Morphometric analyses of non-invasive fecal samples of the Korean long-tailed goral (Naemorhedus caudatus) for species and age identification

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ABSTRACT

The Korean long-tailed goral (Naemorhedus caudatus) is at risk of population decline due to habitat loss and fragmentation. Therefore, it is essential to ascertain its presence and/or the identity of individuals of the goral using non-invasive fecal samples for its conservation and management. In this study, we examined the morphology of fecal samples to provide the baseline data that can be used to distinguish species and age of goral individuals. We detected a significant difference in the length-to-width ratios of feces among the five ungulate species found in Korea. Also, we detected a significant difference in the length-to-width ratios of feces of gorals depending on the age groups. To assess the accuracy of species and age identification based on the fecal morphology, we conducted a series of blind comparison between the mean length-to-width ratios of the fecal pellets and the reference mean ratio values of the fecal pellets. Using 20 fecal pellets, our results showed 73%–86% probability of correct identification of the three species (gorals, goats, and roe deer), and 83%–90% probability of correct identification of >5 year-old goral individuals. The use of fecal morphometric analyses will be useful for the studies of Korean ungulate species, particularly the endangered gorals.

Introduction

Non-invasive tracking, the oldest science (Liebenberg 1990), is an important method to study wildlife that is difficult to capture, or even observe (Taberlet et al. 1999). It can be also used to study rare, endangered, or cryptic fauna (Fernando et al. 2003). Low cost and easy accessibility make it a useful tool for field studies, even though it requires experts with sensitive field skills (Chame 2003). Using footprints is probably one of the easiest tracking methods. However, other factors (e.g. soil characteristics, vegetation, local climate, etc.) could affect the tracking results (Chame 2003). In contrast, fecal samples are the clearest and most easily recognizable sign, if the original shape is maintained through time (Liebenberg 2000; Chame 2003). Thus, fecal samples can be useful for identification of the animals and provide valuable data to understand the local community structure.

For population studies of wild animals, species identification is essential to understand the distribution of species in natural communities (Halfpenny 1986; Foran et al. 1997; Chame 2003). Chame (2003) conducted a study on species identification of terrestrial mammals, including ungulates, using a morphometric description of feces. This study suggested that fecal shape and size could be useful for species identification. Determination of age class is also important to estimate the age structure of free-ranging animals, and this can be done by measuring the length or width of fecal pellets (Ball 2000). According to Ball (2000), pellets with a small mean length could be considered to be from calves or yearlings while large pellets could be considered to be from adults.

There are four wild ungulate species on the Korean peninsula: long-tailed gorals (Naemorhedus caudatus), Siberian roe deer (Capreolus pygargus), water deer (Hydropotes inermis), and Siberian musk deer (Moschus moschiferus). In addition, some domestic goats (Capra aegagrus) have been accidentally released into the wild. Fecal size and shape are known to be species-specific, but accurate identification of species can be very difficult without detailed examination and baseline data. In particular, feces of gorals, roe deer, and feral

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goats look very similar. For population studies on the endangered long-tailed goral, which is at risk of population decline due to human disturbance (IUCN 1996; Hutton and Dickson 2000; Ministry of Environment of Korea 2002, 2004), it is essential to distinguish the goral from other ungulates so that goral-specific data can be collected. To date, studies on species and age identification using feces have rarely been carried out for the long-tailed goral (except a couple of molecular studies by Kim et al. 2008, 2009).

In this study, we examined three different morphometric parameters (length, width, and length-to-width ratio) of the fecal pellets of the five ungulates (gorals, roe deer, goats, water deer, and musk deer) found in Korea. In addition, we examined the three morphometric parameters of the fecal pellets of captive gorals with known age information to detect any difference in the fecal morphology between different age groups.

Materials and methods

Fecal samples and sampling sites

Fecal samples of wild and captive gorals (n = 940 pellets, 620 from the wild and 320 from the captive populations), and wild roe deer (n = 210), water deer (n = 150), and musk deer (n = 90) were collected for this study (Table 1). Fecal samples of goats (n = 210) were collected from semi-captive individuals near farms. Most fecal samples were naturally dried, and then measured under ambient temperature. Fecal samples of captive gorals, collected from a farm in Yanggu, South Korea, were stored at −70°C, dried, and then measured. Fecal samples of wild gorals, roe deer, and water deer were collected together with domestic goats in South Korea, while those of musk deer were collected in Primorsky, Russia. Species and age identity of the fecal samples was confirmed by field experts using supporting evidence (e.g. the shape and/or color of the hairs found with the fecal pellets, direct behavioral observation of defecation, etc.) or molecular analyses (mitochondrial DNA sequences used in our previous study; Kim et al. 2008). For age identification, we only used the fecal samples collected from captive gorals with known age information. Fecal samples without supporting evidence were not used in the analyses.

Species difference in the fecal morphology of Korean ungulate species

We compared the size of fecal samples of the five ungulate species (Table 2). Captive goral feces were excluded for this comparison. The number of fecal pellets used for statistical analysis was 620 (26 individual deposits), 210 (7), 210 (7), 150 (5), and 90 (5) for gorals, roe deer, goats, water deer, and musk deer, respectively.

Age difference in the fecal morphology of gorals

We compared the size of fecal samples collected from the goral individuals with known age kept in a farm. In total, 320 fecal pellets (16 individual deposits, n = 20 for each) were used (60 for <1 year, 60 for 1–2 year, 200 for >5 year; Table 3). No samples were available for the age between two and five.

Morphometric analyses of feces

We measured the length and width of fecal samples with a Vernier caliper, and then calculated the ratio between the two size measures (length/width). Maximum, minimum values and 95% confidence interval of the three parameters were calculated for each species and age group (Tables 2 and 3). Also, Pearson correlation coefficients between the length and width of fecal pellets were calculated for each species and age group (Tables 2 and 3). One-way ANOVAs were performed to detect difference in the length-to-width ratios of fecal samples among five ungulate species and three age groups of gorals (Table 4). All data were analyzed by using SPSS version 12.0 (ESRI Inc., U.S.A.).

In order to assess the accuracy of species and age identification based on the fecal morphology, we randomly selected 5–20 fecal samples from each species and age group, and compared the length-to-width ratio of the fecal samples with the mean reference values of the feces. We assumed that the fecal sample belong to the specific species or age group if the value closely matches the reference mean values of the species or age group. Probabilities of successful assignment for species and age groups were calculated.

Results

Before conducting the morphometric analyses, we evaluated the accuracy of visual identification. First, we
showed the field experts a photo of unmarked fecal deposits of the five species (Figure 1(A)). Four out of seven field experts (members of the Korean Wildlife Conservationist Group) correctly identified all species (57%). All experts correctly distinguished the feces of the goral or water deer (100%) from the others, but only four or six experts correctly distinguished the feces of the roe deer (57%), goat (57%), and musk deer (86%) from the others. Second, we showed the same field experts another photo of unmarked fecal deposits of the goral, goat, and roe deer (Figure 1(B)). Out of seven experts, only one correctly identified the three feces (14%). One expert correctly identified the feces of the goral (14%) from the others. Two and three experts correctly identified the feces of the goat (29%) and roe deer (43%), respectively.

Species difference in the fecal morphology of Korean ungulate species

Shapes of feces of the five species were mostly unique (Figure 1(A)), but sometimes similar (Figure 1(B)). Also, the lengths (Figure 2(A)) and widths (Figure 2(B)) showed a wide overlap among the five species. Among the five ungulate species, the water deer and musk deer could be easily differentiated due to their unique fecal shapes and sizes (Figure 1(A)). However, the rest looked very similar (Figure 1(B)). The feces of the goral were the largest and those of the musk deer were the smallest in terms of both length and width (Table 2). Frequency distributions of the length and width of the feces overlap among the five species; specifically, the musk deer and goat had the peak frequency at 9–10 mm in length (Figure 2(A)), and the roe deer and goat showed the peak frequency at 7–8 mm in width (Figure 2(B)). On the other hand, when we considered the length-to-width ratio of the feces, the feces of the musk deer showed the highest ratio and those of the goat showed the lowest (Table 2). There was a significant difference in the fecal length-to-width ratio among the five ungulate species (one-way ANOVA, $F_{4,125} = 197.466, P < 0.001$; Table 4). The correlation between the length and width of the feces ranged from 0.577 (the water deer) to 0.794 (the roe deer) (Table 2).

Age difference in the fecal morphology of gorals

For the gorals, we detected age difference in the fecal size. The size tended to increase with age; the feces of the individuals younger than one year were the smallest and those of individuals older than five years were the largest (Table 3). Similarly, it appeared the length-to-width ratio of the fecal samples changed with age; the feces of the individuals younger than one year showed the lowest length-to-width ratio and those of the individuals older than five years had the largest (Table 3). The age

### Table 2. Shape and size of the fecal pellets of the five ungulate species.

| Species      | No. fecal pellets | No. fecal deposits | Mean length (95% C.I., mm) | Mean width (95% C.I., mm) | Mean L/W (95% C.I.) | Correlation coefficient |
|--------------|-------------------|--------------------|----------------------------|---------------------------|---------------------|-------------------------|
| Goral        | 620               | 26                 | 13.140 (12.933–13.347)     | 8.454 (8.362–8.546)       | 1.553 (1.534–1.571) | 0.687***                |
| Roe deer     | 210               | 7                  | 11.897 (11.538–12.257)     | 6.736 (6.591–6.881)       | 1.760 (1.727–1.794) | 0.794***                |
| Goat         | 210               | 7                  | 9.271 (9.752–10.098)       | 5.666 (7.671–7.912)       | 1.653 (1.259–1.300) | 0.582***                |
| Water deer   | 150               | 5                  | 8.923 (8.909–9.948)        | 5.281 (5.520–5.812)       | 1.635 (1.613–1.694) | 0.773***                |
| Musk deer    | 90                | 3                  | 8.532 (8.188–8.876)        | 4.281 (4.207–4.356)       | 1.983 (1.923–2.043) | 0.735***                |
| Total        | 1280              | 48                 | 11.631 (11.475–11.788)     | 7.443 (7.353–7.534)       | 1.584 (1.544–1.600) |                         |

Note: L: length; W: width; C.I.: 95% confidence interval; correlation coefficient: Pearson correlation coefficient between the length and width. *** denotes for $P < 0.001$.

### Table 3. Shape and size of the fecal pellets of gorals by the age groups.

| Age      | No. fecal pellets | No. fecal deposits | Mean length (95% C.I., mm) | Mean width (95% C.I., mm) | Mean L/W (95% C.I.) | Correlation coefficient |
|----------|-------------------|--------------------|----------------------------|---------------------------|---------------------|-------------------------|
| <1 year  | 60                | 3                  | 9.660 (9.372–9.948)        | 5.662 (5.486–5.837)       | 1.719 (1.662–1.773) | 0.479***                |
| 1–2 years| 60                | 3                  | 13.167 (12.871–13.463)     | 7.252 (7.065–7.438)       | 1.835 (1.770–1.900) | −0.066NS                |
| >5 years | 200               | 10                 | 14.611 (14.401–14.822)     | 7.320 (7.264–7.375)       | 1.999 (1.970–2.029) | 0.256***                |
| Total    | 320               | 16                 | 13.412 (13.155–13.669)     | 6.996 (6.904–7.088)       | 1.916 (1.889–1.943) |                         |

Note: Significance levels of the correlation coefficients are noted as *** for $P < 0.001$ and NS for $P > 0.5$. 
The difference in the length-to-width ratio was significant (one-way ANOVA, \( F_{2,317} = 43.493, P < 0.001 \); Table 4). The correlation between the length and width of the feces ranged from 0.256 (>5 year) to 0.479 (<1 year) but the correlation between length and width was not apparent in the feces of the individuals between one and two year old (Table 3).

**Assessment of the fecal morphology as the means for species and age identification**

We compared the mean length-to-width ratio of the fecal pellets \((n = 5, 10, 15, \text{ or } 20)\) in each individual deposit with the reference mean length-to-width ratio in Tables 2 and 3. Among gorals, roe deer and goats, the percentage of correct species identification based on the length-to-width ratio was 73%–86% when 20 fecal pellets were used (Figure 3(A)). Percentage of correct age group assignment of gorals based on the length-to-width ratio was generally low (33%–90%), but it was higher with the older individuals (83%–90%, Figure 3(B)).

**Discussion**

Although molecular techniques have emerged in the field of population ecology, tracking techniques based on non-invasive samples are still useful due to accessibility and cost-effectiveness. Fecal samples are often the most easily collectable source of information for studying rare, elusive species (Putman 1984). However, to retrieve useful information, proper identification of the species should be conducted. As the easiest method, visual identification is known to have high error rates (more than 50%–66%) even by field experts; thus, it is highly unreliable (Halfpenny 1986). Also, it has been thought that species identification based on the diameter of feces is not possible (Weaver and Fritts 1979). On the contrary, our results show that the morphology of feces can be useful to identify Korean ungulate species. Moreover, our results suggest that the age determination based on fecal morphology is possible, at least in gorals, but more data would be needed to provide the

| Identification | Sum of squares | df | Mean squares | F   | P value |
|----------------|---------------|----|--------------|-----|---------|
| Species        |               |    |              |     |         |
| Between groups | 41.605        | 4  | 10.401       | 197.466 | <0.001 |
| Within groups  | 67.160        | 1275 | 0.053     |     |         |
| Total          | 108.765       | 1279 |           |     |         |
| Age            |               |    |              |     |         |
| Between groups | 4.111         | 2  | 2.056        | 43.493 | <0.001 |
| Within groups  | 14.982        | 317 | 0.047     |     |         |
| Total          | 19.093        | 319 |           |     |         |

**Figure 1.** Photos of fecal samples from the Korean ungulate species. A: common fecal shapes of the goral, roe deer, goat, water deer, and musk deer. B: similar fecal shapes of the goral, goat, and roe deer.
guidelines for reliable age determination. Particularly, age determination based on fecal morphology would be useful because no molecular tool is available for this purpose. We believe that our data, even in its preliminary nature, can provide useful baseline data for species identification of Korean ungulates and age determination of the goral.

According to our results, the length and width of the feces per se could not be utilized for species identification due to overlaps in fecal size distribution among the five species. Instead, length-to-width ratio could serve as the means to identify each species. The shape and size of goral fecal pellets can be very similar to those of roe deer and goats, but our results suggest that even these three species could be fairly accurately distinguished using the length-to-width ratio of the fecal pellets.

We found clear age differences in the fecal samples of goral from a farm. In a previous study, Chame (2003) also reported that the fecal size of various ungulate species (e.g. bison, buffalos, domestic cattle, etc.) varies with age. In our study, younger gorals had smaller feces and smaller length-to-width ratios than older ones. The size increase in the feces with age can be due to body size increase with age. The data that we provide in this study is not sufficient for age determination of the goral because the data from 2 to 4 year-old individuals were not available. Also, the accuracy of age determination based on the fecal morphology was somewhat low between <1 year old and 1–2 year-old individuals. However, the older individuals could be recognized with the accuracy of 83%–90%. Currently, it is not clear whether age determination of gorals base on the fecal morphology could be made among each age group (e.g. between 2–3 year-old and 3–4 year-old individuals) with similar accuracy. More data collection should be made to ascertain the possibility.

For the comparisons among the species, we used the data of the fecal samples collected from wild gorals and excluded those of individuals from the farm. The two types of feces showed a slight difference in the length-to-width ratio (data not shown). This difference may have been caused by a different amount of food and/or different food sources. Captive gorals are usually fed on sufficient human-provided food, such as rice and alfalfa hay, but wild gorals mostly fed on insufficient natural food, such as leaves of graminoids and pine trees. Scott (1941, 1943) mentioned that fecal size varied in proportion to the amount of food consumed. Weaver and Fritts (1979) indicated that fecal diameter may be related to diet composition. However, it is not clear how this feeding difference results in the size variation in the feces. Changing the food sources and/or their amount and examining any change in fecal size

Figure 2. Considerable overlapping patterns in fecal pellet sizes from the five ungulate species. Both mean length (A) and width (B) of pellets in each individual deposit were calculated for each species, and frequencies of each class of length and width were computed.

Figure 3. Probabilities of successful species (A) and age (B) identification of fecal samples through assignment test.
and shape would reveal the influence of the food condition on the fecal morphology.

In conclusion, this study provides baseline data and preliminary results of non-invasive approach for species and age identification of ungulates, especially goral.

To use this approach, it is important to collect as many fecal samples as possible with correct information on species and age identity as the reference; we recommend to collect at least 20 pellets from each individual deposit. If one uses this method with prior knowledge of environmental conditions (e.g. elevation, habitat type, presence of hairs and tracks on or near the fecal deposits), the accuracy and reliability of species and age identification could be greatly enhanced. This method for non-invasive sampling and analysis of fecal samples can be useful in the studies of endangered ungulate species.

Acknowledgments

We thank the members of the Korean Wildlife Conservationist Group for their participation in our blind tests.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was funded by the Research Institute of Veterinary Science and the Brain Korea 21 Program for Veterinary Science, Seoul National University, and the Ministry of Environment of Korea.

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