A multifaceted approach to the study of plant-eating in feline carnivores

Hiroto Yoshimura

1. Introduction

Mammals are commonly categorized into three dietary groups: carnivores, omnivores, and herbivores. As obligate carnivores, members of the Felidae family primarily consume animal flesh, with their morphology and physiology not attuned to digest plants. However, various felids have been observed eating plants in both wild and captive settings. This behavior, seemingly contradictory to their natural diet, have been largely unexplored. Gaining insights into the plant-eating behavior of felids not only offers a deeper comprehension of the evolution of dietary traits but also enhances our understanding of the intricate relationship between carnivores and plants. In this series of studies, my aim is to establish a foundation for investigating this intriguing behavior in felids, employing various methodologies from literature reviews to molecular-based analyses.

2. Materials and methods

2-1 Multispecies analysis

I conducted a literature search using Web of Science targeting dietary research articles of 41 extant felid species. In all, 316 records from 213 studies of 24 felids were used in the analyses. I evaluated the effect of six environmental attributes: island, mean monthly precipitation, mean maximum daily temperature, mean minimum daily temperature, mean monthly normalized difference vegetation index (NDVI), and season (spring, summer, autumn, winter, dry, wet) on the frequency of plant occurrence using two-part binomial models. Additionally, phylogenetic distance, sample type and body mass were included.

2-2 Behavior observation and scat analysis

The subject animals were 13 snow leopards (7 females, 6 males) kept in six zoos in Japan. The behavioral observation was conducted on 11 individuals. Their behavior was continuously recorded while they were in the outside enclosure.

The collection of scat samples was conducted for 10 individuals. The scat samples were freeze-dried overnight, then weighed, and washed in tap water with 1 mm mesh to pick out undigested matters, hair, plants including pieces of wooden benches and other material (e.g., gravel). The contents from each sample were packed in airtight plastic bags, then freeze-dried overnight and weighed. To test the quantitative relationship among scat sample contents, the amount of plant matter in a scat sample, the amount of plant matter contained in the scat sample evacuated before hair was excreted, and the amount of plant matter contained in the scat sample evacuated after hair was excreted were set as fixed effect and the amount of hair in the scat sample was set as the objective variable. A generalized linear mixed model (GLMM) with gamma distribution and identity link function was applied. Sampling unit was set as random effect in GLMMs.

2-3 Diet analysis

The study area was Sarychat-Ertash Reserve (42°02′N 78°25′E) in Kyrgyzstan. We collected scats from November 2017 to May 2019 and September 2022 to May 2023. We opportunistically collected 150 fecal samples in total. The host species were genetically identified. Sex identification was conducted for snow leopard samples. A DNA metabarcoding was conducted targeting one vertebrate and four plant barcode regions using an Illumina MiSeq. The sequence data was filtered and converted into presence/absence data. We used the randomForest package of R to determine which plant genera best discriminated whether a sample came from snow leopard or other sympatric mammals. A post hoc probabilistic co-occurrence analysis was conducted to show which taxa are simultaneously present in the same fecal samples of predators. The sex difference of diet composition was visualized using non-metric multidimensional scaling (NMDS). Constrained analysis of principal coordinates (CAP) was performed to evaluate dietary composition differences between male and female snow leopard, accounting the effect of sampling season and spatial autocorrelation.

3. Results

1. Multispecies analysis

The estimated frequency of plant occurrence varied substantially, from 0.005 [Pampas cat (*Leopardus colocola*)] to 0.749 [southern tigrina (*Leopardus guttulus*)]. Within the 18 variables considered, log- transformed body mass (MAP = −0.814 [−1.452, −0.302], EAP = −0.881 [−1.586, −0.164]) had a significant effect on the frequency of plant occurrence.

3-2 Behavior observation and scat analysis

Behavioral observation was conducted for a total of 417 hours from September 2018 to October 2019, and 398 hours were used for the analysis. Plant-eating behavior was observed in 10 out of 11 individuals. Three individuals were each observed vomiting once. In addition, there was no significant relationship between the amount of plant matter contained in scats and the amount of hair in scats.

3-3 Diet analysis

We applied a DNA metabarcoding method on 90 snow leopard fecal samples. We found that argali (*Ovis ammon*), the largest prey ungulates, were consumed only by male snow leopards, though the difference was not statistically significant. Among the three most common plant families in snow leopard scat, Poaceae and Asteracae were often consumed by various mammals, while Tamaricaceae (the genus *Myricaraia*) was primarily consumed by snow leopards. The genus *Myricaria* frequently appeared in samples lacking any animal prey DNA. We also observed a significant difference in plant composition between male and female snow leopards (permutation test, $p<0.05$), and potentially between sampling seasons ($p=0.09$).

4. Discussion

This series of studies is the first attempt in the world to investigate the plant-eating behavior in felids. By compiling data from published articles, this study conducted multi-species comparisons that unveiled a correlation between species body mass and

the frequency of plant occurrence. The hair evacuation hypothesis, a predominant hypothesis about adaptive significance of plant-eating behavior was tested with captive snow leopards. Contrary to the common assumption, plant ingestion did not induce vomiting or hair evacuation in the scat of captive snow leopards. Molecular techniques were utilized to determine the dietary plants of wild snow leopards in Kyrgyzstan, revealing that the representative plant genus negatively co-occurred with prey. The data also suggested a potential difference in dietary plant composition between the sexes.

By challenging existing paradigms and setting the stage for future research, this series of studies acts as a catalyst in the field of wildlife science. Further exploratory studies, along with hypothesis testing, are necessary to understand the complex drivers and adaptive significance that lead feline carnivores to consume plants.