Notes

Consumption of Truffles and other Fungi by the American Red Squirrel (*Tamiasciurus hudsonicus*) and the Eastern Chipmunk (*Tamias striatus*) (Sciuridae) in Northwestern Ontario

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Faecal matter collected from the American Red Squirrel (*Tamiasciurus hudsonicus*) and the Eastern Chipmunk (*Tamias striatus*) on the campus of Lakehead University in Thunder Bay, Ontario, was examined for the presence of consumed fungal tissue. A total of 14 faecal samples were collected from live-trapped animals over six trapping periods (19 June to 25 September 2010). Eight samples contained intact remains of spores representative of hypogeous truffle fungi found in the genera *Elaphomyces, Gautieria, Hymenogaster, Hysterangium,* and *Leucangium*, as well as spores of epigeous mushrooms found in the orders Boletales and Pezizales and possibly in the family Tricholomataceae of the Agaricales. The results of this brief survey suggest not only the importance of mycophagy in the diet of American Red Squirrels and Eastern Chipmunks in the boreal mixed wood forests of northwestern Ontario but also the important role played by sciurids in vectoring spores of truffle fungi in this region.

Key Words: hypogeous fungi, *Tamiasciurus hudsonicus*, *Tamias striatus*, Sciuridae, mycophagy, truffles, *Elaphomyces*, *Gautieria*, *Hymenogaster*, *Hysterangium*, *Leucangium*, Boletales, Pezizales, Tricholomataceae, Agaricales, Ontario.

Mycophagy, or the consumption of fungi, is a worldwide phenomenon found among small to large mammals, including humans (Maser et al. 2008). Members of the family Sciuridae (mainly tree squirrels and chipmunks) are the most frequently observed mammalian mycophagists (Fogel and Trappe 1978). The availability and variety of fungi, including truffles, in forest ecosystems when in season and the minimal foraging efforts needed to locate them are what make fungal fruiting bodies a common and valuable food source to sciurids (Cork and Kenagy 1989; Maser et al. 2008). The American Red Squirrel (*Tamiasciurus hudsonicus* Erxleben) alone has been documented eating 89 species of fungi (Fogel and Trappe 1978), and it is well known that the American Red Squirrel, along with other tree squirrels, dries fungi in tree branches or in the sun before storing them elsewhere (Fogel and Trappe 1978; Lurz and South 1998; Vernes and Poirier 2007; Maser et al. 2008). Many sciurids are known to cache fungi over the winter making fungal fruiting bodies a significant component of a sciurid's diet year round (Maser et al. 1985; Currah et al. 2000; Vernes et al. 2004).

When sciurids dig up and ingest fruiting bodies of hypogeous (fruiting underground) truffle fungi, they also act as vectors for spores, since the spores are indigestible and are excreted intact in faeces (Johnson 1996; Maser et al. 1978). Another means of spore dispersal occurs when fruiting bodies are dug up by sciurids and broken open, releasing spores into the air and onto the fur and paws of the animal (Johnson 1996). For this reason, truffle fungi are dependent on sciurids and other foragers for spore dispersal (Fogel and Trappe 1978; Johnson 1996; Maser et al. 2008). It has been suggested that co-evolution has occurred between hypogeous truffle fungi and their vectors (Maser et al. 2008).

Studies on mycophagy by sciurids in Canada are limited and have included the work of Anderson (2003) in coastal British Columbia, Sidlar (2012) in central British Columbia, Currah et al. (2000) in northeastern Alberta, Vernes et al. (2004) in New Brunswick, and observations by Buller (1922) in Manitoba and adjacent northwestern Ontario. As American Red Squirrels were observed digging up truffle fungi on the Lakehead University campus, we decided to investigate fruiting body consumption and the diversity of fungal species eaten, based on spores found in sciurid faeces.

Study Area and Methods

Live-trapping of sciurids occurred every two to three weeks from 19 June to 25 September 2010, for a total of six sampling periods. No specific species were targeted, but we focused on diurnal species. Three transects were set up, each containing four Sherman live traps. Each transect was approximately 100 metres in length, with each trap in the transect approximately 30 metres apart. Peanut butter on a piece of plain cracker

was provided as a food source. When trapping occurred during dry and/or hot conditions, a slice of apple was used instead to supply water.

Traps were always set before sunrise, and were checked approximately four and a half hours later. If any animals were captured, identifications were made before release.

Trapping occurred in natural forested areas of the Lakehead University campus in Thunder Bay, Ontario, in proximity to the McIntyre River. The forest cover in these areas is conifer dominant mixed woods composed mainly of White Spruce (*Picea glauca* (Moench) Voss), Balsam Fir (*Abies balsamea* (L.) Mill.), Jack Pine (*Pinus banksiana* Lamb.), and Trembling Aspen (*Populus tremuloides* Michx.). The forest cover is characteristic of the transition zone between the boreal forest region (B.9 Superior) (Rowe 1972) and the Great Lakes St. Lawrence Forest Region (L.11 Quetico) (Rowe 1972).

Protective gloves and a surgical mask were worn during handling of any traps containing captured animals and faecal matter. Faecal material collected from the traps was transported from the field in small plastic bags and frozen as soon as possible. Each of the faecal samples was assigned a unique three-digit code indicating the trapping date, the trap line, and the trap number.

Samples were dried in aluminum weighing boats at 100°C for 48 hours. They were then weighed, ground up with a mortar and pestle, and added to 70% ethanol (ethyl alcohol) at a ratio of 0.01g dry weight to 0.2 mL ethanol. From these samples, spore counts were completed using a hemacytometer. Faecal material was mounted in Melzer's reagent (Castellano et al. 1989) and examined using a Nikon Eclipse E400 phase contrast light compound microscope. Spore morphology was used for identification at the genus (or family) level utilizing key taxonomic manuals (e.g., Castellano et al. 1989; Trappe et al. 2007).

Results and Discussion

Only two sciurid species were trapped over the entire trapping period: the American Red Squirrel (4 faecal samples) and the Eastern Chipmunk (10 faecal samples). As no animals were enumerated, it was possible that some of the faecal samples were from repeat captures. Other sciurids occur in forested areas in and around Thunder Bay. These species include the Northern Flying Squirrel (*Glaucomys sabrinus* Shaw), which is a nocturnal species (Banfield 1974); the Least Chipmunk (*Tamias minimus* Bachman); and the Eastern Gray Squirrel (*Sciurus carolinensis* Gmelin), which appears to be more restricted to urban settings. Of the samples collected, all of those from American Red Squirrels contained fungal spores; however, only 4 out of the 10 Eastern Chipmunk samples contained fungal spores.

Of the faecal samples that contained spores, total spore concentrations ranged from 75 million spores per gram of faecal sample dry weight (sample 1-2-2) to 332.5 million spores per gram of faecal sample dry weight (sample 1-1-2). Recognizable taxa varied considerably in concentration per faecal sample (Table 1).

Eight distinctive spore types were identified (Table 1). Of these, five clearly belonged to genera of hypogeous truffle fungi: *Elaphomyces, Gautieria, Hymenogaster, Hysterangium* and *Leucangium* (known formerly as *Picoa*). These fungi have been reported in the literature from studies done elsewhere in North America and Europe (Maser and Maser 1988; Currah et al. 2000; Bertolino et al. 2004; Vernes et al. 2004).

TABLE 1. Taxa of fungi and their spore concentrations found in faecal pellets collected from live-trapped sciurids in Thunder Bay, Ontario, between 19 June and 25 September 2010.

Sample no.	Date faeces collected	Sciurid species	Spore types found	Spores/g dry weight collected (millions)
1-1-2	19 June 2010	Eastern Chipmunk	unknown	325.0
			Leucangium sp.	5.0
			Hymenogaster sp.	2.5
1-2-1	19 June 2010	American Red Squirrel	*Hysterangium* sp.	162.5
			Tricholomataceae	60.0
			Hymenogaster sp.	17.5
			Elaphomyces sp.	7.5
1-2-2	19 June 2010	Eastern Chipmunk	*Hysterangium* sp.	60.0
			Leucangium sp.	15.0
1-3-2	19 June 2010	American Red Squirrel	*Hysterangium* sp.	115.0
			Pezizales	7.5
			Gautieria sp.	5.0
			Elaphomyces sp.	2.5
3-3-4	31 July 2010	American Red Squirrel	Boletales	147.5
4-1-2	14 August 2010	American Red Squirrel	unknown	147.5
4-2-3	14 August 2010	Eastern Chipmunk	unknown	197.5
			Boletales	15.0
6-2-4	25 September 2010	Eastern Chipmunk	unknown	92.5

Three remaining recognizable spore types encountered included those representative of the basidiomycete order Boletales. However, it was difficult to determine whether the spores belonged to an epigeous (fruiting above ground) mushroom genus (e.g., *Suillus*) or to a hypogeous truffle genus (e.g., *Rhizopogon*) within this order, as spore morphology is similar in both groups. The spores belonging to a representative of the ascomycete order Pezizales were larger and more conspicuous than other spores in the faecal samples, but they could not be identified to the genus level. Spores similar to those produced by some members of the epigeous mushroom family Tricholomataceae were also found. However, there were several spore types encountered that could not be identified and were labelled as unknown (Table 1).

The results of this brief survey suggest not only the importance of mycophagy in the diet of American Red Squirrels and Eastern Chipmunks in the boreal mixed wood forests of northwestern Ontario but also the important role played by sciurids in vectoring spores of truffle fungi in this region. Further studies with other sciurid species are required, in addition to the need for testing of spore viability after passage through the digestive tract.

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