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Chapter 10

FUNGAL TOXINS RELEVANT TO ANIMALS — THE CASE OF AMANITA

Nobuko Tuno[1], Naoya Osawa[2]· and Chihiro Tanaka[3]

[1]Laboratory of Ecology, Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa 920-1192 Japan

[2]Laboratory of Forest Ecology, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502 Japan

[3]Laboratory of Env. Mycoscience, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502 Japan

ABSTRACT

A wide variety of secondary metabolites occur in fungi and several are toxic to both invertebrates and vertebrates. The evolutionary history and ecological role of fungal toxins, however, remain largely unexplored pending the organization of structured investigations. The fruitbodies of fungi are structures for sexual reproduction that form unique epigeal stages facilitating interactions with mobile terrestrial organisms living above the ground. In terms of fungal fitness such interactions can be neutral, negative or positive. Many invertebrates and vertebrates are known to feed on mushrooms and toadstools. So far, most available natural history studies of fungivorous invertebrates are on those that inhabit in fungi for their developing stage and as such can be specifically recognized. On the other hand, fewer cases of vertebrates have been reported since they do not live in the fruitbodies and it is hard to discriminate predators. The influence of predation on fungal fitness can be variable given the combination of fungal and animal characteristics and timing. The most critical predation in terms of fungal fitness is the one occurring on the initial immature and mature stages of fungi. It is well known that many of the secondary metabolites present in mushrooms are harmful or toxic to animals in general, though little specific information is available. We planed investigate further the different aspects of animal interactions with mushrooms using the genus *Amanita* to clarify the relevance of mushroom poisons. However, we recognized that the paucity of information of mobile fungal consumers on fungi those can be the most important consumers affecting on fungal host fitness. Different procedure are needed to trace

spore feeding insects and mature fruitbodies eating animals since they are not breeding in fruitbodies but they can be the most important predators in terms of fungal host fitness. But this kind of information is scarce in worldwide. To clarify the ecological significance of fungal secondary metabolites we suggest focusing on fungal consumption by mobile animals in the functional stages of macrosporocarps.

INTRODUCTION

It has been suggested that predation as a selective force has shaped the chemical defense systems of fungi [1], but this raises the question of which types of organisms are predators of fungi. Exploitation of fungi by insects occurred early in evolution as feeding on dead or decaying material is probably the primitive condition among the class Insecta[2]. Some insects breed in the poisonous fruitbodies raising the question of whether symbiotic relationships are mediated by fungal toxins.

The most harmful and visually conspicuous mushrooms are found among the agarics belonging to the genus *Amanita*; its roughly1000 species include the death cap and fly agarics. Yet this genus also contains several edible and even palatable species for humans, such as Caesar's mushroom (*A. caesaria*). Species of *Amanita* produce a spectrum of chemical compounds that affect the survival and performance of animals and their physiology and molecular biology have been well studied. Using *Amanita* as a model group we focused on the ecological aspects of fungal secondary metabolites in the interactions between fungi and animals. *Amanita* mushrooms may avoid predation by generating poisons. However, poisonous mushrooms may also serve as a refuge for fungivorous insects that are less harmful to host fungi, or they may facilitate mutualism with some vertebrates that can act as dispersal agents for fungi. But first we examined the possible relationships between fungi and other organisms.

Relationships between Fungi and Animals

Fungi, such as mycorrhizal fungi, litter decomposers, and wood rotting fungi depend on other organisms-the living or dead bodies of plants or animals-for their nutrition. Fungal hyphae are mainly found underground or inside substrates, where they are exposed to unavoidable contact with symbiotic organisms such as soil-dwelling insects, mites, *etc*. In addition, many fungi grow macrosporocarps above the ground as sexual reproductive organs to disperse spores into the air. In this environment, fungi come into contact with more mobile organisms such as flying insects and mammals. Fungus-insect interactions range from complete dependence of entomopathogenic fungi on insects to complete dependence of strict fungivorous insects on fungi accommodating a wide spectrum in between [3]. For fungi aboveground contacts with animals may be transient compared to the lifelong contact with organisms underground or in the substrate. Some wood rotting fungi may have a lifespan lasting many years, but even for such species the spore-discharging period is quite limited[4]. Therefore, through evolutionary time one would suggest that fungi must have developed defense systems to avoid predation of their reproductive organs. Yet the great diversity and

variety of sizes, colors, and odors of mushrooms seem aimed at attracting mobile animals, much the same way as flowers and fruits/nuts for the vectors of higher plants. It is likely that strong selective powers have resulted in this rich array of mushroom characteristics *via* interactions with animals in the epigeal mushroom stage.

There is a wide degree of variation in the degree of contribution to fungal fitness over mushroom parts and fruit body mature stages. In this context, the significance of fruit body predation will have different influences on fungal fitness according to feeding habits. Table 1 lists the possible impacts of fungivory of immature, mature, and decomposing fruit bodies on the fungal host. From the perspective of the fungus, fungivory is important only while the fruit bodies are functional, *i.e.*, in the immature and mature stages, rather than when they reach the dysfunctional decomposing stage[5]. We separated fungi–animal interactions according to degree of fruit body maturity and part of fruit body preyed upon (Table 1). We also estimated the type of predation by two different animal taxa, *i.e.*, mammals and insects. They may differ in their feeding preferences as to part and stage of maturity of the fruit body. In studies of the microfeeding patterns of fungivory it was reported that insect larvae generally feed on parts of fruit bodies, [6, 7] whereas mammals may feed unselectively on the whole fruitbody. Fungivorous insects show a large degree of variation in feeding preferences over the whole range of fruit body maturity to the decomposing stage [7], although little information is available regarding mammalian feeding habits on decaying fruit bodies.

Predation of immature fruitbodies will be simply negative for fungal fitness, with gradation of whole fruitbody predation as the most negative and selective/partial predation as the least one which do not disturb fruitbody-growth.

Table 1. Estimated fungivory influence on host fungi

Mature degree of fruitbody	Predation type	Infulence on fungal fitness
Immarure	Whole body	negative
	Cap	negative
	Partial feeding except for cap	negative/neutral
Mature	Whole body	negative/positive
	Cap	negative/positive
	Spore	negative/positive
	Partial feeding except for cap	neutral
Decomposing	all parts	neutral

Predation on mature fruitbodies is not necessarily negative for fungal fitness since those predators may disperse spores by adhesion or *via* digestive tracts [8-12]. In this case, fruit bodies are analogous to fruits, providing an attractant and reward for animals. Spore dispersal in most epigeal fruit bodies is airborne [13]. However, fungi that are unable to discharge spores into the air, *e.g.*, hypogeous fungi and stinkhorns [14], use animals as vectors for spore dispersal. Hypogeous fungi have evolved from epigeous forms to adopt to arid or freezing environments [9]. Thus, hypogeous forms are evolutionarily driven by the abiotic

environment but require biotic agents for dispersal. Among the epigeal sporocarps, stinkhorns are dispersed by flies [10]. Stinkhorns recruit passing insects, even non-mycophagous species, as spore dispersers, paying the cost of the odor and mucilaginous gleba instead of having substantial flesh [11]. There may be advantages to recruiting animals for spore dispersal in addition to airborne dispersal even for epigeal fungi that can release spores into the air. Wood-rotting fungi utilize dead logs, which are an unpredictable patchy resource. The presence of a guild structure was reported in wood-rotting fungal communities, and the differences in coarse woody debris preference among wood-rotting fungi also structured the communities [15]. Hence, it is likely a more successful strategy for wood-rotting fungi to use animal agents than airborne dispersal, as spore-feeding insect guilds may be present in decaying logs harboring spore-discharging macrofungi [12, 16]. There may be important advantages for spores of wood-rotting fungi to pass through the digestive tracts of insects. One species of *Ganoderma* cannot germinate without passing through the guts of tropical fly larvae [17]. Spores of *Ganoderma applanatum* are dormant until exposed to high temperatures of 30°C to 40°C for 24 to 48 h [18]. At warm temperate to subtropics, *Mycodrosophila* flies apparently not only fed on spores but excreted them [12, 16]. Analogous to fruiting plants that utilize animals as seed dispersers, *G. applanatum* may provide insects with some nutritional substance. Plant pathological studies have shown that extracellular mucilage or adhesives consisting of polysaccharides and proteins or glycoproteins are common on fungal germlings. Scanning electron microscopy showed a layer of coating on the surface of the spores of several species that was cleared in the recta of *Mycodrosophila* flies (Tuno, 1999; Figure 1AB). This coating, thought to be the outer cell wall, may prevent germination, similar to the pulp of many kinds of fruits. In addition, digestion of the outer cell wall by insects may promote spore germination. However, *Trametes palisotii* spores in spore-feeding beetles of Endomychidae appear to be destroyed and digested (Tuno unpublished; Figure 1C). The fate of spores is determined by a combination of the physical features of the spores themselves and the morphological features of the mouthparts of insect predators (suctorial or mandibulate). Some wood-rotting fungi yield different types of spores, *i.e.*, proterospores and normal spores [19]. Proterospores are formed exclusively at the beginning of sporulation, and other spores are developed later by the same fruit body. Proterospores germinate readily, whereas other spores must first pass through the gut of a fly larva. Proterospores are therefore well adapted to dispersal by air, whereas other spores are adapted to dispersal by animals [19]. Hypogeous mycorrhizal fungi depend on animals for spore dispersal [8, 9, 14]. There may be advantages to animal-borne spore dispersal in epigeous ectomycorrhizal fungi; however, the contribution of animal consumption to host fungal fitness has not been examined.

Predation of decaying fruit bodies has no effect on fungal fitness, as such bodies are nonfunctional except for some perennial bracket fungi. The maturation of these spores and the fruit body does not necessarily coincide; a soft and ephemeral fruit body may be decomposing but contain mature spores. In this case, predation will influence fungal fitness and should be considered as feeding on the mature stage.

Therefore, the influence of predation on fungal fitness can be negative or positive given the combination of fungal and animal characteristics and timing. The fungal host should discourage undesirable predators. First, it should avoid predators that feed on young fruit bodies as long as the predation disturbs fruit body maturation. Second, it should avoid predators that are able to digest spores. Diverse groups of animals prey on fruit bodies, such

as mammals, slugs, and insects [8, 20-26]. Among these, predation by those that may disperse spores to favorable substrates for colonization or enable spores to germinate will be encouraged.

Ecological Significance of Fungal Poison

We have examined some possible interactions between fungi and animals. Here, we consider the significance of poisons in fungal fruit bodies in this context. Fungal poisons may be neutral, or they may play a role in predation avoidance or in mutualism. Fungal toxins may be produced in the course of necessary metabolism or may be artifacts, as sporocarps accumulate heavy metals and other toxic substances [27]. However, to our knowledge, there have been no studies differentiating neutral and biologically interactive aspects of fungal toxins.

The predation avoidance hypothesis predicts that poison concentration may vary among mature stages and fruit body parts in a manner consistent with relative contribution to fitness. Few data are available regarding the microdistribution of poison with regard to fruit body part and stage of maturity, except among the fly agaric, *Amanita muscaria*. This fruit body has traditionally been used for the eradication of flies and other insects [28]. Fruit bodies of *A. muscaria* contain ibotenic acid [29]. Figure 2A classifies the degree of maturity and division of the fruit body; changes in ibotenic acid concentration with stage of maturity are shown in Figure 2B. These data indicate which parts of the fruit body and which stage of maturity are the most poisonous. The highest concentrations were detected in the caps that produce spores in the immature stages. These observations suggest that ibotenic acid is produced selectively and distributed specifically in the immature stages and in the caps as a defense against predation. The concentration of ibotenic acid increased and then decreased according to maturation stage, and mature fruit bodies did not show the highest level of protection. It is interesting that Stømer and Janak [28] reported a lower concentration of ibotenic acid in spores than in caps of *A. muscaria*.

Next we examined whether mutualism is mediated by fungal chemical compounds. Mutualism between fungi and consumers can be viewed from two perspectives—that of the fungus and that of the animal. Fungal toxins could originally defend against predation, although they may have been involved in selecting rather than avoiding predators. Studies in temperate regions have suggested that chemical defense in fruit bodies more likely evolved as a defense mechanism against mammalian consumers rather than invertebrates[30]. However, many types of fruit body are significantly preyed upon by invertebrates in tropical forests[5]. Nonmycophagous *Drosophila melanogaster* were reared in medium with fruit body powder to examine the effects of fungal toxins on insects. Approximately half of the fungal species inhibited fly growth: 81 of 127 species [31] and 79 of 175 species [27]. Both of these studies indicated that far fewer fungal species had similar effects on mycophagous insects [27, 31]. Similar results were reported with regard to the application of amanitin, which is harmful to all eukaryotes [32], or ibotenic acid, which is harmful to insects [33]; *i.e.*, mycophagous species were unaffected by these poisons, whereas nonmycophagous species showed high mortality rates. Fungivorous insects generally utilize a variety of fungal species, but there are still feeding preferences [5, 16, 34]. North American fruit flies inhabiting *Amanita* fruit bodies may have been selected to become tolerant to or to prefer fruit bodies that are seldom infested

by nematodes [35]. *Pluerotus* fruit bodies feed on nematodes [36] and are themselves the primary food source for some fruit fly species in some parts of Japan [37]. Kimura (1980) suggested that the primary fungivorous habit may have evolved because of competition or parasitism [37].

In summary, the relationships between fungi and the animals that feed on their fruit bodies vary greatly from immature to mature stages of sporocarps, and thus the significance of chemical defense differs by stage: (1) immature sporocarps should be most poisonous; (2) predation of mature sporocarps may be promoted unless the spores are destroyed, therefore becoming less poisonous; and (3) consumption of decomposing sporocarps does not affect fungal fitness. It is necessary to focus on predation of immature and mature stages to investigate the significance of fungal poisons. A number of dipteran families inhabit fruit bodies [7, 21, 38, 39], and their larval growth is often restricted to a single fungal species, in contrast to mammal fungivores, which usually feed on multiple fungal bodies or species. Under these conditions, it does not take long for flies to acquire tolerance against fungal toxins. Fungivorous insects are able to digest mycelia, although spores are generally unharmed, which may be an adaptation to promote dispersal and maintain inoculum potential [40].

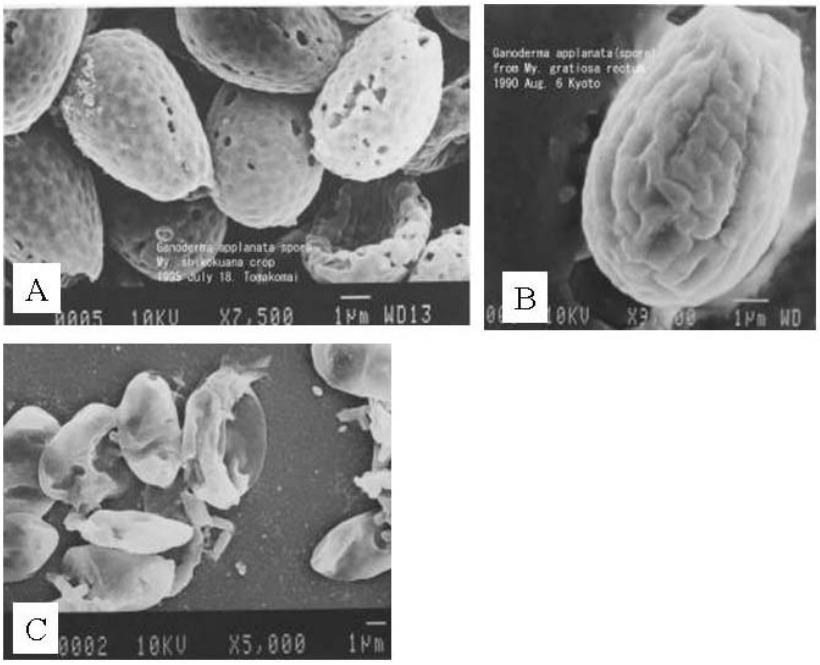

Figure 1A. *G. applanata* spores in *Mycodrosophila*'s crop (Drosophilidae, Diptera), spores are before digestion. Fig. 1B. *G. applanata* spores in *Mycodrosophila*'s rectum, spores are after digestion and the outer tick coating appear to be removed. Fig. 1C. *Trametes palisotti* spores in a beetle's digestive tract (Endomychidae, Coleoptera), The spores appear to be destroyed.

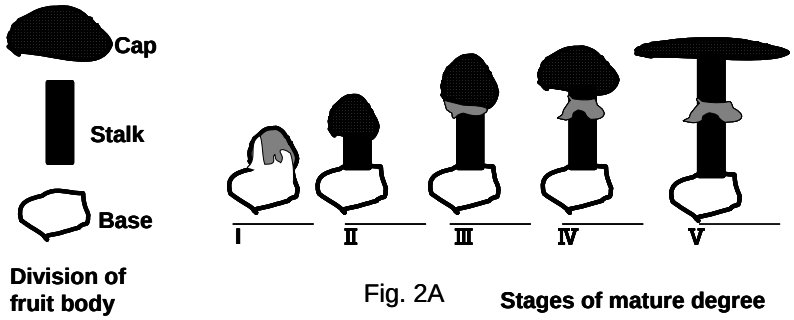

Fig. 2A

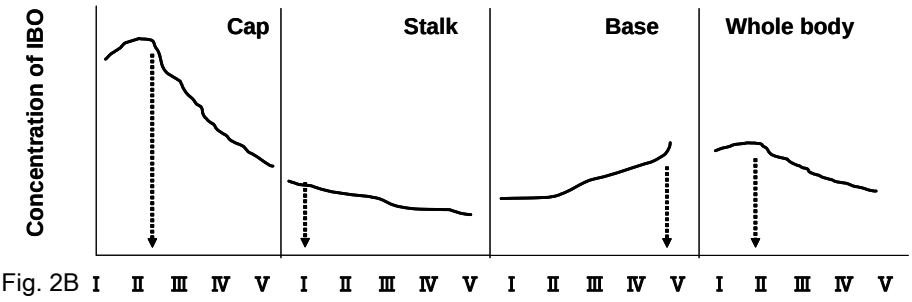

Fig. 2B

Figure 2A. Classification for mature degree, and division of fruit body, *Amanita muscaria* in Tsunoda et al 1993. Fig. 2B Changes of Ibotenic acid concentration in the parts for the stages, *Amanita muscaria.* Data was modified from Tsunoda et al 1993.

Amanita muscaria, easily recognized by its red cap adorned by white volva scales, is the best known of all mushrooms in the world. It is distributed in Europe, North America and Asia, and has been introduced into other parts of the world like New Zealand as a mycorrhizal partner on the roots of imported trees. We plan to investigate animal consumption of *Amanita* among continents to clarify the evolutionally process.

The Biogeography of Fungivorous Insects on the Fly Agaric Amanita muscaria – Case Study in New Zealand

The flora and fauna of New Zealand are unique, as the country contains many ancient "Gondwanan" elements. There are 2500 plant species in New Zealand, among which 80% are endemic[41]; 95% of a total of 18000 insect species are endemic to the area [42]. Several species of various zoological taxa are distributed in New Zealand: *e.g.*, there are 20 species of butterfly [41] (*cf.* 290 species in Japan[43]) and only two native mammal species (two bats[41]). These unique biogeographical distributions of flora and fauna imply that (1) there are many vacant niches for flora and fauna in New Zealand, and (2) these niches may provide new habitats for many exotic species. Yet the invasion of exotic species and human activities may destroy the delicate balance among the relatively few endemic flora and fauna; *e.g.*, there

were 60 species of native birds before human settlement, of which 40% have become extinct[41].

The unique flora and fauna of New Zealand may also contribute to its unique fungi. Among the fungi, the genus *Amanita* is one of the most well-known macrofungal genera. *Amanita* species inhabit mainly forests and develop small- to large-size fruit bodies[44]. At present, *ca*. 500 *Amanita* species have been described[45]. Most *Amanita* species are ectomycorrhizal fungi[44] associated with birch (*Betula*), pine (*Pinus*), fir (*Abies*), and larch (*Larix*)[46]. These observations suggest that there may be strong relationships among plants and *Amanita* species. Seventeen species belonging to the genus *Amanita* have been recorded in New Zealand: *A. australis*, *A. inopinata*, *A. karea*, *A. mappa*, *A. mumura*, *A. muscaria*, *A. nauseosa*, *A. nehuta*, *A. nigrescens*, *A. nothofagi*, *A. pantherina*, *A. pareparina*, *A. pekoides*, *A. phalloides*, *A. pumatona*, *A. rubescens*, and *A. taiepa* [47]. *Amanita muscaria* has a wide geographic distribution, occurring in Europe; Asia; Africa; Australia; New Zealand; and North, Central, and South America. *A. muscaria* was first recorded in New Zealand in 1937, and its distribution expanded from 1970 to 1980. *A. muscaria* is now distributed throughout New Zealand[47] .

Some *Amanita* species (*e.g.*, *A. phalloides*, *A. verna*, and *A. virosa*) contain deadly peptides (amanitins, phallotoxins, and virotoxins)[44]. Others (*e.g.*, *A. muscaria* and *A. pantherina*) contain ibotenic acid, muscimol, and muscarin[44], which are hallucinogenic. Although these agents are not normally fatal to humans, they are fatal to many types of insects. These observations imply that certain types of fungivorous insects can utilize the *Amanita* species, and therefore characteristic fungivorous communities are observed on the *Amanita* species. In fact, characteristic drosophilid communities hasz reported on *Amanita* fungi[24, 48, 49].

The molecular approach to determining the phylogeny and biogeography of *A. muscaria* was first reported by Oda *et al.* [50], who indicated three geographic clades (*i.e.*, Eurasian, Eurasian-alpine, and North American groups). Furthermore, Geml *et al.* [46] recently conducted phylogenetic and nested clade analyses and determined the phylogeographic history of the species complex. Although phylogenetic analyses confirmed the existence of the three clades, representatives of all three groups were found to occur sympatrically in Alaska, suggesting that they represent cryptic phylogenetic species with partially overlapping geographic distributions rather than allopatric populations. They also suggested that the Siberian–Beringian populations later evolved into separate species and expanded their range in North America and Eurasia, although the precise mechanisms for the maintenance of the three phylogenetic species in Alaska were less well documented. The fungivorous insects feeding on the three species of *A. muscaria* in Alaska may play a role in maintaining the phylogenetic species through the reproductive isolation of spore dispersal. However, there have been no studies of the geographical and phylogenetic species-related variations of the fungivorous insects that feed on *A. muscaria*.

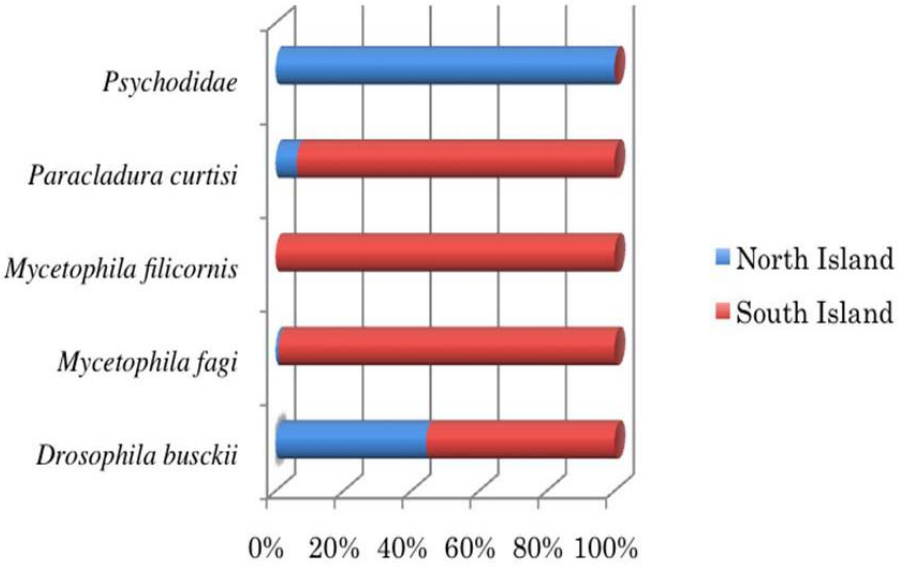

Figure 3. The percentage of major fungivorous insects on *A. muscaria* on North and South Island in New Zealand.

From this biogeographical viewpoint, fungivorous insects that feed on *A. muscaria* in New Zealand are intriguing because *A. muscaria* invaded New Zealand only recently, and we can investigate the actual evolutionary manifestations of the spreading processes for both the fungus and the related fungivorous insects. Furthermore, the invasion process of *A. muscaria* may be uneven throughout New Zealand, and therefore differences in fungivorous communities may be clarified in relation to biogeographical, vegetation-related, and *Amanita* phylogenetic variations. Another interesting point is whether the fungivorous insects feeding on *A. muscaria* are endemic and/or exotic; *i.e.*, whether exotic fungivorous insects that feed on *A. muscaria* also invaded toward New Zealand, and/or whether endemic fungivores feeding mainly on the genus *Amanita* expanded their niche and utilized endemic *A. muscaria*.

Based on the results of a DNA phylogenic study, Arensburger *et al.* [51]clarified that New Zealand cicadas form two well-defined clades: one clade groups with Australian taxa and the other with New Caledonian taxa. These results suggest that cicada species likely colonized New Zealand in two or more invasions. They also suggest that there are different biogeographical histories between the North and South Islands of New Zealand, implying that there are differences in the community structure of fungivorous insects between the two islands invaded recently by *A. muscaria*.

We conducted field research on the community structure of the fungivorous insects feeding on *A. muscaria* at various biogeographical locations (North *vs*. South Island) in New Zealand. For this purpose, the fruit bodies of *A. muscaria* from endemic and exotic host plants (endemic species: *Nothofagus* [Fagaceae]; exotic species: *Betula* [Betulaceae], and *Pseudotsuga* and *Pinus* [Pinaceae]) were collected from April to May 2005 and 2006 at 15 locations on the North and South Islands. Fungivorous insects were collected in insect traps from *A. muscaria* fruit bodies (one to three combined) and identified at the species level.

Figure 3 shows the percentages of major fungivorous insects from *A. muscaria* in New Zealand. *Drosophila busckii* was equally observed on the North and South Islands;

Mycetophila fagi, *M. filicornis*, and *Paracladura curtisi* were mainly observed on the South Island; and Psychodidae were mainly observed on the North Island as fungivores feeding on *A. muscaria* (Figure 2). There was a significant difference between the North and South Islands (χ^2-test for number of individuals: $d.f. = 4$, $\chi^2 = 363.425$, $P < 0.0001$; Figure 3).

Fungivorous insects are generally recognized as polyphagous species [51-53]. Hanski [53] proposed two hypotheses, the quantity and qualitative hypotheses, to explain why polyphagy is widely observed in fungivorous insect communities. The quantity hypothesis suggests that polyphagy is due to the low predictability of occurrence of fungal fruiting bodies[54], whereas the qualitative hypothesis suggests that the differences in chemical traits between host species are not major barriers to wider host use. Agaric fruit bodies contain insecticidal compounds that affect nonfungivorous insects[32], and toxicity differs among fungal species[27, 31]. These results suggest that these fungal insecticides do not affect those fungivorous insects that are adapted to them[27, 31, 32, 53] and thus the fungivores have wide host ranges. Burns [6] divided fungivorous insects into two groups based on their food habits: (1) primary fungivores, *e.g.*, Mycetophilidae and Phoridae, which feed on fungal tissues; and (2) secondary fungivores, *e.g.*, Drosophilidae and Psychodidae, which feed on microbes living on the fruit body. The European *Drosophila*, included within the fungal breeding species[6, 55], can be conveniently divided into two groups based on resource utilization patterns: (1) "obligate" fungal species (*D. phalerata*, *D. transversa*, *D. kuntzei*, *D. limbata*, *D. testacea*, *D. histrio*, *D. cameraria*, and *D. confusa*) and (2) "facultative" fungal species (*D. subobscura*, *D. funebris*, *D. busckii*, and *D. replete*)[56].

Our study also showed that there were significant differences in fungivorous insects feeding on *A. muscaria* in various forests between the North and South Islands (Figure 1). These observations suggest that there are differences in the evolutionary history of interaction between insects and *A. muscaria*. Each population of *M. fagi*, *M. filicornis*, and *P. curtisi* on the South Island may have a longer evolutionary history than those on the North Island, and thus certain populations of endemic fungivorous species *M. fagi*, *M. filicornis*, and *P. curtisi* on the South Island can utilize *A. muscaria* as their hosts, with the reverse being true on the North Island. The evolutionary differences between the North and South Islands were also determined by phylogenic studies of the large flightless takahe (*Porphyrio hochstetteri*;)[57] and giant weta (Deinacrida)[58].

Plants that are endemic to the North Island in New Zealand are predominantly woody, whereas those on the South Island are distinctly less so[59]. The distribution of local endemics (*i.e.*, endemic dicotyledonous plants and insects) is remarkably uneven (largely divided into two areas in the North Island and three in the South Island)[42]. These observations suggest that the possibility may still exist of differences in habitats of fungivorous insects feeding on the genus *Amanita* between the North and South Islands of New Zealand, because ectomycorrhizal fungi such as *Amanita* have a strong relationship with plants. However, we sampled endemic and exotic vegetation over a wide area in New Zealand.

Our study showed that Psychodidae was dominant on the North Island, whereas *M. fagi*, *M. filicornis*, and *P. curtisi* were observed mainly and/or only on the South Island. Furthermore, *D. busckii* was observed equally on both the North and South Islands. The different fauna may be interpreted as that fungivorous species are dominant in the south island whereas the niche is vacant in the north island being occupied by universal or saprophagous species. We found that *Drosophila bizonata* and Phorid species were dominant

in central Japan[24, 64] whereas, *Hirtodorosophila quadrivittata* species groups were dominant in northern Japan [65, 66] in *Amanita* species. It appears that *Amanita* species are colonized by fungivorous species with broad food preferences. Mycetophilidae, with *ca.* 3000 species recorded around the world, are most abundant in humid areas, especially moist woodland areas, and many larvae live in fleshy or woody fungi or in dead wood, under bark, or in the nests of birds or squirrels[60]. Burns[6] reported that *M. fagi* and *M. filicornis* are primary fungivores, which means that their larvae may feed on the fungal tissue of *A. muscaria*. *M. fagi* and *M. filicornis* are endemic species in New Zealand and are common fungus grants that are abundant in native forests and scrub as well as in suburban garden habitats (Toft, personal communication). Therefore, the endemic fungus grants *M. fagi* and *M. filicornis* expand their niches toward the exotic *A. muscaria* mainly on the South Island. This study clarified that the exotic *D. busckii* emerged from the exotic *A. muscaria*. *D. busckii* is a cosmopolitan saprophagous species[61] and is regarded as a secondary[6] and "facultative" [55] fungal species, although less ecological information for *D. busckii* was obtained on *A. muscaria*. Furthermore, the survival and pupal sizes of fungivorous *Drosophila* species *D. bizonata*, *D. angularis*, and *D. brachynephros* were unaffected by ibotenic acid and/or musimol, which are present in *A. muscaria*, whereas those of frugivorous species *D. immigrans* and *D. melanogaster* were negatively affected[33]. These observations suggest that there are large variations in tolerance toward ibotenic acid and musimol among *Drosophila* species based on food habits. These circumstantial and fractional lines of evidence suggest that the larvae of *D. busckii* feed on microbes living on *A. muscaria* that may be decaying and/or less toxic. Our study showed that the endemic species *P. curtisi* emerged from exotic *A. muscaria*. *P. curtisi* belongs to the family Trichoceridae, with only *ca.* 100 species recorded around the world; 25 of these species are recorded in the genus *Paracladura* in Asia and New Zealand, a few are found in extreme southern South America, and a single species is found in western North America[62]. The distribution patterns imply that *P. curtisi* may be a type of ancient Gondwanan element. Less ecological information is available regarding *P. curtisi*, but early larvae of Trichoceridae are scavengers found in a variety of habitats, especially in decaying leaves and vegetables, mature fungi, stored roots and tubers, burrows of rodents, and other comparable materials[62]. This suggests that *P. curtisi* is a secondary fungivore that feeds on the microbes living on decaying *A. muscaria*. Psychoidids are widely distributed in many niches [63], however, there is no information available on their fungal poison tolerance.

There is no data to evaluate the significance of the fungivores on the host *A. muscaria*. There was few spore feeding insects during our sampling. Different procedure are needed to trace spore feeding insects and mature fruitbodies eating animals since they are not breeding in fruitbodies but they can be the most important predators for fungal host. But these kind of information is scarce in worldwide not only from New Zealand. The poison in the genus of *Amanita* has comparatively well studied[27-29, 31-33, 67], however, so few mobile animals predation except for dipteran larvi has documented. Animal predations' contribution has been overlooked even on *Amanita muscaria*. It is recommended to clarify animal predation in immature and mature stage of the fungi in their world wide distribution since it appears to yield poison to defend animals but the significance can be adverse.

CONCLUSION

Fungus-insect interactions range from complete dependence of entomopathogenic fungi on insects to complete dependence of strict fungivorous insects on fungi accommodating a wide spectrum in between. Among the antagonistic fungus-consumer relationships, the consumption by insects has received considerable attention and these studies have generally been skewed towards a zoo-centric point of view. This bias has resulted in other effects of fungivory being largely overlooked and few complementary studies have been undertaken. However, the most critical consumers are those feeding on fruitbodies in its immature stage affecting it in a negative way and mobile animals feeding on the mature stage that possibly affect fungi in positive way. We again recognized that the paucity of information of mobile fungal consumers on fungi.

To clarify the ecological significance of fungal secondary metabolites we suggest to focus on fungal consumption in the initial immature and mature stages. Many studies have been carried out to investigate fungal consumers by rearing emergent adults from fruitbodies but new sampling methods needs to be developed for the study of spore feeding animals. Our survey on *Amanita muscaria* in New Zealand, where the species newly invaded, found a different fauna between the South and North Island and those are further different from the fauna living on another *Amanita* species in Japan. These results suggests to us that the fungivorous fauna is actually reflecting the local fauna, even in similar fungal host species, and that we need to carefully select the study sites in order to reveal more general trends.

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