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Does fungus consumption by the woodland jumping mouse vary with habitat type or the abundance of other small mammals?

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Abstract: Fungi are important in the diet of many small mammal species, but patterns of fungus consumption (mycophagy) in eastern forests of North America have received little attention. Examination of stomach contents of the woodland jumping mouse, *Napaeozapus insignis*, revealed that fungi were an important dietary component in both eastern hemlock and mixed mesophytic habitats. Jumping mice in both forest types consumed mostly Glomalean fungi (primarily from the genera *Glomus* and *Endogone*), in agreement with previous studies. Mice also consumed fungi from the genera *Elaphomyces* and *Melanogaster*, previously unreported in the literature. Fungi from the genus *Hymenogaster* were only found in mice from eastern hemlock habitats. *Melanogaster* spores occurred more frequently in jumping mice from sites in which deer mice, *Peromyscus maniculatus*, were abundant, whereas Glomalean fungi were less frequent in the diet of *N. insignis* when deer mice were abundant. Overall frequency of spores in the diet of jumping mice was negatively related to the abundance of deer mice, suggesting that interactions between species may shape patterns of mycophagy.

Résumé : Les champignons constituent une partie importante du régime alimentaire de plusieurs espèces de petits mammifères, mais les patterns de consommation de champignons (mycophagie) ont été peu étudiés dans les forêts de l'est de l'Amérique du Nord. L'examen des contenus stomacaux de souris-sauteuses des bois, *Napaeozapus insignis*, montre que les champignons sont un élément important de leur régime alimentaire, tant dans les habitats de pruches de l'Est que dans les forêts mixtes mésophytiques. Comme l'ont indiqué des études antérieures, les souris-sauteuses consomment surtout des glomales (particulièrement des genres *Glomus* et *Endogone*). Ils mangent aussi des champignons des genres *Elaphomyces* et *Melanogaster*, ce qui n'avait jamais été signalé dans la littérature. Les champignons du genre *Hymenogaster* sont utilisés seulement dans les habitats de pruches de l'Est. Les spores de *Melanogaster* se retrouvent plus fréquemment chez les souris-sauteuses aux sites où les souris sylvestres, *Peromyscus maniculatus*, sont abondantes, alors que les glomales sont moins fréquentes. La fréquence totale des spores dans le régime des souris-sauteuses est en relation négative avec la densité des souris sylvestres, ce qui laisse croire que les interactions entre les deux espèces structurent les patterns de mycophagie.

[Traduit par la Rédaction]

Introduction

Fungi are an important food for small mammals (Fogel and Trappe 1978; Maser et al. 1978; Johnson 1996). By consuming fungi (mycophagy), small mammals may also be important in the dispersal of mycorrhizal fungi that affect plant communities (Johnson 1996). However, most examinations of species- and habitat-specific patterns of mycophagy in North America have focused on western forests (e.g., Fogel and Trappe 1978; Maser et al. 1978; North et al. 1997; Pyare and Longland 2001). Moreover, although mammals may consume a wide variety of fungi (Maser et al. 1978; Johnson 1996), little is known about how the abundance of potential competitors may affect fungus consumption, despite suggestions that mycophagy may be driven by competitive interactions (Pyare and Longland 2001).

Napaeozapus insignis is a small, saltatorial rodent that inhabits high-elevation mixed mesophytic and hemlock-dominated forests throughout the northeastern United States and southeastern Canada (Whitaker and Wrigley 1972). The

Received 12 August 2002. Accepted 24 January 2003. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 2 May 2003.

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 doi: 10.1139/Z03-035

importance of fungi in the diet of *N. insignis* has been reported (Whitaker and Wrigley 1972; Linzey and Linzey 1973), but comparisons of mycophagy among habitats have not been performed in eastern forests. Additionally, it is not known if patterns of fungus consumption by *N. insignis* are negatively related to the abundance of other small mammals, suggestive of competitive interactions. *Napaeozapus insignis* is well suited for this examination because it may be found in association with other mycophagous small mammals: deer mice (*Peromyscus maniculatus*; Fogel and Trappe 1978; Maser et al. 1978); red-backed voles (*Clethrionomys gapperi*; Orrock and Pagels 2002); and northern short-tailed shrews (*Blarina brevicauda*; Whitaker 1962).

Our objectives were (*i*) to document the frequency of fungal spores in the diet of *N. insignis*; (*ii*) to determine if patterns of mycophagy differ between eastern hemlock and mixed mesophytic habitats; and (*iii*) to determine if fungal consumption by jumping mice is related to the abundance of conspecifics or other small mammals.

Materials and methods

Study sites

Animals were captured as part of a larger project within a 160 km^2 portion of the George Washington and Jefferson National Forest in the southern Appalachian Mountains (Orrock et al. 2000). Small mammals were sampled from 13 sites on a portion of the Allegheny Mountain between Hightown and Mountain Grove, Virginia, between 38°28′–38°02′N and 79°40′–79°50′W. All sites were circular (11-m radius) and located in either eastern hemlock or mixed mesophytic forests. Mixed mesophytic forests were characterized by mature yellow birch (*Betula alleghaniensis*), green ash (*Fraxinus pennsylvanica*), and American basswood (*Tilia americana*). Eastern hemlock forests were dominated by hemlock (*Tsuga canadensis*), with occasional yellow poplar (*Liriodendron tulipifera*) and birch (*Betula* spp.). All sites were located at least 250 m from each other.

Small mammal sampling

Sampling was conducted from July to September 1996 and from May to July 1997. Pitfall arrays connected with a 0.3 m high drift fence (see Orrock et al. 2000) and Sherman live traps (H.B. Sherman Traps, Inc., Tallahassee, Fla.) were used to sample small mammals at each site. A complete sampling session lasted 5 days. At four sites, five additional days of sampling were conducted with Museum Special snap traps (Woodstream Corporation, Lititz, Pa.). Collected specimens were identified to species and sex and mass were determined; they were then fixed in 10% formalin and stored in the Virginia Commonwealth University Mammal Collection.

Abundance of small mammals at each site was estimated using the number of unique individuals captured (M_{t+1} sensu Slade and Blair 2000). Because two trapping sessions were conducted at four sites, M_{t+1} was divided by the number of trapping sessions to yield captures adjusted for sampling effort, as the number of traps was the same among all sites.

Mycophagy quantification

Stomach contents were removed from each animal. Slides were created in a manner similar to that of Castellano et al. (1989): forceps were dipped into a vigorously homogenized stomach sample suspended in formalin, closed, and withdrawn to remove a random sample of material. Each slide contained two mounts: one created using water and PVLG (Omar et al. 1979) and one with Melzer's reagent, which produces diagnostic reactions with some spores (Castellano et al. 1989).

Because the sampling technique did not strictly control the amount of material on each slide, we used relative frequency of occurrence (hereinafter referred to as frequency) of each spore type as our metric of dietary abundance. We determined frequency by examining 50 random fields of view from each mount made with Melzer's reagent at 40× magnification. Two stained mounts were examined per individual animal, resulting in 100 fields of view per individual *N. insignis*. Frequency was calculated as the proportion of fields that contained spores of a specific type. Total frequency was calculated as the proportion of fields that contained any spore. Total frequency could never be greater than the sum of the individual frequencies but could be less (e.g., if many fields contain more than one spore type). In addition to spores, we also quantified the frequency of plant material (starch granules, pollen grains, and herbaceous material) and insect material (e.g., exoskeleton fragments and wing scales).

Spores were identified using Castellano et al. (1989). Spores in the genera *Glomus*, *Endogone*, *Gigaspora*, and *Sclerocystis*, all within the phylum Zygomycota, were classified as Glomalean fungi because of the ecological similarity of these fungi and the difficulty in correctly classifying Glomalean spores (Alexopoulos et al. 1996). Identifications were checked against herbarium specimens and further reviewed by mycologists at Oregon State University (D. Luoma and K. Jacobs) and Iowa State University (R. Healy). Taxonomy of spore types follows Alexopoulos et al. (1996).

Statistical methods

We compared the frequency of consumption of spore types between mixed mesophytic and eastern hemlock forests using multivariate analysis of variance (MANOVA; Scheiner 2001) with habitat type as a fixed effect. MANOVA is more appropriate than ANOVA because the types of fungi consumed by *N. insignis* at a site are not likely to be independent. To determine if the presence of *C. gapperi*, *P. maniculatus*, *B. brevicauda*, or other *N. insignis* affected fungal consumption, we entered the abundance of each species into the MANOVA model as a covariate. If a significant covariate was found using MANOVA, we evaluated its importance in affecting total mycophagy using Pearson correlation analysis (Zar 1996). To determine if differences existed between the frequency of mycophagy and the frequency of plant and insect material, we used paired *t* tests (Zar 1996).

Because multiple jumping mice were collected from three sites used in the analyses, mean values were used as dependent variables for these sites, resulting in 13 observations for all analyses. This conservative approach was used because we could not assume that samples obtained from the same site at the same time were independent (Zar 1996). Performing the same analyses with nonpooled data did not change the qualitative results. All spore frequencies were arcsin squareroot transformed to improve normality (Zar 1996). Analyses were conducted using SAS software (SAS Institute Inc. 2000).

Table 1. Mean frequency (±SE) of occurrence of plant material, insect material, and fungal spores in the diet of six *Napaeozapus insignis* from six mixed mesophytic sites and 16 *N. insignis* from seven eastern hemlock sites in the southern Appalachians.

	Habitat type	
Food type	Eastern hemlock	Mixed mesophytic
Plant	0.20 (0.09)	0.16 (0.11)
Insect	0.05 (0.01)	0.07 (0.04)
Fungi		
Ascomycetes		
Elaphomyces	Trace	0.02 (0.01)
Basidiomycetes		
Hymenogaster	0.14 (0.07)	0
Melanogaster	0.02 (0.01)	0.03 (0.01)
Zygomycetes		
Glomalean[a]	0.24 (0.05)	0.27 (0.09)
Unknown fungi	0.03 (0.02)	0.07 (0.02)
Total fungi	0.36 (0.06)	0.29 (0.08)

Note: Total frequency of spore types (Total fungi) may be less than the sum of the constituent types because spores of more than one type of fungi may be present in the same field of view. Cells for which mean frequency of occurrence was <0.01 are indicated by "Trace".

[a]Includes *Glomus*, *Endogone*, *Gigaspora*, and *Sclerocystis*.

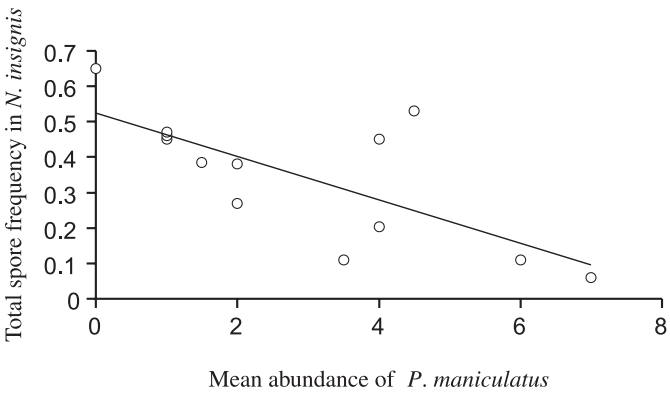
Results

A total of 2200 fields of view was examined from 22 *N. insignis* taken from 13 sites. All *N. insignis* had consumed fungi. Of the 4245 spores detected, 4148 (98%) were identified. Spores of imperfect fungi were also present (106 spores) but were most likely incidentally ingested with other food items and were not retained for analysis. Spores were as frequently encountered as plant material (paired t test, df = 12, $t = 1.42$, $P = 0.18$; Table 1). Spores were more frequently encountered than insect material (paired t test, df = 12, $t = 5.35$, $P < 0.001$; Table 1). Plant material was also more frequently encountered than insect material (paired t test, df = 12, $t = 2.26$, $P = 0.04$; Table 1).

Patterns of *N. insignis* mycophagy did not significantly differ between mixed mesophytic and eastern hemlock habitats (MANOVA, Pillai's trace = 0.40, $F_{[5,6]} = 0.81$, $P = 0.58$). Glomalean spores were the most frequently encountered spore type in both habitats (Table 1). *Hymenogaster* spores were found in the six *N. insignis* captured in eastern hemlock stands but not in the 16 *N. insignis* captured in mixed mesophytic stands (Table 1). Spores of the genera *Elaphomyces* and *Melanogaster* were found in *N. insignis* from both habitats. Unknown spore types were also encountered, especially in mice from mixed mesophytic habitats (Table 1).

The abundance of *P. maniculatus* was a significant covariate in the MANOVA analysis (Pillai's trace = 0.96, $F_{[5,6]} = 28.65$, $P < 0.001$). There was a positive relationship between *P. maniculatus* abundance and the frequency of *Melanogaster* spores ($r = 0.69$, df = 11, $P < 0.01$) found in *N. insignis*. There was a negative relationship between the abundance of *P. maniculatus* and the frequency of Glomalean spores ($r = –0.61$, df =11, $P < 0.03$) found in *N. insignis*. Total spore frequency was also negatively related to the abundance of *P. maniculatus* ($r = –0.73$, df = 11, $P < 0.01$; Fig. 1).

Fig. 1. Relationship between mean total spore frequency in *Napaeozapus insignis* stomachs and the mean number of *Peromyscus maniculatus* from six mixed mesophytic and seven eastern hemlock sites in the southern Appalachians. Trend fitted by least-squares regression.



Discussion

The comparable frequency of plant and fungal material indicates that fungi are an important dietary component of woodland jumping mice in the southern Appalachians, in agreement with other studies (Whitaker 1962; Linzey and Linzey 1973). Patterns of mycophagy did not differ between mixed mesophytic and eastern hemlock forests, probably because jumping mice consumed mostly Glomalean fungi, which have a broad host range (Alexopoulos et al. 1996).

Our results support other evidence that *N. insignis* primarily consumes Glomalean fungi (Whitaker 1962; Whitaker and Wrigley 1972; Linzey and Linzey 1973; Maser et al. 1978; Ovaska and Herman 1986). Whitaker (1962) also found *Hymenogaster* in *N. insignis* from Ithaca, N.Y., although the type of forest where the animals were captured was not mentioned. To our knowledge, our study is the first to document the presence of *Elaphomyces* and *Melanogaster* in the diet of *N. insignis*. *Melanogaster* and *Elaphomyces* both have a broad host range that includes many hardwood species (Castellano et al. 1989) and may have been readily available in both habitats. Indeed, Loeb et al. (2000) found *Elaphomyces* to be the most common fruiting hypogeous fungi in their study area in the southern Appalachians.

Because they are often found in association, Whitaker and Wrigley (1972) suggested that *P. maniculatus* is the most likely competitor of *N. insignis*. Data from the 13 sites used in this study and 336 additional sites in the study area support this positive association (χ^2 test, $\chi^2 = 6.41$, df = 1, $P = 0.01$; McShea et al. 2003). Yet abundance of both species was not correlated at the 13 sites examined ($r = 0.17$, df = 11, $P = 0.59$) or at all of the sites in the study area in which the species are found together ($r = 0.05$, df = 55, $P = 0.69$). This, coupled with the shifts in mycophagy by *N. insignis* that we observed (Fig. 1), suggests that *N. insignis* may reduce competitive interactions by shifting patterns of mycophagy as *P. maniculatus* becomes more abundant.

Overall mycophagy trends are also consistent with the dietary preferences of small mammals in the southern Appalachians. Although *B. brevicauda* consumes some fungi

(Whitaker 1962; Linzey and Linzey 1973), it consumes primarily invertebrate and other animal material (Linzey and Linzey 1973). *Clethrionomys gapperi* consumes mostly ectomycorrhizal fungi (Orrock and Pagels 2002), rather than the Glomalean fungi consumed most often by *N. insignis*. *Peromyscus maniculatus* consumes primarily Glomalean fungi (Whitaker 1962; Maser et al. 1978), consistent with our results: as *P. maniculatus* increased, fewer Glomalean and more *Melanogaster* spores were found in *N. insignis*.

Our findings must be considered with the caveat that our sample sizes were limited, samples were taken at different times, and the 13 sampling sites had been harvested at different times. However, time of sampling and time since harvest were not significant when used as a fixed effect (time of sampling) or covariate (time since harvest) in MANOVA analyses, reducing the likelihood that our results are confounded by time of sampling and stand age. Our data also cannot evaluate the ultimate mechanism responsible for the change in fungus consumption by *N. insignis* as *P. maniculatus* becomes more abundant. For example, changes in fungal availability, shifts in microhabitat use, or direct competition for fungi could all produce the trends that we observed. However, across both habitat types, total fungal frequency was not correlated with habitat characteristics measured as part of another study (Orrock et al. 2000): elevation, aspect, soil field capacity, leaf litter, herbaceous vegetation, woody debris, or shrub and tree density ($df = 11$, all $P > 0.14$). Ultimately, our limited sample sizes and the large number of variables preclude an in-depth analysis of habitat-specific relationships between microhabitat variables and mycophagy, but this would be a profitable area for future work.

Napaeozapus insignis may not be useful as an indicator of fungal availability for rare mycophagists in the southern Appalachians, as may be the case for *C. gapperi* (Orrock and Pagels 2002), primarily because *N. insignis* consumes mostly Glomalean fungi. However, our data suggest that *N. insignis* and *P. maniculatus* may be useful for a more rigorous, in-depth examination of how competitive interactions and habitat characteristics affect fungus consumption. Moreover, because *N. insignis* is often found along riparian corridors (Whitaker and Wrigley 1972), its role in spore dispersal also deserves closer inspection.

Acknowledgements

We are indebted to R. Healy, L. Tiffany, D. Luoma, K. Jacobs, E. Cázares, J. Whitaker, L. Nicolai, and S. Semones for assistance with spore identification and methodology. The manuscript benefited from review by E. Damschen, J. Pastor, G. Curler, and two anonymous reviewers. M. Fine and G. Peters assisted with laboratory facilities and logistical matters. The U.S. Department of Agriculture Forest Service provided access to study sites. The staff at Virginia Power's Bath County Pumped Storage Hydroelectric Facility, especially biologist S. Bell, facilitated data collection. This study was supported in part by funding to J. Pagels from the U.S. Department of Agriculture Forest Service, George Washington and Jefferson National Forests, the U.S. Fish and Wildlife Service, and the Virginia Department of Game and Inland Fisheries Non-game Program.

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