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A Microhistological Technique for Analysis of Food Habits of Mycophagous Rodents

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Abstract

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We present a technique, based on microhistological analysis of fecal pellets, for quantifying the diets of forest rodents. This technique provides for the simultaneous recording of fungal spores and vascular plant material. Fecal samples should be freeze dried, weighed, and rehydrated with distilled water. We recommend a minimum sampling intensity of 50 fields of view for each of three slides per sample. Absolute and relative percentage of frequencies per sample can then be calculated.

Keywords: Diets, fecal analysis, food habits, *Glaucomys sabrinus*, mycophagy, rodents, *Tamias townsendii.*

Contents

Introduction

Most forest rodents consume mature fruiting bodies (sporocarps) of mycorrhizal fungi, a process known as mycophagy, and egest viable spores. Principal mycophagists in the Pacific Northwest include Townsend's chipmunk (*Tamias townsendii*) and the northern flying squirrel (*Glaucomys sabrinus*), both of which consume only fungi at certain times of the year. Fungi may fruit above ground (mushrooms) or below ground (truffles). Forest rodents prefer truffles, which emit a strong odor when mature.

Forest rodents consume other foods as well. Northern flying squirrels eat various nuts, buds, staminate cones, fruits, insects, and sap (Maser and others 1985, Wells-Gosling and Heaney 1984). Chipmunks also consume common fruits, leaves, and seeds (Maser and others 1981, Tevis 1953). Douglas squirrels (*Tamiasciurus douglasii*), well known for their consumption of conifer seeds, are also mycophagous (Fogel and Trappe 1978, Maser and others 1978). Temporal and spatial variations in relative proportions of diet items are ecologically important.

Food habits of rodents are usually studied by analyzing stomach contents or fecal pellets. Stomachs generally contain masticated hyphal material, spores, and other food items (Stienecker and Browning 1970, Tevis 1952, Ure and Maser 1982). Relative dietary contributions of different types of fungi, as well as of seeds, fruits, and leaves, can be quantified and compared. Soft fungal and plant material are usually digested; fungal spores, lichens, arthropods, and undigestible vascular plant parts can be identified, however. Spores pass through intact and provide the means for analyzing the fungal portion of the diet (Maser and others 1978; McIntire 1984, 1985; Stienecker and Browning 1970). Structural components, such as cutinized epithelial cells, stomata, seeds, and pollen, can be used to identify the higher plants that were ingested (Johnson and others 1983).

A major advantage of analyzing fecal pellets instead of stomach contents is that animals are not sacrificed; activities and food habits can be examined simultaneously. Patterns of fungal and plant abundance can be related to the animals' selection of microhabitats, home ranges, and foraging strategies. Also, pellets represent a greater time interval after food is eaten than do stomach contents; dietary components found in pellets may more broadly represent the overall diet than do stomach contents alone.

Microhistological techniques for analysis of herbivore diets have been well developed (Johnson and others 1983, Robel and Watt 1970, Smith and Shandruk 1979, Ward 1970, Westoby and others 1976). Generally, these techniques involve quantifying recognizable structures in samples from stomachs or pellets. Each microscope field is considered a sampling unit according to the following assumptions: (1) diet components are randomly distributed on a slide, (2) components are the same average size, (3) dry-weight bulk densities of component taxa are the same (Johnson 1982), and (4) the most common component appears in less than 86 percent of the fields (Curtis and McIntosh 1950). These assumptions should be met before particle densities (Sparks and Malechek 1968) are calculated. Each taxonomic unit is then recorded in each of specific numbers of microscope fields. These techniques give reliable results when correction factors for differential digestibilities are used to calculate the final diet estimates (Dearden and others 1975, Leslie and others 1983, Neal and others 1973).

Few investigators have determined the relative importance of fungi and higher plants in herbivores' diets concurrently. Fungi in the diet complicates fecal analyses. Spores often are not seen at the low magnification used for counting vascular plant fragments. Few workers have any knowledge of spore or hyphal characteristics; often fungi are ignored or are lumped into the single category of "fungus." Quantifying the composition of a sample containing spores and plant fragments is difficult because these components differ greatly in size and violate the assumption of components of equal sizes. At present, more than one technique must be used to ensure completeness (Johnson and others 1983). Under ideal conditions, a single technique would be used to identify most diet components, common or rare, to similar taxonomic levels and to quantify these components in a statistically appropriate manner. The results would then represent the diet for the period of interest.

The purpose of this study was to develop a technique that would accurately quantify the fungal and plant portions of sciurid diets. Our specific objectives were to qualitatively compare ovendrying of pellets with freeze drying, four liquids for rehydrating dried pellets, and several staining techniques for differentiating fungal from plant material and to determine an adequate sampling intensity per fecal sample. Sampling intensity is the number of fields per slide and the number of slides per sample needed to detect 80 to 90 percent of the components of the sample if time constraints are realistic. We used our new technique to analyze the contents of pellets taken from northern flying squirrels and Townsend's chipmunks that were live trapped in an old-growth Douglas-fir stand.

Methods and Materials

While developing our technique, we used pellets taken from live-trapped Siskiyou chipmunks (*Tamias siskiyou*) and golden mantled ground squirrels (*Spermophilus lateralis*) from Klamath County, Oregon. In the field, pellets were immediately placed into individual vials with 3-percent Formalin.[1] Both species are mycophagous and egest pellets similar to those of the Townsend's chipmunk and northern flying squirrel. Ground squirrels do consume a variety of foods, so different diet components were available for observation and analyses. Siskiyou chipmunks are primarily mycophagous.

Drying and Rehydrating Pellets

A total of 20 vials with pellets (samples) were frozen at 0 °C. After 24 hours, the vials were transferred to the freeze dryer for 48 hours and were then removed and immediately weighed to the nearest 0.0001 gram. Twenty other samples were ovendried at 70 °C for 24 hours. The ease of grinding and rehydrating ovendried pellets was subjectively compared with that for freeze-dried pellets. The number of diet components per field of view was estimated and the numbers of freeze-dried pellets were compared. After the pellets were dried, a glass rod was used to grind the pellets in the vials. Subsamples from two fecal samples were rehydrated with different liquids: Tween (a commercial wetting agent), potassium hydroxide (KOH), Melzer's reagent, and distilled water (H_2O).

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[1] The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture of any product or service to the exclusion of others that may be suitable.

To judge the applicability of Curtis and McIntosh's (1950) assumption of an 86 percent or lower frequency of *Rhizopogon* spores, we microscopically examined subsamples that were rehydrated with 0.2, 0.4, 0.5, 1.0, and 1.5 cubic centimeters of H_2O. We noted the relative abundances of spores and of plant fragments.

Clearing and Staining

We examined reactions of fecal material and herbaceous plants to heat, clearing with Hertwig's solution (Johnson and others 1983), and staining in various combinations. Fecal samples were selected from the group of 20 and rehydrated with 10 drops of water. Herbaceous material was boiled in hydrochloric acid (HCL) and water for 2 hours to simulate digestion. One drop of pellet solution or a small portion of the herb mixture was placed on a glass slide. Treatments consisted of adding Hertwig's solution and boiling the mixture, adding a dye and boiling, adding Hertwig's and dye and boiling, and adding Melzer's reagent in combination with the other dyes. Dyes used were cotton blue, safranin, Gram's iodine, phloxine B, trypan blue, and Melzers' reagent. Control slides had no Hertwig's solution or dyes and were not heated. Two drops of polyvinyl alcohol (PVA, a mounting medium, Johnson and others 1983) were added to the mixture, which was then covered with a 22- by 40-millimeter cover slip. Five slides were made for each treatment. We then subjectively compared the degree of specific staining of fungal and plant material, the effects of clearing on identifying fungi and plant structures, and the effects of clearing on staining.

Sampling Intensity

Ten fecal samples were freeze dried and weighed. Five slides for each of five samples were prepared. Six slides for each of the five other samples were also prepared. Melzer's reagent or cotton blue was used, and Hertwig's solution was not.

For the first three samples (15 slides), diet components in 25 systematically selected fields of view per slide were recorded under a magnification of 500X. Components were categorized as fungal spores (to genus), fungal tissue, and plant tissue. Occasionally, plant material could be identified as grass, herb, seed, or pollen. An object that was not fungal but only assumed to be plant material was classified as an unknown plant; unidentifiable spores and structures were categorized as unknown. Very small (one field of view), amorphous objects were ignored; only obvious fungal or plant structures were recorded. If necessary, parts of large objects occurring in a field were identified by reducing the magnification to 125X. Fifty fields per slide were observed for each of the remaining seven samples (40 slides). For each slide, the percentage of frequency (Sparks and Malecheck 1968) for each diet component was calculated. Each value was divided by the sum of all values to obtain the relative frequency of each component (Holechek and Gross 1982, Mueller-Dombois and Ellenberg 1974). Frequency data were grouped for analysis into three categories: those recorded in 25 fields per slide, in 50 fields, and in all slides combined. For each component in each category, we calculated the mean relative frequency and the first field of occurrence as well as the associated coefficients of variation (Sokal and Rohlf 1981).

To examine the degree of similarity among slides per sample, we calculated similarity indices for every combination within a sample. We used Spatz' modification of Jaccard's index as given by Chambers and Brown (1983):

$IS_{sp} = R \times [MC / (MA + MB + MC)] \times 100,$

where

IS_{sp} = the index;

R = the small percentage of frequencies of diet components common to both slides, divided by the larger values, summed over all common objects, and divided by the total number of components in both slides;

MC = the sum of the percentage of frequencies of all components common to both areas;

MA = the sum of the percentage of frequencies of all components in one slide;

MB = the sum of the percentage of frequencies of all components in the second slide.

Spatz' index is sensitive to rare components occurring in only one of the two slides, which results in lower values than those produced by other indices, if rare components are present (Chambers and Brown 1983).

Technique Evaluation

After analyzing the results of the above methodology, we determined an appropriate procedure. This procedure was then evaluated from samples taken from Townsend's chipmunks and northern flying squirrels captured in an old-growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) stand in Coos County, Oregon, in the spring of 1986. Ten samples from each species were randomly selected, freeze dried, and weighed. Three slides per sample were prepared; only Melzer's reagent and PVA were used. Spores, hyphae and other fungus tissue, vascular plant structures and tissues, pollen, amorphous bits of matter, and unknowns were recorded in 50 fields per slide. Values for frequency and similarity were calculated as above.

Results and Discussion

Drying and Rehydrating Pellets

Freeze drying was vastly superior to ovendrying. The pellets were dry, yet evidently not affected by the freeze-drying process; they rehydrated completely. Ovendried pellets were very hard and difficult to grind even after extensive time in water or Tween. Although fungal spores were visually unaffected by the heat and could be identified, some of the sample was "baked" onto the inside of the glass vial and thus was not available for identification. Ovendrying pellets at lower temperatures for longer times was not tested, however. No qualitative differences were noted between freeze-dried and undried pellets, so all subsequent samples were freeze dried.

No differences were observed for Tween, KOH, Melzer's reagent, and water as wetting agents. Therefore, water was used to rehydrate all subsequent samples.

Differences were great between observed densities of common and rare components at all dilutions. Some spore types were so numerous in fecal samples that dilution to the optimal level suggested by Curtis and McIntosh (1950) would either preclude the observation of many rare components or require an unrealistic number of fields or slides to be examined (see Holechek and Vavra 1981). Thus, particle densities were not calculated because the criterion of a maximum frequency of occurrence for any single item could not be met.

Clearing and Staining

Treating the drops of pellet solution and the plant material with Hertwig's solution had little effect on most observed materials. Plant material was not completely cleared of pigments, and fungal material was not affected.

No stain was totally effective in differentiating fungal material from plant material. Cotton blue and trypan blue did stain some hyphae, but staining was not consistent nor specific. Melzer's reagent in combination with other dyes produced dark coloration that was not specific; also, this combination caused confusion because some spores are identified based on their reaction in Melzer's reagent alone. Thus, Melzer's reagent without Hertwig's solution was the best stain. Continued work on this problem is warranted, however.

Sampling Intensity

Sampling intensity is a function of the number of fields examined per slide and the number of slides examined per sample. Slides represent random subsamples of each fecal sample. Fields represent random samples of these subsamples. Common diet components should appear within the first few fields on the first slide. Rare components may occur in any field.

Number of fields per slide—In the 10 samples, hypogeous fungal genera were the dominant food type (table 1). Most common components exhibited minimal variation in relative frequency per slide. As the number of fields per slide increased from 25 to 50, little difference in relative frequency was observed (table 1). Differences in coefficient of variation (CV) values for some items probably were due to intersample variations in occurrence.

Table 1—Total fields of occurrence of fungi genera[a]

Diet component	25 fields per slide (n = 15 slides)			50 fields per slide (n = 40 slides)			All slides (n = 55)		
	Total fields	Mean RF	CV	Total fields	Mean RF	CV	Total fields	Mean RF	CV
			Percent			*Percent*			*Percent*
Fungi:									
Rhizopogon	329	36.8	32.6	1,106	32.3	46.8	1,435	33.9	42.0
Balsamia	179	21.2	59.4	682	21.4	69.8	861	21.9	66.9
Nivatogaster	13	6.6	59.5	147	6.3	92.2	160	6.4	85.2
Melanogaster	17	3.8	49.2	289	22.6	60.0	306	15.8	89.3
Hysterangium	114	10.4	43.8	71	4.6	84.3	185	7.6	66.6
Leucophelps	94	10.8	57.6	274	15.1	139.1	368	14.0	131.9
Geopora	35	7.4	73.3	6	0.8	37.3	41	4.9	108.5
Hydnotria	3	1.7	68.1	81	6.4	70.6	84	5.7	79.0
Hymenogaster	2	1.0	1.0	—			2	1.0	1.0
Gautieria	110	16.5	30.4	34	3.9	82.8	144	8.4	86.2

Table 1—Total fields of occurrence of fungi genera[a] (continued)

Diet component	25 fields per slide (n = 15 slides)			50 fields per slide (n = 40 slides)			All slides (n = 55)		
	Total fields	Mean RF	CV	Total fields	Mean RF	CV	Total fields	Mean RF	CV
			Percent			*Percent*			*Percent*
Octavianina	1	3.6	0	1	1.5	0	2	2.5	41.7
Gastroboletus	—			294	32.1	6.0	294	32.1	6.0
Radiigera	—			1364	44.8	57.9	1364	44.8	57.9
Glomus	—			143	11.7	90.9	143	11.7	90.9
Leucogaster	—			2	1.3	29.6	2	1.3	29.6
Fungal tissue	37	4.0	26.6	88	4.4	141.9	125	4.3	124.5
Plant:									
Plant tissue	7	3.3	54.4	91	3.1	64.5	98	3.2	54.4
Grass	4	.9	25.6	3	2.0	14.8	7	1.3	41.3
Herb	3	3.2	11.3	—			3	3.2	11.3
Seed	2	3.1	13.9	14	1.7	66.2	16	2.0	58.2
Pollen	1	1.0	0	3	1.2	33.5	4	1.1	31.2
Unknown:									
Plant	6	1.5	48.4	6	1.5	84.5	12	1.5	70.1
Spore	66	6.8	58.2	201	14.5	135.3	267	11.9	139.2
Structure	40	5.3	64.6	177	5.6	101.4	217	5.5	93.8

— = not recorded in this category.
[a] Relative frequency (RF) and associated coefficient of variation (CV) for 3 samples at 25 fields per slide, 7 samples at 50 fields per slide, and all slides.

In the three datum categories, most diet components exhibited variation in the first field of occurrence (table 2). Although all components were observed before the 25th field on a slide, 19 of 24 components had maximum values greater than 25 over all slides. *Rhizopogon*, the most common component overall (table 1), varied greatly because of significant differences in number between the first five samples and the second five samples. Thus, at least 50 fields per slide should be examined to maximize the probability of recording rare objects, especially when samples from different species, locations, or time periods are analyzed.

Table 2—Mean 1st field of occurrence for recorded components of diets of *Tamias siskiyou* and *Spermophilus lateralis*[a]

Diet component	25 fields per slide (n = 15 slides)				50 fields per slide (n = 40 slides)				All slides (n = 55)			
	Mean	CV	Min	Max	Mean	CV	Min	Max	Mean	CV	Min	Max
		Percent	*Fields*			*Percent*	*Fields*			*Percent*	*Fields*	
Fungi:												
Rhizopogon	1.1	23.4	1	2	3.6	209.4	1	30	2.7	228.3	1	30
Balsamia	1.5	79.8	1	5	5.5	135.4	1	22	4.3	151.1	1	22
Nivatogaster	12.2	21.6	8	15	6.4	98.6	1	20	7.8	79.3	1	20
Melanogaster	6.1	76.2	2	18	7.8	131.8	1	31	7.9	114.4	1	31
Hysterangium	4.3	157.5	1	25	14.1	99.7	1	39	9.0	133.0	1	39
Leucophelps	8.8	104.5	1	25	9.4	93.4	1	31	9.2	96.2	1	31
Geopora	5.6	70.2	1	15	24.2	65.3	4	41	12.5	107.9	1	41
Hydnotria	9.7	27.2	6	12	14.7	73.1	2	44	14.0	72.6	2	44
Hymenogaster	14.5	65.5	5	24	—				14.5	65.5	5	24
Gautieria	1.5	74.5	1	4	21.8	58.3	1	43	14.7	96.4	1	43
Octavianina	22.0	0	22	22	49.0	0	49	49	35.5	38.0	22	49
Gastroboletus	—				1.0	0	1	1	1.0	0	1	1
Radiigera	—				5.8	150.5	1	27	5.8	150.5	1	27
Glomus	—				15.3	92.7	1	44	16.3	92.7	1	44
Leucogaster	—				28.5	2.8	22	35	28.5	22.8	22	35
Fungal tissue	7.2	68.1	2	18	14.6	93.2	1	44	13.0	96.9	1	44
Plant:												
Plant tissue	9.0	59.6	3	18	16.1	67.4	1	46	15.2	69.7	1	46
Grass	12.5	32.5	8	19	24.5	10.2	22	27	16.5	40.6	8	27
Herb	10.3	82.2	2	22	—				10.3	82.2	2	22
Seed	15.0	6.7	14	16	25.6	52.5	8	47	23.2	54.4	8	47
Pollen	8.0	0	8	8	33.6	24.9	25	45	27.3	48.7	8	45
Unknown:												
Plant	10.0	63.6	2	18	25.2	68.0	3	49	18.3	83.9	2	49
Spore	7.9	88.5	1	25	15.7	94.7	1	49	13.1	101.5	1	49
Structure	8.5	82.6	1	21	12.1	71.6	1	33	11.1	75.5	1	33

— = not recorded in this category.
[a] Associated coefficient of variation (CV) and minimum (Min) and maximum (Max) values for 3 samples at 25 fields per slide, 7 samples at 50 fields per slide, and all samples.

Number of slides—The majority of diet components were observed in the first slide per sample (table 3). Of 126 first occurrences per sample for all objects over all samples, 87 (69.1 percent) were recorded for the first slide, 17 (13.5 percent) for the second, 6 (4.8 percent) for the third, 2 (1.6 percent) for the fourth, and 7 (5.6 percent) for the fifth. After the second slide for all samples was sampled, 104 (82.5 percent) of the 126 occurrences were recorded; after the third slide, 110 (87.3 percent) were recorded.

Table 3—Slide of 1st occurrence of fungal genera per sample for diet components

	Sample									
	25 fields			50 fields						
Diet component	1	2	3	4	5	6	7	8	9	10
Fungi:										
Rhizopogon	1	1	1	1	1	1	1	2		
Balsamia	1	4[a]	1	1	1	1	1	1		2
Nivatogaster	5[a]	2		1	1		1			
Melanogaster	2	2[a]		1		1	2[a]			
Hysterangium	1	3	1	3[a]	1		1			1[a]
Leucophelps	1	2		1	1	1	3			1
Geopora	1	1			1[a]	1				6[a]
Hydnotria	2	3[a]			1	5[a]		1		1
Hymenogaster	1									
Gautieria	1	5		1	1[a]	5[a]				1
Octavianina		1[a]								6
Gastroboletus						1				
Radiigera									1	1
Glomus					2			1	1	1
Leucogaster					5[a]					3
Fungus[b]		2	1	1	1	1	1	1	1	1
Plant:										
Grass	2		1[a]							1
Herb	1									
Seed	1	1	3[a]	4						
Pollen	1[a]					6[a]				2
Plant tissue	1[a]	1	1[a]	2	2	1	1	1	1	1
Unknown:										
Plant	1	1	1	1	1	2	3	1	2	1
Spore	1	1	1	1	1	2	3	1	2	1
Structure	1	1	1	1	1	1	1	1	1	1

[a] Component was observed only on this slide.
[b] Fungal tissue and hyphal strands.

Mean similarity values for all pairs of slides within a sample varied greatly, but not significantly, among samples (fig. 1). Mean value per pair was consistent among different pairs (fig. 2).

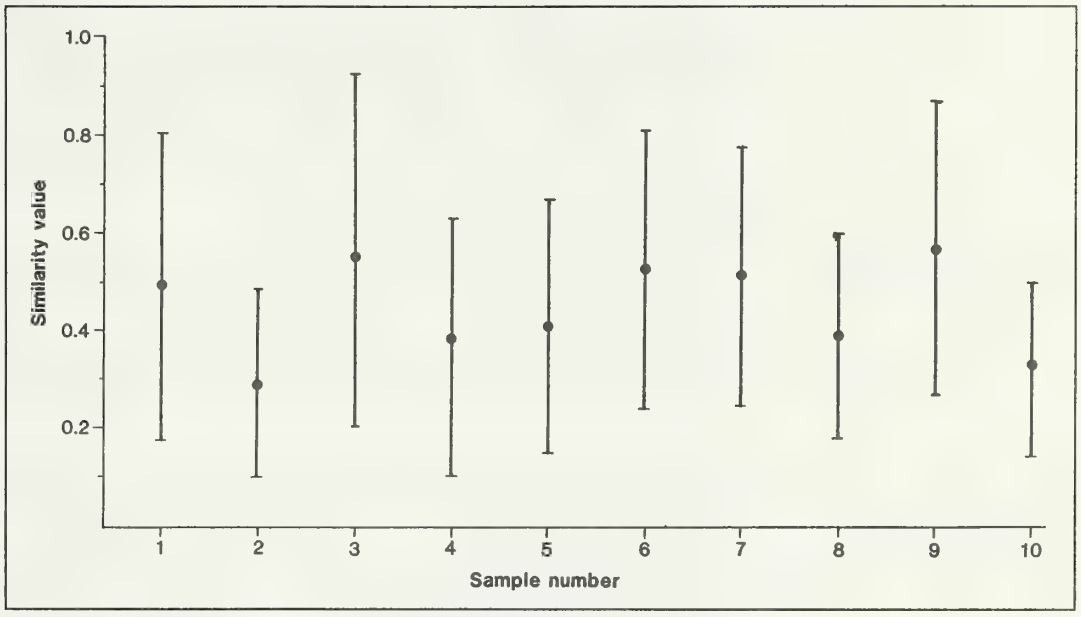

Figure 1—Mean values of Spatz' modification of Jaccard's index of similarity (IS_{sp}), for random subsamples of fecal samples used in developing the technique.

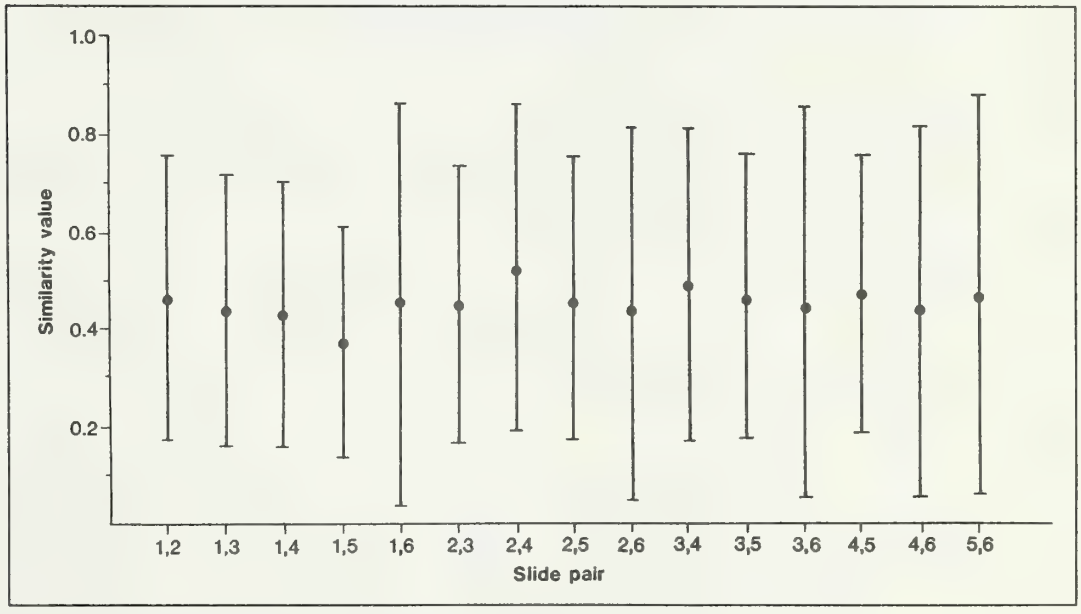

Figure 2—Mean values of Spatz' modification of Jaccard's index of similarity (IS_{sp}), with sample sizes for all possible combinations of paired random subsamples (slide combinations) of fecal samples used in developing the technique.

Generally, the first and second slides, and the second and third slides were more similar than the first and third slides. An acceptable degree of accuracy (87.3 percent) is achieved after three slides per sample have been analyzed. More slides would return little additional information for the amount of time invested. Differences among samples and not among slides within samples, as well as observer error, are primary considerations when a particular level of precision and reality in sampling is desired; see Williams (1987) for a discussion.

Technique Evaluation

Our technique revealed differences between diets of flying squirrels and chipmunks (table 4). Flying squirrels consumed almost twice as many (12 versus 7) fungal genera as did chipmunks. *Melanogaster* and *Rhizopogon* were common in both diets. Less common fungi in diets of flying squirrels were absent from diets of chipmunks, however. Plant tissue and all unknowns were more frequent in chipmunks' diets.

Common diet components were recorded for the first slide (table 5). For flying squirrels, 92.4 percent of all first occurrences were recorded by the first slide; 96.2 percent were recorded by the second. Six genera were first observed in the second slide. *Picoa* was observed only in the second slide of the fourth sample. All fungi were recorded by the second slide. Nonfungal components were more common in the second and third slides; 18.0 and 10.2 percent of nonfungal first occurrences were recorded for the second and third slides, respectively.

For chipmunks, 78.1 percent of all first occurrences were recorded by the first slide; 84.9 percent were recorded after the second was analyzed. For all samples, four of the six recorded genera were first observed in the second or third slides (table 5).

Table 4—Total fields and slides (n) of occurrence, with mean relative frequency (RF) and associated coefficient of variation (CV) per slide for components in 10 samples of diets of northern flying squirrel and Townsend's chipmunk[a]

	Flying squirrel				Townsend's chipmunk			
Diet component	Total fields	n	Mean RF	CV	Total fields	n	Mean RF	CV
				Percent				*Percent*
Fungi:								
Melanogaster	1,114	30	22.3	48.5	372	15	30.0	93.7
Rhizopogon[b]	1,022	29	21.1	45.0	803	20	37.1	37.5
Russulaceae[b]	867	30	17.5	40.7	240	23	16.1	105.3
Martellia	470	26	10.0	81.6	10	3	2.3	50.1
Hymenogaster	285	17	10.1	76.9	17	6	2.5	24.1
Hysterangium	249	12	9.9	80.5	198	6	21.1	60.7
Elaphomyces	122	12	4.9	61.7	0	0		
Gautieria	78	3	26.2	6.5	0	0		
Octavianina	71	8	4.4	99.1	0	0		
Balsamia	37	5	5.4	63.8	1	1	1.9	
Leucophelps	3	3	.6	16.9	0	0		
Picoa	1	1	.6	0	0	0		
Fungus[c]	142	24	3.2	96.9	24	14	2.6	81.4

Table 4—Total fields and slides (n) of occurrence, with mean relative frequency (RF) and associated coefficient of variation (CV) per slide for components in 10 samples of diets of northern flying squirrel and Townsend's chipmunk[a] (continued)

Diet component	Flying squirrel				Townsend's chipmunk			
	Total fields	n	Mean RF	CV	Total fields	n	Mean RF	CV
				Percent				*Percent*
Plant:								
Plant tissue	42	16	2.0	127.6	117	25	7.3	102.1
Pollen	4	2	1.5	1.1	1	1	.9	
Unknown:								
Undefined	466	27	1.1	48.5	429	30	28.0	78.8
Spore	37	14	1.5	66.6	79	10	7.7	87.6
Structure	22	12	1.1	58.8	198	20	7.6	111.0

[a] 3 slides per sample at 50 fields per slide.
[b] May contain related genera whose spores are indistinguishable.
[c] Fungal tissue and hyphal strands.

Table 5—Slide of 1st occurrence per sample for components in 10 samples from diets of northern flying squirrels (1st number of pair) and Townsend's chipmunks (2d number of pair)

Diet component	Sample									
	1	2	3	4	5	6	7	8	9	10
Fungi:										
Melanogaster	1,1	1,3	1,1	1,1	1,*	1,*	1,*	1,*	1,1	1,1
Rhizopogon[a]	1,1	1,1	1,1	1,1	1,*	1,1	1,3	1,1	1,*	1,1
Russulaceae[a]	1,1	1,1	1,1	1,*	1,1	1,1	1,1	1,1	1,*	1,1
Martellia	1,*	1,1	1,*	1,*	1,*	1,*	1,*	1,*	1,*	1,*
Hymenogaster	1,1		*,1	1,*	1,*	1,*	1,*	2,*	1,*	
Hysterangium		1,1	*,3	2,*		1,*			1,*	2,1
Elaphomyces			1,*		1,*	1,*	2,*		1,*	
Gautieria								1,*		
Octavianina						1,*	1,*	2,*	1,*	
Balsamia							1,*	1,*	*,2	
Leucophelps				1,*			2,*			
Picoa				2,*						
Fungus[b]	1,2	1,1	1,1	1,1	1,*	1,*	1,2	1,1	1,*	
Plant:										
Plant tissue	1,1	1,1	1,1	1,1	1,1	1,1	*,1	2,1	*,1	1,1
Pollen	3,*							3,*		
Unknown:										
Undefined	2,1	1,1	1,1	1,1	1,1	1,1	1,1	1,1	1,1	1,1
Spore	1,1	3,1	1,1	2,*	2,1	1,1	1,2	2,1	1,*	*,3
Structure	2,1	1,1	1,3	1,1	1,2	1,1	3,3	2,1	1,2	1,*

* = not recorded in this category.
[a] May contain related genera whose spores are indistinguishable.
[b] Fungal tissue and hyphal strands.

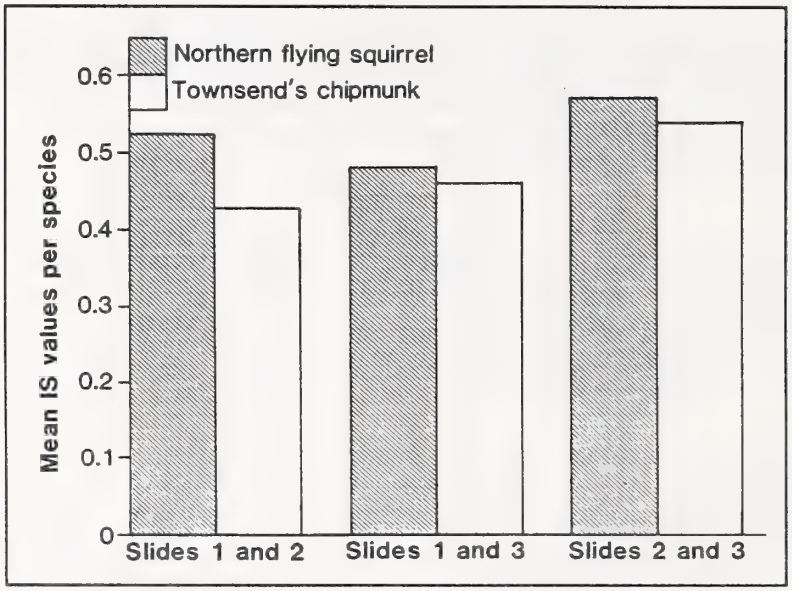

Figure 3—Mean values of Spatz' modification of Jaccard's index of similarity (IS_{sp}), for random subsamples per slide pair for northern flying squirrel and Townsend's chipmunk fecal samples.

Average similarity values per slide pair were greater for flying squirrels than for chipmunks (fig. 3). The second and third slides were most similar for both sciurids.

Conclusions

The following technique is recommended for quantifying the diets of mycophagous rodents:

1. Freeze 20 to 40 samples (fecal pellets in glass vials) at 0 °C for 24 hours. Place frozen samples into a vacuum-freeze dryer (with lids of vials on loosely) for 48 to 72 hours.

2.Weigh the samples to the nearest 0.0001 gram immediately after removal from the freeze dryer.

3. Grind all pellets in the vial with a glass stirring rod. Rehydrate with 20 drops of distilled water (more or less water, depending on the average size and the number of pellets for all species to be compared; the number should be consistent among categories of comparison).

4.Thoroughly mix the contents of the vial; place one drop of fecal solution on a glass slide. Add one drop of Melzer's reagent and three to five drops of PVA (Johnson and others 1983). Mix and cover with a 22- by 40-millimeter cover slip. The solution should extrude beyond the cover slip to prevent excess shrinking of contents while drying. Allow the slide to air-dry 48 to 96 hours before examining it. Make three slides per sample.

5. Record each diet component per slide in each of 50 systematically selected fields of view at 400X or 500X; large objects should be examined at 100X or 125X if necessary. Fungal spores should be recorded to genus and plant material to type (grass, herb, shrub, seed, flower, and so forth) or lower (genus, species) if possible. Other objects should be described as completely as possible. Repeat for the two remaining slides.

Previous studies of mycophagists' diets have been descriptive or have focused on fungi or plant material or both. Few attempts were made to determine adequate sampling intensities. Tevis (1953), Stienecker and Browning (1970), and Stienecker (1977) examined stomachs of sciurids from California and reported high volumes of hypogeous fungi at particular times of the year. These authors reported their overall results in detailed categories, of which "fungi" or "fungus" was one. The latter two papers did list four genera of truffles, however, and implied that these were the only hypogeous fungi present. McKeever (1964) listed fungi as a food category for Douglas' squirrels but gave little information about his analysis technique. Maser and others (1978) examined stomachs and fecal pellets from a variety of small-mammal species. Their study was meant as a survey; thus, sampling rigor was ignored. Ure and Maser (1982) used a point sampling method on two slides per sample to statistically analyze *Clethrionomys* diets. Data were presented as broad categories of food types; no fungal genera were given. McIntire (1984) compared frequencies of occurrence for fungal genera in one subsample but ignored other diet components. Hayes and others (1986) approximated numbers of spores for observed genera but also ignored the remainder of the diet. Maser and others (1985) presented detailed diet categories for the northern flying squirrel, including data for fungal genera, but merely visually estimated percentages by volume for each on a single slide.

The quantification of mycophagous diets via fecal analysis presents a unique challenge. The vast number of basidiomycete fungal spores that occur in some samples contrasts markedly with that of rare ascomycetes and plant fragments. Size differences among spores and plant material can be great. Thus, some assumptions pertaining to the analysis of herbivore diets are not always valid when diets of mycophagous rodents are analyzed. Accurate results are possible, however, when these limitations are considered. Additional concerns that should be addressed by future research include a difference in digestibility among diet components and in time of passage through the gut.

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We present a technique, based on microhistological analysis of fecal pellets, for quantifying the diets of forest rodents. This technique provides for the simultaneous recording of fungal spores and vascular plant material. Fecal samples should be freeze dried, weighed, and rehydrated with distilled water. We recommend a minimum sampling intensity of 50 fields of view for each of three slides per sample. Absolute and relative percentage of frequencies per sample can then be calculated.

Keywords: Diets, fecal analysis, food habits, *Glaucomys sabrinus*, mycophagy, rodents, *Tamias townsendii*.

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