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CHAPTER 19

PREY ANALYSIS OF SHORT-EARED OWL WITH MOLECULAR GENETIC TECHNIQUE

By

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ABSTRACT

DNA of prey present in owl pellets may provide a valuable source of information for dietary studies. This study intended to identify prey species of short-eared owl (*Asio flammeus*) designated as natural monument No. 324 in South Korea, using molecular genetic methods. We used 170 bones from 18 pellets to identify species. A total of nine species were identified as short-eared owls' prey. Small mammals constituted the highest percentage (89.9%): *Apodemus agrarius* (51.5%), *Micromys minutus* (23.6%), *Mus musculus molossinus* (8.3%), *Crocidura shantungensis* (5.3%) and *Rattus norvegicus* (1.2%). We also were able to identify not only small mammals but birds and an amphibian: Japanese quail (*Coturnix japonica*), Vinous-throated parrotbill (*Paradoxornis webbianus*), and eastern turtle dove (*Streptopelia orientalis*), and Kori-salamander (*Hynobius yangi*). This study will greatly enhance the ability of prey identification for conservation management of short-eared owls in the future.

Key words: *Asio flammeus*, *Apodemus agrarius*, cytochrome *b* gene, *Hynobius yangi*, owl pellet,

INTRODUCTION

The short-eared owl (*Asio flammeus*) is a medium-sized owl in the order of Strigiformes. It is included in Appendix II of Convention on International Trade in Endangered Species (CITES) and listed as natural monument No. 324 by the Ministry of Culture and Tourism, Korea. The short-eared owl is active during day time and prefers open habitats such as coastal tidal and brackish marshes, inland fields, pastures, and grasslands, whereas most owls are mainly nocturnal and distributed in forest zones (Clark 1975).

Owl pellets are accumulations of the undigested portions of prey, which are regurgitated and ejected through the mouth in compact units (Taberlet & Fumagalli 1996). The bones, including the skull and mandibles of even the most delicate parts of small mammals or bird species, are found in the pellets (Poulakakis *et al.* 2005). Therefore many studies have utilized pellet analysis to identify food items (Hendrickson & Swan 1938; Lyman *et al.* 2003; Petty 1999; Randle & Austing 1952; Smith *et al.* 1972; Twente & Baker 1951). However, the conventional method may be erroneous and have low efficiency. Over the last 15 years, many methods and experimental strategies have been developed for the retrieval and analysis of genuine ancient DNA sequences (Orlando *et al.* 2003; Paabo *et al.* 1988; Yang *et al.* 1996;), and have succeeded in identifying of food analysis using extraction of DNA from bones. Therefore, in this study, we attempted to identify the prey of short-eared owls from pellets using the molecular genetic method, which is effective and accurate.

METHODS

Collection of pellets

Pellets from the short-eared owl were collected on the Dalsung wetland in Daegu, South Korea which is at the junction of Nakdong and Kumho rivers (Fig. 1). Flora such as reed canary grass (*Phalaris arundinacea*), fleabane (*Erigeron Canadensis*), curly dock (*Rumex crispus*), and waterfowl such as mallard (*Anas platyrhynchos*), pintail (*Anas acuta*), green-winged teal (*Anas crecca*) and spot-billed duck (*Anas poecilorhyncha*) were common locally (Lee 2003). A total of 71 pellets were collected within 50m of short-eared owls' roosting sites in April and May 2002 and May 2003. We randomly selected bones from each pellet because there were dozens of bone fragments. We chose lower mandible of the same direction, and femur of the same direction. Also we chose all unusual bones. We sampled 64 pieces of bones from 7 pellets out of 23 pellets collected in April 2002, 50 pieces from 5 pellets in May 2002 and 56 pieces from 6 pellets in May 2003 (Table 1). The total number of bone fragments was 170.

In addition, a trap survey was conducted in 2002 (Lee 2003) to identify small mammals in this area. It was conducted using the Sherman trap and Museum snap trap from May 28th to June 12th in 2002 for 15 consecutive days.

Genetic analysis

Bone samples were used for DNA extraction. The samples were carefully cleaned using 70% EtOH and ground to fine powder using micropestle (Eppendorf). The DNA extraction procedure followed the DNeasy® tissue kit (QIAGEN).

PCR amplification was performed in a 20μℓ volume containing 2μℓ of 10X BSA (promega), 2μℓ of 10X buffer (iNtRON), 1.6μℓ of dNTP (iNtRON), 1μℓ of each primer, and 0.2μℓ of *Taq* polymerase (iNtRON). The optimal thermocycler program was: 45 cycles of 94 ℃ for 1 min, 51 ℃ for 1 min, 72 ℃ for 2 min. We used the following primer sequences : L14841 (5'-AAA-AAG-CTT-CCA-TCC-AAC-ATC-TCA-GCA-TGA-TGA-AA-3') and H15149 (5'-AAA-CTG-CAG-CCC-CTC-AGA-ATG-ATA-TTT-GTC-CTC-A-3') (Kocher *et al.* 1989).

The PCR products were purified using Zymoclean Gel DNA Recovery kit (ZYMO RESEARCH) and sequenced using an Automatic DNA sequencing machine (ABI 3700, PE Applied Biosystems). Prey species were identified by comparison with sequence data from GenBank and personal data.

RESULTS

A total of nine species were identified as short-eared owls' prey. The predominant species of prey were small mammals: Eurasian field mouse (*Apodemus agrarius*), European harvest mouse (*Micromys minutus*), house mouse (*Mus musculus molossinus*), and Norway rat (*Rattus norvegicus*) of Muridae, and Asian lesser white-toothed shrew (*Crocidura shantungensis*) of Soricidae. We could also identify three bird species and an amphibian: Japanese quail (*Coturnix japonica*) of Phasianidae, eastern turtle dove (*Streptopelia orientalis*) of Columbidae, Vinous-throated parrotbill (*Paradoxornis webbianus*) of Panuridae and Kori salamander (*Hynobius yangi*) of Hynobiidae (Table 2).

Relatively large prey species such as Japanese quail and Norway rat were found in only one pellet (Table 2). We were able to identify the nine prey species from an analysis of 169 DNA samples out of 170 bone samples (99.4%).

Small mammals constituted the highest percentage (89.9%): Eurasian field mouse (51.5%), European harvest mouse (23.6%), Japanese wild mouse (8.3%), Asian lesser white-toothed shrew (5.3%) and Norway rat (1.2%). Birds (7.1%) and amphibian (3%) were also identified (Fig. 2).

Discussion

Kori salamander (n = 2 pellets) and Japanese quail, eastern turtle dove and vinous-throated parrotbill of birds (n = 5 pellets) were found in only a few samples. However, we determined that amphibians and birds are important prey species because these came from a relatively large number of pellets (n= total 7 of 18 pellets). It seems that it is possible to identify reptile and insects as their prey like the existing studies (Banks 1965; Alvarez-Castaneda *et al.* 2004).

Of the short-eared owls' prey species, Kori salamander was especially important as it was reported in this study. Kori salamander was reported as a new species in 2003 (Kim *et al.* 2003), because it is distributed only in Kori, Korea, and it differs from the Korean salamander (*Hynobius leechii*) which distributed broadly in South Korea. However, in this study shows short-eared owl preyed on Kori salamander.

In this study, Eurasian field mice was a dominant species similar to the trap survey of small mammals (37/39 individuals caught) (Lee 2003). However, we identified five mammal species whereas Sherman and snap traps revealed only 2 species (37 individuals of Eurasian field mice and 2 white-toothed shrew, *Crocidura lasiura*). The white-toothed shrews were not identified in the pellet study whereas this species was identified in previous trap survey. We believe that the short-eared owl avoid preying white-toothed shrews due to their strong smell from the stink gland at the lateral part of body (Ministry of Education 1967). Therefore it seems that it is possible for short-eared owl to avoid them as their prey species. Another possibility is that since we did not completely examine the whole owl pellets there may be a chance to find the white-toothed shrews among the pellets not examined. However, we believe that there is a little chance of identifying the shrew species in the remaining owl pellets considering the species list in the study area.

Our method using DNA in bones was proved to be more accurate than the conventional trap method and also was non-invasive sampling method. Presumably our method using owl pellets

demonstrated the value of examining the owl pellets to indicate the species present in a region.

There were hundreds of pieces of bone in a pellet. So paired elements of bones (mandibles, femur, etc.) were selected and the largest number of elements from either left- or right-hand side was chosen to reduce the cost. In our study, we only spent about 10 dollars for each species (bone) and had a reliable result (Table 3). To have fast and inexpensive results we are trying to use PCR-Restriction Fragment Length Polymorphism (RFLP) and Denaturing Gradient Gel Electrophoresis (DGGE). With those methods identification of all the bones in a pellet would be possible and faster result would be expected.

This study for the first time in continental Asia demonstrated that molecular genetic technique can be a useful tool for identification of food items in owl pellets and will greatly enhance the ability of identification and conservation of short-eared owls in the future.

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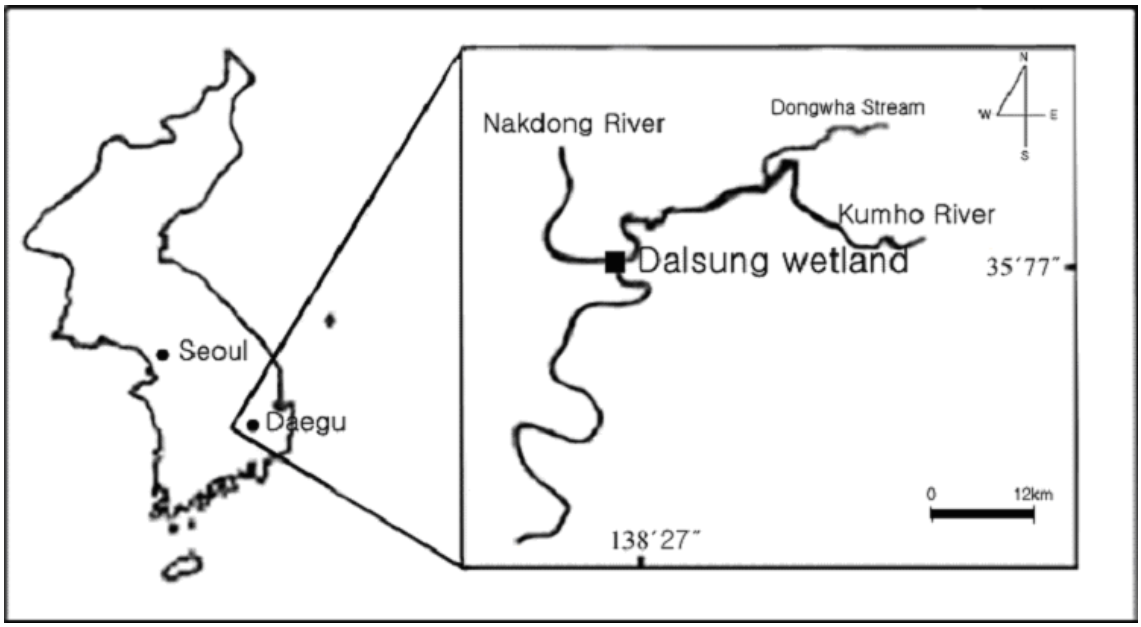

Fig. 1. Map showing study area in Daegu, South Korea. Pellets of short-eared owl were collected within 50m of roosting sites

Table 1. The number of samples used for molecular analysis from short-eared owl pellets in April and May 2002 and May 2003

Pellet of owl		Prey species								
		Crocidura shantungensis	*Apodemus agrarius*	*Microtus minutus*	*Mus musculus molossinus*	*Rattus norvegicus*	*Coturnix japonica*	*Streptopelia orientalis*	*Paradoxornis webbianus*	*Hynobius yangi*
April 2002	1	-	-	-	-	+(2)	-	-	-	-
	2	-	-	+(10)	+(2)	-	-	-	-	-
	3	-	+(6)	+(1)	+(2)	-	-	-	-	-
	4	-	+(21)	+(7)	-	-	-	-	-	-
	5	-	-	-	-	-	+(1)	-	-	-
	6	+(3)	+(1)	+(5)	+(1)	-	-	-	-	-
	7	-	-	+(1)	+(1)	-	-	-	-	-
May 2002	1	-	+(3)	+(2)	+(5)	-	-	-	-	-
	2	-	-	-	-	-	+(2)	-	-	-
	3	+(2)	+(3)	-	-	-	-	-	-	+(4)
	4	+(2)	+(13)	-	+(3)	-	-	-	-	+(1)
	5	+(2)	+(2)	+(5)	-	-	-	-	-	-
May 2003	1	-	+(3)	+(4)	-	-	-	+(1)	-	-
	2	-	+(6)	-	-	-	-	-	-	-
	3	-	-	-	-	-	+(3)	-	-	-
	4	-	+(26)	-	-	-	-	-	-	-
	5	-	+(3)	+(3)	-	-	-	-	-	-
	6	-	-	+(2)	-	-	-	-	+(5)	-
TOTAL		4 (9)	11 (87)	10 (40)	6 (14)	1 (2)	3 (6)	1 (1)	1 (5)	2 (5)

+ Species present in each pellet, – species absent.

Numbers in parenthesis means number of samples analyzed.

Table 2. Nine prey species of short-eared owl identified using molecular genetic analysis.

Sampling date	Total Samples		
	Total No. of pellets collected	No. of pellets used in this study	No. of pieces of bone for molecular analysis
2002. 4	23	7	64
2002. 5	30	5	50
2003. 5	18	6	56
Total	71	18	170

Fig. 2. Percentage of species composition in short-eared owl pellets by molecular genetic analysis.

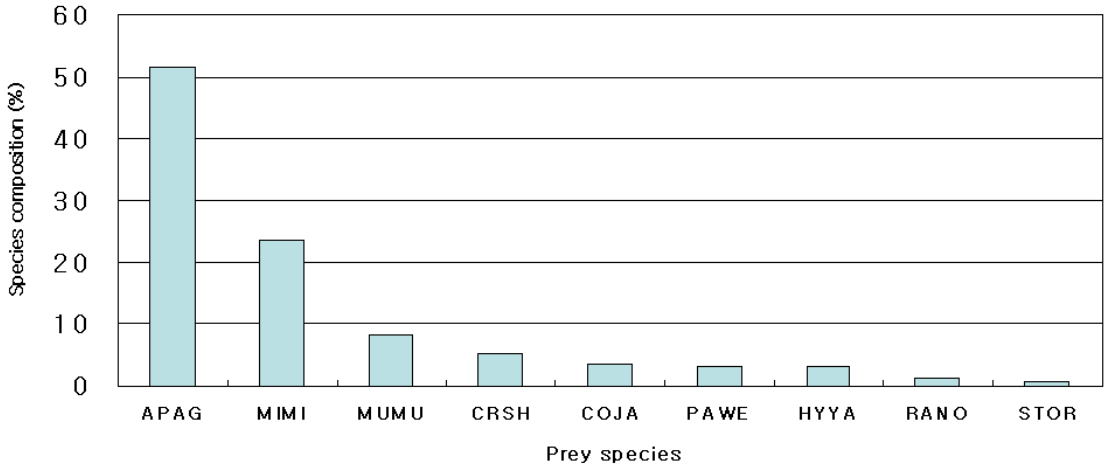

Table 3. Total cost of molecular analysis for each bone (species) (unit: US dollars)

Experiment	Price per sample	Company
DNA extraction	$ 2.4	Qiagen
Gel purification	$ 2.0	Zymo Research
PCR	$ 0.3	iNtRON
Sequencing	$ 5.0	ABI 3700
Total	$ 9.7	