative sequence of the Japanese serow (AB05698) using the program ClustalX 1.81 (Thompson et al. 1997).

Two randomly chosen pellets were analyzed for grit content. They were oven-dried at 105°C for 24 hours just before the analyses in order to measure water content. After being ground in a mortar, they were burned in an electric Muffle furnace (EYELA TMF-3200) at 500°C for more than 2 hours, and weighed to the nearest 0.0001 g.

The botanical composition of the fecal contents was analyzed by crushing sample pellets, suspending them in water, and counting plant fragments using the point quadrat method (Stewart 1967). Plant fragments were classified to four categories: graminoids, broad-leaved plants, vascular tissue, and unidentifiable plant tissue.

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Results and discussion

The pellets had cylindrical shapes with a slight taper at the ends. They were light gray, rather than black or dark brown as in ordinary deer pellets. They lacked the minute surface roughness commonly found after drying (Fig. 1). Apart from the color and surface texture, there were no particular differences in appearance between these pellets and typical sika deer pellets. Decomposition and fungal growth were not apparent.

We successfully amplified and sequenced 142 bp of the mitochondrial control region of \textit{Ashio}1 and obtained the same result from the replicate. This sequence was identical to the mitochondrial control region sequence from a Japanese sika deer from Hyogo Prefecture on the Honshu mainland (AB012374, with the highest score (281) in the BLAST homology search). Nucleotide sequences of \textit{Ashio}1 (142 bases), sika deer (142 bases from AB012374), and serow (146 based from AB055698) were aligned in order to distinguish their genetic difference. The genetic difference between \textit{Ashio}1/sika deer and serow was 8.45% (12/142 bp), a percentage much greater than intraspecific differences among Japanese serow populations (2.91%, Okumura personal comm.). Consequently, \textit{Ashio}1 was identified as a sika deer.

The mean length with S.E. of the pellets (n = 25) was 14.2 ± 1.4 mm. Their maximum and minimum diameters were 12.0 ± 0.4 mm and 11.3 ± 0.4 mm, respectively. These measures fall within the range of size variation of sika deer pellets. Dry weight of the pellets was 1.162 ± 0.132 g, considerably heavier than typical sika deer pellets (Table 1). The grit contents of the two pellets were 86.9% and 87.0% (W/W). Our sample has one of the highest grit contents known in ungulate feces, although literature references which would allow comparison are few. Fecal pellets of high grit content can reflect the loss of the organic matrix through some biotic or abiotic processes, but the pellets we studied were hard, solid and heavy. Holl and Bleich (1987) reported that the grit content in the mountain sheep (\textit{Ovis canadensis nelsoni}) feces varied seasonally and peaked at 33.8% in June. This high content of fecal grit indicates frequent use of mineral licks by sheep. They use mineral licks in June when the moisture content of forage species is highest, and they need to compensate for mineral loss caused by the increase in water turnover rate. Skipworth (1974) stated that most use of mineral licks by Alberta bighorn sheep (\textit{Ovis canadensis}) occurred in May through July, with an average of 30.1% acid insoluble residue in the feces during June and July. Hebert and Cowan (1971) found that mountain goats (\textit{Oreamnos americanus}) use

![Fig. 1. The deer pellets with unusual appearance (left) and others with normal appearance (right) collected nearby on the same date.](image)

<table>
<thead>
<tr>
<th>Deer status</th>
<th>Month</th>
<th>Dry weight (g)</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>captive</td>
<td>March</td>
<td>0.227 ± 0.009 (P: 0.05)</td>
<td>Takatsuki et al. 1981</td>
</tr>
<tr>
<td>captive</td>
<td>April</td>
<td>0.236 ± 0.041 (P: 0.05)</td>
<td>Takatsuki et al. 1981</td>
</tr>
<tr>
<td>captive</td>
<td>August</td>
<td>0.314 ± 0.018 (P: 0.05)</td>
<td>Takatsuki et al. 1981</td>
</tr>
<tr>
<td>captive</td>
<td>October</td>
<td>0.237 ± 0.014 (P: 0.05)</td>
<td>Takatsuki et al. 1981</td>
</tr>
<tr>
<td>captive</td>
<td>January</td>
<td>0.175 ± 0.013 (SD)</td>
<td>Togari and Kunishige 2001</td>
</tr>
<tr>
<td>captive</td>
<td>April</td>
<td>0.186 ± 0.011 (SD)</td>
<td>Togari and Kunishige 2001</td>
</tr>
<tr>
<td>captive</td>
<td>June</td>
<td>0.169 ± 0.004 (SD)</td>
<td>Togari and Kunishige 2001</td>
</tr>
<tr>
<td>captive</td>
<td>July</td>
<td>0.230 ± 0.002 (SD)</td>
<td>Togari and Kunishige 2001</td>
</tr>
<tr>
<td>captive</td>
<td>October</td>
<td>0.257 ± 0.027 (SD)</td>
<td>Togari and Kunishige 2001</td>
</tr>
<tr>
<td>captive</td>
<td>December</td>
<td>0.209 ± 0.025 (SD)</td>
<td>Togari and Kunishige 2001</td>
</tr>
<tr>
<td>free-ranging</td>
<td>unknown</td>
<td>1.162 ± 0.132 (SE)</td>
<td>current material</td>
</tr>
</tbody>
</table>
natural earth licks in the spring (males) and early summer (females). The same pattern may occur in cervid species, because sheep, goat and deer are all ruminants. Seip and Bunnel (1985) reported that Stone’s sheep (Ovis dalli stonei) feces contained up to 88% ash in summer due to the regular use of mineral licks. Given the limits of their report, comparisons are difficult, but the similarity of reported grit contents suggests that our sample may also reflect the use of mineral licks.

The botanical composition of the fecal contents was dominated by vascular tissue (19%) and unidentifiable plant tissue (70%). Graminoids comprised 9%, and broad-leaved plants had the smallest share (2%). The percentage of broad-leaved plants is considerably smaller than the corresponding figures in literatures. For example, Padmalal and Takatsuki (1994) reported that the leaves of dicotyledons comprised from 23.7% to 43.6%, for different ages and sexes, of fecal contents in April, and from 10.2% to 23.4% in July on Kinkazan Island. It casts doubt on the supposition derived from the discussion on mineral lick use, if the scantiness of broad-leaved plants indicates that the pellets in question were defecated in winter or very early spring, when leafy food items are least available. There is, however, another possibility, which is more likely. The observed amount of broad-leaved plants may have been significantly lower than it really was, because these plant fragments must have been too fragile to survive the friction with the overwhelmingly abundant grit during the process of fecal pellet formation in the intestine. In addition, the contribution of broad-leaved plants in fecal contents can be as small as 0.88% or even less in July (Horino and Kuwahata 1986). A large part of the unidentifiable plant fragments in the pellets may have been broad-leaved plants, because they were very small particles. If so, it is not necessary to reject the possibility that the pellets were defecated in late spring or early summer, when deer live mainly on forbs of high moisture content.

If the high grit content in the pellets we analyzed was a result of typical feeding patterns and physiological processes, then pellets of that high grit content should be observed frequently, but this is the first reported case for sika deer. The current case may have been a very rare, but quite normal event. Given the lack of similar reports, however, we can also not deny the possibility that these pellets were produced by some abnormal cause, such as disease, starvation, or other kinds of very high stress.

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References


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