sabrinus, and North American Red Squirrels, *Tamiasciurus hudsonicus*, in a Secondary Hardwood Forest of Southern Ontario

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Through deployment of artificial nest boxes, we examined the composition of cavity nest materials used by Northern Flying Squirrels (*Glaucomys sabrinus*) and North American Red Squirrels (*Tamiasciurus hudsonicus*) in a secondary hardwood forest of southern Ontario, Canada. We collected 32 nests of known species association and found that 85.7% of *G. sabrinus* nests and 77.8% of *T. hudsonicus* nests were constructed almost entirely of shredded bark from Eastern White Cedar (*Thuja occidentalis*). Mean nest depth across all samples was 12.2 cm and showed no significant difference between species or between spring and summer nests. We review the antiparasitic properties of *T. occidentalis* and suggest that the use of shredded cedar bark by *G. sabrinus* and *T. hudsonicus* to line nest cavities may be a behavioural adaptation, which reduces ectoparasite loads in the nest environment.

Key Words: Northern Flying Squirrel, *Glaucomys sabrinus,* North American Red Squirrel, *Tamiasciurus hudsonicus,* ectoparasites, nest box, nest material, nest-protection hypothesis, thermoregulation, Eastern White Cedar, *Thuja occidentalis,* Ontario.

Tree cavities are a critical resource for many tree squirrels in forest ecosystems, providing sites for raising young, avoiding predators, and improving thermoregulation (Collias 1964; Weigl 1978; Wiebe 2001). Tree cavities are generally most abundant in old growth forests where large diameter trees and well-decayed snags are commonplace (Holloway and Malcolm 2006; Holloway and Malcolm 2007). Cavities also may form in live trees where the entry of rot has been facilitated by disease, deformities, broken limbs or woodpecker excavations. Where tree cavities are not readily available, many tree squirrels use external nests (dreys) or subterranean nests, although evidence is emerging that tree cavities are preferentially selected when available (Bakker and Hastings 2002). Nest materials used in cavity nest construction may not only act to increase ambient temperature in the nest environment and provide comfort for nest occupants, but may also reduce ectoparasite loads and repel moisture in these dark, damp microclimates (Stapp et al. 1991; Hemmes et al. 2002.) However, despite their importance, tree cavities and cavity nests are rarely incorporated into studies of tree squirrels (Fokidis and Risch 2005).

The nocturnal Northern Flying Squirrel (*Glaucomys sabrinus*) and the diurnal North American Red Squirrel (*Tamiasciurus hudsonicus*) are both occasional cavity nesting, arboreal rodents common in temperate and boreal forests of North America. Both species are primarily associated with conifer-dominated forests, although in many areas of eastern North America they inhabit hardwood and mixed hardwood-conifer forests (Flyger and Gates 1982; Holloway and Malcolm 2006; Patterson 2008). Both *G. sabrinus* and *T. hudsonicus* have been found to use cavities in trees more intensively than external or subterranean shelters, especially for natal nests (Layne 1954; Bakker and Hastings 2002; Holloway and Malcolm 2007).

Nest materials used by arboreal squirrels have been found to vary geographically (Muul 1974). However, most reports describing nest materials employed by squirrels have been qualitative in nature and have focused on dreys. *Tamiasciurus hudsonicus* dreys have been noted to include grape bark, deciduous leaves, dried grasses, moss, feathers, fur and soft inner bark (Layne 1954). Similarly, dreys of *G. sabrinus* have been found to consist of dried grasses, shredded bark, mosses, twigs and lichens (Rust 1946; Mowrey and Zasada 1984). Cavity nests have been described quantitatively only once for *G. sabrinus* (Hayward and Rosentreter 1994). Using nest boxes, these authors found that *G. sabrinus* in the northern Rocky Mountains of central Idaho and western Montana primarily constructed cavity nests from arboreal lichens, especially of the genera *Bryoria* and *Letharia*. We are not aware of studies that have quantified cavity nest materials of *T. hudsonicus*; however, Hayward and Rosentreter (1994) suggest a preference for grasses and shredded bark. Published data on *G. sabrinus* and *T. hudsonicus* nest materials, and associated seasonal differences and depths, in the Great Lakes-St. Lawrence forest region of eastern North America do not exist. In general, the study of nest material use and function in mammals has received very little attention by researchers and thus warrants investigation.

In order to gain a more complete understanding of the habitat requirements for *G. sabrinus* and *T. hudsonicus* in northern secondary hardwood forests, we characterize cavity nest materials used by these two species in a large network of nest boxes in southern Ontario, Canada. We hypothesized that *G. sabrinus* and *T. hudsonicus* would preferentially select nest materials that favoured cavity nest occupation (i.e., parasite reduction, moisture repellency). Nest depth measurements were investigated as a function of the type of nest material and season (spring and summer); we also assessed seasonal variation in nest material composition. We predicted that nest depths would exhibit interseasonal variability if nest construction was primarily to enhance thermoregulation, such that deeper nests would be constructed under colder temperature regimes. Given the prevalence of shredded cedar bark in the nests of both *G. sabrinus* and *T. hudsonicus,* we discuss the potential antiparasitic and thermoregulatory benefits of Eastern White Cedar (*Thuja occidentalis*) bark.

Study Area

The study was conducted in Bruce and Grey counties, Ontario, Canada (43°59' to 45°12'N, 80°22' to 81°39'W), which together encompass an area of about 22 000 km^2. Nest boxes were placed in trees growing at various elevations, ranging from approximately 200 m to 450 m above sea level. Approximately 22% of the landscape is currently composed of closed-canopy secondary forests, which are dominated by Sugar Maple (*Acer saccharum*), White Ash (*Fraxinus americana*) and American Beech (*Fagus grandifolia*). Other common tree species include Eastern White Cedar, Eastern Hemlock (*Tsuga canadensis*), aspen (*Populus tremuloides, P. grandidentata*) and White Birch (*Betula papyrifera*) (Patterson 2008). Small stands of Eastern White Pine (*Pinus strobus*) and spruce (*Picea glauca, P. mariana, P. rubens*) occur throughout the area, often in plantations.

Materials and Methods

A total of 266 nest boxes were installed between 2002 and 2004 as part of a larger study being conducted by SJP on *G. sabrinus*. Sampling effort varied among years due to logistical constraints. In 2002, 154 boxes were installed; an additional 36 boxes were installed in 2003 and the final 76 boxes were installed in 2004. All nest boxes were placed at heights of 3-4.5 m on trees that had a diameter at breast height >17 cm. Following Carey (2002), nest boxes were established at a density of three nest boxes/ha to allow for a balance between occupancy rates and monitoring effort. Three or more boxes were placed at each of 70 sites across the study region.

Boxes were constructed following Sonenshine et al. (1973), except for the door which was top mounted and could be flipped up to remove any animals and nest material. Our entrance hole was also of larger diameter (4 cm) to permit use by *G. sabrinus* and *T. hudsonicus,* but exclude Eastern Grey Squirrels (*Sciurus carolinensis*). All boxes were mounted to the tree trunk and any tree branches were removed from below the nest box.

All boxes were intensively checked twice per year during the day: once in the spring and once again in autumn. Spring checks were conducted between 12 May and 12 June following the onset of parturition, whereas autumn checks were conducted between 12 October and 12 November. Cursory visual nest box inspections were conducted monthly to monitor the condition of the boxes, but no sampling was done on these occasions. These visual inspections indicated that neither *G. sabrinus* nor *T. hudsonicus* used the nest boxes in late autumn and winter (early December – late February), presumably because individuals abandoned the nest boxes for larger, better insulated natural cavities or subnivean nests. Therefore, nests found at the spring inspection had been constructed between early March and the date of inspection in spring (hereafter termed "spring" nests), whereas those found at the autumn inspection had been constructed between the spring and autumn inspection dates (hereafter termed "summer" nests). Where nests were present and occupied at time of inspection, we recorded the location, number and reproductive condition of occupants and nest depth. Samples of nest material were collected from all nests for identification of materials. In cases where the nest box was not occupied at the time of inspection, the entire nest was removed. In cases where the nest box was occupied at the time of inspection (14% of the nests), a small sample of nest material was initially removed and the entire nest was subsequently removed during targeted monthly nest box examinations following the departure of nest occupants. We removed nest material not only to examine nest materials, but also because Hayward and Rosentreter (1994) observed that nest boxes were typically not occupied in successive years if used nest material was present. Nest depth was always determined during the initial investigation and measured *in situ* along the vertical dimension from the internal base of the nest box to the top of the nest. Nest volume was determined by multiplying the nest depth by the internal width and length dimensions of the nest box.

Results

Nest Box Occupation

Between 2002 and 2005, we found 224 occupied and unoccupied nests. Of these, 32 were occupied when checked (14 *G. sabrinus* and 18 *T. hudsonicus*). Females were found occupying the nest boxes in 12 cases for *G. sabrinus* and 14 cases for *T. hudsonicus*. Lone males were occasionally found occupying the nest boxes (two *G. sabrinus* and four *T. hudsonicus*).

Nest Materials of *G. sabrinus* and *T. hudsonicus*

Twelve of the 14 boxes occupied by *G. sabrinus* and 14 of the 18 boxes occupied by *T. hudsonicus* contained >90% shredded cedar bark by volume. One *G. sabrinus* nest was composed entirely of fibreglass insulation and another was composed of >80% shredded deciduous bark. Trace amounts of peat moss, dried grasses, cedar leaves and twigs also were found in occupied *G. sabrinus* nests. Two *T. hudsonicus* nests were composed entirely of dried grasses and two nests contained >80% deciduous leaves. Trace amounts of shredded plastic, bird feathers, animal fur, dried grasses and deciduous leaves also were found in occupied *T. hudsonicus* nests.

Nest materials did not appear to differ between seasons (t = -1.892, P = 0.06), with shredded cedar bark appearing as the most prominent nest material in both spring (84.5%) and summer (79.3%). Small sample sizes for male nests did not allow for robust comparisons between male and female nest materials.

Nest Depth

Mean depth of nests occupied by *G. sabrinus* was 12.1 cm (SD = 2.4, range = 6.7 to 15.3 cm) and for *T. hudsonicus* was 12.3 cm (SD = 2.1, range = 9.1 to 17.8 cm), although the difference was not statistically different (t = 0.31, P = 0.76). We found no statistical difference between spring (*G. sabrinus* mean = 11.9 cm; *T. hudsonicus* mean = 12.6 cm) and summer (*G. sabrinus* mean = 11.1 cm; *T. hudsonicus* mean = 12.7 cm) nest depths for either species for occupied nests (*G. sabrinus*: t = 0.55, P = 0.30; *T. hudsonicus*: t = 0.17, P = 0.43). Small sample sizes for male nests did not allow for robust comparisons between male and female nest depths.

Discussion

The prominence of cedar bark in *G. sabrinus* and *T. hudsonicus* nests may be explained by at least two, nonmutually exclusive, hypotheses: nest-protection and thermoregulation. The nest-protection hypothesis has received much attention from ornithologists to explain the use of green vegetation in avian nests. Initially suggested by Wimberger (1984) and tested by Clark and Mason (1985), the nest-protection hypothesis posits that animals exploit the antiparasitic properties of certain plant species that emit specific volatile compounds (Dawson 2004). All plants contain secondary metabolites that are used as a defense against disease and herbivory (Clark and Mason 1988) and, when these plants are used as nesting materials, certain compounds may reduce ectoparasite loads in the nest environment. Numerous studies have supported the nest-protection hypothesis in birds (Wimberger 1984; Rodgers et al. 1988; Fauth et al. 1991; Lafuma et al. 2001). The nest protection hypothesis has found support on at least one occasion for small mammals: Hemmes et al. (2002) reported that Dusky-footed Wood Rats (*Neotoma fuscipes*) placed California Bay (*Umbellularia californica*) leaves around their nest sites. Laboratory tests revealed that when incubated with torn *U. californica* leaves for 72 h, survival of flea larvae was reduced by 74% compared to controls. By using shredded Eastern White Cedar bark as a primary nest material, *G. sabrinus* and *T. hudsonicus* also may be limiting exposure to nest-borne ectoparasites. Phytochemical analysis of *T. occidentalis* bark has revealed 22 volatile compounds, including monoterpenes, fenchene, camphene, camphor, carvacol and paracymene (Shaw 1953; Witte et al. 1983; Yatagai et al. 1985; Keita et al. 2001), several of which have noted antiparasitic or insecticidal properties (Adams et al. 1988; Adams 1993; Keita et al. 2001).

Support for the nest protection hypothesis is bolstered by the use of lichens in nests of *G. sabrinus* found in western North America. Antiparasitic effects have been described for several species of lichen. Hayward and Rosentreter (1994) found that *G. sabrinus* selected lichens containing the secondary compounds norstictic acid, fumarprotocetraric acid, vulpinic acid and atranorin, all of which have been attributed with varying degrees of antiparasitic or antimicrobial effects (Giez et al. 1994; Tay et al. 2004; Yilmaz et al. 2004). These various studies support the possibility that the use of *T. occidentalis*, as well as several lichen species, by *G. sabrinus* and *T. hudsonicus* may be a behavioural adaptation for ectoparasite control in the nest environment. Further testing of the antiparasitic effects of *T. occidentalis* volatile oils and secondary compounds on known *G. sabrinus* and *T. hudsonicus* ectoparasites is required.

An alternative hypothesis is that shredded cedar bark may offer greater insulative properties than other available materials. Hayward and Rosentreter (1994) suggested the same hypothesis when characterizing the primary use of lichens as nest material by *G. sabrinus*. This hypothesis is based on the finding by Stapp et al. (1991) that nests composed of plant fibres allowed Southern Flying Squirrels (*Glaucomys volans*) to reduce their energy expenditure when experimentally subjected to cold temperatures. We are not aware of studies on the thermal properties of cedar bark, lichens or other nest materials found in this study. A common assumption is that nest materials with greater thermal properties are favoured, and/or that nest depth would peak, during colder periods. Contrastingly, we found that shredded cedar bark was used equally in spring and summer and that nest depth did not vary between the two seasons. This suggests that cedar bark may not be chosen solely for thermoregulatory purposes; however, we are unable to reject the thermoregulation hypothesis outright without further empirical testing.

We dismiss a third potential hypothesis that squirrels choose the most widely and readily available materials for use as nest substrate. Patterson (2008) found that deciduous trees accounted for approximately 80% of the tree composition in our study area by basal area, while Eastern White Cedar accounted for less than 10% of the total forest composition on average, by basal area. Deciduous leaves and deciduous tree bark are therefore more greatly available than *T. occidentalis* bark in our study area. At some sites where cedar bark nest material was found to occur, Eastern White Cedar trees accounted for only 2% or less of the total tree composition (Patterson 2008). On one occasion during this study we observed a single female *G. sabrinus* travel over 500 m to the nearest *T. occidentalis* tree to acquire cedar bark for nest construction. Mosses, lichens, and grasses are also widely available throughout the study area (J. Patterson, unpublished data) and yet were rarely found as nest materials in this study.

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