Deadly Amanita Mushrooms as Food:

A Survey of the Feeding Preferences of Mycophagous Diptera from Across North America, with Notes on Evolved Detoxification.

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Life within the fungus among us!

Overview: *The genus* Amanita *is well-known for their typically large, showy, and ubiquitous mushrooms. Additionally, the genus is infamous for being toxic to mammals, and especially humans, that consume these mushrooms. While most species of* Amanita *probably are not toxic, a handful of species are responsible for 95% of the fatal mushroom poisonings in North America and they are feared worldwide. The mode of action of* Amanita *toxins in mammalian cells is well-known. Paradoxically, many disparate groups of invertebrate animals consume* Amanitas, *along with other mushrooms, with impunity. True flies are among the most successful mycophagous animals; mycophagy has likely arisen many times within the order and is found in several families of flies. Recently, tolerance to* Amanita *toxins (primarily α-amanitin) was elucidated in mycophagous species of Drosophilidae. It is not known how many other groups of Diptera have species tolerant to* Amanita *toxins and if the same mechanisms are involved as those in the Drosophilidae. This current report on the Diptera of* Amanitas *of North America is a brief synopsis of a larger manuscript in preparation for publication later this year. The purpose of this study was to find out how widespread* Amanita *mycophagy is among species of Diptera and to determine if there are feeding preferences for or against species considered toxic.*

Fungi are found in virtually every ecological niche on the planet. And the sporophores of many groups of macrofungi (in plain English: "mushrooms" of the Basidiomycota and Ascomycota) are food sources for a bewildering diversity of animals on the planet, in addition to humans. Most groups of mushrooms serve as hosts of mycophagous Diptera, the true flies.

(It's probably worth pointing out to the non-entomologically inclined that all flying insects are not true flies. All insects, by definition, in addition to having a three-segmented body, have two pairs of wings—except for Diptera, which have only a single pair of wings; think house flies, fruit flies, and mosquitos.)

Despite the frequency and diversity of Diptera that inhabit mushrooms, mycophagous species mostly remain poorly known. Although the coevolved associations among mushrooms and their insect symbionts is fascinating, they have caught the attention of few researchers over the years. (As mycophiles and mushroom hunters frequently encounter mushroom-eating insects, and may be curious as to the nature of the association and wonder just what these insects are, I will cite ample references that the reader may seek out for additional information.) No comprehensive studies of mushroom flies have been conducted across North America, however some regional studies have been published (Bunyard, 2003; Bunyard and Foote, 1990a). In Europe, there have been a few large-scale studies involving a large diversity of mushroom host species and fly species (Ševčík, 2006; Yakovlev, 1994; Hackman and Meinander, 1979; Buxton, 1960) as well as reviews of previous work (Krivosheina, 2008). However, most such ecological studies were conducted several decades ago (Shorrocks and Wood, 1973; Papp, 1972; Valley et al., 1969; Pielou and Verma, 1968; Pielou, 1966; Pielou and Mathewman, 1966; Buxton, 1960) and often gave only anecdotal accounts of adult flies occurring on mushrooms (Graves and Graves, 1985; Valley et al., 1969; Patterson, 1943), not verifying true mycophagy. Some studies have included flies that emerged solely from decaying mushrooms (thus, possibly only scavenging) (Frouz and Makarova, 2001) and therefore did not establish a strong ecological association (e.g., food substrate, site of overwintering, etc.) with fungal sporocarps. Many had improper

mushroom identification (based on current taxonomy concepts), or made no attempt to identify mushrooms to species, focusing only on dipteran identification. Some studies have made no attempt to identify to species the flies or mushroom hosts (Hosaka and Uno, 2012).

The ecology and life cycle of mushroom-feeding species has been mostly overlooked by previous studies and currently little is known about larval stages, feeding preferences, seasonality, or geographic range for numerous species (Bunyard and Foote, 1990a; 1990b; Graves and Graves, 1985; Buxton, 1960). The larval stages of many of the rarer species of mycophagous flies have never been described (Bunyard, 2003) and most reports infer that mycophagous flies are probably generalists and not specific to any species of fungus, as fungal hosts are considered too patchy and/or ephemeral; or are scavengers, feeding on all sorts of decaying organic material (Krivosheina, 2008; Hackman and Meinander, 1979; Jaenike, 1978a; 1978b) in addition to mushrooms. Occasional accounts of oligophagous and specialist species (and even monophagy) are likely artifacts of insufficient sampling (Hanski, 1989). It is likely that very few mycophagous Diptera truly are obligate consumers of mushrooms. Many, if not most, are facultatively mycophagous, and able to utilize a wide array of fresh or rotting plant and fungal material (Hackman and Meinander, 1979). Some have suggested that many species found in mushrooms are predacious on other dipteran larvae there (Krivosheina, 2008). Undoubtedly, mycophagy within the Diptera has arisen several times and it has been postulated that mycophagy probably arose from ancestral detritivores within many, or even most groups (Bruns, 1984).

Previous studies have not focused on Amanitas

Previously, fruitbodies from the Basidiomycota and Ascomycota were

Figure 1. Unidentified species of Family Mycetophilidae. Photo courtesy of F. Rhoades.

Figure 2. Mycophagous species of Drosophilidae reared from Amanitas; a) *Drosophila falleni*; b) *Mycodrosophila dimidiata*; c) *Leucophenga varia*. Photos courtesy of F. Rhoades.

surveyed as hosts for mycophagous Diptera (Bunyard, 2003; Bunyard and Foote, 1990a). Adult flies were reared from sporocarps representing most major commonly-occurring groups of eastern North America (belonging to 30 families of basidiomycete and 11 families of ascomycete macrofungi). Furthermore, all major groups of known mycophagous Diptera were represented in those surveys: Drosophilidae, Anthomyiidae, Asteiidae, Heleomyzidae, Chloropidae, Phoridae, Mycetophilidae, Sciaridae, Tipulidae, Trichoceridae, Cecidomyiidae, and Platypezidae, as well as mushroom scavengers of decaying mushrooms, including Psychodidae, Ceratopogonidae, Lonchaeidae, Stratiomyiidae, Sphaeroceridae, and Sarcophagidae.

During these surveys, and those conducted by other authors, it is noteworthy to see species of *Amanita* mushrooms listed as hosts for mycophagous flies, as this group is notoriously toxic to humans. *Amanita muscaria*—one of the most common mushrooms of North America (indeed, the world)—is known as the "Fly Agaric," reportedly due to its historic use as a fly killer around human dwellings (for a review of history and toxicology, see Michelot and Melendez-Howell, 2003). Despite the claims that the Fly Agaric earned its common name as a result of historical use as a fly-killer, the consensus based on science is that this mushroom is likely the most preferred host of mushroom-consuming flies in North America, and globally.

To date, there is a paucity of data on insect mycophagy of Amanitas. No comprehensive study has been undertaken

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Mushroom	Section of genus	Location
A. aprica	Amanita	Chelan Co., Washington 48.0739°N 120.6741°W
		Mt. Hood, Clackamas Co., Oregon 45.4198°N 121.5706°W
A. gemmata	Amanita	Mendocino Co., California 39.3556°N 123.4715°W
A. multisquamosa	Amanita	Jefferson Co., Colorado 39.4219°N 105.7730°W
A. muscaria	Amanita	Victoria, Vancouver Island, BC 48.4113°N 123.3631°W
		Ventura Co., California 34.2420°N 118.9068°W
		The West Woods, Newbury, Ohio 41.4565°N 81.3023°W
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		Chelan Co., Washington 48.0739°N 120.6741°W
		The West Woods, Newbury, Ohio 41.4565°N 81.3023°W
		West Branch SP, Ravenna, Ohio 41.1427°N 81.1129°W
A. pantherina	Amanita	Forest Co., Wisconsin 46.1394°N 89.2881°W
		San Bernardino Co., California 34.2506°N 117.1923°W
A. vernicoccora	Caesareae	San Bernardino Co., California 34.2506°N 117.1923°W
A. fulva	Vaginatae	Forest Co., Wisconsin 46.1394°N 89.2881°W
A. protecta	Vaginata	San Louis Obispo Co., California 35.4744°N 121.0007°W
A. rhacopus (=A. borealisorora)	Vaginatae	Monroe Co., Pennsylvania 41.2191°N 753964°W
		Oneida Co., Wisconsin 45.3237°N 90.2816°W
A. sinicoflavia	Vaginatae	Great Northern Peninsula, Newfoundland
A. vaginata	Vaginatae	Rock Island SP, Door Co., Wisconsin 45.4182°N 86.8192°W
A. velosa	Vaginatae	Lompoc, Santa Barbara Co., California
A. whetstonae	Amidella	Rock Creek Park, Washington, D.C. 38.9912°N 77.0917°W
A. canescens	Lepidella	Perry Co., Arkansas 34.8772°N 93.0461°W
A. magniverrucata	Lepidella	Santa Cruz Co., California 37.0118°N 122.0537°W
		San Louis Obispo Co., California 35.4744°N 121.0007°W
A. polypyramis	Lepidella	Butler Co., Maryland 39.3676°N 76.5664°W
A. ravenellii	Lepidella	Montgomery Co., Virginia 37.2876°N 80.4781°W
A. silvicola	Lepidella	Mendocino Co., California 39.3556°N 123.4715°W
A. thiersii	Lepidella	Butler Co., Maryland 39.3676°N 76.5664°W
		Montgomery Co., Virginia 37.2876°N 80.4781°W
A. zayantensis (=A. baccata)	Lepidella	Santa Cruz Co., California 37.0118°N 122.0537°W
A. bisporigera	Phalloideae	Brown Co., Wisconsin 44.8853°N 88.2966°W
		Cobb Co., Georgia 34.0044°N 84.5497°W
		Forest Co., Wisconsin 46.1394°N 89.2881°W
A. magnivelaris	Phalloideae	Bayfield Co., Wisconsin 46.3881°N 91.3362°W
A. ocreata	Phalloideae	Lompoc, Santa Barbara Co., California
		Santa Cruz Co., California 37.0118°N 122.0537°W
		Lompoc, Santa Barbara Co., California
		Ventura Co., California 34.2420°N 118.9068°W
		San Bernardino Co., California 34.2506°N 117.1923°W
A. phalloides	Phalloideae	Victoria, Vancouver Island, BC 48.4113°N 123.3631°W
		Sonoma Co., California 38.4117°N 122.9558°W
A. suballiacea	Phalloideae	Bayfield Co., Wisconsin 46.3881°N 91.3362°W
A. augusta	Validae	Santa Cruz Co., California 37.0118°N 122.0537°W
A. brunnescens	Validae	Forest Co., Wisconsin 46.1394°N 89.2881°W
		The West Woods, Newbury, Ohio 41.4565°N 81.3023°W
A. flavoconia	Validae	Forest Co., Wisconsin 46.1394°N 89.2881°W
A. flavorubens	Validae	The West Woods, Newbury, Ohio 41.4565°N 81.3023°W
A. novinupta	Validae	Mendocino Co., California 39.3556°N 123.4715°W
		Santa Cruz Co., California 37.0118°N 122.0537°W
A. rubescens	Validae	Perry Co., Arkansas 34.8772°N 93.0461°W
		The West Woods, Newbury, Ohio 41.4565°N 81.3023°W

Table 1. *Amanita* species and collection sites discussed in this survey.

for this group of fungi due to several reasons. Proper identification of Amanitas can be difficult and the genus includes hundreds of species in North America; of the few previous studies including Amanitas, misidentification was common (e. g., *Amanita phalloides*, a nonnative species, has been listed in the Great Lakes region but is known to occur only on the West and East Coast, and was only introduced into North America during the last century). Many species of *Amanita* are endemic to small regions, thus missed in all studies not inclusive for all North America. The biggest limitation to any comprehensive ecological study of *Amanita* is in the generally ephemeral and sporadic nature of mushroom fruitings: fruitbodies are present for a brief period of time (usually just a few days) and fruitings are difficult to predict in years of unfavorable weather (e.g., too dry/wet or too cold / hot). Fruitings of any one species may not happen in a given year or even consecutive years. While ID to species can be difficult, gross morphology allows pretty easy identification of Amanitas below the level of genus. The genus *Amanita* is divided into seven Sections, historically based on morphology, chemistry (specifically, class of toxins, reviewed below), and more recently supported by DNA sequence analysis.

A 30-year survey of mushroom flies in North America

Fresh mushrooms were collected from 1987-2017; collection sites ranged

Figure 3. Toxic Amanitas; a) *Amanita muscaria* var. *flavivolvata* (Section *Amanita*); b) *Amanita muscaria* var. *guessowii* (Section *Amanita*), photo courtesy J. Hammond; c) *Amanita phalloides* (Section *Phalloideae*); d) *Amanita suballiacea* (Section *Phalloideae)*; e) *Amanita magniverrucata* (Section *Lepidella*), page 44.

from as far north in the Northeast as Newfoundland, to as far south as Georgia; across the Midwest and Great Plains; throughout the Rocky Mountains; from the Southwest to as far north in the Pacific Northwest as Vancouver Island. Most of the sites were collected multiple years.

For rearing adult flies from fungal hosts, special rearing chambers were constructed as previously described (Bunyard and Foote, 1990a) and consisted of the bottom of a petri dish (10 X 100 mm) to which had been added moistened vermiculite. The upper portion of the rearing chamber consisted of rigid clear plastic tubing (90 mm diameter) cut to various lengths. To the top end of each tube was glued a fine polyester mesh material. Fungal sporocarps were placed on the vermiculite substrate, and the upper portion of the chamber placed securely over the fungus, into the petri plate. The rearing chambers allowed the fungal specimens to remain in a somewhat natural condition. It was necessary to moisten the vermiculite substrate periodically to prevent desiccation of fungal material. As the sporocarps decayed, the substrate absorbed any excess moisture produced.

To avoid incidental occurrences of Diptera with the fungi (for example, resting or hiding in crevices) only adults which emerged from larvae occurring within the fungus were counted. Following emergence, adult flies were kept alive for at least 24 hrs to allow for exoskeleton hardening (to facilitate identification) and

3e.

studies from North America and Europe), *Amanita muscaria* is most "myco-licious" to mushroom-feeding flies. The Fly Agaric routinely supports the most numbers of individual flies, as well as highest diversity of flies, from all mushroom taxa examined. This is no doubt due to a number of factors including this mushroom's typically large size (specimens can measure one foot across), its ubiquity and regularity of fruiting (it is found all across North America and commonly seen, even in very dry years), and its gregarious nature (wherever it fruits, it is usually in very large numbers).

And what about those flies? As is the case across fungal taxa (Ševčík, 2010; Bunyard, 2003; Yakovlev, 1994), the most commonly encountered groups of Diptera utilizing Amanitas are Mycetophilidae, Drosophilidae, and Phoridae, both in numbers of individuals and numbers of species. You have seen mycetophilids—they are typically the large (comparably) maggots inside of mushrooms that have the "black caps" on their heads. As adults they are rather interesting, adorned with large bristles about their legs and bodies (Fig. 1). Mycetophilids are much more difficult to complete their lifecycles indoors, and thus identification to species is often not possible (Bunyard, 2003). Drosophilids are reared from just about any mushroom collection. Mycophagous drosophilid species are found in several genera of the Drosophilidae, including *Drosophila*, *Mycodrosophila*, and *Leucophenga* (Fig. 2). Also common in Amanitas are heleomyzids, phorids, sciarids, plus scavenging species including psychodids and several muscoids.

It should be pointed out to others considering undertaking surveys of mycophagous arthropods—I encourage you to do so, there is a paucity of such information—but it's no easy task; much of the time collections will result in no successful rearings (even though you have seen larvae within the mushrooms); even under the best of circumstances, you are subjecting the immature insects to unnatural conditions; premature rotting or drying out is also a hazard.

A discussion of Amanita toxins

The Amanitas are likely the most infamous group of mushrooms. The poisonous reputation of Amanitas is understandable, as the vast majority (90% worldwide) of mushroom fatalities

then killed in alcohol. Adult flies were dried and pinned for microscopic examination and identification.

All species of Amanita host mycophagous Diptera … but not all families of mycophagous flies utilize Amanitas as food

This study includes data on species of mycophagous Diptera that emerged from 31 species of *Amanita* mushrooms collected over several decades from across North America (Table 1). Of particular interest were known toxic species of Sections *Amanita*, *Lepidella*, and *Phalloideae* (Fig. 3) as it was presumed that these mushrooms might be less suitable hosts for different fauna than other species regarded as nontoxic. Some common species were collected on numerous occasions and from many different locations over multiple years; some species in this study are rarely encountered, poorly known, or collected on a single occasion.

The most notable finding was that some families of Diptera seen as polyphagous and previously reared from many different mushroom taxa (Bunyard, 2003; Bunyard and Foote, 1990a) were not found to utilize Amanitas as hosts (Anthomyiidae, Chloropidae, Tipulidae, Trichoceridae, Platypezidae, and Asteiidae). Furthermore, several species of flies commonly known as scavengers of decaying mushrooms (Lonchaeidae, Sphaeroceridae) also are not known to utilize Amanitas.

All species reared from Amanitas in this study have been seen previously from other genera of fungi (Bunyard, 2003; Bunyard and Foote, 1990a), thus no specialization was seen. Mushroom fruitbodies do not seem to be a limiting resource as it was most common to see more than a single species of fly emerge from a single fruitbody. Also, in general, larger fruitbodies yielded more adult flies overall. Predictably (based on previous

is due to *Amanita* species from the Section *Phalloideae* (Bunyard, 2012), and mostly due to a single species, *A. phalloides.* Poisoning records are kept and tabulated annually by the North American Mycological Association. Other than dogs, extensive data is not kept for other animals. *Amanita* species of this group produce highly toxic substances collectively called amatoxins (or amanitins). But, just as all Amanitas are not toxic, not all toxic Amanitas produce amatoxins. Toxic properties are seemingly restricted to four separate Sections of the genus (Sections *Amanita*, *Lepidella*, *Validae*, and *Phalloideae*). Furthermore, there are four known classes of toxic compounds produced by Amanitas: amatoxins, isoxazole derivatives (muscimol, ibotenic acid), allenic norleucine, and hemolytic toxins.

Amatoxins. Amanitins, or "amatoxins," (α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, and related compounds) are a group of bicyclic polypeptides produced by *Amanita* species in Section *Phalloideae* (including the "Death Cap," *A. phalloides*, and several entirely white species known as "Destroying Angels," *A. ocreata*, *A. bisporigera*, *A. suballiacea*, *A. magnivelaris*, and others) that were first isolated and characterized by the Wielands (for a review, see Wieland, 1986). The mode of action of amatoxins is the blockage of functionality of the enzyme RNA polymerase II which is responsible for transcription of DNA into messenger RNA (mRNA). There are two other classes of toxins of less importance in species of *Phalloideae* Amanitas. These are phallotoxins (bicyclic peptides like amatoxins) and virotoxins (monocyclic peptides); for a review see Wieland (1986). These compounds are highly toxic but in animal studies were not absorbed from the intestine and therefore not felt to play a significant role in human toxicity. Phallotoxins have not been shown to be toxic to cells in vitro and when injected into animals. Furthermore, different species of Section *Phalloideae* produce different amounts of these toxins, with *A. phalloides* thought to produce the highest levels of amatoxins—on average, twice the concentration of amatoxins in any other species (Benjamin, 1995), which

may account for far more deaths being attributed to that species (as well as the fact that they are widely encountered).

Amatoxins are heat stable, are not inactivated in the mammalian digestive tract, and are rapidly absorbed into the bloodstream and across the plasma membrane. A lethal dose of α-amanitin may be as little as 0.1 mg per kg of human body weight, thus it would take only 6-7 mg of the toxin to kill someone. *Amanita phalloides* have been shown to contain from about 0.5 to 1.5 mg α-amanitin per g of tissue; since a single mushroom cap can weigh 50-60 grams (or much more), an average sized specimen could kill several people (Benjamin, 1995).

It interesting to note that amatoxins are thought to have evolved independently multiple times within fungi, as these cyclopeptides are produced by some other wild mushrooms not closely related to Amanitas. The small brown-spored wood rot mushroom *Galerina marginata* (=*G. autumnalis*), the tiny brown-spored lawn mushroom *Conocybe* (=*Pholiotina*) *filaris*, and a number of small white-spored woodland Lepiotas, including *L. subincarnata (=L. josserandii), and L. brunneoincarnata* are known to produce amatoxins. Galerinas and Lepiotas have caused human deaths. The quantity of amatoxin in 15-20 *Galerina* caps is equivalent to that in one *A. phalloides* cap (Berger and Guss, 2005). That these compounds have evolved more than once seems to suggest they are functionally important (and selected for) in fungi, although no real functionality has been shown.

Isoxazole Derivatives (Muscimol and Ibotenic acid). Ibotenic acid (α-amino-4[-hydroxy-isoxazol-2]yl acetic acid) and its decarboxylation products muscimol (and to a lesser extent muscazone) are produced by species of Section *Amanita* (including *A. muscaria*, known as the "Fly Agaric"). Another toxin, muscarine is potentially dangerous, but present in barely detectable levels in these same mushrooms.

Ibotenic acid was first isolated and purified by researchers in Japan and takes its name from "Ibotengutake" which is the Japanese name for *Amanita strobiliformis*, a misapplied name for a common toxic mushroom found there. The Japanese mushroom has since been renamed *Amanita ibotengutake* and *A. strobiliformis* is now thought to occur only in Europe. Ibotenic acid, muscimol,

and other isoxazol derivatives target the brain (isoxazols are similar structurally to γ-aminobutyric acid, GABA), binding to GABA receptors there and disrupting neurotransmission, resulting in inebriation and loss of bodily control. In humans, there are no reliably documented cases of death from toxins in these mushrooms in the past 100 years (Beug et al., 2006).

Allenic Norleucine. Allenic norleucine is produced by members of Section *Lepidella*. Allenic norleucine (amino-hexadienoic acid), is a renal (kidney) toxin, having a lethal dose of 100 mg/kg body weight in guinea pigs (Pelizzari et al., 1994; Chilton and Ott, 1976; Chilton et al., 1973). Human deaths have been documented.

Hemolytic toxins. The fourth group of *Amanita* toxins is the hemolysins produced by species of two Sections of the genus. Hemolysins seem to be the least problematic to humans and are not of much concern to the public or clinicians. Phallolysins are produced by *A. phalloides* and close relatives; rubescenslysins are known from species of the Section *Validae*. Hemolysins are well-studied. As their name implies, upon contact with erythrocytes (red blood cells), hemolysins cause those cells to lyse or burst (hemolysis), releasing hemoglobin into the blood plasma. There are no cases of impact upon humans from mushroom-produced hemolysins. This is because hemolysins are not heat stable; moderately acidic conditions (pH 4) also quickly degrades these toxins.

Mycophagous Diptera

Many arthropods (particularly Diptera and Coleoptera) have coevolved associations with fungi. Fungal fruit bodies contain a complete set of nutrients needed for the development of both phytophagous and zoophagous insects (Martin, 1979); thus many species of dipterans are able to develop in different decomposing organic substrates including fungal (Krivosheina, 2008), or have evolved a mycophagous life style from an ancestral phytophagous habit.

Sitta and Süss (2012) determined that arthropods including Diptera associated with fungi can be placed into one of four groups based on their lifestyle. (1) Primary fungivores (or primarily mycophagous) are thought to feed exclusively on fungi, mostly fruitbodies but some mycophagous arthropods feed on fungi mycelium; they infest young fruiting bodies often

causing considerable damage and may exhibit preference for fungal hosts of a single family or genus. Some arthropods are symbiotically tied to fungi, not only feeding on the fungus but may cultivate it (in the case of some ants and termites) and may have specialized body parts to transport the fungal partner to the oviposition site (as with some bark beetles and wood wasps) (Bunyard, 2015). (2) Secondary fungivores (or secondarily mycophagous) are exclusively mycophagous and live on decaying hosts (thus considered saprobes, but specific to fungi) without damaging young fruiting bodies and are almost always polyphagous. (3) Detritivores are similar to secondary fungivores, but in contrast are not exclusively mycophagous as they can feed on a large variety of decaying matter; they are not found in young fruiting bodies but several studies have shown that adults may lay eggs on young mushrooms with the eggs not hatching until the mushrooms begin to decay. Some dipterans are known to utilize a wide array of decaying organic matter but have been shown in the lab to preferentially oviposit on fungal material (Bunyard, 1990b). (4) Predators (coleopteran and dipteran) are frequently found associated with mushrooms; they are extremely widespread, and for some of them, the larvae are obligate predatory in the final instar, whereas they are fungivorous in earlier instars (Sitta and Süss, 2012). Other authors have reviewed mycophagous arthropods and place them into more or fewer groups based on lifestyle (Krivosheina, 2008; Ševčík, 2006; Bruns, 1984).

Regarding trophic status, most mycophagous Diptera species are probably polyphagous, thus able to complete their life cycles in most mushroom fruitbodies encountered (Lacy, 1984a; Jaenike, 1978a; 1978b). Some oligophagous associations have been discussed, where some or all mycophagous species of a family are restricted (or mostly so) to a particular group of fungi (Ševčík, 2006). Examples of the latter are species of Platypezidae associated almost entirely with species of *Agaricus* (Bunyard, 2007) and species of Anthomyiidae with boletes (Bruns, 1984). Monophagy among mycophagous Diptera rarely has been described and unsubstantially supported with data (Bunyard, 2003). Trophic status of mycophagous insects is thought to follow the same patterns of phytophagous insects,

where species feeding on ephemeral annual species are more typically polyphagous; perennial plants host more monophagous insects (Lacy, 1984a).

Habitat selection of mycophagous Diptera depends primarily on the occurrence of host fungi, especially in oligophagous species, which follow the distribution of their host (Ševčík 2006). Fungi that produce mushrooms are either mycorrhizal and restricted where they occur, geographically, by their host tree species (e. g., only at certain elevations or in close proximity to water) or those fungi grow saprobically, and often less restricted on where they occur, with many being commonly found across North America in forests as well as in urban areas. Amanitas are nearly all mycorrhizal; only a few basal species are saprobic. Saprobic Amanitas are poorly known, but one species seems to be rapidly expanding its host range, possibly as a result of Global Climate Change (Bunyard, 2013). The distribution of mycophagous Diptera is influenced by abiotic factors (climate, elevation, and other ecological factors at the locality), thus the same fungus species may host different insect species in temperate lowland forest than in extreme peat-bog in high mountains (Ševčík, 2006).

The distribution of mycophagous Diptera is influenced by biotic factors, including antifeedant properties and chemical defenses that the mushrooms may exhibit. Some fungal species avoid damage / mycophagy by their physical makeup: many species of bracket fungi (mostly within the family Polyporaceae) have a hard, woody texture that is difficult for many arthropods to consume (Courtney et al., 1990). In general, tougher woody bracket fungi (mostly polypores) are dominated by mycophagous beetles; softer fleshy fungal fruitbodies like agarics (gilled stipitate mushrooms) and boletes (pored stipitate mushrooms) are dominated by mycophagous Diptera (Bunyard, 2016). Some beetles found within the tissues of fleshy mushrooms may be predacious species in search of mycophagous fly larvae. A factor determining host suitability (ephemeral agarics and boletes vs. perennial polypores), is length of larval period. Diptera have a short larval period and are better suited to agarics and boletes which may persist for a few days only (Yamashita and Hijii, 2003; Lacy, 1984b; Hackman and Meinander, 1979). Coleoptera typically have a long larval

period and more formidable chewing mouthparts, thus are more adapted for life within woody polypores which may persist for an entire season or many years on tree hosts (Lacy, 1984b; Pielou and Verma, 1968). *Amanita* species are more ephemeral than many other groups of commonly-seen agarics (e. g. *Cantharellus*, *Lactarius*, *Tricholoma*), as well as boletes, coral mushrooms, and other ubiquitous groups (Yamashita and Hijii, 2004).

Many macrofungi produce a wide array of toxic metabolites. Although the defensive properties of mushrooms that have evolved to deter insects have been poorly investigated, Mier et al. (1996) examined qualitatively which mushroom groups were most insecticidal. Their findings were somewhat limited by virtue of not being carried out under natural conditions; the researchers fed mushroom extracts to arthropods in a completely artificial fashion. Clearly, the ability to detoxify secondary metabolites of mushrooms is widespread throughout taxa of arthropods, and in particular mycophagous Diptera; this ability seems to have evolved more than once in this group. This is of particular interest when looking at flies and whether or not they can utilize species of Amanitas. Few previous studies have considered amatoxins and mycophagous flies (Jaenike, 1985; Jaenike et al., 1983), with no previous studies attempting to comprehensively examine the entire genus, with data on individual groups of toxic species as suitable host for mycophagous Diptera. What is known is that mushrooms considered toxic to humans are attractive hosts (and thus not toxic) to many groups of mycophagous Diptera. Amatoxin-containing Amanitas (Section *Phalloideae*, e. g., *A. phalloides*) may be less attractive to some groups of Diptera (Ševčík, 2006), or even toxic, but many groups are able to utilize these mushrooms. Indeed 13 species of Diptera have been reared from *A. phalloides* in Europe (Hackman and Meinander, 1979; Papp, 1972; Buxton, 1960). This species may become less toxic as it decays. Conversely, some wild mushrooms highly sought as food by humans, may be mostly avoided by mycophagous flies. For example, most species of *Agaricus* ("meadow mushrooms" etc.) are rarely infested by larvae of dipteran families other than Phoridae or Platypezidae (Ševčík, 2006; Bunyard, 2003; Hackman and Meinander, 1979). Hanski (1989) found the genus

Agaricus to be the most toxic group when tested on Diptera. The "puffballs" including *Calvatia* spp. and *Lycoperdon* spp. are also rarely infested with fly larvae (Ševčík, 2006; Bunyard, 2003). The Ascomycota (notably the genera *Morchella* ("morels"), *Gyromitra*, and *Helvella*) are rarely seen (Ševčík, 2006; Bunyard 2003).

Despite the production of toxins described above, the Amanitas are cited as one of the most attractive groups of mushrooms to host mycophagous Diptera by all researchers familiar with the subject (Ševčík, 2006; Bunyard, 2003; Yakovlev 1994).

The evolution of tolerance to amatoxins in the Family Drosophilidae

Arguably, the most successful insects, evolutionarily-speaking, is the Family Drosophilidae. Worldwide, there are more than 4,000 species in 74 genera of drosophilids. Drosophilids can be found in just about every terrestrial habitat on the planet; around the home they are familiar as the "fruit flies" that hover around fruit (although to entomologists, drosophilids are known as "pomace flies" or "vinegar flies;" "true" fruit flies belong to the Family Tephritidae). Mycophagy is no exception to drosophilids. More rearings of Drosophilidae were recorded in this study than those of any other dipteran family. In terms of frequency of occurrence, numbers of individuals, and numbers of species involved, drosophilids seem to be the flies most successful at utilizing Amanitas as host mushrooms—including species deadly poisonous to humans. How do they do it? Read on…

The Family Drosophilidae came from a common ancestor that was detritivorous (Courtney et al., 1990) and became selective for rotting substrates supportive of yeast growth, especially fruits. Today, most species of drosophilids feed on decaying fruit material, some are scavengers, and a few feed on fungi. However, it is likely that even the mycophagous drosophilids are feeding on the microbiota of yeast and bacteria within the context of the mushrooms (Lacy, 1984b). Phylogenetic evidence suggests that mycophagy has arisen more than once within the Drosophilidae (Courtney et al., 1990; Lacy, 1984a). All the species reared in this study (*Drosophila busckii*, *D. falleni*, *D. putrida*, *D. neotestacea*, *D. quinarian*, *D. recens*, *D. subquinaria*,

D. suboccidentalis, *D. tripunctata*, *Mycodrosophila claytonae*, *M. dimidiata*, *Leucophenga varia*, and *Scaptomyza graminum*) are known to be mycophagous, detrivorous, or both.

How some mycophagous insects avoid being poisoned by Amanitas in Section *Phalloideae* has been the subject of speculation for a long time. Jaenike postulated that amatoxin tolerance was highly selected for as it might be nematocidal to *Howardula* nematodes that parasitize and render sterile many other mycophagous Diptera. This makes for an interesting story, evolutionarily (one that is still cited to this day), but was later found to be untrue. Wilson (2001) speculated that some Diptera had evolved resistance to amatoxins through genetic mutations of the DNA coding for the target site of amatoxins (RNA polymerase II genes) or by up-regulation of general detoxification enzymes (cytochrome p450s or glutathione S-transferases, or both). These are the same genetic and biochemical mechanisms that underlie resistance to host defenses by pest species. This also is how organisms with powerful defense toxins avoid poisoning themselves. Alas, this also is mostly incorrect in explaining how mycophagous Diptera avoid intoxication by amatoxins.

Stump et al. (2011) performed an elegant study to determine the mechanism of amatoxin tolerance in species of mycophagous Drosophilidae. For their study, they chose eight mycophagous species that are tolerant to amatoxins along with one drosophilid species that is not a mushroom feeder and, thus, susceptible to amatoxins. They performed DNA sequence analysis for the RNA polymerase II gene (the target site of amatoxins) for all the species in the study and concluded there are no differences in the DNA sequences of this gene for any of the species examined. Thus a genetic mutation in the gene sequence for the target site of amatoxins (RNA polymerase) is not the source of amatoxin tolerance. They next performed two feeding assays on the flies, spiking their food with commercially-purchased amatoxin. For one food + amanitin assay they also included a chemical to block the function of cytochrome p450 (cyt p450); for the other assay they added a chemical to block glutathione S-transferase (GST). What they found was that in blocking GST there was no change. Mycophagous flies could

consume the toxin with impunity. When they blocked the cyt p450, toxin tolerance was now blocked and the mycophagous species perished just as the control species did. Stump et al. (2011) concluded that the mycophagous drosophilid species had evolved a cyt p450 mechanism which confers detoxification of amatoxins which makes available a very large food source. Furthermore, the authors speculate that this cyt p450 system may serve as a general detoxification system for mushroom species (e. g. *Cortinarius* or *Omphalotus*) with other classes of toxins.

Literature Cited

Benjamin, D.R. 1995. *Mushrooms: Poisons and Panaceas.* W.H. Freeman and Company, New York. 422 pp.

Berger, K.J., and D.A. Guss. 2005. Mycotoxins revisited, part I. *Journal of Emergency Medicine* 28 (1): 53–62.

Beug, M.W., M. Shaw, and K.W. Cochran. 2006. Thirty-plus years of mushroom poisoning: summary of the approximately 2,000 reports in the NAMA Case Registry. *McIlvainea* 16 (2): 47–68.

Bruns, T.D. 1984. Insect mycophagy in the Boletales: fungivore diversity and the mushroom habitat. Pp. 91–129 in: *Fungus – Insect Relationships: Perspectives in Ecology and Evolution*, Q. Wheeler and M. Blackwell, Editors, Columbia University Press, New York.

Bunyard, B.A. 2016. Mysterious mushrooms and intriguing insects. *Omphalina* 7(6): 13–15.

Bunyard, B.A. 2015. A tripartite relationship between a woodrot fungus, a wood-boring sawfly, and the giant ichneumonid wasp. *Fungi* 8(1): 14–20.

Bunyard, B.A. 2013. A dangerous *Amanita* mushroom is taking North America by storm. Is Global Warming to blame? *Fungi* 6(2): 26–29.

Bunyard, B.A. 2012. The deadly Amanitas of North America: amatoxin and phallotoxin concentration in *Amanita bisporigera* spores. *Fungi* 5(3): 33.

Bunyard, B.A. 2007. Legerdemain in the fungal domain: the use and abuse of insects by fungi. *American Entomologist* 53(4): 238–241.

Bunyard, B.A. 2003. Biodiversity and ecology of mycophagous Diptera in northeastern Ohio. *Proceedings of the Entomological Society of Washington* 105(4): 847–858.

Bunyard, B. and B.A. Foote. 1990b. Biological notes on *Drosophila guttifera* (Diptera: Drosophilidae), a consumer of mushrooms. *Entomological News* 101(3): 161–163.

Bunyard, B. and B.A. Foote. 1990a. Acalyptrate Diptera reared from higher fungi in northeastern Ohio. *Entomological News* 101(2): 117–121.

Buxton, P.A. 1960. British Diptera aassociated with fungi. III. Flies of all families reared from about 150 species of fungi. *Entomologist's Monthly Magazine* 96: 61–94.

Chilton, W.S., and J. Ott. 1976. Toxic metabolites of *Amanita pantherina*, *A. cothurnata*, *A. muscaria*, and other *Amanita* species. *Lloydia* 39(2–3): 150–157.

Chilton, W.S., L. Decato, and M. Malone. 1973. The unsaturated norleucines of *Amanita solitaria*, chemical and pharmacologic studies. *Lloydia* 36: 169–173.

Courtney, S.P., T.T. Kibota, and T.A. Singleton. 1990. Ecology of mushroom-feeding Drosophilidae. *Advances in Ecological Research* 20: 225–274.

Frouz, J. and O.L. Makarova. 2001. Succession of communities of Diptera larvae in decaying fungi. *Biologia Bratislava* 56: 191–197.

Graves, R.C. and A.C.F. Graves. 1985. Diptera associated with shelf fungi and certain other micro-habitats in the Highlands Area of western North Carolina. *Entomological News* 96: 87–92.

Hackman, W. and M. Meinander. 1979. Diptera feeding as larvae on macrofungi in Finland. *Annales Zoologici Fennici* 16: 50–83.

Hanski, I. 1989. Fungivory: fungi, insects and ecology. Pp. 25–68 in: *Insect-Fungus Interactions*, N. Wilding, N.M. Collins, P.M. Hammond and J.F. Webber, Editors, Academic Press, London.

Hosaka, K., and K. Uno. 2012. A preliminary survey on larval diversity in mushroom fruit bodies. *Bulletin of the National Museum of Nature and Science, Series B*, 38(3): 77–85.

Jaenike, J. 1978b. Resource predictability and niche breadth in the *Drosophila quinaria* species group. *Evolution* 32: 676–678.

Jaenike, J. 1978a. Host selection by mycophagous *Drosophila*. *Ecology* 59: 1286–1288.

Jaenike, J. 1985. Parasite pressure and the evolution of amanitin tolerance in *Drosophila*. *Evolution* 39: 1295–1301.

Jaenike, J., D.A. Grimaldi, A.E. Sluder, and A.L. Greenleaf. 1983. α-amanitin tolerance in mycophagous *Drosophila*. *Science* 221: 165–167.

Krivosheina, N.P. 2008. Macromycete fruit bodies as a habitat for dipterans (Insecta, Diptera). *Entomological Review* 88: 778–792.

Lacy, R.C. 1984b. Ecological and genetic responses to mycophagy in Drosophilidae (Diptera). Pp. 286–301 in: *Fungus – Insect Relationships: Perspectives in Ecology and Evolution*, Q. Wheeler and M. Blackwell, Editors, Columbia University Press, New York.

Lacy, R.C. 1984a. Predictability, toxicity, and trophic niche breadth in fungus-feeding Drosophilidae (Diptera). *Ecological Entomology* 9: 43–54.

Martin, M.M. 1979. Biochemical implications of insect mycophagy. *Biological Reviews* 54: 1–21.

Michelot, D., and L.M. Melendez-Howell. 2003. *Amanita muscaria*: chemistry, biology, toxicology, and ethnomycology. *Mycological Research* 107(2): 131–146.

Mier, N., S. Canete, A. Klaebe, L. Chavant, and D. Fournier. 1996. Insecticidal properties of mushroom and toadstool carpophores. *Phytochemistry* 41: 1293–1299.

Papp, L. 1972. Systematical and ecological investigations on fly pests of fungi in Hungary, II. Sphaeroceridae and Asteiidae (Diptera). *Annales Historico-Naturales Musei Nationalis Hungarici* 64: 315–317.

Patterson, J.T. 1943. *Studies in the Genetics of Drosophila III. The Drosophilidae of the Southwest.* The University of Texas Press, Austin, TX. 327 pp.

Pelizzari, V., E. Feifel, M.M. Rohrmoser, G. Gstraunthaler, and M. Moser. 1994. Partial purification and characterization of a toxic component of *Amanita smithiana*. *Mycologia* 86: 555–560.

Pielou, D.P. 1966. The fauna of *Polyporus betulinus* (Bulliard) Fries [Basidiomycetes: Polyporaceae] in Gatineau Park, Quebec. *Canadian Entomologist* 98: 1233–1237.

Pielou, D.P. and W.G. Mathewman. 1966. The fauna of *Fomes fomentarius* (Linnaeus ex. Fries) Kickx growing on dead birch in Gatineau Park, Quebec. *Canadian Entomology* 98: 1308–1312.

Pielou, D.P. and A.N. Verma. 1968. The arthropod fauna associated with the birch bracket fungus, *Polyporus betulinus* in eastern Canada. *Canadian Entomology* 100: 1179–1199.

Ševčík, J. 2010. *Czech and Slovak Diptera associated with fungi.* Slezské zemské muzeum, Opava, 112 pp.

Shorrocks, B. and A.M. Wood. 1973. A preliminary note on the fungus feeding species of *Drosophila*. *Journal of Natural History* 7: 551–556.

Sitta, N., and L. Süss. 2012. Insects parasitizing edible ectomycorrhizal mushrooms, pp. 335–353. In: A. Zambonelli and G. M. Bonito (eds.), *Edible Ectomycorrhizal Mushrooms, Soil Biology.* Springer-Verlag, Berlin.

Stump, A.D., S.E. Jablonski, L. Bouton, and J.A. Wilder. 2011. Distribution and mechanism of α-amanitin tolerance in mycophagous *Drosophila* (Diptera: Drosophilidae). *Environmental Entomology* 40(6): 1604–1612.

Valley, K., T. Wearsch, and B.A. Foote. 1969. Larval feeding habits of certain Chloropidae (Diptera). *Proceedings of the Entomological Society of Washington* 71: 29–34.

Wieland, T. 1986. *Peptides of Poisonous Amanita Mushrooms.* (Springer Series in Molecular Biology) Springer-Verlag, New York. 256 pp.

Wilson, T.G. 2001. Resistance of *Drosophila* to toxins. *Annual Review of Entomology* 46: 545–571.

Yakovlev, E.B. 1994. *Palearctic Diptera Associated with Fungi and Myxomycetes.* Karelian Research Center, Russian Academy of Sciences Forest Research Institute, Petrozavodsk, 128 pp.

Yamashita, S., and N. Hijii. 2007. The role of fungal taxa and developmental stage of mushrooms in determining the composition of the mycophagous insect community in a Japanese forest. *European Journal of Entomology* 104: 225–233.