Detecting nesting trees of Siberian flying squirrels (*Pteromys volans*) using their feces

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Forty-three species of flying squirrels are known worldwide (Goldingay 2000). They frequently use tree cavities for daily rest, reproduction and/or wintering (Taulman 1999; Hanski et al. 2000; Nakama and Yanagawa 2009; Shafique et al. 2009). Cavity trees are thus crucial resources for these species. Recent advances in research on the northern flying squirrel (*Glaucomys sabrinus*) and the southern flying squirrel (*G. volans*) have revealed that they prefer large dead trees for building their nests (Meyer et al. 2005; Holloway and Malcolm 2007; Hough and Dieter 2009) and are selective for tree species (Hackett and Pagels 2003; Menzel et al. 2004; Holloway and Malcolm 2007).

Siberian flying squirrels (Pteromys volans) also nest inside tree cavities; however, not much is known about their preference for tree types. Nest sites of P. volans have been studied in some regions, including Finland, where they often nest inside tree cavities of the common aspen (Populus tremula) (Hanski et al. 2000). In Japan, P. volans does not show any preference in the diameter at breast height or between dead and living trees in small woods (Asari et al. 2009). Radio telemetry research has been used to identify the nesting trees of P. volans (Hanski et al. 2000; Asari et al. 2009) in the same way as G. sabrinus and G. volans. This method, however, requires considerable labor, and catching the squirrels may disturb them. Inserting CCD cameras to peer into tree cavities to confirm their presence (Muraki and Yanagawa 2006) also risks disturbing the individuals in their nests, in some cases causing them to move elsewhere.

Tree cavities are also used for breeding and resting by small mammals such as mice and bats, as well as many species of birds (Kotaka and Matsuoka 2002; Muraki and Yanagawa 2006). Peering into cavities using cameras may disturb these animals, increasing the risk of their abandoning their nest-building activities, especially during breeding periods. These facts prompted us to develop a method of finding cavity trees used as nests by *P. volans*, other small mammals and birds without causing serious disturbance.

Feces tends to accumulate at the foot of nesting trees used by *P. volans* (Kadosaki 2001). In addition, *P. volans* usually defecates on the tree trunk near its nesting cavity (Kadosaki 2001). This can be used as an indication that the cavity tree is inhabited by this species (Hanski et al. 2000). Finding trees being used for nesting simply by looking for feces at the foot of trees with cavities is unlikely to affect the animals very much, but the accuracy of this method of confirming the presence of squirrels has so far not been determined. In this study, we verified the accuracy of this method.

Study areas and methods

First, we compared the number of nests built in cavity trees between those with and without accumulations of feces of *P. volans* at their base. Then, from among those with feces, we compared the nesting rate (presence of individuals) between cavity trees with new feces and those without. See below for details.

Study areas

The research was conducted in forests stretching between Obihiro City and Nakasatsunai Village (42°42'– 42°52'N, 143°8'–143°11'E) in Hokkaido, Japan. Searched forest area was approximately 42 ha. Number of covered animals will be approximately 40 to 50, because their home-range sizes are 2.98 ha for males and 1.28 ha for females (Asari 2008). This area is known to be inhabited

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by *P. volans*, with an average of 5 nests per individual (Asari and Yanagawa 2008; Tojo and Yanagawa 2008). The forests are composed mainly of Korean pine (*Pinus koraiensis*), eastern white pine (*Pinus strobus*), Japanese larch (*Larix leptolepis*), Japanese white birch (*Betula platyphylla*), Manchurian walnut (*Juglans mandshurica*) and Japanese emperor oak (*Quercus dentata*).

Feces search

First, we searched cavity trees in the forest. Mean height of searched tree cavities was 2.4 (range: 0.2–14.0) m. To distinguish cavity trees with or without accumulated feces at their base, we looked for the feces on the ground within a 1 m radius of the foot of each cavity tree from May to October 2009. The stool of P. volans is shaped like a straw bag, measuring 4-15 mm in length and 2-5 mm in diameter, making it relatively easy to identify (Kadosaki 2001). A cavity tree with one or more pieces of feces underneath was classified as a tree with accumulated feces, while a tree with no piece within 1 m was classified as one without feces. Since dung beetles increasingly break up the feces of herbivorous animals from spring to summer (Ikeda et al. 2002), we conducted research in 3 seasons-spring (May to June), summer (July to August) and autumn (September to October): once per season for most trees, but for some trees more than once per season.

It was impossible to identify the exact age of feces found at the foot of cavity trees, so we searched for new feces along with older accumulations of feces. Plastic umbrellas (80 cm in diameter) were placed upside down at the foot of trees where the feces tended to be densest (Fig. 1). If one or more pieces of feces were found in an umbrella one day after placing it, it was classified as "feces collected," whereas the classification as "feces not collected" was made if none were found.

Verification of cavity occupancy and analyses

The presence of *P. volans* inside tree cavities was confirmed using a small CCD camera (HOGA: PX/ 167SP/1L-5m) and a fiberscope (iPROS Corporation: High-brightness LED One Hand Free scope 1.5M). The presence of squirrels inside tree cavities was confirmed on the same day that feces had been found at the foot of the trees. A cavity tree with a confirmed presence of *P. volans* was defined as a "nested" tree, while one without a confirmed presence was defined as "non-nested." However, for a tree with multiple cavities, a tree with a confirmed presence in any of the cavities was defined as



Fig. 1. An umbrella placed at the base of a cavity tree, to trap newly-produced feces of *Pteromys volans*. The white arrow indicates a cavity occupied by *P. volans*.

"nested". If we were unable to observe any presence inside tree cavities using these tools, we observed whether the flying squirrels came out of the nests during one hour after sunset (the time they are most likely to leave their nests) (Yamaguchi and Yanagawa 1995), but on only one day at the site of the study. A tree was defined as "nested" if *P. volans* was seen coming out of the nest at this time, while a tree was defined as "nonnested" if no *P. volans* was spotted.

To test whether the feces at the foot of cavity trees can be used as an indicator for nesting by *P. volans*, we compared in each season the number of trees used for nesting with that of non-nested trees among the cavity trees with and without feces accumulation. This analysis was performed using Fisher's exact test. To test whether the feces deposited on the day of confirming nesting can be used as a marker for *P. volans*, we compared in each season the number of cavity trees with and without new feces, using the same test.

Results and discussion

Relationship between feces at the foot of cavity trees and nesting cavities

A total of 177 different cavity trees were studied: 146 in the spring, 116 in the summer and 121 in the autumn. Of these trees, sixty-five cavity trees were searched three

Table 1. The number of observed cavity trees and trees nested by *P*. *volans*. The proportions of nested trees are indicated in parentheses. *P*-values by Fisher's exact test are shown to compare the nesting rates in each season between trees with and without accumulated feces at their base.

Accumulated	Presence		Absence		D voluo
	п	nesting	п	nesting	
Spring	29	12 (41.4)	117	0 (0.0)	< 0.001
Summer	24	10 (41.7)	92	2 (2.2)	< 0.001
Autumn	24	10 (41.7)	97	3 (3.1)	< 0.001

seasons. Twenty-nine cavity trees with accumulated feces were found in the spring, while 24 each were found in the summer and the autumn. The remaining trees had no feces. The number of cavity trees nested in by *P. volans* was 12 in the spring, 12 in the summer and 13 in the autumn. They climbed obliquely upward from their nesting cavity and defecated on the tree trunk near the cavity. The comparison between the cavity trees with the accumulated feces and those without shows that the number of nests was much greater in those with accumulated feces than in those without. The nesting rate in trees with the accumulated feces was approximately 40% in each of the 3 seasons (Table 1).

The above results indicate that feces at the foot of cavity trees can be a marker for the nesting of *P. volans* in every season tested. The nesting rate inside tree cavities with accumulated feces was almost the same in all the seasons, indicating that the results will be almost the same in any season except for winter. One of the reasons for the nesting rate being at only approximately 40% may be the fact that each individual has maltiple nests and moves to a new nest every 20 days (Hanski et al. 2000), which could lead to the finding of old feces of an individual which has already left the nest.

Relationship between new feces and nesting cavities

We tried to collect new feces using umbrellas placed at the foot of 22 cavity trees with pre-existing feces in the spring, 24 in the summer and 20 in the autumn from a total of 42 different cavity trees. Umbrellas were placed under three identical trees through the seasons. New feces were collected at seven cavity trees in the spring, 10 in the summer and 8 in the autumn. No new feces were found in the remaining trees with previously accumulated feces. Based on the average $\pm SD$, the number of feces collected was 3.3 ± 3.6 (range: 1–11) in the spring, 4.3 ± 2.5 (1–9) in the summer and 3.8 ± 3.7 (1–

Table 2. Numbers of observed cavity trees and trees nested by *P*. *volans*, in the experiment to trap newly-produced feces at the base of cavity trees that already had accumulated feces. The proportions of nested trees are indicated in parentheses. *P*-values by Fisher's exact test are shown to compare the nesting rates in each season between presence and absence of new feces caught at the foot of cavity trees.

New feces –	Presence		Absence		D voluo
	п	nesting	п	nesting	-value
Spring	7	6 (85.7)	15	1 (6.7)	< 0.001
Summer	10	10 (100.0)	14	0 (0.0)	< 0.001
Autumn	8	8 (100.0)	12	0 (0.0)	< 0.001

12) in the autumn. Seventy-two percent (18/25) of the trees had 1–4 pieces of feces.

A comparison of the number of nests of *P. volans* between the cavity trees with and without new feces shows that in all seasons, the number of nests in the trees with new feces was much greater than those without (Table 2). Of cavity trees with new feces, the percentage of nested trees remained high at 85%–100% (Table 2).

These results indicate that new feces can be employed as a marker for cavity trees nested in by *P. volans* in any season other than winter. Cavity trees with accumulated feces where new feces are also collected can be identified, with high accuracy, as cavity trees nested in by *P. volans*. Since the percentage of nesting in the cavity trees with new feces did not change greatly with the seasons, it can be concluded that the results will be equally useful in all seasons other than winter when snow has fallen.

The methods employed in this study can reduce the amount of labor required for the conventional method of catching and tracking flying squirrels (Hanski et al. 2000; Asari et al. 2009). They are also less likely to have a negative impact on the wide variety of mammals and birds that use tree cavities (Kotaka and Matsuoka 2002; Muraki and Yanagawa 2006). Placing umbrellas under all cavity trees, regardless of the presence or absence of feces, would be inefficient, because nesting rate of cavity trees without accumulated feces were low percentage.

We conclude that finding the feces of *P. volans* accumulated under the cavity trees and collecting new feces with umbrellas under them is an effective and accurate method for detection of cavity trees nested by *P. volans*, and will not disturb any animals nested there.

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