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Nest material usage in the small Japanese field mouse (*Apodemus argenteus*) on Hokkaido Island, Japan

Ayumu Teruuchi, Masatoshi Sato & Tatsuo Oshida*

ABSTRACT. Mammals that build the nests utilize various nesting materials, such as leaves. However, the selectivity of these materials has not been studied in detail for each species. In this study, we tested whether the dominant nesting resources within habitats are more frequently used for nest building by the small Japanese field mouse (*Apodemus argenteus*), a semi-arboreal species endemic to Japan. We established two study sites in Hokkaido, Japan, with different vegetation types: one in a natural deciduous broad-leaved forest in Ashoro and the other in a secondary mixed conifer-broadleaf forest in Obihiro. Using nest boxes, we collected nesting materials (leaves) and compared them between the two study sites. For tree leaves, *A. argenteus* primarily used *Quercus crispula* and *Betula platyphylla* in Ashoro and Obihiro, respectively. These results suggest that this mouse frequently used the dominant nesting resources in the habitat. *Sasa nipponica*, a commonly observed floor plant in Hokkaido, was frequently used at both study sites, suggesting that it could be a stable nesting resource for *A. argenteus* in different habitats. This study showed that the combination of tree and floor plant leaves may be more effective for nest building than using either tree or floor plant leaves alone.

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Использование гнездового материала малой японской полевой мышью (*Apodemus argenteus*) на острове Хоккайдо, Япония

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РЕЗЮМЕ. Млекопитающие, которые строят гнезда, используют различные гнездовые материалы, в том числе, листья. Однако выбор этих материалов не был подробно изучен для разных видов. В этом исследовании мы исследовали, используются ли доминирующие гнездовые ресурсы в пределах местообитаний для строительства гнезд малой японской полевой мышью (*Apodemus argenteus*), полудревесным видом, эндемичным для Японии. Мы создали два участка для исследования на острове Хоккайдо, Япония, с разными типами растительности: один в естественном лиственном широколиственном лесу в Асёро, а другой во вторичном смешанном хвойно-широколиственном лесу в Обихиро. Используя гнездовые ящики, мы собрали гнездовые материалы (листья) и сравнили их между двумя местами исследования. Из листьев деревьев, *A. argenteus* в основном использовал *Quercus crispula* и *Betula platyphylla* в Ашоро и Обихиро соответственно. Это свидетельствуют о том, что для обустройства гнезда часто были использованы доминирующие ресурсы в среде обитания. *Sasa nipponica*, обычно на Хоккайдо растение нижнего яруса, часто использовалось в обоих местах исследования, что предполагает, что оно может быть стабильным ресурсом для гнездования для *A. argenteus* в различных средах обитания. Это исследование показало, что сочетание листьев древесных и травянистых растений может быть более эффективным для строительства гнезда, чем использование листьев либо древесных, либо травянистых растений по отдельности.

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Introduction

Nests provide many mammalian species with essential spaces for resting and environmental protection (Deeming, 2023), and give benefits of survival (Sealander, 1952) and reproductive success (Gaskill *et al*., 2013). Some mammalian species use natural materials to build their nests. For instance, the hazel dormouse, *Muscardinus avellanarius* (Linnaeus, 1758) uses leaves (Čanády, 2015); the European ground squirrel, *Spermophilus citellus* (Linnaeus, 1766) exclusively uses fresh fescue (Gedeon *et al*., 2010); the northern flying squirrel, *Glaucomys sabrinus* (Shaw, 1801) primarily uses arboreal lichens (Hayward & Rosentreter, 1994); and the Japanese giant flying squirrel, *Petaurista leucogenys* (Temminck, 1827) mainly uses shredded bark (Ando & Shiraishi, 1983). The nesting materials may confer good insulation and provide appropriate structural support, but detailed studies on the selection of nesting materials for mammals are limited (Deeming, 2023).

Arboreal small rodents provide valuable models for studying nesting material selectivity. The small Japanese field mouse, *Apodemus argenteus* (Temminck, 1844), which is an endemic species to Japan (Nakata *et al*., 2015; Denys *et al*., 2017), typically inhabits underground nests. These hole nests are found near tree roots, under fallen trees, and under rocks and have various functions such as food storage, refuge, nursing, and resting (Setoguchi, 1981). However, during the breeding season, it transports nesting materials, primarily leaves from the ground to tree cavities to construct nests (Teruuchi *et al*., 2021; Kikuchi & Oshida, 2023, Fig. 1) for raising its young (Sekijima, 2001; Suzuki & Yanagawa, 2012), although the hole nests are also used for raising on the ground by some individuals. This behaviour allows for the analysis of the nest materials collected into arboreal nests (tree cavities) (Ando, 2005).

On Honshu Island, Japan, *Apodemus argenteus* does not exhibit a preference for specific tree species as nesting materials (Ando, 2005). However, in the *Abies sachalinensis*-dominated mixed forest on Hokkaido Island, Japan, this mouse shows a preference for leaves of *Quercus crispula* and frequently uses bamboo leaves (*Sasa* spp.) (Ito *et al*., 2023). These observations suggest that *A. argenteus* populations in different habitats may exhibit varying selectivity for nesting materials, likely influenced by differences in the availability of nest materials each habitat provides.

We hypothesized that *A. argenteus* exhibits different nesting material selectivity depending on habitat conditions, specifically utilizing dominant nesting resources available within each habitat for nest building. To test this hypothesis, we compared the nesting materials between two habitats with different vegetation types. This study aimed to examine the nesting material selectivity of *A. argenteus* and provide new insights into the factors influencing its nest-building behaviour.

Materials and methods

Study sites

In this study, we established two study sites (Fig. 1). One site was located in a natural deciduous broadleaved forest in the Kyushu University Hokkaido Experimental Forest (43.517° N, 143.883° E), Ashoro, Hokkaido, Japan. The forest primarily comprised *Q. crispula*, *Betula platyphylla*, and *Acer pictum*, with the forest floor primarily covered with *Sasa nipponica* (Okano, 1994). In 2023, the annual precipitation was 738.0 mm, and the mean annual air temperature ranged from 2.3° to 15.0° (Japan Meteorological Agency, 2023). The other site was established in a mixed conifer-broadleaf forest (42.87° N, 143.267° E) in Obihiro, Hokkaido, Japan. The dominant trees included *Alnus japonica*, *Ulmus davidiana*, *B. platyphylla*, and *Quercus dentata*, with *S. nipponica* covering the forest floor. In 2023, the annual precipitation was 808.0 mm, and the mean annual air temperatures ranged from 4.2° to 14.8° (Japan Meteorological Agency, 2023). Although both study sites differed in vegetation, *B. platyphylla* and *S. nipponica* were dominant plants in common.

To identify dominant nesting materials in both study sites, 10 one-meter square plots were randomly placed within each study site to survey fallen leaves that are possible nesting materials. For each plot, we collected surface leaves (litter) and determined their species names. For each plant species, we recorded the number of leaves. In addition, the leaves were dried at room temperature for a few weeks and weighed separately by species.

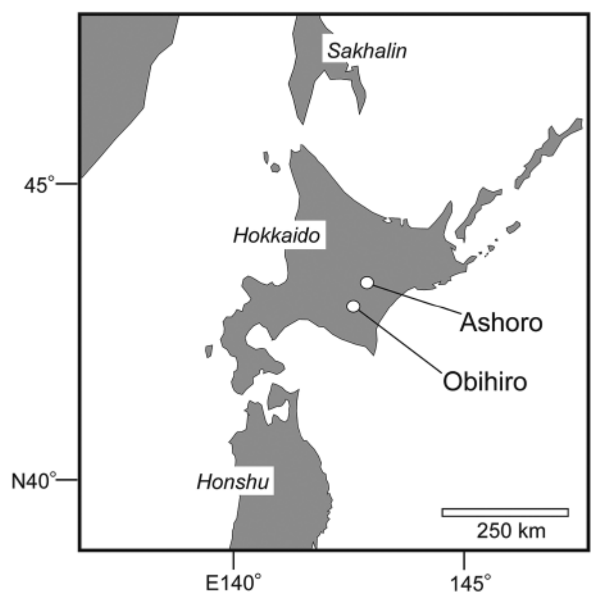

Fig. 1. Study sites (Ashoro and Obihiro) in Hokkaido, Japan.

Collection of nesting materials

The study periods were from May 15 to October 16, 2023, in Ashoro and from May 30 to November 14, 2023, in Obihiro. To collect nesting materials of *A. argenteus*, we constructed wooden nest boxes with internal dimensions of 9 × 12 × 22 cm and entrance dimensions of 4 × 4 cm, following Yanagawa (1994). At each study site, 60 nest boxes were attached to tree trunks at a height of approximately 1.0 m above the ground, following Suzuki & Yanagawa (2012). The nest boxes were numbered from 1 to 60. In both study sites, three line transects were established, spaced approximately 20–30 m apart. Nest boxes were placed along each transect at intervals of approximately 15–30 m. We monitored whether *A. argenteus* used these nest boxes several times per month during the study periods. After *A. argenteus* had finished using a nest box, we collected their nesting materials accumulated inside the nest boxes (Fig. 2).

Analysis of nesting materials

The nesting materials (leaves) were dried at room temperature for several weeks. The total amount of dry nesting materials collected from each nest box was weighed.

To assess the different status of nesting materials, we primarily compared the mean weights of the nesting materials between Ashoro and Obihiro. For this analysis, after testing for normality using the Shapiro-Wilk test, we applied either a parametric or non-parametric test as appropriate. Due to the condition of the leaves (almost all leaves were fragmented), we extracted 2.5 g identifiable partial leaves from the total nesting materials collected in each nest box for further statistical processing. Leaf species were then visually identified. For each plant species, we recorded the number of leaves and their corresponding weights. Moreover, we categorized leaves into either tree or floor plants.

Because *A. argenteus* carries only one leaf at a time (Kikuchi & Oshida, 2023), we considered the number of leaves to be a reliable indicator for assessing differences in nesting material selectivity, taking into account the availability of plant materials. In each study site, we compared nesting materials with available leaves detected on the ground. In addition, we compared the numbers of leaves between Ashoro and Obihiro using a χ^2 test to test for regional differences in the ratio of plant species used as nesting materials. Statistical analyses were conducted using JMP version 11.0 (SAS Institute Inc., 2013), with a significance level set at 5%.

Results

In Ashoro, we identified 11 plant species in available materials collected on the ground;

Fig. 2. Nest box occupied with nesting materials consisting of bamboo (*Sasa nipponica*) leaves.

Q. crispula was most commonly detected, followed by *S. nipponica* (Table 1). In Obihiro, we identified eight plant species in available materials collected on the ground; *U. davidiana* was most commonly detected, followed by *S. nipponica* (Table 1).

Table 1. Number of leaves collected in one-meter square plots established on ground in study sites. Weight of leaves (in grams) is in parentheses. Hyphens indicate no leaves.

Study site	Plot number	*Quercus crispula*	*Acer pictum*	*Betula platyphylla*	*Ulmus davidiana*	*Alnus japonica*	*Quercus dentata*	*Populus suaveolens*	*Hydrangea petiolaris*	*Kalopanax pictus*	*Aesculus turbinata*	*Acer amoenum*	*Tilia maximowicziana*	*Sasa nipponica*
Ashoro	1	105 (37.03)	–	–	–	–	–	–	–	–	–	5 (0.32)	–	14 (2.19)
	2	84 (38.39)	2 (0.30)	–	–	–	–	–	–	–	–	–	–	24 (3.79)
	3	75 (33.60)	3 (0.93)	–	–	7 (1.17)	–	–	–	11 (8.91)	–	3 (0.30)	–	8 (0.80)
	4	63 (33.16)	–	–	–	3 (0.36)	–	–	–	12 (13.88)	–	–	–	15 (2.47)
	5	74 (32.66)	2 (0.17)	–	–	4 (0.44)	–	3 (0.62)	–	5 (4.18)	–	–	1 (0.46)	21 (3.35)
	6	41 (18.51)	26 (4.58)	12 (1.33)	10 (1.48)	21 (1.51)	–	4 (1.80)	–	8(15.03)	–	–	3 (0.59)	14 (1.88)
	7	85 (33.99)	6 (1.05)	–	–	5 (0.41)	–	–	–	4 (1.34)	–	2 (0.16)	8 (3.80)	8 (0.92)
	8	109 (45.01)	–	–	–	–	–	–	–	–	–	–	–	25 (3.24)
	9	124 (38.10)	–	–	–	–	–	–	–	–	–	–	–	26 (3.10)
	10	84 (36.21)	–	–	–	–	–	–	–	18 (12.55)	–	–	–	27 (3.55)
	Total	844 (346.66)	39 (7.03)	12 (1.33)	10 (1.48)	40 (3.89)	–	7 (2.42)	–	58 (55.89)	–	10 (0.78)	12 (4.85)	182 (25.29)
Obihiro	1	–	–	11 (1.65)	11 (1.81)	53 (12.75)	–	8 (2.01)	–	–	–	–	–	15 (1.51)
	2	–	–	47 (5.73)	13 (2.21)	56 (15.11)	–	–	–	–	–	–	–	12 (1.03)
	3	–	–	58 (7.69)	35 (6.49)	13 (2.14)	–	–	–	–	–	–	–	43 (4.60)
	4	–	–	46 (8.10)	40 (8.07)	17 (2.76)	–	–	–	10 (5.25)	–	–	–	1 (0.12)
	5	–	–	–	78 (13.04)	19 (2.71)	–	–	–	2 (0.41)	17 (4.81)	–	–	55 (5.89)
	6	–	–	2 (0.48)	29 (6.27)	18 (3.65)	2 (1.55)	37 (13.45)	–	–	20 (3.47)	–	–	14 (1.72)
	7	–	–	–	96 (15.28)	8 (1.49)	1 (0.71)	2 (0.84)	–	5 (0.48)	–	–	–	43 (4.70)
	8	–	–	–	49 (8.42)	44 (7.62)	4 (2.72)	14 (4.99)	–	–	–	–	–	38 (4.81)
	9	–	–	–	43 (8.00)	2 (0.27)	22 (33.96)	6 (2.25)	–	–	–	–	–	32 (3.20)
	10	–	–	–	31 (5.83)	34 (5.51)	10 (9.60)	1 (0.31)	–	1 (0.04)	–	–	–	65 (10.35)
	Total	–	–	164 (23.65)	425 (75.42)	264 (54.01)	39 (48.54)	68 (23.85)	–	18 (6.18)	37 (8.28)	–	–	318 (37.93)

Apodemus argenteus built nests in 12 nest boxes at Ashoro and 14 nest boxes at Obihiro (Table 2). The Shapiro-Wilk test indicated that the weights of nesting materials in both Ashoro and Obihiro followed a normal distribution (w = 0.97, $p = 0.938$ in Ashoro; w = 0.95, $p = 0.565$ in Obihiro). The mean weight of nesting materials in Ashoro (27.59 ± 11.42 g ($\bar{x}$± SD)) did not significantly differ from that in Obihiro (22.55 ± 10.73 g ($\bar{x}$ ± SD)) (*t* test: $t = -1.160$, *d.f.* = 24, $p > 0.2$).

Table 2. Weight of nesting materials (in grams) collected by *Apodemus argenteus* into nest boxes.

Study site	Nest box number	Weight of nesting material
Ashoro	1	18.8
	5	24.2
	7	32.9
	10	23.0
	13	31.0
	14	20.8
	15	42.1
	19	25.5
	24	38.0
	51	48.2
	57	19.7
	59	6.9
Obihiro	1	27.6
	6	5.1
	9	10.9
	10	17.9
	11	17.7
	12	17.8
	17	32.3
	18	44.6
	20	35.8
	22	29.9
	37	16.1
	41	16.7
	54	27.8
	55	15.5

In Ashoro, we identified four plant species in nesting materials (*Q. crispula*, *A. pictum*, *Calyptranthe petiolaris*, and *S. nipponica*). Notably, *B. platyphylla* was not detected. *Quercus crispula* was found in all nests and was the most dominant species, followed by *S. nipponica* and *A. pictum* (Table 3). Unfortunately, it was difficult to statistically evaluate plant species between nest materials and available leaf resources found on the ground due to inadequate data. However, in both categories, *Q. crispula* accounted for more than 60%. In Obihiro, we identified six species (*B. platyphylla*, *U. davidana*, *A. japonica*, *Quercus dentara*, *Populus suaveolens*, and *S. nipponica*) in nest boxes (Table 3). *Sasa nipponica* was observed in all nests and was the most commonly used material, followed by *B. platyphylla*. The other four species were poorly represented in the nest material. Two nest boxes (nos. 37 and 41) were occupied by only *S. nipponica*. Unfortunately, it was difficult to statistically evaluate plant species between nest materials and available leaf resources found on the ground, due to an inadequate data set in nest materials.

Nest material species such as *Q. crispula*, *A. pictum*, and *C. petiolaris* were only found at Ashoro, while *B. platyphylla*, *U. davidana*, *A. japonica*, *Q. dentara*, and *P. suaveolens* were only observed at Obihiro. The number of leaves of *C. petiolaris*, *U. davidana*, *A. japonica*, *Q. dentara*, and *P. suaveolens* was insufficient for statistical analysis for comparison between the two study sites (Table 3). However, we compared the number of leaves of trees and floor plants between the two study sites. The ratio of trees and floor plants differed significantly between the two study sites (χ^2 test: $\chi^2 = 81.604$, $p < 0.01$).

Discussion

The mean weights of the nesting materials collected from each nest box did not differ significantly between Ashoro and Obihiro. Although the capacity of the nest box may have limited the amount of materials collected, this result suggests that the arboreal nests of *A. argenteus* were constructed with similar amounts of materials across different habitats independently of the different plant species available for *A. argenteus*.

In term of tree leaves, *A. argenteus* primarily used *Q. crispula* in Ashoro and *B. platyphylla*, the third most common species in Obihiro. Therefore, our hypothesis that dominant nesting resources within the habitat are utilized for nest building seemed to be supported, although the most common *U. davidiana* was not used so much in Obihiro. *Apodemus argenteus* has a narrow home range, ranging from 871 to 1 241 m^2 (Setoguchi, 1981), which may restrict its ability to forage for nesting materials beyond this range. For instance, the harvest mouse *Micromys minutus* (Pallas, 1771) constructs spherical nests using grasses (Iwasa, 2015), with the species of grasses varying depending on the habitat conditions (Hata, 2011). Similarly, *A. argenteus* appears to use opportunistically available nesting materials within its limited home range.

In this study, we found that the ratio of tree leaves to floor plants (*S. nipponica*) differed between the two study sites. In Ashoro, the number of tree leaves was almost double that of the floor plant leaves. In contrast, the number of floor leaves was significantly greater than that of tree leaves in Obihiro. Considering the floor plants, *S. nipponica* could be the primary nesting material in Obihiro. In Ashoro, as reported by Ito *et al*. (2023), this mouse might prefer *Q. crispula*, which is the primary nesting material of choice when sufficiently present. Among the tree leaves, *B. platyphylla* was used most frequently in Obihiro but that was not observed in nesting materials in Ashoro, despite being abundant there. This mouse may have preferred *Q. crispula* to *Betula* spp. in Ashoro. *Apodemus argenteus* feed on the acorns of *Q. crispula* (Ando, 2005; Nakata *et al*., 2015; Denys *et al*., 2017). Therefore,

Table 3. Number of leaves found in nest boxes used by *Apodemus argenteus*. Weight of leaves (in grams) is in parentheses. Hyphens indicate no leaves.

Study site	Nest box number	Trees								Floor plant
		Quercus crispula	*Acer pictum*	*Betula platyphylla*	*Ulmus davidiana*	*Alnus japonica*	*Quercus dentata*	*Populus suaveolens*	*Calyptranthe petiolaris*	*Sasa nipponica*
Ashoro	1	16 (1.47)	–	–	–	–	–	–	–	6 (0.32)
	5	10 (1.45)	1 (0.13)	–	–	–	–	–	–	7 (0.56)
	7	21 (1.31)	–	–	–	–	–	–	–	7 (0.25)
	10	3 (0.54)	5 (0.54)	–	–	–	–	–	8 (0.73)	–
	13	11 (1.60)	2 (0.19)	–	–	–	–	–	–	5 (0.37)
	14	14 (1.34)	1 (0.13)	–	–	–	–	–	–	2 (0.15)
	15	12 (1.80)	2 (0.31)	–	–	–	–	–	–	3 (0.18)
	19	10 (1.06)	2 (0.24)	–	–	–	–	–	–	–
	24	1 (0.11)	13 (1.30)	–	–	–	–	–	–	1 (0.05)
	51	11 (1.23)	–	–	–	–	–	–	–	8 (0.72)
	57	13 (1.93)	–	–	–	–	–	–	–	3 (0.18)
	59	5 (0.50)	–	–	–	–	–	–	–	1 (0.04)
	Total	127 (14.34)	26 (2.84)	–	–	–	–	–	8 (0.73)	43 (2.82)
Obihiro	1	–	–	15 (1.52)	2 (0.16)	–	–	–	–	4 (0.32)
	9	–	–	3 (0.27)	–	–	–	–	–	28 (2.22)
	10	–	–	11 (0.98)	–	–	–	–	–	12 (0.75)
	11	–	–	4 (0.38)	1 (0.19)	2 (0.18)	–	–	–	13 (1.07)
	12	–	–	15 (1.02)	–	–	–	–	–	22 (0.99)
	17	–	–	1 (0.10)	–	2 (0.50)	–	4 (0.69)	–	14 (1.14)
	18	–	–	6 (0.88)	–	–	–	–	–	7 (0.40)
	19	–	–	11 (0.97)	–	–	–	–	–	17 (1.38)
	20	–	–	14 (1.16)	2 (0.28)	–	–	–	–	15 (0.93)
	22	–	–	28 (2.27)	–	–	–	–	–	2 (0.17)
	37	–	–	–	–	–	–	–	–	28 (1.49)
	41	–	–	–	–	–	–	–	–	24 (2.46)
	54	–	–	3 (0.18)	–	–	4 (0.44)	–	–	16 (0.93)
	55	–	–	2 (0.12)	–	–	–	–	–	27 (2.19)
	Total	–	–	113 (9.85)	5 (0.63)	4 (0.68)	4 (0.44)	4 (0.69)	–	207 (16.44)

Q. crispula may serve as an important resource for both nesting and feeding.

Apodemus argenteus frequently used *S. nipponica* at both study sites. Although no *S. nipponica* leaves were found in two nest boxes in Ashoro (Table 3), this floor plant was more frequently used for nest construction than the tree leaves of *A. pictum* and *C. petiolaris*. In Obihiro, *S. nipponica* was the most frequently used species for *A. argenteus*. Ito *et al*. (2023) reported that the mice frequently used *Sasa* spp. as nesting material. *Sasa* spp. are commonly found as dominant floor plant species in the forests of Hokkaido (Yamanaka, 1983; Okitsu, 2002). Therefore, *A. argenteus* may be able to reliably obtain this material from various vegetation types in Hokkaido. When *A. argenteus* builds nests, *Sasa* spp. may be an important key resource.

Our results indicate that *A. argenteus* uses the leaves of the dominant tree species in its habitat as a nesting resource but does not rely solely on these leaves. The mouse also frequently uses the dominant forest floor species, regardless of habitat. This combination of tree and floor plant leaves may be more effective for nest construction than relying on either tree or floor plant leaves alone.

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